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INFECTION OF OUTPLANTED DOUGLAS-FIR
SEEDLINGS BY VERTICICLADIELLA WAGENERI
(BLACK STAIN ROOT DISEASE) WHEN
PLANTED AROUND INFECTED
DOUGLAS-FIR STUMPS

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Figure 1. Roots of infected overstory become source of infection for seedlings planted within several years after harvest.

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METRIC CONVERSIONS

1 ft	=	0.305 m
1 in	=	2.54 cm
1 ac	=	0.405 ha
1 ft	=	0.093 m
1 ft	=	0.028 m
1 °F	=	0.556 °C
1 mi	=	1.609 km

Abstract

More than 25,000 2-0 Douglas-fir seedlings were planted around stumps of recently harvested Douglas-fir and coast redwood. The Douglas-fir stumps had previously been graded from non-infected to light-through heavy black stain root disease infection levels.

Seedling infection levels were highest around Douglas-fir stumps having light to heavy infection levels, while infection levels were lowest around apparently uninfected Douglas-fir and redwood stumps. There was no difference in level of seedling infection between the clearcut and the selection cut test areas.

INTRODUCTION

Verticicladiella wagneri Kendr., the cause of black stain root disease, (BSRD), was first reported as a pathogen on Douglas-fir, in 1967 (3). Since that time, the fungus has been reported with increasing frequency in Douglas-fir throughout most of the range of the host (2,5,8,12,14). The increased incidence of the disease in Douglas-fir may be related to greater awareness and detection or to an increase in plantation management and stand disturbances (6,7,8,9), or both. In either case, the level of infection in Douglas-fir along the northern coast of California is cause for concern (13).

To date, most of the published research reports have dealt with the disease in pine, which is caused by a variant of V. wagneri that may differ in several respects from that which infects Douglas-fir (8). As a result, relatively little is known about the biology/epidemiology of the fungus in Douglas-fir, and even less can be said about its control. Hylastes nigrinus, a root-feeding bark beetle, is probably the major aerial vector of the fungus. V. wagneri apparently has little capacity to move through soil to cause infection of unwounded roots of Douglas-fir (11,16), although the variant in pine may do so (10).

Some of the more pertinent questions needing study include the following: can the fungus infect outplanted seedlings around stumps of trees that were infected prior to harvest; if so, how long can the fungus persist to infect outplanted seedlings; can the fungus persist in infected roots and seedlings to spread and infect the Douglas-firs as they grow into saplings and larger trees; and can differences in silvicultural harvesting methods influence the incidence of infection of the seedlings? The current studies reported herein were designed to provide information in these areas.

METHODS

Study design involved establishment of seedling plots around stumps of freshly harvested trees of Douglas-fir and coast redwood representing several infection severities in both a clearcut and a selection cut. Trees in the selection cut were harvested from mid-March through mid-June, 1981. Those in the clearcut were harvested from mid-August through early October, 1981. Both harvests occurred in adjacent areas of the same stand. Stand age was 80 years, and composition of the commercial species by volume was 45% Douglas-fir, 43% coast redwood, and 12% grand fir. Average tree diameter at breast height (1.3 M) was 67 cm. The aspect varied from N30°E to N30°W, and slope was 0-50%. Soil type was Hugo. Stocking in the selection cut was reduced from approximately 86.4 sq. m./ha (226 stems) of basal area to 45 sq. m./ha (108 stems).

Plots were established around selected Douglas-fir and redwood stumps in the clearcut and around Douglas-fir only in the selection cut. Stumps selected in the clearcut were (i) redwood (a non-host species), (ii) apparently uninfected Douglas-fir, (iii) lightly-infected Douglas-fir, (iv) moderately-infected Douglas-fir, and (v) heavily-infected Douglas-fir. In the selection cut there were just two types of plots: (i) apparently uninfected Douglas-fir, and (ii) infected Douglas-fir.

The different BSRD stump infection severities, chosen to determine if there might be a correlation with seedling infection, were based on the amount of characteristic *V. wagneri* staining on freshly-cut stumps. It had previously been observed through excavation of roots of infected trees that the presence of BSRD in roots is usually accompanied by disease symptoms at stump height. Earliest disease expression on stump surface is in the form of pathological heartwood, which often precedes the actual presence of the distinctive "black stain" (1).

Infection level of the Douglas-fir stumps used in this study is based upon the percentage of the surface area of a freshly recut stump that has the characteristic pathological heartwood/black stain symptoms found in known BSRD infected trees. Each stump was recut after completion of the logging operation to provide a fresh surface which was immediately sprayed with sodium hypochlorite (Clorox), a process that facilitates observations of stain patterns (1). Examinations were made immediately, and the percentage of xylem circumference with stain was recorded. If no staining or unusual heartwood formation was observed, the stump was considered apparently uninfected; stain around less than 10% of the circumference was categorized as light infection, 10-25% was moderate infection and >25% was heavy infection.

Two-year-old Douglas-fir bareroot seedlings from Ben Lomond Nursery (CDF) were planted in plots during late January and early

February, 1982, 4 to 10 months after the logging operation. Plots of approximately 440 Douglas-fir seedlings (21 rows x 21 columns) at 60 cm spacing with the stump at the center were established around each of ten stumps of each type. With the exception of the moderately diseased plots on the clearcut, all seedlings were examined on six occasions: July-August 1982, February 1983, May 1983, November 1983, May 1984, and May 1985. The moderately diseased plots did not differ from other disease categories in the early examinations and were skipped in May 1983 and May 1984. Thus, they are excluded from the comparison analyses.

During the first three examinations, each plot location (usually 441 seedlings/plot) was visited and the seedlings were recorded as missing, or living/dead (including obviously dying). The dead seedlings were pulled from the ground, and the major roots were examined. If no disease or insect attack was evident, a zero (0) was recorded. If insect activity was present, an (I) was recorded; if the typical stain of V. wagneri was present, an (S) was recorded; and in the case of both, an (IS) was recorded. To attempt to determine why the "0" seedlings died was considered to be beyond the scope of the study.

RESULTS

Initial occurrence of seedling mortality associated with V. wagneri infection appeared 6-7 months after planting (Figure 2). Mortality on plots surrounding infected stumps increased substantially 12 months after planting. Mortality increased on the redwood and apparently uninfected plots, but at a much slower rate during this time. The rate of mortality continued to accelerate through 15 months, but has slowed over the following 12 months of observations. Most of the insect-related mortality appeared to occur during the first 12-18 months after planting.

The percentage of seedlings that died in the selection cut without infection by V. wagneri or obvious insect attack increased from 2.6 percent after 6 months to 7.0 percent in 39 months from planting. Most of such mortality occurred within the first year and was due to poor planting. In the clearcut, the mortality in this category was similar; 4.7 percent after 6 months to 7.2 percent in 39 months after planting.

The percentage of missing seedlings, based on the number of potential planting sites averaged about 11 in the selection cut and 13 in the clearcut. Most of the missing seedlings could be explained on the basis of obstructions such as logs and stumps which precluded planting.

INFECTION (PERCENT MORTALITY)

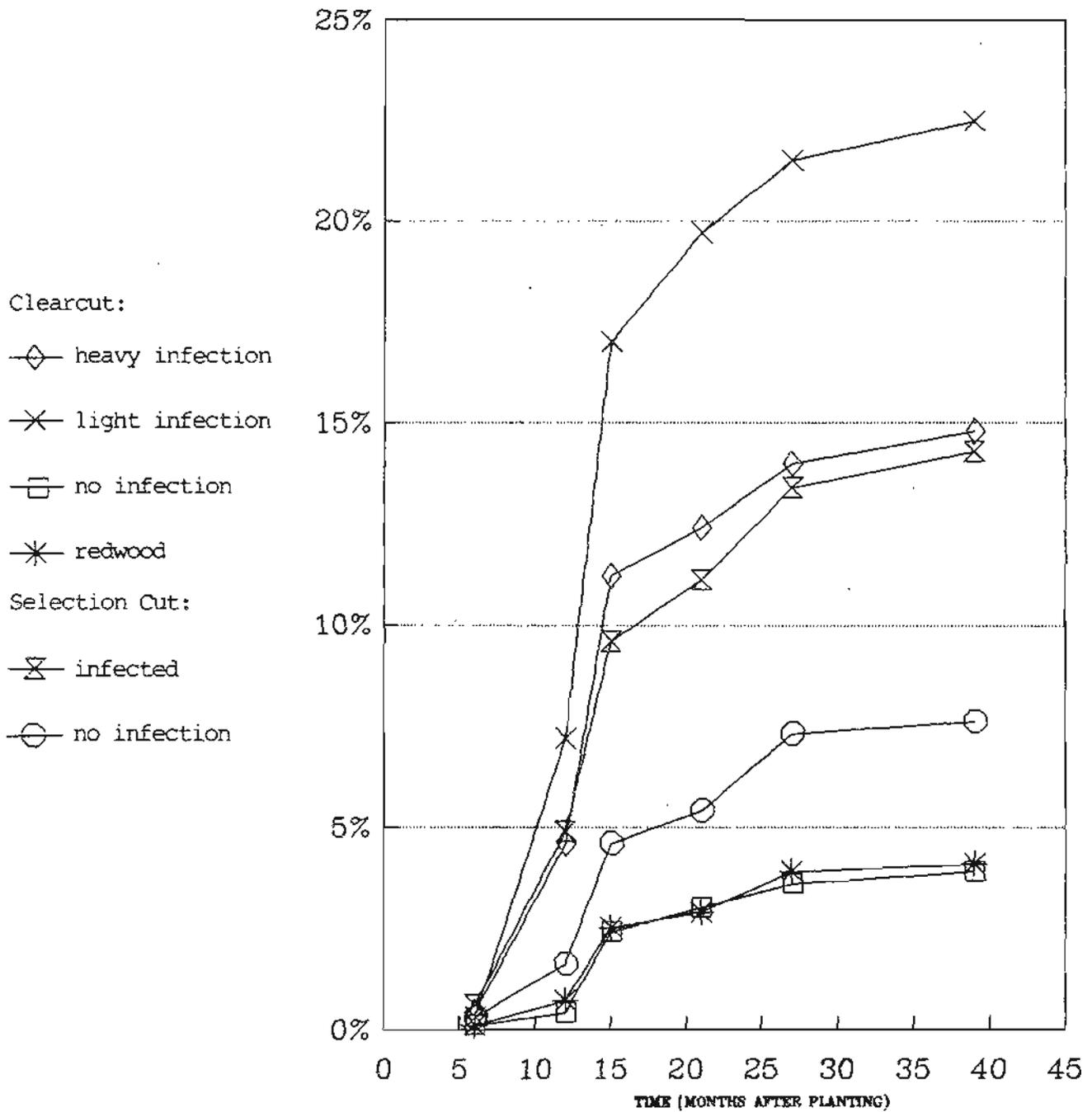


Figure 2. Development of seedling mortality caused by *V. wagneri* infection on the selection and the clearcut planting sites through 39 months after planting in Feb. 1982.

Seedling mortality attributed to *V. wagneri* in the plots varied considerably (Table 1). Plots surrounding uninfected Douglas-fir and redwood stumps in the clearcut had the lowest average mortality at 3.9 and 4.1 percent respectively (Table 2). These plots had significantly less infection (0.05 level of probability) than plots surrounding apparently healthy trees in the selection cut. There was no significant difference between the infected Douglas-fir in the selection cut and the heavily infected Douglas-fir in the clearcut. However, highest seedling mortality occurred in plots surrounding lightly infected Douglas-fir in the clearcut. This difference was significantly higher than any other infection level in this study.

Table 1. Ranges of mortality caused by *V. wagneri* over the 10 plots of the six study types.

Silvicultural Method	% Seedlings Killed
Selection Cut:	
Non-infected D-f	0.8 - 21.1
Infected D-f	1.6 - 54.2
Clearcut:	
Redwood	0.3 - 14.4
Non-infected D-f	0.3 - 15.4
Lightly Infected D-f	7.7 - 44.0
Heavily Infected D-f	1.0 - 64.4

Table 2. Douglas-fir seedling mortality and cause during the first 29 months after planting.

Silvicultural Treatment	No. of Seedlings Planted ^{1/}	Total Seedling Mortality No. %	Seedling Mortality Groups									
			(1) Non-Insect or Black Stain		(2) Insects Only		(3) Insects and Black Stain		(4) Black Stain Only		Total Mortality caused by Black Stain ^{2/} (columns 1 + 4)	
			No.	%	No.	%	No.	%	No.	%		No.
Selection Cuts												
Non-Infected D-f	1983	350 19	200	7.0	166	4.2	190	4.8	114	2.9	304	7.6 B
Infected D-f	1915	1019 26	284	7.2	175	4.4	111	7.9	249	6.4	560	14.2 C
Total	3898	1769 37	564	7.1	341	4.2	301	5.2	363	4.6	864	10.9 C
Clearcuts												
Redwood	1699	971 36	281	9.5	488	11.7	51	1.4	190	7.7	151	6.1 A
Non-Infected D-f	1541	811 21	256	7.2	418	11.8	49	1.4	89	2.5	137	5.9 A
Lightly Infected D-f	1726	1678 45	321	8.6	520	14.0	112	6.9	505	13.6	837	22.5 B
Heavily Infected D-f	1626	1331 37	380	10.8	405	11.2	236	6.5	100	6.7	536	14.0 C
Total	14593	4791 31	1318	9.0	1612	13.4	167	4.6	994	8.8	1661	13.4 C

^{1/} The numbers of missing seedlings, and those seedlings not planted because of obstructions at the seedling planting spot are not included in these summaries.

^{2/} Differences between treatments having the same upper case letter are insignificant at the 0.05 level.

DISCUSSION

Preliminary studies of infection of outplanted ponderosa pine (*Pinus ponderosa*) seedlings had yielded very low levels of infection during the first 2-3 years of observation (Cobb and Goheen, unpublished). Based on those results, we decided to plant more than 25,000 seedlings in 70 plots for this study in Douglas-fir. The high level of infection, up to 64 percent in one plot, and the rapidity with which it became evident were unexpected.

To establish this study, the clearcut stand was intensively surveyed to find a sufficient number of well-placed Douglas-fir stumps for each of the four infection categories previously noted. The examination of 307 Douglas-fir stumps gave the following data on BSRD infection severity: heavy - 9.1%, moderate - 4.2%, light - 7.5%, non-infected - 79.2%. Therefore, relatively high mortality levels could be expected around some 20% of the stumps, with lesser mortality rates around the remainder and away from stumps on this study site.

Comparisons of the infection percentages for the various treatments 39 months after planting (Table 2) yields several observations worth noting. First, there is no real difference between the overall level of infection between the plots on the selection cut and the clearcut: around eleven percent for each silvicultural method. Second, infection levels around infected stumps on the selection cut are not significantly different than around heavily infected stumps in the clearcut. Third, infection levels around lightly infected stumps in the clearcut is significantly higher than any other combination of silvicultural method and stump source. However, the infection level associated with non-infected stumps in the selection cut was significantly higher than that found on either the redwood or non-infected Douglas-fir stumps in the clearcut.

The apparent inconsistencies with regard to seeding infection are probably not due to site differences or seedling host susceptibility, but probably that while survey for black stain presence can be accomplished through stump assay, the true infection status of the stump root system is not known. The data (Table 2) does seem to indicate that black stain symptoms at stump height reveals high potential for planted seedling mortality associated with that stump.

There appears to be a "background" level of infection potential around stumps with no black stain symptoms, or stumps of non-host species associated with host stumps. This "background" level of potential infection may be due to any one or more of several factors, namely: an unrecognized infected root system, infected roots from adjacent trees or stumps penetrating the plot, and chemotaxic attraction of *Hylastes nigrinus* to the harvesting

area together with random attack of Douglas-fir stump and tree roots and planted seedlings. It is not known whether planted seedlings are more susceptible to BSRD, at least during the first several years, than are natural seedlings.

The redwood stump plots were initially included in the study both to serve as a further uninfected (non-host) check and to preclude increased activity by insects, especially H. nigrinus, that might be attracted to severed or wounded Douglas-fir trees. As can be seen from the data, there were no real differences in insect activity around the two stump types in the clearcut. On the basis of infected seedling distribution, at least some of the infection of seedlings around redwood stumps appeared to be due to penetration of the plot by infected roots. However, there were several instances where the infected seedlings were far removed from any known or suspected inoculum source. These seedlings were closely examined, and infection appeared to originate at a site of feeding or gallery construction by H. nigrinus on several of them.

A study by Goheen (4) indicated that V. wageneri usually survives in ponderosa pine roots for only a few months after the host tree dies. Wagener and Mielke (15) report a similar observation, although they found one case that may have involved fungus survival for 10 years. Our data indicate that the fungus remained viable in stump roots and/or soil and could infect seedlings for at least 24 months after logging. Some of the seedlings examined in May, 1984 appeared to have been infected after the end of the 1983 growing season.

The data in Table 2 shows that for this study, there is no difference in overall black stain seedling mortality between a clearcut or a selection cut. The important difference is that following a clearcut site regeneration often means that seedlings must be planted, and if Douglas-fir is used, high seedling mortality may occur around some stumps. The severity of the problem will, of course, depend upon the root infection level at the time of overstory harvest and the infection level of adjacent stands with various levels of H. nigrinus populations that may be attracted to the clearcut.

The levels of infection by V. wageneri in some plots probably are high enough to be of concern to the forest manager. They indicate that there will be areas of variable size, depending upon the amount of infection in the original stand, where stocking will be less than adequate. However, if the fungus ceases to be viable before the overall mortality average due to infection exceeds 15-20 percent, the stocking may still be at an acceptable level. Without long-term studies to determine the capabilities of V. wageneri to persist and to spread, it is still difficult to predict the potential impact of this pathogen on planted seedlings.

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