Symptoms and Fungal Associations of Declining *Chamaecyparis nootkatensis* in Southeast Alaska

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ABSTRACT

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The roots of 35 dying and healthy Alaska yellow-cedar trees (*Chamaecyparis nootkatensis*) were excavated to study symptoms and organisms associated with decline and death. Dying fine roots and necrotic lesions on roots and boles were common on cedars with declining crowns. Of 1,864 isolations, 1,047 from both healthy and dying cedars yielded fungi. However, when cedar seedlings were inoculated with the 11 most commonly isolated fungi, only *Cylindrocarpon didymum* caused necrotic lesions, and no fungi killed seedlings. Vesicular-arbuscular mycorrhizae and *Mycelium radicis atrovirens* were common (83 and 79%, respectively) in cortical cells of 42 fine root samples. *Phytophthora gonapodyides* was recovered in soil samples from beneath cedar trees but too infrequently to be considered responsible for mortality. These results suggest that pathogens are not the primary cause of Alaska yellow-cedar decline.

Alaska yellow-cedar (*Chamaecyparis nootkatensis* (D. Don) Spach) is a slow-growing, valuable forest tree that ranges from Prince William Sound in Alaska, south through British Columbia, to south of the Oregon-California border (8). In undisturbed areas of southeast Alaska, old-growth stands of Alaska yellow-cedar are suffering from a spectacular mortality problem (1) that began before the turn of the century (14,16). Recent studies on the forest dynamics of mortality have shown that Alaska yellow-cedar is the principal victim and that the boundaries of mortality have spread locally (15), suggesting possible involvement of a pathogen.

A preliminary evaluation of crown symptoms and the role of pathogens in this decline suggested involvement of root or root-related problems. Entire crowns of affected trees fade slowly, with foliage gradually thinning, or die quickly, with all foliage turning shades of brown without thinning (28). In addition, necrotic lesions occur on the boles and roots of some dying Alaska yellow-cedars (28). By excavating affected cedars, Shaw et al (28) observed that many coarse roots were dead and that *Armillaria* spp. and basal scars were common on dead and dying trees; they could not confirm any pathogen involvement with tree decline, however.

The objective of this study was to determine which microorganisms are associated with dying Alaska yellow-cedar and to evaluate their roles in decline. A concentrated effort was made to determine whether any species of *Phytophthora* were present, because necrotic lesions on roots and root collars of dying cedars suggested similarity to a serious disease of Port Orford cedar (*C. lawsoniana* (A. Murr.) Parl.) in southwest Oregon (24) caused by *P. lateralis* (Tuck. & J. A. Milb.). The concentration of dead and dying Alaska yellow-cedar trees on wet sites and local patterns of spread (15) also suggest a *Phytophthora* disease. Similarly, the pathogenicity of *Armillaria* sp. was investigated because it was commonly present on dead and dying Alaska yellow-cedars (28) and because elsewhere it frequently kills forest trees (32).

MATERIALS AND METHODS

Root and crown symptoms. Duff and soil were removed from around root collars and adjacent primary roots of 35 dying or apparently healthy Alaska yellow-cedars. The root diameter and proportion of circumference with living phloem (light color) were measured. From these measurements, an overall proportion of live and dead root surface area entering the root collar was calculated. Bark was removed, and the proportion of live and dead tissues was observed at the root collar, on the bole just above entering roots, and at breast height (1.37 m up the bole).

Three live primary roots were randomly chosen from each tree and, along with their secondary roots, were excavated by hand until dead portions were reached or until root diameters were less than 1 cm. Root diameter, proportion of circumference alive, and presence of fine roots (<2-mm diameter) were determined at 15-cm intervals along each root. Up to 10 fine roots were rated as alive or dead by observing the color of the cortex beneath the epidermis. Sampling intervals along roots that lacked fine roots were also tallied. Differences between healthy and declining trees in the percentage of living fine roots and the percentage of sampling intervals lacking fine roots were tested, using Student's *t* test, for three different size classes of coarse roots.

Height, diameter at breast height, signs of *Armillaria* spp. (i.e., mycelial fans and rhizomorphs), and presence of basal scars (10,12) were recorded for each tree. Crowns were rated for percentage of foliage fullness and proportion of different colors of retained foliage (28), and an overall "percent green" was calculated. Cedars referred to as "healthy" had crowns with percent green exceeding 75%. Crowns were also inspected for insects and symptoms of disease on foliage, branch, and bole tissues.

Fungal isolations. Isolations were attempted mostly from fine roots or from lesions on roots, stems, or branches of 31 of the excavated cedars. Fine roots were surface-sterilized for 30–60 sec in 1% sodium hypochlorite, rinsed twice in sterile water, blotted dry with sterile paper, and placed in petri plates containing one of the media described below. Most other tissues were not surface-sterilized before isolation. Stem lesions were collected by removing, with a chisel, a patch (4 cm per side) of sapwood and attached bark. The patches were then split along the cambium to expose clean sapwood and phloem surfaces, and chips of these were removed with a sterilized scalpel and placed on agar media. Root

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and branch lesions were treated similarly.

Media used were potato-dextrose agar (PDA), 1.5% water agar, and 1.5% malt extract agar (with 2 ppm of benomyl); streptomycin (100 ppm) was added to each to reduce bacterial contamination. Two media selective for *Phytophthora* spp. were also used, both consisting of 1.5% cornmeal agar, 40 ppm of pimaricin, 200 ppm of vancomycin, and 100 ppm of penicillin G, with hymexazol (25 ppm) added to one to reduce *Pythium* contamination (20). To preserve antibiotic effectiveness, the *Phytophthora* media were stored in the dark and used within 7 days of preparation. Isolated fungi were routinely transferred to PDA for identification or storage.

Fine root observations. Forty-two samples of fine roots (<2-mm diameter) were collected from 26 Alaska yellow-cedars ranging from healthy trees growing some distance from mortality sites to trees in the final stages of decline. Fine roots were fixed in Formol-acetic alcohol (FAA), cleared with warm potassium hydroxide to remove pigments, stained with trypan blue in lactophenol (22), and sectioned lengthwise by hand. Sections were squash-mounted in lactophenol and examined microscopically for presence of mycorrhizal fungi and infections by other fungi.

Sampling for *Phytophthora* spp. Baiting (7) for species of *Phytophthora* was conducted around 69 Alaska yellow-cedar trees over a 3-yr period. Soil, fine roots, and organic matter were combined from three locations around the base of each tree. The organic fraction was separated by flotation wet-sieving, then placed in the bottom of doubled Styrofoam cups for baiting (18). Foliage baits of Alaska yellow-cedar (2–3 cm long) were flooded for 7 days above the organic material in each double cup, then transferred to selective medium. Isolations were also attempted directly from symptomatic tissues (e.g., dying fine roots, root and stem lesions) by placing small, nonsterilized pieces onto the *Phytophthora*-selective medium.

Pathogenicity testing. Pathogenicity of the 11 most frequently isolated fungi was determined by inoculating Alaska yellow-cedar seedlings (about 20 cm tall) grown in cold frames at Corvallis, Oregon. Inoculations were done in the fall (October) and spring (April), and for most fungi, 10 seedlings were inoculated. Each fungus was grown on PDA and induced to sporulate by exposing the cultures to near-ultraviolet illumination (approximately 360 nm) at temperatures ranging from 12 to 28 C (17). Cultures were examined microscopically for presence of spores and tested for viability by transferring pieces onto fresh media just before being used as inocula. All inocula were sporulating except *Armillaria* sp. and *Mycelium radicis atrovirens* Melin, and all were viable. Media containing each fungus were cut into 1-cm squares and placed adjacent to 1-mm-wide wounds just below the root collar of seedlings. One fungus, *Dermea (Gelatinosporium)* sp., which was isolated exclusively from aboveground tissues of cedars, was also inoculated onto branchlets. Each inoculation site was wrapped in damp cheesecloth, and 1-mil plastic was placed over the cheesecloth and fastened with twist ties.

After 7 mo, inoculations were examined and lesion lengths were measured. Reisolations were attempted from all lesions. Differences in lesion lengths above and below inoculation points on inoculated and control trees were tested using Student's t test ($P = 0.05$).

Inoculations with *Armillaria*. Roots of 16 mature, healthy Alaska yellow-cedars were inoculated with an isolate of *Armillaria* sp. obtained from a dead Alaska yellow-cedar. Branch segments (about 3×10 cm) from red alder (*Alnus rubra* Bong.) were colonized with *Armillaria* as described by Shaw (26). Uncolonized segments of alder served as controls. A control segment and a colonized segment were placed in contact with live roots (1- to 4-cm diameter) on each tree. One-half of the inoculated roots (including controls) were wounded at the inoculation point by removing bark and outer sapwood. All inoculations were covered with soil and duff, reexcavated after 2 yr, and examined for symptoms and signs of infection by *Armillaria*.

Root wounding. Roots on mature cedars were wounded to determine whether lesions similar to those on symptomatic trees could be induced by the death or damage of distal tissues. Individual roots on five live cedars at each of three sites (total = 60 roots) were treated by: 1) wounding through the cambium for one-half of the root circumference, 2) girdling through the cambium for the entire root circumference, 3) wounding through the cambium and sapwood for one-half of the root circumference, or 4) not wounding (control). Roots were covered with soil and duff. After 2 yr, roots were uncovered and measured for lesion development and extent. Isolations from developed lesions were attempted to determine if the same fungi were present as in lesions on declining trees. Differences in lesion length according to type of wounding treatment were tested using a one-way analysis of variance (ANOVA, $P = 0.05$).

Stem lesions and square patch implants. Vertical stem lesions with darkened phloem commonly arise from dead roots on declining Alaska yellow-cedars (14,28). When these lesions were encountered, a chisel (4 cm wide) was used to remove square portions from the upper margin of the lesion, one square (4 cm per side) from above the lesion (with healthy phloem and sapwood), and three or four squares from below the top margin of the lesion. Fungal isolations were attempted as described above. The square from the upper margin of the lesion was used directly as a form of inoculum by being placed into a same-sized section on a nearby healthy cedar. Seventeen "implants" were obtained from necrotic lesions and 23 from healthy cambium (controls). Each implant was covered with petroleum jelly and left for 2 yr, at which time lesion development and size were determined and isolations were attempted. Differences in length of lesions between control and necrotic implants were tested using Student's t test ($P = 0.05$). Differences in frequencies of fungi isolated from lesions were compared by a chi-square test ($P = 0.05$). The species of fungi isolated from developing lesions were compared with those obtained from lesions on declining trees.

RESULTS

Root and crown symptoms. The entire crown of an affected tree declined and died as a unit, not as individual, scattered branches. Proximal (older) foliage died first, often changing from green to yellow to brown. In slowly declining cedars, the dead proximal foliage abscised before the distal foliage died, leaving a crown with a thin appearance. In cedars that died quickly, all foliage died concurrently, leaving a relatively full but red or brown crown.

Symptoms in the roots of declining cedars included a high proportion of dead roots of all sizes, missing fine roots, lesions on large-diameter coarse roots, and cambial lesions extending vertically from dead coarse roots up the bole. Significantly more fine roots were dead on the living coarse roots of declining trees (38%) than on those of healthy trees (7%) (Table 1). Fine roots (live or dead) occurred most commonly on small coarse roots and least often on large coarse roots of both healthy and declining trees. Fine roots attached to dead coarse roots were never alive.

Small coarse roots from distal parts of the root systems of cedars in the early stages of crown decline were frequently dead; these dead roots were often located in a water-saturated, black organic muck. As crown decline advanced, the proportion of dead small coarse roots increased. Large coarse roots were also dead or had necrotic cambial lesions that had apparently spread from smaller, more distal roots. These lesions sometimes advanced vertically up the bole, occasionally reaching almost to the top of a dying cedar.

Bole lesions occurred on 11 of 35 trees (nine lesions reached above breast height on boles) and affected only trees with crowns in an advanced stage of decline (Table 2). As the crown declined, more of the cambium on the major roots died,

then more of the cambium on the root collar, and finally the cambium on the bole (Table 2). Once on the bole, lesions did not progress distally from the bole to limbs, nor did necrotic lesions develop from limbs up or down the bole. In the final stages of tree death, patches of phloem on the bole not connected to the vertical lesions appeared mottled or necrotic and dark brown. The cambium in the upper portions of the bole and on limbs was generally the last tissue to die.

An unusual bark malformation was common on small branches (1- to 3-cm diameter) of both declining and healthy cedars. Although bark on small branches is normally smooth, the bark on affected branches appeared rough, with shallow fissures running parallel to the axis of the branch. Branches were slightly swollen along the 2–5 cm length of affected tissue. The phloem, cambium, and sapwood of these rough areas appeared live and undamaged, although bark was somewhat thicker than on adjacent unaffected areas. No fungal fruiting bodies were associated with these symptoms.

Fungal isolations. Of the 1,864 isolations, 1,047 (56%) yielded fungi, 486 (26%) showed no growth after 3 wk of incubation, and 331 (18%) yielded bacteria or airborne fungi. Of the fungi isolated, 812 isolates were assigned to a genus, species, or unidentified group based on microscopic observations of spore or hyphal characteristics (Table 3). Most fungi isolated from dying trees were also isolated from apparently healthy cedars (Table 3). Fungal species were not particularly associated with certain stages of crown decline but were associated more closely with specific symptomatic tissues (Table 3). For example, most isolates of *M. radicis atrovirens* were from fine roots and most isolates of *Phialophora melinii* (Nannf.) Conant were from bole lesions. Two fungi, *Cryptosporiopsis* sp. and *Cylindrocarpon didymum* (Harting) Wollenweb., were commonly isolated from all types of symptomatic tissues: fine roots, root lesions, and stem lesions.

Fungi isolated from bole lesions followed a distributional pattern—some were common near the top of lesions, others were more common farther down. Attempts at isolations from 4 cm above the top of lesions were generally unsuccessful. *Cryptosporiopsis* sp. occurred most often at the upper margin of the lesion but was absent 12 cm below the lesion top. *Cylindrocarpon* sp. and *Sporidesmium* sp. peaked at 4 and 8 cm, respectively, below the lesion top. *P. melinii* peaked at the sampling point farthest below the lesion top; isolations several meters below lesion tops often yielded this fungus. *Polyporus elegans* Bull.:Fr., a sapwood decay fungus that frequently produced basidiocarps on the boles of recently killed Alaska yellow-cedars, also was isolated some distance below the top of lesions. This distribution of fungi in stem lesions was not consistent from lesion to lesion.

Lesions appeared to be actively spreading up the boles on declining cedars, as live tissues adjacent to lesions did not develop callus tissue. Lesions were consistently connected to one or more dead roots from which they appeared to originate. The same complement of fungi was isolated from root lesions and bole lesions, with *Cryptosporiopsis* sp., *Cylindrocarpon* sp., and *M. radicis atrovirens* being the most common (Table 3). *Gelatinosporium* sp. was an exception—it was sometimes isolated from bole lesions but not from root lesions.

Phytophthora was not isolated directly from Alaska yellow-cedars, despite numerous attempts with a *Phytophthora*-selective medium. *Seiridium cardinale* (W. Wagener) Sutton & Gibson, another potential pathogen of *Chamaecyparis* species (30), was isolated from the top of one callused basal scar. A new, undescribed species of *Apostrasseria* was common on regeneration of Alaska yellow-cedar at some sites but was not found on mature trees.

Fine root observations. Vesicles and arbuscules of VA mycorrhizae were observed in cortical cells (Fig. 1A and B) from 83% of the fine roots examined. These structures were equally common on healthy and dying trees (Table 2) and were present in fine roots that had recently died but had not yet deteriorated.

Another fungus commonly (79% of samples) infected cortical cells of fine roots (Fig. 1C–E). Brown, septate hyphae were frequently observed in individual cortical cells of live fine roots. Once fully colonized, cells contained brown material and resembled cortical cells of strawberry (35) and several tree species (34) colonized by *M. radicis atrovirens*. These infected cortical cells were equally common in live, healthy cedars some distance from mortality areas and in dying cedars in areas with many dead cedars (Table 2). Infected cortical cells were also observed in dead fine roots.

Sampling for *Phytophthora* spp. Four isolates of a *Phytophthora* sp. were recovered by baiting soil collected under four of the 69 cedar trees sampled. All four isolates were recovered from areas suffering decline along Peril Strait—two beneath declining cedars and two beneath apparently healthy cedars with full, green crowns.

Pathogenicity testing. In spring inoculations, *C. didymum* was the only fungus to cause lesions on seedlings (Table 4) and was reisolated from two of the eight lesions. The small wounds made during inoculation had all callused over, or nearly so, on seedlings lacking lesions. No seedling inoculated with *C. didymum*, or any other fungus, died.

Regardless of treatment, lesion development was common in fall inoculations (Table 4). Inoculation wounds developed callus tissue within 7 mo, and no seedlings died. Fungi were generally not reisolated.

Inoculations with *Armillaria*. None of the 16 trees inoculated with *Armillaria* sp. developed lesions, but on one tree the fungus colonized a dead secondary root adjacent to the inoculum piece. All wounding treatments callused shut. After the 2-yr incubation, most pieces of inoculum appeared viable, with white and tan mycelium and emerging rhizomorphs.

Root wounding. Lesions developed on 28 of 45 (62%) of the wounded roots of mature cedars. Severe wounding (e.g., girdling the entire circumference or cutting through sapwood) caused more and longer lesions, but differences among wounding treatments were not significant. Lesions and wounds without lesions were callusing 2 yr after injury, and nonwounded roots lacked lesions. Except for the developing calluses, lesions on wounded roots resembled those on declining cedars, with dead, brown phloem and tan to dark-stained sapwood. *Leptographium* sp. was the most frequently isolated (43% of lesions) fungus from this stained sapwood, followed by the other fungi commonly

Table 1. Condition and presence of fine roots (<2-mm diameter) on three size classes of coarse roots from eight healthy and 23 declining Alaska yellow-cedar trees

	Live fine roots (%)[a]		Intervals with fine roots missing (%)[b]	
Coarse root size class	Healthy	Declining	Healthy	Declining
Over 3-cm diameter	92 ± 14	63 ± 38	52 ± 32	56 ± 28
From 1- to 3-cm diameter	93 ± 7[c]	63 ± 27[c]	35 ± 23	48 ± 26
Less than 1-cm diameter	93 ± 12[c]	61 ± 20[c]	20 ± 18	29 ± 20
All coarse roots	93 ± 10[c]	62 ± 18[c]	32 ± 23	42 ± 24

[a] Values are mean percentage (±SD) of live fine roots. Fine roots were determined to be live based on color of cortex tissues.
[b] Values are mean percentage (±SD) of 15-cm sampling intervals along coarse roots that lacked fine roots.
[c] Significant difference between healthy and declining Alaska yellow-cedars based on Student's *t* test ($P = 0.05$).

Table 2. Characteristics of excavated Alaska yellow-cedars whose symptomatic tissues were used for fungal isolation[a]

Tree no.	Diam. (cm)	Height (m)	Basal scar[b]	*Armillaria* present	Crown rating[c]	Living cambium (% circumference)			Fine root observations	
						Roots	Root collar	Bole	VA mycorrhizae	*Mycelium radicis atrovirens*
202	30.2	15.8	−	−	1.00	100	100	100	+	+
222	34.9	17.3	−	−	0.95	100	100	100	+	+
301	25.5	13.1	+	−	0.95	86	100	100	+	−
212	18.3	10.1	+	−	0.90	100	100	100	+	+
316	18.1	10.7	+	−	0.90	69	100	100	NS	NS
315	17.8	11.9	+	−	0.90	66	100	100	NS	NS
205	50.8	22.8	+	−	0.80	78	100	100	+	+
304	15.9	9.1	+	−	0.77	100	100	100	+	+
311	5.1	2.8	−	−	0.72	72	100	100	+	+
217	16.4	12.1	−	−	0.68	98	100	100	NS	NS
302	38.0	18.2	+	+	0.60	76	92	100	NS	NS
308	27.6	16.8	+	+	0.60	94	100	100	+	+
213	13.5	11.4	−	+	0.53	86	100	100	+	+
309	16.8	14.0	+	−	0.49	82	100	100	+	+
206	21.1	15.4	+	+	0.48	78	100	100	−	+
215	31.4	17.1	+	+	0.48	78	100	100	+	+
210	19.4	9.1	+	−	0.45	89	100	100	+	+
314	17.9	11.6	−	−	0.42	84	84	74	+	+
201	13.7	10.2	−	−	0.42	62	42	100	NS	NS
306	27.0	16.7	−	−	0.41	100	100	100	NS	NS
312	26.6	12.0	+	−	0.36	52	53	58	+	+
207	23.6	16.0	+	−	0.36	47	87	100	+	+
313	11.0	8.2	−	−	0.30	20	40	80	NS	NS
208	46.9	21.3	+	−	0.26	40	46	27	+	+
307	22.6	14.5	−	−	0.18	13	13	25	+	−
305	23.0	15.3	−	−	0.16	0	0	0	+	+
204	17.8	10.9	+	−	0.16	0	85	100	+	+
310	21.4	13.6	−	−	0.14	32	40	34	+	+
303	17.0	11.1	+	−	0.10	0	0	74	NS	NS
211	20.7	11.8	−	−	0.09	70	100	100	−	+
221	33.7	17.1	−	+	0.06	33	32	43	NS	NS
218	29.4	14.5	+	−	0.00	62	85	100	+	+
219	19.9	7.7	−	+	0.00	16	100	100	NS	NS
214	12.0	7.7	−	+	0.00	0	4	100	NS	NS
203	10.7	8.7	−	+	0.00	0	4	100	NS	NS

[a] Trees are arranged in descending order by crown rating. Nonnumerical values: + = present, − = absent, NS = not sampled.
[b] Most basal scars are caused by bears (10,12); portions of root collar or bole with a scar were not used in calculating the percentage of circumference with living cambium.
[c] The product of the proportion of foliage remaining (relative to a tree with a full crown) and the proportion of remaining foliage that is green; rating ranges from 1.00 for a healthy tree to 0.00 for a tree with a bare or brown crown.

Table 3. Frequency of isolation of fungi from different types of tissue on declining and healthy Alaska yellow-cedar trees

Fungus[a]	Isolation frequency of verified fungi		No. of trees from which isolated		Frequency of isolation from tissue type (%)						
	No.	%[b]	Declining ($n = 23$)	Healthy ($n = 8$)	Fine root	Root lesion	Root collar	Stem lesion	Bole scar	Sap stain	Branch
Mycelium radicis atrovirens	235	29	21	7	75	11	1	11	1	1	0
Cryptosporiopsis sp.	89	11	15	5	42	33	0	26	0	0	0
Gelatinosporium sp.	79	10	5	3	0	0	2	70	0	0	28
Armillaria sp.	83[c]	10	11	0	0	16	29	48	7	0	0
Sporidesmium sp.	75	9	12	5	35	3	4	52	3	4	0
Cylindrocarpon didymum	70	9	11	3	50	12	1	34	1	1	0
Phialophora melinii	50	6	8	3	2	2	2	64	18	12	0
Unknown basiodiomycete no. 1	31	4	7	2	1	3	3	66	3	23	0
Unknown basidiomycete no. 2	29	4	4	1	28	0	14	55	3	0	0
Apostrasseria sp.[d]	15	2	0	0	0	0	0	0	0	0	0
Spegazzinia tricholophilia	11	1	6	1	18	0	27	27	27	0	0
Polyporus elegans	6	1	2	0	0	0	0	17	83	0	0
Leptographium sp.	5	1	1	1	0	0	0	0	20	80	0
Total	778										

[a] Accounting for less than 1% of fungi isolated were *Ceratocystis* sp. (from scars), *Ditangium* sp. (from scars), *Gliocladium* sp., *Septonema secedens*, *Penicillium* sp., *Sporothrix* sp., *Gnomoniella* sp., *Botrytis cinerea*, *Seiridium cardinale*, and *Verticillium* sp.
[b] Based on 778 isolations (from 1,864 attempts) yielding fungi that were identified.
[c] Seventy-five were from mycelial fans or rhizomorphs.
[d] Isolated from dying shoots on Alaska yellow-cedar regeneration; not found on mature trees that were root-excavated.

found in necrotic lesions on declining cedars. In 18% of the induced lesions, no fungi were recovered.

Stem lesions and patch implants. After 2 yr, nine of the 23 (39%) cedar trees with patch implants from declining cedars had developed new lesions; seven of 17 (41%) control implants also developed lesions, however. Although implants from lesions induced longer lesions on average than implants from healthy trees (12.5 and 8.6 cm, respectively), the difference was not significant. Induced lesions resembled stem lesions on declining cedars, except that all induced lesions developed callus tissue after 2 yr. The fungi isolated from induced lesions were similar to those isolated from lesions on declining cedars (Table 3), except that *Leptographium* sp. was more common in induced lesions.

DISCUSSION

The crowns of declining Alaska yellow-cedars die as a unit, either slowly or relatively quickly, rather than as isolated branches. This symptomatology suggests a belowground, root-related problem. Trees in early stages of crown decline have dead and missing fine roots as well as root lesions that spread from small distal roots into and along larger proximal roots to the root collar. In the final stages of decline, vertical lesions spread from dead roots up a tree's bole. If a pathogen is primarily responsible for killing these trees, then it is likely to be located in one or more of these necrotic tissues. Vertical bole lesions do not, however, appear to be caused by fungal activity, as no fungi were consistently isolated from them. Only one fungus, *C. didymum*, caused stem lesions in inoculation trials. Implanted pieces of healthy bark caused as many lesions as implanted pieces of necrotic bark. Indeed, wound-induced lesions resembled natural lesions and were colonized by a similar array of fungi.

Lesions on the larger roots and boles of declining cedars were oriented mostly along the root axis or vertically on boles and did not spread circumferentially or develop a girdling pattern typical of lesions induced by a pathogen. For example, bole lesions on *C. lawsoniana* caused by the aggressive pathogen *P. lateralis* spread circumferentially and typically extend only about two bole diameters above the ground (36).

Rudinski and Vite (25) induced narrow, vertical stain columns in *C. lawsoniana* by introducing dye into one root. They suggest that the narrow, vertical stain, similar to the configuration of necrotic lesions on dying Alaska yellow-cedars, is a result of sapwood anatomy. In their study, similar treatments to other conifers caused wider or more spiraling lesions than those on *Chamaecyparis*. These findings suggest that the necrotic lesions common on declining cedars are secondary symptoms rather than direct results of pathogen activity. The lesions perhaps result from the death of smaller roots occurring earlier in tree decline than stem lesions or severe crown decline.

None of the 50 species of fungi (9,11) isolated or collected from Alaska yellow-cedar in our studies can alone be considered primarily responsible for the extensive mortality. Most of the fungi that were consistently collected from dying cedars were also found on healthy Alaska yellow-cedars. Only *C. didymum* showed any pathogenicity; it caused

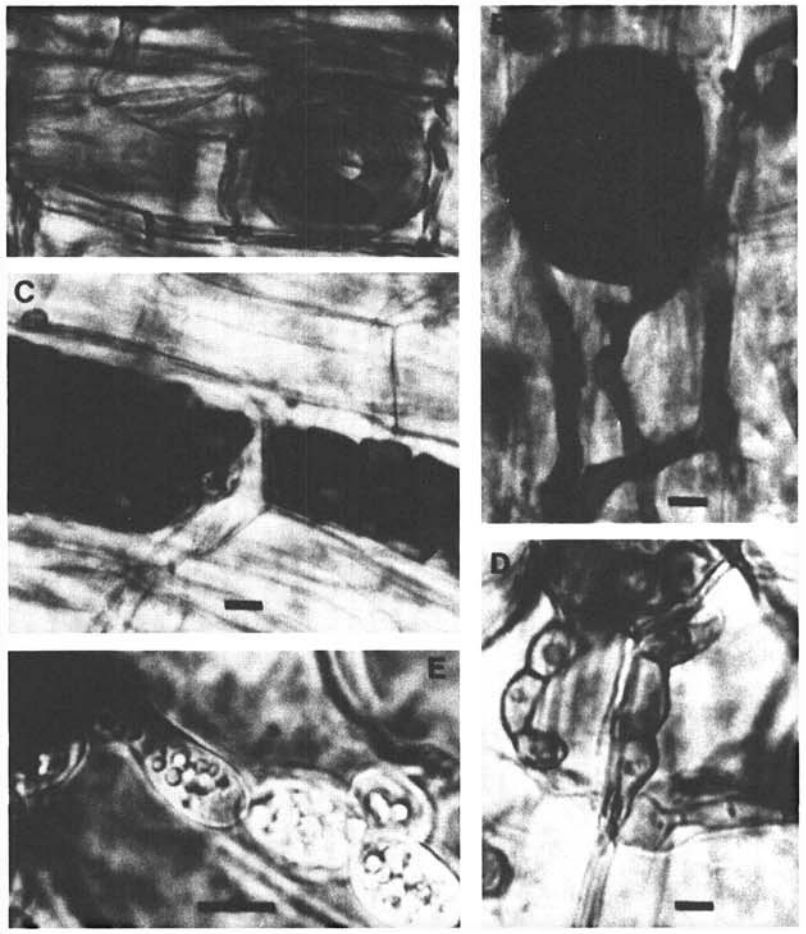

Fig. 1. Light micrographs of fungi in cortical cells of fine roots on Alaska yellow-cedar: **(A)** Hyphal coil and **(B)** vesicle of VA mycorrhizae; **(C and D)** infection of cortical cells, probably by *Mycelium radicis atrovirens*; and **(E)** hyphae of *M. radicis atrovirens* in culture. Scale bars = 10 μm.

Table 4. Pathogenicity of fungi isolated from declining Alaska yellow-cedar trees on 2-yr-old wound-inoculated seedlings of Alaska yellow-cedar

	Spring inoculations		Fall inoculations	
Fungus	**Lesions produced[a]**	**Mean lesion length (cm)**	**Lesions produced[a]**	**Mean lesion length (cm)**
Cylindrocarpon didymum	8/10	5.61	11/15	1.90
Phialophora melinii	0/10	0	8/10	1.23
Armillaria sp.	0/10	0	6/10	0.68
Gelatinosporium sp.	0/10	0	6/10	0.60
Gelatinosporium sp.[b]	0/5	0	3/11	0.48
Branch control[b]	0/5	0	3/7	0.76
Sporidesmium sp.	0/10	0	1/4	0.63
Leptographium sp.	0/10	0	4/5	0.56
Mycelium radicis atrovirens	0/10	0	4/8	0.42
Cryptosporiopsis sp.	0/10	0	4/10	0.71
Apostrasseria sp.	0/10	0	13/20	1.52
Ceratocystis sp.	0/10	0	...[c]	...
Seiridium cardinale	0/10	0	...	...
Control	0/10	0	4/10	0.50

[a] Number of inoculations yielding lesions after 6 mo/number of inoculations attempted.
[b] *Gelatinosporium* sp. and some control inoculations were made on branches in addition to root collars of seedlings because this fungus was always isolated from aboveground parts of mature cedar trees.
[c] Not done.

necrotic lesions but did not kill any inoculated cedar seedlings.

M. radicis atrovirens was commonly isolated from fine roots and other tissues and was most likely the dark-colored fungus we observed in the cortical cells on nearly all fine roots of Alaska yellow-cedar. None of the 235 isolates in this study sporulated, even though they were grown on many different media and exposed to near-ultraviolet light at different temperatures. Thus, their relationship to other members of this heterogeneous taxon, such as *Phialocephala dimorphospora* Kendrick (3,23), *P. fortinii* Wang & Wilcox, *Phialophora finlandia* Wang & Wilcox, and *Chloridium paucisporum* Wang & Wilcox (31), is uncertain.

Although *M. radicis atrovirens* has been repeatedly isolated from roots of various forest trees and is reportedly ubiquitous in boreal forest soils (3), the nature of its parasitism on conifers is unclear. Different taxa form associations with trees that apparently range from ectomycorrhizal, ectendomycorrhizal, pseudomycorrhizal, to pathogenic (34). Wilhelm et al (35) demonstrated the pathogenicity of *M. radicis atrovirens* on strawberry and believed it weakened plants for secondary infection by other organisms. The fungus has been isolated from another Cupressaceous host, *Juniperus communis* L. (19), but has not been previously reported on Alaska yellow-cedar or from Alaska. The association of *M. radicis atrovirens* with healthy cedars and its lack of pathogenicity suggest it is not a primary pathogen on cedar.

Considerable confusion exists concerning the taxonomy of *A. mellea* (Vahl:Fr.) P. Kummer because it is now being segregated into several species (32,33). This situation may clarify the varying pathogenicity and host ranges of *Armillaria* (21). Some Alaskan isolates of *Armillaria* sp. from Alaska yellow-cedar have a partial affinity with *A. cepaestipes* Vel. subsp. *pseudobulbosa* Romagn. & Marxmuller (27; C. G. Shaw III, *unpublished*). Our isolates from Alaska yellow-cedar are probably North American Biological Species (NABS) V (Geral McDonald, *personal communication*), which, along with NABS IX, is known to be common in the region (29) and is considered to be, at best, a weak pathogen (21).

In our inoculation tests, *Armillaria* sp. neither initiated lesions nor killed roots. In another study (29), isolates of *Armillaria* sp. from dying Alaska yellow-cedars readily produced rhizomorphs but failed to infect cedar seedlings. This *Armillaria* sp. appears to be a common but distinctly secondary pathogen on declining cedars. It colonized roots that were dead or dying and occasionally hastened the death of declining trees by killing the cambium at the root collar. *Armillaria* behaves similarly in forests in the eastern United States (32).

Gelatinosporium sp. was commonly isolated from bole lesions, where it was twice associated with its presumed teleomorph, *Dermea* sp. (9,11). It was also isolated from cedar branches, particularly from rough-appearing bark with shallow fissures that occurred on branches of seemingly healthy trees. Although the fungus was frequently isolated, no fruiting bodies of *Gelatinosporium* or *Dermea* were associated with this symptom, and no other fungus was isolated. Interestingly, another species of *Dermea, D. rhytidifomans* Funk & Kuijt, causes a bark disease of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) in British Columbia (2). Although symptoms are similar, the disease of subalpine fir occurs on boles of trees of all sizes, causing deep fissures in the bark and often leading to tree death. The effects of *Dermea* sp. on Alaska yellow-cedar are less severe, i.e., shallow fissures in bark, occurrence on small-diameter branches, no death of underlying phloem or cambium, and no apparent effect on tree vigor.

Ironically, members of both *Phytophthora* and *Seiridium*, genera known to cause serious diseases in *Chamaecyparis* elsewhere, were found associated with Alaska yellow-cedar in southeast Alaska but were rare and probably caused little or no damage. We isolated *S. cardinale* only once, from a bear-caused scar. This species varies in pathogenicity from an aggressive pathogen to a saprophyte (30); the isolate from Alaska yellow-cedar lacked pathogenicity in inoculation studies.

The *Phytophthora* isolated from baits, recently identified as *P. gonapodyides* (Petersen) Buisman (E. M. Hansen, *unpublished*), is probably not the cause of Alaska yellow-cedar decline. The same species, referred to as *P. drechsleri*-like (5,7) because of similar morphology and electrophoretic protein patterns, was recovered from undisturbed watersheds lacking significant tree mortality in Oregon (5).

Root and crown symptoms of dying Alaska yellow-cedar trees are somewhat similar to symptoms of Port Orford cedar attacked by *P. lateralis*. With the latter, however, trees die quickly without crown thinning, necrotic lesions girdle boles circumferentially, and lesions typically do not spread higher than two stem diameters above the roots on the bole. Because *P. gonapodyides* was isolated only by baiting from soil around cedar trees and was recovered only four times out of several hundred attempts, it could be viewed as an uncommon component of the mycoflora in stands of Alaska yellow-cedar. The efficiency of this baiting technique is not known for *P. gonapodyides*, but the procedure has worked successfully for isolating *P. lateralis* from soil around dying Port Orford cedar (6). *P. gonapodyides* recovered in our study was never isolated directly from Alaska yellow-cedar tissues even though several hundred attempts were made using media selective for *Phytophthora*. This, combined with its apparent lack of pathogenicity on cedar (7), suggests that Alaska yellow-cedar may not even be a host for *P. gonapodyides*.

In recent sampling for *Pythium* spp. at six locations throughout southeast Alaska, including sites with extreme decline (4), five species were commonly recovered, but recovery rates were not associated with mortality sites or with soil samples from beneath dying trees. Similarly, root-feeding nematodes are neither sufficiently prevalent at nor restricted to sites of dying cedars to be primary inciters of Alaska yellow-cedar decline (13).

The lack or dysfunction of beneficial mycorrhizae does not appear to contribute to Alaska yellow-cedar decline, since vesicular-arbuscular mycorrhizae were common in the cortical cells of live fine roots on both declining and healthy trees, as long as the fine roots were living.

Decline of Alaska yellow-cedar is probably not incited by a single pathogenic agent. Our studies, by necessity, have not included all types of plant-pathogenic agents. Bacteria, mycoplasmas, and viruses were not studied because none has been shown to cause significant mortality to conifers in natural stands. Suspect pathogenic fungi were found (e.g., *C. didymum, Armillaria* sp., and *Dermea* sp.), but none killed unstressed cedar seedlings. Necrotic lesions on large roots and boles of declining cedars probably are not caused by pathogens killing tissue at their leading margins; similar lesions were produced by mechanical wounding. Pathogenic fungi, along with the *Phloeosinus* bark beetle (28), can be concluded to have only secondary or contributing roles in Alaska yellow-cedar decline. On the basis of accumulated negative results from an exhaustive search for a primary pathogen, it now seems most likely that the primary cause of Alaska yellow-cedar decline is some abiotic factor.

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