

## Studies on the incidence of coniferous needle endophytes in the Pacific Northwest

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The incidence of internal fungal infections has been scored in coniferous needles from 19 hosts sampled in over 200 sites dispersed throughout western Oregon and southern Washington. *Abies grandis*, *A. magnifica*, *Picea sitchensis*, *Pseudotsuga menziesii*, and *Sequoia sempervirens* have proved congenial hosts for needle blade endophytes; petiole fungi are common in all species of *Picea* and *Tsuga* sampled. An undescribed taxon in the Hemiphaciaceae, *Chloroscypha* spp., *Cryptocline* spp., *Leptostroma* spp., *Naemacyclus* spp., *Phomopsis* spp., *Phyllosticta* sp., and several unidentified Coelomycetes with *Phoma*-like spores were the dominant fungal taxa in the coniferous hosts sampled. The observed patterns of species dominance and diversity suggest that the true population of endophytes has been inadequately sampled in the present study and that an order of magnitude more intensive sampling might be required for real patterns of dominance and diversity to emerge. Many endophytes are restricted to a single coniferous host or to a restricted group of hosts. When similarity coefficients between coniferous species are computed on the basis of their internal needle microfloras, the resultant taxonomic groupings appear similar to those derived from consideration of conventional morphological criteria. Comparison of endophyte incidence with host distribution patterns for *Pseudotsuga menziesii* reveals that infection rates decrease at high elevations and dry sites.

### Introduction

Symptomless fungal infections have been described recently from living needles of Douglas fir in Oregon (Bernstein and Carroll 1977) and from the foliage of several conifers in western Europe (Carroll *et al.* 1977). Both of these studies were based on small samples of needles collected from a few restricted sites. Intensive studies on the ecology of needle endophytes were felt to be justified only if such fungi proved to be widespread with regard to both host and habitat. Consequently, the present extensive survey was undertaken to document the incidence of needle endophytes in a variety of host conifers over a heavily forested region in western Oregon and southwestern Washington.

### Materials and Methods

#### Field Sites and Sample Selection

Samples were taken from a number of Research Natural Areas, from reference stands in the H. J. Andrews Experimental Forest, and from a large number of arbitrarily chosen incidental collection sites scattered throughout the region. Research Natural Areas sampled included the following: Abbott Creek, Ashland, Bagby, Bluejay, Brewer Spruce, Bull Run, Camas Swale, Canyon Creek, Cedar Flats, Cherry Creek, Coquille River, Fox Hollow, Goodlow Mountain, Little Sink, Lost Forest, Meeks Table, Metolius, Mill Creek, Mohawk, Nesko-win Crest, Ochoco Divide, Olallie Ridge, Persia M. Robinson, Port Orford Cedar, Pringle Falls, Sister Rocks, Wildcat Mountain, Wheeler Creek, and Wind River. Detailed descriptions of these areas are provided by Franklin *et al.* (1972). Within each Research Natural Area samples were taken from two to six arbitrarily chosen sites dispersed as evenly throughout the area as access and topography would permit. Within the H. J. Andrews Experimental Forest samples were collected from reference stands 1, 2, 4, 5, 8, 10, 11, 16, and 18 (see Zobel *et al.* 1976) as well as from several incidental sites adjacent to clear-cuttings. All other samples were collected from incidental sites. The locations of all sites are shown in Fig. 1.

Within each site at least one collection of needles was taken from each coniferous species present. Because of the difficulties in separating needles from twigs, samples were not regularly taken from *Thuja plicata*, *Calocedrus decurrens*, *Chamaecyparis lawsoniana*, *Chamaecyparis nootkatensis*, or *Juniperus* spp. Individual branches were cut from the lower canopy of arbitrarily chosen trees with a 13-m pole pruner and were labeled for future identification with tags. To discourage spurious infections, branches were not enclosed in any kind of bag after collection (for further discussion see Millar and Richards 1974; Bernstein and Carroll 1977). All branches were returned to the laboratory within 48 h of the time they were collected and were stored overnight at 6°C prior to culturing the following day.

#### Culture Methods

Needles were chosen for culturing on the basis of age-class. Needle age was determined by counting in sequence twig segments, each delimited by a set of terminal bud scars produced in previous years. Thus, the current season's growth at the tips of the branches was considered to be age-class 1 foliage, although it might vary in actual age from 0 to 12 months, depending on the season in which it was collected (bud burst in these forests typically occurs between May 15 and June 30). Needles attached to twig segments below the first set of terminal bud scars were considered as age-class 2 needles, the next set were age-class 3, etc.

Needles were dipped briefly in 90% ethanol to wet the surface and were then surface sterilized for 10 min in a solution of 65% commercial Chlorox. Needles were cut with a sterile scalpel into two, four, or eight segments depending on needle size, and the segments were transferred in serial order to 120-mm Petri plates containing 2% malt extract agar. Normally 20 segments from five individual needles were incubated in a single plate. Plates were incubated at 20°C with a 12-h dark-light cycle under fluorescent lights. Isolation of fungi from plates to 2% malt agar slants was carried out by direct transfer of conidia or mycelial fragments.

#### Scoring of Infections

Needle segments were scored for fungal infection at weekly intervals for the 1st month after inoculation and irregularly every 2–4 weeks for a considerable period thereafter. After 6 months plates were generally discarded. Single and multiple infections were scored on each individual needle segment.

# PACIFIC NORTHWEST

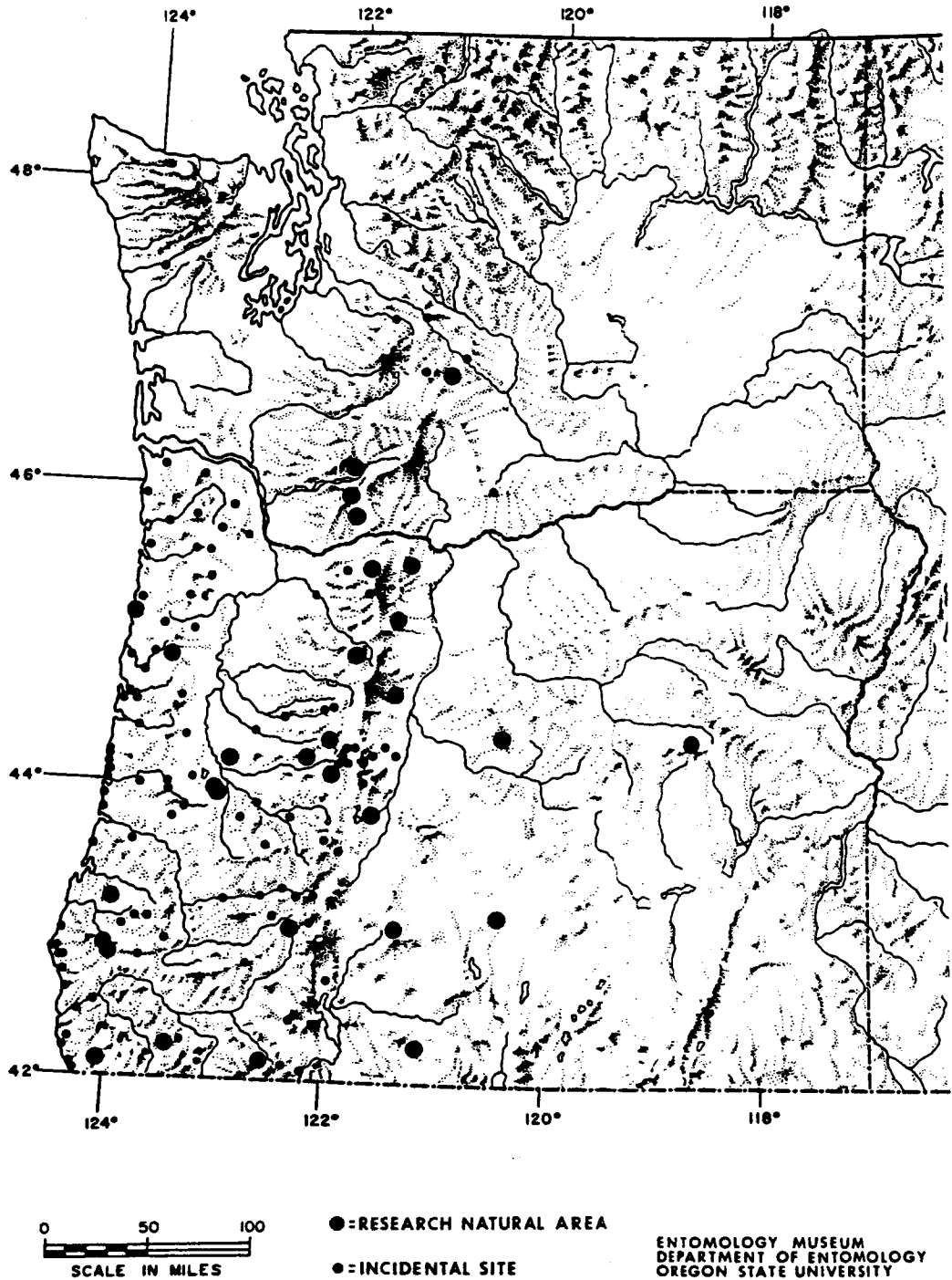


FIG. 1. Location of collecting sites for coniferous foliage (1 mi = 1.609 km).

*Identification and Nomenclature*

Identification of coniferous hosts was based on Hitchcock and Cronquist (1973) and Munz and Keck (1959); nomenclatural citations are as given by these authors. All fungi were initially assigned arbitrary code names based on the host and the order in which they were isolated. Synonymies and identifications of

individual fungal taxa were established subsequently on the basis of cultural characteristics and on the morphologies of fruiting bodies and spores which ultimately developed. Fungi were identified from a variety of sources; where species identifications are available, author citations have been provided. Names for ascomycetous perfect states have been assigned only

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#### Data Reduction and Statistical Analyses

Rates of infection for individual fungal taxa on a given host were calculated by dividing the total number of needle segments infected by a given fungus by the total number of segments incubated. Similarly, overall rates of infection for a given host were derived by dividing the total number of segments infected by any fungus by the total number of segments incubated. Because of the occurrence of multiple infections on some of the segments, the sum of infection rates for individual fungi was sometimes greater than the overall infection rate for all fungi. The incidence of fungi restricted to the petiole of the needle (petiole fungi) was considered separately from the incidence of fungi occurring on all needle segments (blade fungi).

Early in the study samples from several age-classes were taken from each branch. For these early samples a statistical test was used to determine the equivalency of percentages of infection for the different age-classes (Sokal and Rohlf 1969, p. 607). Data from samples whose infection rates were significantly different from infection rates of age-classes 4 or 5 (at the 5% level) were excluded in calculating overall infection rates. Thus, the total sample of needles was kept relatively homogeneous with respect to age-class (see below for further discussion).

In using the endophytic microflora as an indicator of taxonomic affinity among the various coniferous hosts sampled a coefficient of similarity was applied (Curtis 1959). This coefficient is computed as follows: similarity coefficient =  $2w/(a + b)$ , where  $a$  = the sum of distribution frequencies for all fungal species on one host,  $b$  = the similar sum for a second host, and  $w$  = the sum of lower distribution frequencies for fungal species in common between hosts. For the purposes of the above computation a distribution frequency was considered to be the proportion of all collecting sites sampled in which a given fungal species was found on a given host.

Initially, possible associations between rates of needle infection, aspects, elevations, and floristic zones of the collection sites were examined for *Pseudotsuga menziesii* through the use of contingency tables in which Kendall's tau was taken as an appropriate measure of association. For such tables floristic zones of the sampling sites were designated according to Franklin and Dyrness (1973). Infection frequency classes were delimited as follows: 0-5%; 5-25%; 25-50%; 50-75%; 75-95%; and 95-100%. Elevation classes were chosen at 600-ft (1 ft = 0.305 m) intervals from 0 to 6000 ft. The degree of association between rates of infection and elevation within a given floristic zone was measured by computing Kendall's tau, corrected for ties, for the unclassified data. Since both positive and negative correlations were of interest, a two-tailed test was used in assigning significance levels to computed values of tau.

#### Results and Discussion

A synopsis of coniferous hosts and needle infection rates for the entire survey is presented in Table 1. In interpreting these data certain cautions should be stated. The reported frequencies of infection in the sample vary in the degree of certainty with which they may be expected to estimate infection rates in the true populations; for binomially distributed populations the standard deviation varies with both the sample size and the infection rates

themselves (Sokal and Rohlf 1969). Beyond this, rates of infection appeared to vary systematically with the location of the site, with higher rates of infection apparent in wetter sites. Thus, for hosts such as *Pseudotsuga menziesii*, which occur over a wide range of environmental conditions, the infection rates reported in Table 1 have been affected by the locations of the sites sampled.

The above notwithstanding, Table 1 reveals striking differences among coniferous species with regard to their susceptibility to internal needle infection. *Sequoia sempervirens*, *Picea sitchensis*, *Pseudotsuga menziesii*, *Abies magnifica*, and *Abies grandis* have proved congenial hosts for blade fungi, with overall infection rates in excess of 50%. Conifers which typically occur only in high-elevation sites appear to be poor hosts for blade fungi (*Abies amabilis*, *A. lasiocarpa*, *Picea breweriana*, *Picea engelmannii*); *Taxus brevifolia* and *Tsuga heterophylla* also show low incidences of blade infection. Petiole fungi are common in all species of *Picea* and *Tsuga* sampled; they appear infrequently in other coniferous hosts.

The modest survey conducted by Carroll *et al.* (1977) on endophytes in European conifers provides the basis for a few limited comparisons: the European spruce, *Picea excelsa* Link, proved to be a rich source of diverse petiole endophytes, as have all species of *Picea* studied here. The European yew, *Taxus baccata* L., showed uniformly high rates of blade infection by a single fungal species, *Phyllosticta concentrica* Sacc.; *Taxus brevifolia* in the Pacific Northwest, in contrast, showed only low infection rates by a diverse group of blade fungi.

Table 2 presents information on the relative abundances of the most common individual fungal taxa for the various coniferous hosts and distribution frequencies for the fungi. Only those fungi which accounted for 1% or more of the total infections on a given host have been included; for intensively sampled conifers such as *Pseudotsuga menziesii* a majority of fungal species (80-90%) were seen infrequently or only once, and thus have not been reported here. Most of the common fungi have been identified at least to genus. Many of those fungi referred to only by code name in the table produce small, hyaline, single-celled spores in either stromata or pycnidia and could be classified only as 'Phoma-like' in the absence of expert opinion. A number of the commonly isolated genera have been reported previously as endophytes in needles or evergreen leaves: these include *Phyllosticta*, *Cryptocline* (previously reported as *Cryptosporiopsis*), *Leptostroma*, *Naemacyclus*,

Host
<i>Abies amabilis</i>
<i>Abies concolor</i>
<i>Abies grandis</i>
<i>Abies lasiocarpa</i>
<i>Abies magnifica</i>
<i>Abies procera</i>
<i>Picea breweriana</i>
<i>Picea engelmannii</i>
<i>Picea sitchensis</i>
<i>Pinus attenuata</i>
<i>Pinus contorta</i>
<i>Pinus lambertiana</i>
<i>Pinus monticola</i>
<i>Pinus ponderosa</i>
<i>Pseudotsuga menz.</i>
<i>Sequoia sempervirens</i>
<i>Taxus brevifolia</i>
<i>Tsuga heterophylla</i>
<i>Tsuga mertensiana</i>

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TABLE 1. Synopsis of coniferous hosts and overall infection rates

Host	No. sites	No. trees	No. needles	No. petiole segments	No. blade segments	% infected needles (all fungi)	% petiole segments infected with petiole fungi	% petiole segments infected with blade fungi	% blade segments infected with blade fungi
<i>Abies amabilis</i>	25	27	426	426	1278	20.4	2.4	5.4	7.2
<i>Abies concolor</i>	26	27	404	404	1212	49.5	1.0	32.7	36.2
<i>Abies grandis</i>	39	40	587	587	1758	66.6	2.4	52.1	50.9
<i>Abies lasiocarpa</i>	5	6	90	90	270	21.1	0.0	8.9	9.3
<i>Abies magnifica</i>	3	4	87	87	261	85.1	2.4	62.1	54.8
<i>Abies procera</i>	12	12	182	182	498	42.3	0.0	17.6	26.1
<i>Picea breweriana</i>	3	4	102	102	306	36.3	34.0	0.0	1.6
<i>Picea engelmannii</i>	5	7	110	110	330	56.4	52.7	0.9	3.3
<i>Picea sitchensis</i>	24	29	413	407	1209	94.8	34.9	77.2	68.5
<i>Pinus attenuata</i>	2	2	10	10	70	70.0	0.0	0.0	18.6
<i>Pinus contorta</i>	31	35	500	491	1966	46.0	2.7	14.4	14.6
<i>Pinus lambertiana</i>	8	10	71	71	485	67.6	0.0	32.4	33.6
<i>Pinus monticola</i>	5	5	65	65	455	92.3	8.1	28.8	44.0
<i>Pinus ponderosa</i>	19	19	146	146	1012	77.4	14.2	23.5	21.6
<i>Pseudotsuga menziesii</i>	178	208	3300	3300	9898	71.3	9.6	58.0	54.0
<i>Sequoia sempervirens</i>	5	5	102	102	297	100.0	0.0	82.4	97.0
<i>Taxus brevifolia</i>	10	10	183	183	498	24.6	7.3	9.6	10.2
<i>Tsuga heterophylla</i>	20	20	310	310	906	39.0	20.8	9.8	8.2
<i>Tsuga mertensiana</i>	10	10	180	180	520	56.1	19.0	9.4	19.0

69). Beyond this, they systematically show higher rates of infection. Thus, for hosts which occur over a wide range of conditions, the infection has been affected by

Table 1 reveals that coniferous species with internal needle infections are common in all coniferous hosts for which infection rates in excess of 50% occur only in high-altitude hosts for blade infections. *Picea sitchensis*, *Picea magnifica*, and *Pinus contorta* are common in all coniferous hosts: they appear

by Carroll *et al.* in a comparison of the infection rates of European conifers and North American conifers. The European conifers, as have been reported, showed only a few species of blade

on the relative frequency of individual fungal species on different hosts and distribution. Only those fungi which are included in the total infection rate are included: for internal infections, *Pseudotsuga menziesii* (80-90%) were included and thus have not been reported as common fungi have been reported. Many of those fungi named in the table are not included in the table because of the absence of expert classification. Only those fungi which are reported as endophytes are included: these include *Phomopsis*, *Naemacyclus*,

*Phomopsis*, *Geniculosporium*, and *Xylaria* s. imp. (for further discussion and literature review see Carroll *et al.* 1977).

Casual inspection of Table 2 reveals a pattern of species dominance and diversity which has been widely reported for both higher plants and insects: one or two dominant species account for the great majority of records, while most species are seen infrequently or rarely (Preston 1948; Whittaker 1965). This pattern is even more pronounced for the present data set when the many rare species unreported in Table 2 are considered.

Table 2 also reveals a certain degree of fungal specificity, both for host and location on the needle (petiole vs. blade). Thus, *Phyllosticta* sp. 1 and various species of *Cryptocline* occur primarily on *Abies* spp. and *Pseudotsuga menziesii*; PSm 1, an undescribed taxon in the Hemiphaciaceae with polar appendages on the ascospores, occurs only on *Pseudotsuga menziesii*; various species of *Leptostroma* (presumed here to be the imperfect states of *Lophodermium* spp.) predominate on *Pinus* spp. and on *Picea sitchensis*. *Chloroscypha chloromela* is ubiquitous on needles of *Sequoia sempervirens*. While not included in the table, data from occasional samples of foliage from other members of the Cupressaceae (*Calocedrus decurrens*, *Chamaecyparis lawsoniana*, *Juniperus* spp., and *Thuja plicata*) reveal there the widespread occurrence of other species of *Chloroscypha* as endophytes.

The degree of host specificity observed among

needle endophytes may permit endophyte distribution to be used as a measure of taxonomic affinity among the various conifers studied. To test this possibility, distribution frequencies were computed for each of the recognized fungal taxa on a selected group of host conifers and similarity coefficients between each pair of coniferous hosts were calculated (see Materials and Methods above). The resulting matrix is shown in Table 3. Examination of the coefficients for comparison of *Abies grandis* with other coniferous species suggests relatively close affinity between *Abies grandis*, *A. concolor*, and *A. amabilis*, a somewhat more distant relationship between *A. grandis*, *A. procera*, *A. magnifica*, and *Pseudotsuga menziesii*, and a very distant relationship between *A. grandis* and all other species of conifers sampled, including *A. lasiocarpa*. Similarly, *Pseudotsuga menziesii* appears most closely related to *Abies grandis*, somewhat more distantly related to *A. concolor*, *A. magnifica*, *A. procera*, and *A. amabilis*, and very distantly related to other conifers. Reference to Liu's (1971) *Monograph of the Genus Abies* shows that the *Abies* species sampled in this study fall into three distinct groups on the basis of conventional morphological criteria: section *Nobilis* Engelm., emend Liu, containing *A. procera* and *A. magnifica*; section *Grandes* Engelm., emend Liu, containing *A. grandis*, *A. concolor*, and *A. amabilis*; and section *Balsamae* Engelm., emend Liu, containing *A. lasiocarpa*. These same groupings are reflected in Table 3, although the close

TABLE 2. Relative frequencies of observed fungal taxa. Numbers in parentheses below 'Petiole' and 'Blade' refer to overall infection rates and italic numbers are total numbers of segments sampled. Fungi referred to by code name only sporulated but could not be identified; those marked sterile did not sporulate. Infections which could be scored but which could not be recognized or isolated because of slow growth or contamination are noted as 'indeterminate'

Host	Fungal taxa	Proportion of total, observed infections, %		No. sites sampled	No. sites taxon observed	Distribution frequency, %	Host
		Petiole	Blade				
<i>Abies amabilis</i>		(7.5%)426	(7.2%)1278	25			
	<i>Phyllosticta</i> sp. 1	28	56		14	56	
	<i>Cryptocline</i> sp. 1	25	16		9	36	
	<i>Leptostroma</i> sp. Indeterminate	19 2	19 2		7	28	
<i>Abies concolor</i>		(33%)404	(36%)1212	26			<i>Pinus attenuata</i>
	<i>Phyllosticta</i> sp. 1	70	73		18	69	
	<i>Cryptocline</i> sp. 1	22	19		7	28	
	<i>Ag</i> 2	1.3	2.4		1	3.8	
	<i>Ag</i> 19		1.0		4	15	
	<i>Tiarospora</i> sp. Indeterminate	2.7 2.5	2.0 2.5		4	15	<i>Pinus contorta</i>
<i>Abies grandis</i>		(52%)587	(51%)1758	39			
	<i>Phyllosticta</i> sp. 1	60	63		28	72	
	<i>Cryptocline</i> sp. 1	18	20		13	33	
	<i>Cryptocline abietina</i> Petr.	7.2	11		4	10	
	<i>Geniculosporium</i> sp.	3.9	1.2		10	26	<i>Pinus lambertiana</i>
	<i>Xylaria</i> st. imp.	1.3			1	2.6	
	<i>Micropera lunaspora</i> Linder Indeterminate	1.0 4.6	1.9		3	7.6	
<i>Abies lasiocarpa</i>		(8.9%)190	(9.3%)1270	5			
	<i>Cryptocline abietina</i> Petr.	88	63		4	80	<i>Pinus monticola</i>
	<i>Al</i> 5		11		1	20	
	<i>Al</i> 7		11		1	20	
	<i>Al</i> 10 Indeterminate	11 11	11		1	20	<i>Pinus ponderosa</i>
<i>Abies magnifica</i>		(62%)87	(55%)261	3			
	<i>Phyllosticta</i> sp. 1	77	75		3	100	
	<i>Cryptocline abietina</i> Petr.	7.8	3.5		1	33	
	<i>Ag</i> 4	5.3	4.1		1	33	
	<i>Ag</i> 19	1.8	6.2		1	33	
	<i>Geniculosporium</i> Indeterminate	1.8 5.3	8.3		1	33	
<i>Abies procera</i>		(18%)182	(26%)498	12			
	<i>Phyllosticta</i> sp. 1	97	93		7	58	
	<i>Leptostroma</i> sp.		1.5		2	17	
	<i>Ag</i> 19 Indeterminate	3 1.5	1.5		1	8.3	<i>Pseudotsuga mu</i>
<i>Picea breweriana</i>		(34%)102	(1.6%)306	3			
	PCb 11	56			2	67	
	PCb 2 Indeterminate	11 22			1	33	
<i>Picea engelmannii</i>		(54%)110	(3.3%)1330	5			
	PCe 15 (sterile)	25			3	60	
	PCe 18	15			4	80	
	PCe 6 (sterile)	11			1	20	
	PCe 8	4.6			1	20	
	PCe 9	4.6			1	20	
	PCe 10 Indeterminate	4.6 22			1	20	<i>Sequoia sempe.</i>
	<i>Picea sitchensis</i>		(86%)407	(69%)1209	24		
<i>Leptostroma</i> sp.		46	78		19	79	
<i>Phomopsis</i> sp. 1 <i>Phomopsis</i> sp. 2		11 3.5	10 2.0		4 7	17 29	

TABLE 2. (Continued)

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No. sites taxon sampled	Distribution frequency, %	Host	Fungal taxa	Proportion of total observed infections, %		No. sites sampled	No. sites taxon observed	Distribution frequency, %
				Petiole	Blade			
			<i>Ulocladium</i> sp.	6.1			5	21
			<i>Cryptocline</i> sp. 3	1.3			6	25
			<i>Geniculosporium</i> sp.	2.2			4	17
			PCs 2	2.6			2	8.3
14	56		PCs 18 (sterile)	6.1			5	21
9	36		PCs 27 (sterile)	2.4			2	8.3
7	28		<i>Coryneum</i> sp.	1.3	1.0		5	21
			Indeterminate	10	3.9			
		<i>Pinus attenuata</i>		(0%)10	(19%)70	2		
18	69		<i>Naemaecyclus</i> sp.		38		1	50
7	28		<i>Leptostroma</i> sp.		15		2	100
1	3.8		PNa 3		23		2	100
4	15		Indeterminate		15			
4	15	<i>Pinus contorta</i>		(16%)491	(15%)1966	31		
			<i>Leptostroma</i> sp.	41	61		26	84
			PNc 11 (sterile)	10	9.1		4	13
28	72		<i>Cladosporium</i> sp.	7.1	8.8		3	9.7
13	33		<i>Naemaecyclus</i> sp.		4.4		4	13
4	10		Indeterminate	17	8.1			
10	26	<i>Pinus lambertiana</i>		(32%)71	(34%)485	8		
1	2.6		<i>Leptostroma</i> sp.	68	70		8	100
3	7.6		<i>Naemaecyclus minor</i> Butin	28	20		2	25
			PNI 22		2.3		2	25
			Indeterminate	4	3.5			
4	80	<i>Pinus monticola</i>		(34%)65	(44%)455	5		
1	20		<i>Leptostroma</i> sp.	75	94		4	80
1	20		Indeterminate	4.2	1.5			
1	20	<i>Pinus ponderosa</i>		(31%)146	(22%)1012	19		
			<i>Dothichiza pityophila</i> (Corda) Petr.	31	9		13	68
3	100		<i>Leptostroma</i> sp.	16	53		14	74
1	33		PNp 12	13			5	26
1	33		PNp 22				2	11
1	33		<i>Gloeocoryneum cinereum</i> : (Dearn.) Weindlm.		8		1	5.2
1	33		<i>Naemaecyclus</i> sp.		5.6		2	11
			PNp 14		2.2		2	11
7	58		Indeterminate					
2	17	<i>Pseudotsuga menziesii</i>		(58%)3300	(54%)9898	178		
1	8.3		Psm 1	56	72		148	83
			<i>Phyllosticta</i> sp. 1	12	17		80	45
			<i>Cryptocline abietina</i> Petr.	6.8	3.8		34	19
2	67		Psm 88 (sterile)	1.9			20	11
1	33		<i>Geniculosporium</i> sp.	1.8			39	22
			Psm 57	1.6			18	10
			<i>Xylaria</i> st. imp.	1.4			13	7.3
			Psm 62	1.1			11	6.2
3	60		Psm 72 (sterile)		1.7		14	7.8
4	80		<i>Bispora</i> sp.		1.4		21	12
1	20		Indeterminate	9.1	2.6			
1	20	<i>Sequoia sempervirens</i>		(82%)102	(97%)297	5		
1	20		<i>Chloroscypha chloromela</i> Seaver	93	94		5	100
			<i>Cryptocline</i> sp. 2	3.4	1.7		2	40
			<i>Geniculosporium</i> sp.	2.3	1.0		2	40
			Indeterminate	1.1	2.3			
	79							
	17							
	29							

TABLE 2. (Concluded)

Host	Fungal taxa	Proportion of total observed infections, %		No. sites sampled	No. sites taxon observed	Distribution frequency, %
		Petiole	Blade			
<i>Taxus brevifolia</i>		(15%)183	(10%)498	10		
	<i>Phyllosticta</i> spp.	35	32		2	20
	TXbr 20	9.7			1	10
	TXbr 3	6.4			1	10
	TXbr 21	6.4			1	10
	TXbr 15		13		2	20
	Indeterminate	9.7	20			
<i>Tsuga heterophylla</i>		(28%)310	(8.2%)906	20		
	TSh 4	14	20		3	15
	TSh 8	19			7	35
	TSh 23 (sterile)		34		7	35
	<i>Cryptocline</i> sp. 4	6.3			4	20
	TSh 12	6.3	7.6		3	15
	TSh 25	4.2			3	15
	TSh 2 (sterile)	3.2			2	10
	TSh 20 (sterile)		7.6		2	10
	TSh 17		7.6		1	5
	TSh 22 (sterile)		7.6		3	15
		Indeterminate	24	5.8		
<i>Tsuga mertensiana</i>		(28%)180	(19%)520	10		
	<i>Leptostroma</i> spp.	24	70		9	90
	TSm 10 (sterile)	19			4	40
	TSm 1 (sterile)	9.4			1	10
	TSm 8	9.4			3	30
	<i>Phyllosticta</i> sp. 1		11		3	30
	<i>Cryptocline</i> sp. 3	5.7			1	10
	TSm 15	5.7			2	20
	TSm 13		7.6		3	30
		Indeterminate	11	4.8		

relationship between *A. magnifica* and *A. procera* does not stand out clearly in many comparisons. Both species were rather poorly sampled, and more intensive sampling might result in higher similarity coefficients. Coefficients in the range of 0.000–0.200 are probably not significantly different, and therefore Table 3 does not permit conclusions about the taxonomic affinities of *Tsuga mertensiana*, *Tsuga heterophylla*, or *Taxus brevifolia*. These low values probably reflect the general paucity of needle endophytes in these coniferous species. Thus, comparisons of parasitic microfloras may be expected to yield useful taxonomic information only when the parasites themselves are abundant and widespread.

Preliminary computations made early in this study showed drastically different incidences of endophyte infection from one collection site to the next. In general needles collected from high elevations or dry sites showed low incidences of infection while those from low elevations and moist sites showed high incidences of infection. We wished to assess the relative contributions of fungal host

specificity and direct environmental influences towards this variation in infection rates. The consistently low infection rates recorded for *Tsuga heterophylla*, *Abies amabilis*, and *Pinus contorta* over a range of elevations and moisture regimes suggested that host specificity comprises an important factor in determining endophyte distributions: thus, a high proportion of uncongenial hosts at high elevations may account for the low needle infection rates found there.

To test the possible direct effects of environmental influences on endophyte infection rates, correlations between site parameters and infection rates for a single widely distributed coniferous host, *Pseudotsuga menziesii*, were attempted. Although annual precipitation as rain was suspected to be a major determinant of needle infection, precipitation data were not available for most of our collection sites. Consequently, other site parameters which could be measured directly and which might be expected to show a high correlation with precipitation were incorporated in the initial contingency table analysis: these parameters included

TABLE 3. Similarity

<i>Abies grandis</i>	
<i>Abies grandis</i>	1.000
<i>Abies concolor</i>	
<i>Abies amabilis</i>	
<i>Abies magnifica</i>	
<i>Abies procera</i>	
<i>Abies lasiocarpa</i>	
<i>Pseudotsuga menziesii</i>	
<i>Taxus brevifolia</i>	
<i>Tsuga mertensiana</i>	
<i>Tsuga heterophylla</i>	

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TABLE 3. Similarity coefficients for various coniferous species as hosts for needle endophytes. Values can range from 1.000 for complete similarity to 0.000 for complete dissimilarity.

	<i>Abies grandis</i>	<i>Abies concolor</i>	<i>Abies amabilis</i>	<i>Abies magnifica</i>	<i>Abies procera</i>	<i>Abies lasiocarpa</i>	<i>Pseudotsuga menziesii</i>	<i>Taxus brevifolia</i>	<i>Tsuga mertensiana</i>	<i>Tsuga heterophylla</i>
<i>Abies grandis</i>	1.000	0.628	0.527	0.385	0.443	0.075	0.361	0.150	0.151	0.081
<i>Abies concolor</i>		1.000	0.502	0.336	0.517	0.088	0.297	0.091	0.160	0.000
<i>Abies amabilis</i>			1.000	0.207	0.504	0.000	0.242	0.136	0.150	0.052
<i>Abies magnifica</i>				1.000	0.262	0.178	0.247	0.087	0.080	0.044
<i>Abies procera</i>					1.000	0.054	0.223	0.100	0.131	0.000
<i>Abies lasiocarpa</i>						1.000	0.677	0.000	0.000	0.021
<i>Pseudotsuga menziesii</i>							1.000	0.162	0.100	0.071
<i>Taxus brevifolia</i>								1.000	0.062	0.104
<i>Tsuga mertensiana</i>									1.000	0.126
<i>Tsuga heterophylla</i>										1.000

TABLE 4. Significance levels for correlation between elevation and infection rates of *Pseudotsuga menziesii* needles using Kendall's tau as a measure of association. Numbers show the probability of obtaining the observed value of tau if no association exists. Direction of association is shown in parentheses. Levels of association significant at  $P < 0.05$  are marked with an asterisk. Floristic zones as described by Franklin and Dyrness (1973) are denoted here as follows: I, coastal, *Picea sitchensis* zone; II, *Tsuga heterophylla* zone; III, Willamette Valley; IV, mixed conifer and broad-leaved evergreen zone of southwestern Oregon; V, subalpine forest; VI, *Abies grandis*, *Pseudotsuga menziesii* zone east of Cascade crest; VII, *Pinus ponderosa* zone east of zone VI

	Floristic zone						
	I	II	III	IV	V	VI	VII
Blade	(+) 0.780	(-) 0.712	(-) 0.424	(-) 0.020*	(-) 0.656	(-) 0.039*	(+) 0.016*
Petiole	(-) 0.624	(-) 0.212	(-) 0.320	(-) 0.150	(-) 0.126	(-) <0.001*	(+) 0.016*

elevation, aspect, and floristic zone. Both two- and three-way contingency tables showed no significant degree of association between rates of infection and the site parameters examined. Although elevation and infection rate classes were broad, a large number of sampling zeros were encountered in the tables, and these may have obscured significant associations (Fienberg 1970).

Sites were then grouped according to floristic zones (Franklin and Dyrness 1973), and rates of infection were compared with site elevation using Kendall's tau as a measure of association (Table 4). Significant associations were seen only in the high-elevation zones (IV, V, VI, VII). Negative correlations were seen between elevation and endophyte incidence in zones IV, V, and VI, and a

positive correlation between elevation and endophyte incidence was seen in zone VII, the driest zone sampled. These observations are consistent with the notion that precipitation as rainfall may be a factor in endophyte dispersal. In zones I, II, and III the average annual precipitation ranges from moderate to high (80-320 cm) and at any elevation may be adequate for endophyte dispersal. Sites within zones IV, V, and VI may receive a substantial proportion of their precipitation as snowfall; higher sites within these zones will receive more snow and less rain than lower sites, resulting in a negative correlation between endophyte incidence and elevation.

In zone VII lack of rain and relatively open conifer stands may limit the spread of endophytes;

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within this zone precipitation increases and stands become more dense as elevation increases, resulting in a positive association between endophyte incidence and elevation. Many endophytic fungi, when grown in culture, produce masses of gloeoid spores. Such spores are usually considered rain-dispersed propagules (Ingold 1971). Indeed, we have seen endophyte conidia in throughfall samples collected beneath coniferous stands.

Finally, the generality of patterns derived from comparisons of a uniform needle age-class should be considered. To what extent can conclusions based on 4- or 5-year needles be extended to older needle age-classes or to the aggregated foliage of a single tree or stand of trees? In lower elevation moist sites the oldest surviving needles on healthy *Pseudotsuga menziesii* or *Abies grandis* branches usually occur in age-classes 8 and 9. The oldest surviving needles on branches of the same conifer species at higher elevations or in dry sites may be 12–13 years old. Early in our work needles of many age-classes were taken from single branches collected from a number of high-elevation or dry sites. Data for these older age-classes were generally excluded from subsequent analyses (see Materials and Methods). However, when infection rates for the older age-classes are computed and compared with those for the 4- or 5-year needles routinely used, they are found to be consistently higher. Thus, the apparent low infection rates seen at high elevations and dry sites may relate more to delayed onset of endophytic infections than to absolute lower incidences of internal needle fungi.

Age-specific distributions of needles on a single old-growth *Pseudotsuga menziesii* tree (tree 286) have been reported by Pike *et al.* (1977); over half of the needles occur in age-classes 1–3. When the age-specific infection rates computed by Bernstein and Carroll (1977) for this tree and for other trees in the same stand are applied to these needle distribution data, about 75% of the needle segments in the stand are found to be infected. Age-specific needle distributions are not available for *Abies* spp. or *Pseudotsuga menziesii* from high-elevation or dry sites. However, similar calculations can be attempted if the following relative distribution of needles is assumed, starting with age-class 1: 1, 15%; 2, 12%; 3, 12%; 4, 10%; 5, 10%; 6, 9%; 7, 8%; 8, 7%; 9, 7%; 10, 5%; 11, 4%; 12, 1%. Observed infection rates from several of the high-elevation and dry sites can be applied to this distribution to estimate the proportion of infected needle segments in such stands. Even when the upper limits for rates from such sites are used, no more than 30–35% of the total needle segments can be in-

fectured. In many of the stands the overall rates must be much lower. Thus, in spite of difficulties in the use of uniform age-classes, we feel that habitat comparisons are valid.

The documentation and explanation of needle endophyte distribution patterns had constituted a major objective in the present study. Left unanswered are more basic ecological questions on the functional role of needle endophytes and their mode of dispersion. In particular, one may ask, in view of their wide-spread occurrence in needles of certain conifers, whether such fungi form mutualistic associations with their hosts. Possible benefits to the host trees might include antagonism towards pathogenic needle parasites and surface saprophytes, delay of needle senescence, or a decrease in needle palatability for grazing insects. These hypotheses should be amenable to direct experimental test. The present study has shown which host–endophyte pairs might prove appropriate model systems for such experiments. Further studies on the dominant endophytes of *Pseudotsuga menziesii* and *Abies grandis* are currently in progress in this laboratory.

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- BERNSTEIN, M. E., and G. C. CARROLL. 1977. Internal fungi in old-growth Douglas fir foliage. *Can. J. Bot.* 55: 644–653.
- CARROLL, F. E., E. MÜLLER, and B. C. SUTTON. 1977. Preliminary studies on the incidence of needle endophytes in some European conifers. *Sydowia*, 29: 87–103.
- CURTIS, J. T. 1959. The vegetation of Wisconsin: an ordination of plant communities. University of Wisconsin Press, Madison.
- FIENBERG, S. E. 1970. The analysis of multidimensional contingency tables. *Ecology*, 51: 419–433.
- FRANKLIN, J. F., and C. T. DYRNESS. 1973. Natural vegetation of Oregon and Washington. U.S. For. Serv. Pac. Northwest For. Range Exp. Stn. Gen. Tech. Rep. PNW-8.
- FRANKLIN, J. F., F. C. HALL, C. T. DYRNESS, and C. MASER. 1972. Federal Research Natural Areas in Oregon and Washington. U.S. For. Serv. Pac. Northwest For. Range Exp. Stn.
- HITCHCOCK, C. L., and A. CRONQUIST. 1973. Flora of the Pacific Northwest. University of Washington Press, Seattle.

INGOLD, C. T. 1971. University of Toronto, Toronto.

LIU, T. S. 1971. University of Taiwan, Taipei.

MILLAR, C. S., and on the collection of plant communities. *Trans. Br. Mycol. Soc.* 46: 1–10.

MUNZ, P. A., and D. S. S. 1971. University of California, Berkeley.

PIKE, L. H., R. A. 1977. A 400-year-old Douglas fir face area, and 680–699.

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1977. Internal fungi in  
Bot. 55: 644-653.

SUTTON. 1977. Prelimi-  
le endophytes in some  
03.

isconsin: an ordination  
isconsin Press, Madi-

multidimensional con-

73. Natural vegetation  
Serv. Pac. Northwest  
PNW-8.

NESS, and C. MASER.  
reas in Oregon and  
orthwest For. Range

1973. Flora of the  
ngton Press, Seattle.

INGOLD, C. T. 1971. Fungal spores. Clarendon Press, Oxford.  
LIU, T. S. 1971. A monograph of the genus *Abies*. National  
University of Taiwan, Taipei, Taiwan.  
MILLAR, C. S., and G. M. RICHARDS. 1974. A cautionary note  
on the collection of plant specimens for mycological exami-  
nation. Trans. Br. Mycol. Soc. 63: 607-612.  
MUNZ, P. A., and D. D. KECK. 1959. A California flora. Univer-  
sity of California Press, Berkeley and Los Angeles.  
PIKE, L. H., R. A. RYDELL, and W. C. DENISON. 1977. A  
400-year-old Douglas fir tree and its epiphytes: biomass, sur-  
face area, and their distributions. Can. J. For. Res. 7:  
680-699.

PRESTON, F. W. 1948. The commonness and rarity of species.  
Ecology, 29: 254-283.  
SOKAL, R. R., and F. J. ROHLF. 1969. Biometry. W. H. Freeman  
and Co., San Francisco, CA.  
WHITTAKER, R. H. 1965. Dominance and diversity in land plant  
communities. Science, 147: 250-260.  
ZOBEL, D. B., A. MCKEE, and G. M. HAWK. 1976. Relation-  
ships of environment to composition, structure, and diversity  
of forest communities of the central western Cascades of  
Oregon. Ecol. Monogr. 46: 135-156.