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Title: A COMPARISON OF SOIL MICROFUNGI IN FOREST STANDS OF RED ALDER, CONIFER, AND ALDER-CONIFER MIXTURES

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The species composition of soil microfungal populations in adjacent stands of red alder, conifers, and mixed alder conifer correlated strongly with the dominant vascular vegetation. A total of 92 species were isolated: 55 from the alder stand; 45 from the conifers; and 46 from the mixed alder-conifer, with few species (16, 7, and 5 in the three plots, respectively) reaching average frequencies of 50% or higher. Penicillium nigricans, Aureobasidium pullulans, Cephalosporium curtipes, and Cladosporium herbarum were present with high frequency at all sites.

There was little difference in species composition among soil horizons within a stand. Fungi which were dominant in one layer were dominant in the others.

Three isolation techniques: dilution plates, soil plates, and immersion tubes, did not yield significant differences in species composition.

In all three stands, numbers of species were greatest in February and lowest in June, following seasonal maxima and minima of soil moisture.

A Comparison of Soil Microfungi in Forest Stands of Red Alder, Conifer, and Alder-Conifer Mixtures

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I. INTRODUCTION

Ecology of Soil Fungi

Beginning with Waksman (1917) studies of microorganisms have established that filamentous fungi are an important living component of all soils and that the kinds of fungi will vary with different soil types. Observations of Jensen (1931) and Warcup (1951) further suggested that soil fungi live in ecological communities governed by factors such as soil type.

Garrett (1951) first defined ecological groups of soil fungi as "an assemblage of species characterized by some peculiar advantage for pioneer colonization of a particular substrate". He recognized six ecological groups: (1) the root-inhabiting fungi, (2) the saprophytic sugar fungi, (3) the cellulose decomposing fungi, (4) the lignin decomposing fungi, (5) the coprophilous fungi, and (6) the predaceous fungi.

More recent studies (Tresner et al., 1954; Christensen, 1960; Christensen et al., 1962; Christensen and Whittingham, 1965) have attempted to determine the relationship existing between the microfungal populations and the higher plants. These studies have shown that the microfungal population reflects the species composition in the aerial vegetation.

A wide range of techniques have been used in the study of soil fungi; many have been devised to study the population as a whole,

others to study a single organism or group of organisms with some common property (Warcup, 1960). It should be emphasized that the apparent composition of the fungal flora of a soil is dependent on the methods used (Garrett, 1955). The difficulty in devising suitable techniques for the study of soil fungal ecology is twofold: Some of the difficulties are due to the soil's complex structure, its heterogenous nature, and its opacity; other difficulties are due to the fungi's complex mycelial thallus, and their variety of reproductive structures.

Other problems encountered are with differentiating between resting and active phases of organisms, and with measuring in some sense, their activity. As Warcup (1960) has emphasized, most existing isolation methods are inadequate for distinguishing between active and resting structures of fungi in soil. Garrett (1955), Harley and Waid (1955), and Chesters and Thornton (1956) have all pointed out that it is fundamental for many studies to be able to differentiate active from inactive fungi, since it is only in the active mycelial condition that fungi play their part in decomposition and other soil processes.

In the present investigation, the author has attempted to study the ecology of the microfungi in relation to three forest soil types: Red Alder, Conifer, and mixed Alder-Conifer. The forest stands are located on adjacent plots, and the soils under the stands are influenced equally by factors such as climate and parent rock material.

Hence, differences in the higher vegetation would be expected to have the primary influence on the microfungal populations. In an attempt to determine the flora most characteristic of the soils, three isolating techniques have been used. Each technique facilitates the isolation of a diverse flora, which will enable the investigator to more accurately define the dominant fungi of each forest soil.

Description of Study Area

The study area is located about 6.43 kilometers from the ocean, on the Cascade Head Experimental Forest of the Pacific Northwest Forest and Range Experiment Station, USDA Forest, just north of the Salmon River near Otis, Oregon. Topography of the study plots is relatively uniform, gently sloping, and generally southwest in aspect. Elevation is about 183-198.3 meters.

The maritime climate is greatly influenced by the Pacific Ocean and is typical of the coastal fog belt, characterized by equable temperatures, much cloudiness, frequent rains, and summer fog. Normal annual precipitation, almost all in the form of rain, is approximately 222.5 cm. (Madison, 1957). Precipitation is heaviest during November and lighest in July (Bollen et al., 1967). The frequent summer fogs which blanket the region are important, because fog decreases water loss from evaporation and transpiration and, in condensing on and dripping from tree crowns adds to the soil moisture

supply to some degree.

Temperatures are moderate most of the year. Mean annual temperature is approximately 50°F. Days when the temperature is below freezing or above 80°F are infrequent. Seasonal and diurnal fluctuations of temperature are slight (Ruth, 1954).

The adjacent plots support three different cover types, pure alder (conifer removed), pure conifer (alder removed), and a mixture of alder and conifer. The conifers consist of Douglas fir [Pseudotsuga menziesii (Mirb.) Franco], western hemlock [Tsuga heterophylla (Raf.) Sarg.], and sitka spruce [Picea sitchensis (Bong.) Carr.]. Each plot is square; the alder plot being one acre; the other two being 1.5 acres.

The cover types are approximately 35 years old and developed naturally on land previously cleared for agriculture (Berntsen, 1961). The pure stands were established by removing unwanted species when the conifers were eight years old and the alder 11 years old. There remained in the alder stand 733 trees per acre, in the conifer stand 1,148 trees per acre, and in the mixed about 3,000 trees per acre, of which 40 percent were alder and 60 percent conifer (Franklin and Pechanec, 1968).

Franklin and Pechanec (1968) found marked differences in coverage and richness of understory vegetation between the three adjacent plots. Understory vegetation was much better developed

under pure alder than under conifer, with the mixed stand intermediate. They suggested that most of the contrasts could be attributed to differences in canopy density and possibly soil nutrients, especially the nitrogen fixed by the alder nodules.

Substantial coverage by a shrub layer was found only in the pure alder stand. The most abundant shrub species are black elderberry [Sambucus racemosa var. melanocarpa (Gray) McMinn.] and salmonberry (Rubus spectabilis Pursh).

Coverage by an herb layer was comparable in the alder and mixed stands, but was considerably lower in the conifer stand. False lily-of-the-valley [Maianthemum bifolium var. kamtschaticum (Gmel.) Jeps.] and sword fern [Polystichum munitum (Kaulf.) Presl.] were important herbs in all three stands. Candy flower [Montia sibirica (L.) Howell] has a high frequency and coverage in the pure alder and mixed stands, but is nearly absent from the conifer stand.

Coverage by terrestrial cryptogams was considerably higher in the conifer and mixed stands than in the pure alder stand. Eurhynchium oreganum (Sull.) Jaeg. was the most abundant (Pechanec and Franklin, 1968).

Soils in the vicinity are classified as Astoria silty clay loam, representative of the reddish-brown latosol suborder of the great soil groups. Parent material is a tuffaceous siltstone which is included within the Nestucca formation of Eocene age (Franklin et al.,

1968).

Franklin et al. (1968) examined soil profiles in the study plots. The soils are moderately fine textured and moderately well drained. No consistent profile differences were detected among forest types; all were similar in horizon sequence and morphology. The 01 horizon was relatively thin, approximately 2-1/2 cm. of thickness, and was composed of freshly fallen and partially decomposed leaves, needles, and twigs. The 02 horizon, also thin and composed of partially decomposed bits of organic debris with no macroscopically identifiable plant parts, was reported to occur in a discontinuous pattern. The A11 horizon was a very dark brown loam, 5-10 cm. thick. Roots are abundant in all A11 horizons.

Other studies (Franklin et al., 1968; Bollen and Lu, 1968; Bollen et al., 1967) of the plots have shown differences between the three forest types. Bollen et al. (1967) reported that water content of soil samples was directly related to precipitation being highest in winter and early spring. Because organic matter has a greater water holding capacity, the F layer contained more moisture than the A horizons. Under alder, the water holding capacity was higher in spring than in fall in both F layer and A11 horizon. In the mixed stand, water holding capacity was greater in the fall than in the spring in both F layers and A11 horizon.

Soil pH in the alder stand is about one unit lower than in the soil from the conifer stand (Table 1). Bollen et al. (1967) reported alder soils to have a higher acidity at both the F layer and the A11 layer, and that this contrast holds true throughout the seasons. The mixed alder-conifer soils were also reported to have a higher acidity than conifer soils, presumably because of the alder.

Table 1. Average pH of soils from three plots. *

		March	September
Alder	F	3.6	4.1
	A11	3.9	4.4
Conifer	F	5.1	5.3
	A11	5.3	5.5
Mixed	F	3.9	3.8
	A11	4.3	3.9

* From Bollen et al., 1967

Organic matter content was reported by Franklin et al. (1968) for the study site as 37.5% (24.8-56.3) for alder, 28.9% (21.7-37.8) for conifer, and 31.8% (26.2-37.4) for mixed-stand soils. Measuring loss of weight on ignition at 700°C, Bollen et al. (1968) obtained rough estimates of amount of organic matter. The results are presented in Table 2.

Table 2. Loss on ignition (%).

		March	September
Alder	F	62.4	39.6
	All	30.4	27.5
Conifer	F	44.0	41.0
	All	34.1	28.3
Mixed	F	45.0	66.6
	All	28.6	35.0

From both studies, it can be seen that the organic matter content varied considerably with season within each forest type, and from one forest type to the next.

Nitrogen content of surface soils under alder consistently average higher than those under conifers [Bollen et al. (1967); Franklin et al. (1968)]. Kjeldahl nitrogen was measured in both studies. In the F layer and the All layer, alder maintained a higher nitrogen content than conifer, with the largest percentage difference in the F layer. This difference was maintained seasonally.

The carbon-to-nitrogen ratio (C:N) is useful in predicting the rate of microbial decomposition of organic matter (Alexander, 1967). On the alder conifer study site, C:N ratios under alder are always lower than under conifer (Bollen et al., 1967; Bollen and Lu, 1968; and Franklin et al., 1968). Bollen et al. (1967) reported C:N ratios as seen in Table 3.

Table 3. C:N ratio.

		March	September
Alder	F	17	15
	A11	16	15
Conifer	F	22	23
	A11	21	19
Mixed	F	19	19
	A11	19	22

Lower ratios of carbon-to-nitrogen under the alder and the mixed stand are probably caused by higher nitrogen content of the alder litter. Changes in the C:N ratio of soil on the different plots reflected not only composition and period of litter fall, but also the influence of season on microbial activity.

Previous studies comparing soil chemical and biological properties in forest stands of pure red alder, pure conifer, and alder-conifer mixtures at Cascade Head Experimental Forest have established that the presence of alder increases Streptomyces population, total nitrogen, and ammonia and nitrate forms of nitrogen, while pH is lowered. In addition, a destructive root pathogen, Poria weirii, has been found to lack nitrate reductase and therefore can neither utilize the high levels of nitrate-nitrogen present nor compete with many organisms that thrive on nitrate. Consequently, the effect of alder on soil places Poria weirii at a distinct ecological disadvantage. It is hoped that this investigation will broaden the knowledge of the effects of red alder on the complex ecology of microorganisms in forest soil.

II. SAMPLING PROCEDURES

Soil samples were collected throughout the year from February 1968 through September 1969 from three adjacent plots at the Cascade Head Experimental Forest near the Oregon coast: pure red alder, pure conifers and alder mixed with conifers. Under these plots three distinct soil layers are visible and were commonly designated as: the litter layer (L), the fermentation layer (F), and the soil layer (A11). The litter layer is 1 to 2-1/2 cm. in depth, and the soil layer 5 to 10 cm. in depth. The soils were sampled at different horizons, with the first sample always taken 1 to 2 cm. below the surface to prevent air contamination. The samples were collected in sterile 8-dram vials and frozen on the same day, following the procedure of Christensen et al. (1962).

Within each of the three forest types, sampling sites were chosen randomly and samples collected at each of the three visible horizons. Initially, samples were also taken at the A2 horizon, but this layer was found to resemble the A1 layer, so sampling of it was discontinued.

Isolation Techniques

Three isolation techniques were employed: soil dilution-plate technique, soil plates (Warcup, 1950), and immersion tube technique (Gochenaur, 1964).

Soil Dilution Plate Technique

Initially, three sampling sites were chosen within each area, and samples collected at each of the three visible horizons. Thereafter, samples were collected at only one site within each area. During the course of the investigation, 63 soil samples were examined. The microfungi were isolated from the soil by the dilution-plate method. The initial soil suspension was prepared by adding a one-gram sample to 99 ml. of sterile water. After shaking, the suspension was diluted (commonly 1:1,000-1:10,000) to yield a propagule density of 10-30 per milliliter (James and Sutherland, 1939). The dilution was then plated out by the pour-plate technique. One milliliter of each dilution was placed in a sterile petri plate, and approximately 15 ml. of Martin's rose bengal streptomycin medium (Martin, 1950) was added, the plates were then gently rotated to ensure adequate distribution and agar were left to solidify.

Five plates were prepared for each soil sample. Dilution plates were incubated six days. Hyphal tips from 30 sequential colonies arising on the dilution plates prepared from each sample were transferred to tube slants of potato dextrose agar. In addition, any colony which appeared to be morphologically different from the 30 isolates was also isolated. After 14 days, the tube cultures were sorted into presumptive species groups. Each fungus was then examined

microscopically, identified if possible, and cross-matched with isolates from the other populations. Occurrence data was kept for all entities.

Soil Plate Method

Small subsamples (0.005-0.015 gr.) of soil were taken from the main samples by means of a sterile nichrome needle with a flattened tip. The sample was dispersed in a drop of sterile water in the bottom of a sterile Petri dish, Martin's rose bengal streptomycin agar was added, and the particles distributed throughout the medium by rotation of the dish.

Immersion Tube Method

The immersion tube method consists of the removal of a core of soil with a borer and the insertion of an immersion tube in the resulting hole. The immersion tube contains a series of invaginated capillaries that allow exposure of the contents to the soil only at certain sites. One gram of dried soil taken from the forest to be studied is added to each tube; the tube is then immersed in water to moisten the soil, capped, and placed in a carrying container of a slightly larger diameter which is also capped. The whole unit is sterilized in an autoclave at 120°C and 15 psi gage for 30 minutes.

In the field, the immersion tube is placed in firm soil or turf by removing a core of soil of slightly less diameter than the tube. When the core is withdrawn, the tube is inserted immediately to fit tightly against the soil. After seven days incubation, the tubes are withdrawn from the ground, replaced in their containers, and returned to the laboratory where they are immediately processed. The exterior of the tube is cleaned with 70% ethanol, and the contents are removed with a sterile L-shaped inoculation needle. The contents are dispensed among five sterile petri dishes, and approximately 15 ml. of a glucose-ammonium nitrate medium (Gochenaur, 1950) is added to each dish. The soil particles are dispersed by gentle rotation before the medium solidifies.

Analyses of Populations

The frequency of each fungus in a given habitat and its overall abundance were computed using the formula given by Christensen et al. (1962):

$$\text{Frequency of occurrence (\%)} = \frac{\text{sites of occurrence}}{\text{total sites sampled}}$$

Species composition of the three forest units was compared using the formula 2w/(a+b), where "a" is the number of species in one population, "b" is the number of species in the other population, and "w" is the number of species found in both populations (Bray and Curtis, 1957; and Christensen et al., 1962). All microfungal entities were

used in the comparisons. Similarity coefficients were calculated for each population with every other population in the unit.

III. RESULTS

A total of 92 species were isolated from the three stands: 55 from beneath alder, 45 from conifer, and 46 from mixed alder-conifer. Of these, 18 occurred at frequencies of 50% or more and are considered dominants. Many of these organisms have been reported as dominants in other soil types and are known to be ubiquitous in their distribution; others have not been reported as commonly from soils.

Those species restricted to a single sampling site within a stand were commonly represented by a very low number of isolates. These restricted species (31 in alder, 28 in conifer, and 28 in mixed alder-conifer), as a group, were of low frequency, accounting for 59% of the total species, but only 22% of the total isolates. In the alder stand the species restricted to one site accounted for 56% of the total species and 15% of the total isolates. In the conifer stand, 62% were restricted species, but they comprised only 28% of the total isolates. In the mixed alder-conifer stand 60% of the species were restricted to one sampling site, with this accounting for 26% of the total isolates. This is in agreement with Christensen et al. (1962), and Christensen and Whittingham (1965), who find that species restricted to one stand comprise a high percentage of the total species, but account for a low percentage of the total isolates.

Distribution of the 92 species within the alder, conifer, and mixed alder-conifer study plot is shown in Table 4. For alder soil, the species present in all sites were Penicillium nigricans and Trichoderma viride. For conifer soil only Trichoderma viride was present in all sites, and for mixed alder-conifer only Penicillium nigricans. The two species present in all alder sites accounted for almost one-third of all the isolates, indicating their adaptiveness to this habitat by both their presence and abundance. Aureobasidium pullulans, Cephalosporium curtipes, Cladosporium herbarum, Gliocladium salmonicolor, and Penicillium daleae also occurred with high frequency in alder. Aureobasidium pullulans, Cephalosporium curtipes, Penicillium janthinellum, and Penicillium nigricans occurred frequently in conifer soils but with less than 100% frequency. In addition to Penicillium nigricans, the only species to occur with high frequency in the mixed alder-conifer stand was Aureobasidium pullulans.

Taxonomic Distribution of Species

A large majority of the isolates were representatives of the family Moniliaceae (Moniliales) due to the high percentage of Penicillium species, which were abundant in all three stands. Aside from Penicillium, species of Aspergillus, Botrytis, Cephalosporium, Gliocladium, Paecilomyces and Trichoderma occurred commonly in all three soils. In total, 16 genera were isolated representing the

Table 4. Species distribution by presence at sites.

No. sites within a stand for which a species was isolated	Alder			Conifer			Mixed		
	No. of species	No. of isolates	% total isolates	No. of species	No. of isolates	% total isolates	No. of species	No. of isolates	% total isolates
6	2	100	27.7	1	45	11.9	1	57	20.0
5	3	43	12.0	2	40	10.5	1	35	12.3
4	2	36	10.0	2	130	34.4	0	0	0
3	9	83	23.0	2	21	5.6	3	34	11.8
2	8	42	11.8	10	46	12.2	13	83	29.0
1	31	56	15.5	28	96	25.4	28	77	26.9
TOTAL	55	360	100	45	378	100	46	286	100

family Moniliaceae. Aspergillus was represented by six species in the alder stands, four species in the conifer stand, and two species in the mixed stands. The relatively common occurrence of Aspergillus in deciduous forest soils has been reported by Christensen et al. (1962). The genus Penicillium has been reported to be prominent in both conifer-hardwood (Christensen, 1960) and deciduous forest soils (Christensen et al., 1963; Tresner et al., 1954). Although the Trichoderma species represent a large percentage of the total isolates from all three stands, Table 5 shows that they represent a low percentage of the total species.

The family Dematiaceae and the order Mucorales were equally represented in percentages of total isolates. Although most isolates in the Mucorales were members of the Mucoraceae, species of Mortierella (Mortierellaceae) were isolated frequently from alder soil. Neither the family Dematiaceae nor the order Mucorales were represented by many genera.

The influence of the soil environment appears not only in the species distribution, but also appears in the distribution of entire groups such as Penicillia and Mucorales. The relative proportion of Penicillium spp. and Mucorales is approximately equal for conifer and alder soils; however, in the mixed alder-conifer soil there is a sharp increase. This appears to be an enhancement effect of the mixed alder-conifer forest soil.

Table 5 Prominence of special groups.

Group	Alder		Conifer		Mixed	
	No. of species	% total species	No. of species	% total species	No. of species	% total species
Aspergillus	6	10.9	4	8.8	2	4.3
Cephalosporium	5	9.9	2	4.4	2	4.3
Mucorales	4	7.2	4	8.8	6	13.0
Penicillium	13	23.6	11	24.4	16	34.8
Trichoderma	1	1.8	2	4.4	2	4.3
Dematiaceae	4	7.2	5	11.1	2	4.3
Yeast & yeast-like forms	5	9.9	2	4.4	1	2.1

Table 6 is a nearly complete list of the microfungi identified in this survey with their frequencies for each of the three stands and for all three as a whole. Many species are found with high frequency throughout the three stands. Other species are represented by high frequencies in one stand but not in the others. The former group is represented by such species as Aureobasidium pullulans, Cephalosporium curtipes, Penicillium nigricans, and Trichoderma viride. Figures 1, 2, and 3 are lists of fungi which occur most commonly within the three stands. They are arranged in order of decreasing frequency of isolation. Penicillium daleae, Aspergillus subsessilis, Fusidium viride, Gliomastix murorum var. felina, Penicillium thomii, and Trichocladium sp. are all species common to the alder stand, but uncommon or totally lacking from the other two stands. Penicillium janthinellum and unidentified C12-1 on the other hand, occur commonly only in conifer soil.

Not only does alder soil support a greater variety of species, but 30% of these species occur with a frequency of 50% or greater, as compared with frequencies of 15% for conifer and 11% for mixed alder-conifer. Although the mixed alder-conifer soil supports a variety of species (46) comparable to either the alder or the conifer, only one of these species (M11-5) occurs only in the mixed stand soil with a frequency of 50% or greater. The mixed alder-conifer soil has few species unique to it or with frequencies higher than in alder or

Table 6. Frequencies of microfungi isolated[1]

Species	Alder				Conifer				Mixed				Total
	L	F	A11	Stand	L	F	A11	Stand	L	F	A11	Stand	
Absidia capillita van Tieghem									17			17	6
Absidia glauca Hagem.	17			17	17		17	33	17			17	22
Aspergillus brunneo-uniseriatus Singh and Bakshi			14	17	17			17					11
Aspergillus chevalieri (Mangin) Thom and Church			14	17									6
Aspergillus flavipes (Bain. and Sart.) Thom and Church	17			17									6
Aspergillus niger V. Tieghem		17±		17			17	17	33	17		33	22
Aspergillus subsessilis Raper and Fennell	33	17	14	50		17		17	17	17		17	28
Aspergillus sydowi (Bain. and Sart.) Thom and Church					17			17					6
Aspergillus sp. (A22-4)		33		33									11
Aureobasidium pullulans (de Bary) Arnaud.	33	33	43	83	33	50	66	66	33	33	66	83	78
Botrytis cinerea Pers. ex. Fr.	17	17	14	33					17			17	16
Candida sp.	17			17									6
Cephalosporium acremonium Corda.											17	17	6
Cephalosporium curtipes Saccardo	66	66	14	66	33		66	83	33		33	50	67
Cephalosporium humicola Oudemans			14	17									6
Cephalosporium sp. (A3-9)			14	17	17		17	17					11
Cephalosporium sp. (A1-5)	17			17									6
Cephalosporium sp. (A3-14)			14	17									6
Cladosporium herbarum (Pers.) Link	50	33	14	66	33	17	17	33	50	33	33	50	50
Cordana pauciseptata Preuss		17		17									6
Cylindrocarpon radicicola Wollenweber					17			17					6
Eupenicillium pinetorium Stolk							17	17					6
Eupenicillium stolkiae Scott						17	17	17					6
Fusidium viride Grove	17	33	28	50									16
Gelasinospora tetrasperma Dowding									17			17	6
Gliocladium penicilloides Corda		17	14	17		17	17	33	17			17	22
Gliocladium salmonicolor Raillo.	66	17	14	83		33	33	50		17	33	33	56

[1] unidentified species with a single isolation are omitted.

Table 6. Frequencies of microfungi isolated. (Cont.)

Species	Alder				Conifer				Mixed				Total
	L	F	A11	Stand	L	F	A11	Stand	L	F	A11	Stand	
Gliomastix murorum var. felina (March) Hughes	17	17	28	50			17	17		17	17	33	33
Hyalodendron sp.		17	28	17		17		17					11
Monilia geophila Oudemans			14	17									6
Monocillium sp.							17	17					6
Monodictys sp.							17	17					6
Mortierella isabellina (Oudemans) Zycha	33	17	14	33					33			33	22
Mortierella pusilla Oudemans	33	33		33	17	17	17	33		33	17	33	33
Mortierella ramanniana var. angulispora (Naumov) Linnemann			14	17	17			17			17	17	16
Mycogone nigra (Morgan) Jensen											17	17	6
Myrothecium verrucaria (Albertini and Schweinitz) Ditmar									17			17	6
Paecilomyces farinosus (Fr.) Brown & G. Smith			14	17									6
Paecilomyces marquandii (Massee) Hughes			14	17									6
Paecilomyces variotii Bain.						17	17	17	17			17	11
Penicillium aceleatum Raper and Fennell									17			17	6
Penicillium canescens Sopp									17			17	6
Penicillium daleae Zaleski	66	50	28	83	17	17		17	17	17	33	33	44
Penicillium frequentans Westling					17		17	17		17	33	33	16
Penicillium funiculosum Thom							17	17					6
Penicillium italicum Wehmer		17	14	33									11
Penicillium janthinellum Biourge		17	28	50	83	60	50	83			33	33	56
Penicillium jenseni Zaleski	17		14	17					17	17		17	11
Penicillium lanoso-coeruleum Thom			14	17							17	17	11
Penicillium lividum Westling					17			17		33		33	16
Penicillium miczynskii Zaleski										17		17	6
Penicillium nigricans (Bainier) Thom	83	100	57	100	83	50	66	66	66	50	83	100	89
Penicillium ochoro-chloron Biourge									17	17	17	17	6
Penicillium oxalicum Currie and Thom					17			17	17			17	11

Table 6. Frequencies of microfungi isolated. (Cont.)

Species	Alder				Conifer				Mixed				Total
	L	F	A11	Stand	L	F	A11	Stand	L	F	A11	Stand	
Penicillium piscarium Westling			14	17									6
Penicillium purpurogenum Stoll	17		14	17						17		17	11
Penicillium raistrickii Smith	17			17									6
Penicillium rugulosum Thom		33		33			17	17					16
Penicillium simplicissimum (Oudemans) Thom	17		14	33							17	17	16
Penicillium soppi Zaleski						17	17	33					11
Penicillium spinulosum Thom					33		17	33					11
Penicillium steckii Zaleski	17			17					17	17	17	17	11
Penicillium thomii Maire	17	33		50									16
Penicillium variabile Sopp.					17			17			17	17	11
Pestulotia sp.					17			17					6
Rhizopus rhizopodiformis Hesseltine							17	17		17		17	11
Sporotrichum carnis Brooks and Hansford			14	17									6
Sporotrichum epigaeum var. terrestre Daszewska											17	17	6
Sporotrichum pruinosum Gilman and Abbott					33			33					11
Stemphylium botryosum Wallroth						17		17					6
Torula herbarum Pers.			14	17									6
Trichocladium sp.	33	17	28	50	17	17	33	17					22
Trichoderma album Preuss										17		17	6
Trichoderma viride Pers. ex. Fr.	66	83	57	100	50	50	66	100	33	33	17	33	78
Trichoderma sp. (variant #2)							33	33					11
Umbelopsis versiformis Amos and Barnett		17	14	17							17	17	11
Verticillium terrestre (Link) Lindau	17			17	17			17					11
Unidentified A11-1	17	17	14	50									16
Unidentified A11-2	17	17	14	50									16
Unidentified A11-4	43			50	17			33					28
Unidentified M11-12									17			33	11
Unidentified C32-1						17		17					6
Unidentified C12-1						17		50					16
Unidentified M21-2					17			17	17			33	16

Table 6. Frequencies of microfungi isolated. (Cont.)

Species	Alder				Conifer				Mixed				Total
	L	F	A11	Stand	L	F	A11	Stand	L	F	A11	Stand	
Unidentified C31-3					17			33					11
Unidentified M11-5									17	17	17	50	16
Unidentified M22-6										17		33	11
Unidentified A23-1			14	17							17	17	11
Unidentified A12-8		17	14	33									11
Unidentified A4EP5			14	17									6
Unidentified M3EP1	17			17							17	17	11
Mycelia sterila			28	17									6

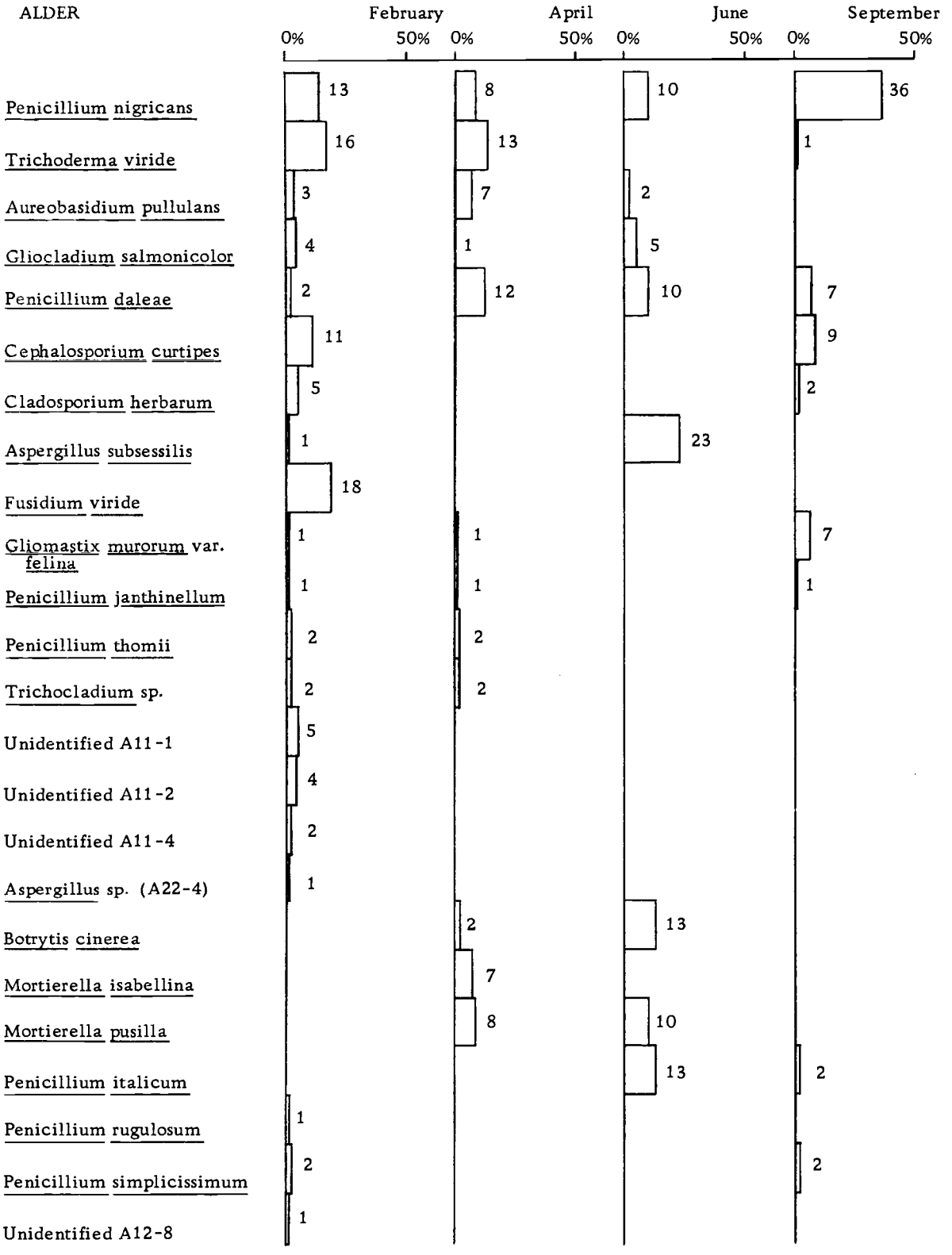

Figure 1. Overall occurrence and seasonal frequencies of selected species in alder soil.

CONIFER

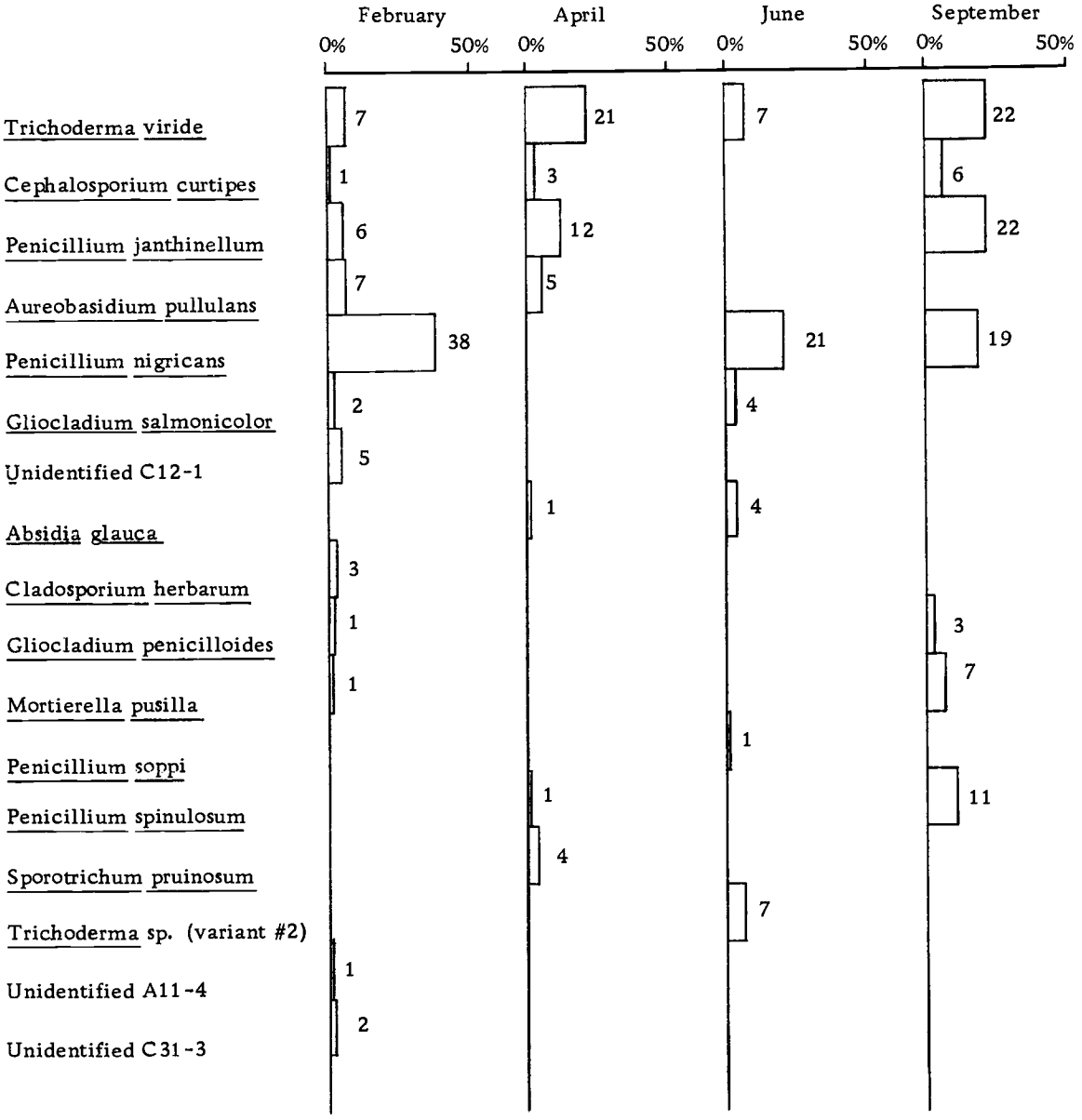

Figure 2. Overall occurrence and seasonal frequencies of selected species in conifer soil.

MIXED ALDER-CONIFER

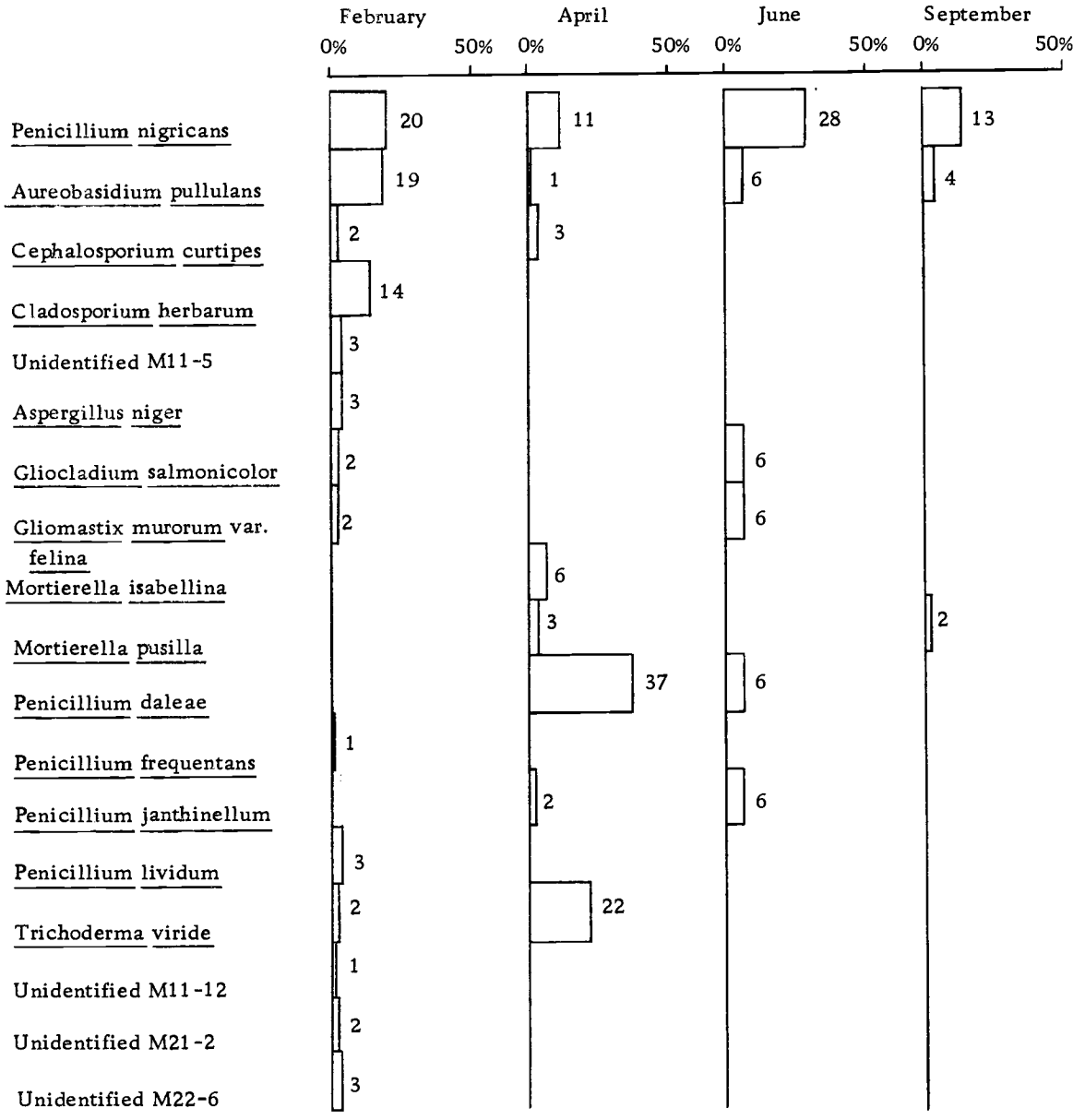

Figure 3. Overall occurrence and seasonal frequencies of selected species in mixed alder-conifer soil.

conifer.

Comparison of Isolation Methods

Three different isolation techniques were used in the expectation that they would reduce the bias introduced by any single technique. Although there were both quantitative and qualitative differences in the populations isolated by the three techniques, these differences were small when compared to the overall sample. Species with high frequencies were commonly isolated by all three methods. Penicillium nigricans, Gliocladium salmonicolor, Trichoderma viride, and Mortierella pusilla were isolated with equal frequency by all three methods. The Dilution Plate Method produced the greatest number of isolates from a given sample. Common species which were isolated only on dilution plates include: Cephalosporium curtipes, Gliomastix murorum var. felina, Cladosporium herbarum, and Mortierella ramanniana var. angulispora. Penicillium janthinellum and Penicillium daleae, both common, were most often isolated by immersion tube or soil plate. Penicillium purpurogenum was isolated only by immersion tube, and Trichocladium sp. and Hyalodendron sp. only by soil plate.

Coefficients of Similarity

Coefficients of similarity were calculated for the three stands using species presence in the formula $2w/a + b$, where a is the number of species in one population, b is the number of species in the other population, and w is the number of species found in both populations. This permitted a comparison of the three stands by species composition of their microfungal populations. Table 7 shows these coefficients of similarity, multiplied by 100, for each pair of populations. All isolates regarded as separate species are included, even unidentified ones. The least similar populations, i.e., those having the lowest coefficient, are the conifer population and the alder population. Based solely upon similarities in microfungal populations, the arrangement of stands corresponds exactly to an arrangement by dominant tree species.

Table 7. Similarity coefficients.

Paired stands	No. of shared species	Similarity coefficients
Alder stand-conifer stand	22	44.00
Alder stand-mixed stand	25	49.50
Conifer stand-mixed stand	22	48.35

Fungi Isolated from the L, F, and A11 Horizons

Organisms characteristic of certain soil horizons were anticipated at the outset of the study. However, species differed but little between the various layers. Within each stand, fungi dominant in any individual horizon were dominant throughout the soil layers. A list of the fungal isolates, with their frequencies in each horizon, appears in Table 6.

The three soils supported equal numbers of species in the litter layer. Approximately half of the species which occurred in the litter layer were present in two or three of the stands, indicating a relatively uniform flora in the litter. Below that, in the F horizon, the number of species in the conifer and mixed alder-conifer soils is lower, whereas in the alder soils, the number remains constant. Below the F horizon there was an increase in the number of species in the A11, especially in the alder soils. The number of isolates from a horizon does not correspond directly with the number of species. Some species produce large numbers of propagules; others, few. In general, the number of isolates increases between the L and F horizons and decreases between the F and A11 horizons.

The relative proportion of Penicillium spp. and Mucorales can be compared at the L, F, and A11 horizons. The percent of total Penicillium species is relatively constant in the L horizon for all

three soils. However, in the F horizon there is a sharp increase in Penicillia in the mixed alder-conifer soils. Likewise, in the A11 horizon, the percent of Penicillium spp. still remains high.

In general, the relative numbers of species of Mucorales decline downward from the L horizon to the A11 horizon, with the exception of the conifer soil in which they increased in the A11 horizon. However, generally for the alder and mixed stands, the percentage of Mucorales decreased with increasing soil depth.

Although no fungi were characteristic of one soil horizon, some were encountered more frequently in one layer than in the others. Aureobasidium pullulans (Figure 4a) was isolated most frequently from the A11 horizon and occurred at each horizon with approximately equal frequency in all of the soil stands. Cladosporium herbarum, Penicillium nigricans, Trichoderma viride, and Penicillium daleae also occurred frequently at each horizon in every soil stand. These fungi were dominant throughout the soil. Cladosporium herbarum and Penicillium daleae (Figure 4c) were most frequent in the L horizon, although P. daleae was dominant only in the alder soils, and C. herbarum was dominant in all three. Penicillium nigricans and Trichoderma viride occurred frequently within all three layers. In the F and A11 horizons of alder soil, P. nigricans appeared most frequently. Cladosporium curtipes was found abundantly in the L and F horizons of alder soil, but very infrequently in the A11 horizon.

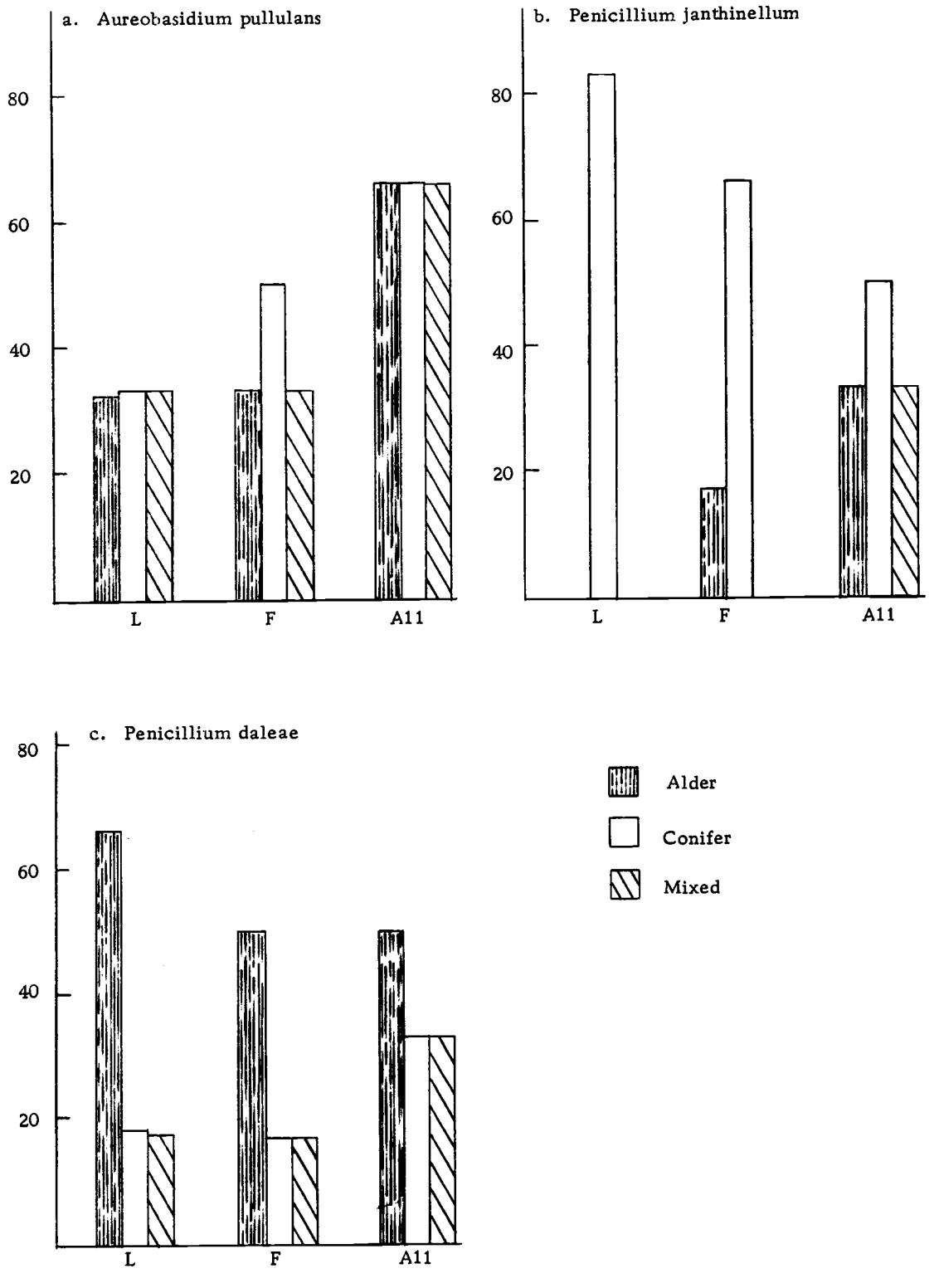

Figure 4. Frequency percentages of selected species at the L, F, and A11 horizon.

In the A11 horizon of conifer, it occurred abundantly, but was either totally lacking or of very low occurrence in the mixed stand. Penicillium janthinellum (Figure 4b) was isolated almost exclusively from conifer soils. In these soils its highest frequency of isolation was in the L and F horizons. Gliocladium salmonicolor appeared also to be characteristic of the L horizon, especially in the alder soils. Aspergillus subsessilis was isolated from all three soils in the F horizon, but was a characteristic member of the L horizon in alder soil. Gliomastix murorum var. felina occurred with equal frequency in each horizon under the alder stand. In the mixed soils it was isolated with the same frequency but only in the F and A11 horizon. In conifer soil, this species appeared only in the A11 horizon, and again with low frequency.

Two representative members of the Mucorales, Mortierella isabellina and Mortierella pusilla, occurred with their highest frequencies in the L horizon. M. isabellina was isolated from all three horizons in alder soil, but with highest frequency in the litter layer. It occurred with the same frequency in the L horizon of the mixed soil, but in no other horizon. It was not present in conifer soils. M. pusilla had a high frequency of occurrence in both L and F horizons of alder soil, but was low in the A11. It was isolated from all three layers of the conifer soils, but with low frequency. M. pusilla occurred only in the F horizon of the mixed soil, with a frequency as high as in the alder stand.

Seasonal Variation in Species Composition

Results of examining seasonal changes in species composition for the three plots are given on Tables 1, 2, and 3. Numbers of species decreased from February to June, increasing from June to September.

Penicillium species and Mucorales show distinct patterns of seasonal distribution in each plot (Figure 5). The numbers of Penicillium spp. to Mucorales appear inverse. When the percent of Penicillium isolates is high, the percent of Mucorales isolates decreases; and when the percent of Mucorales increases, the percent of Penicillia decreases. Under alder, Penicillium spp. decreased from February to April, increased from April to June, and then decreased from June to September (Figure 5a). The opposite effect was observed for Mucorales, with an increase from February to April, a slight decrease from April to June, and then an increase from June to September. This relationship of Mucorales to Penicillia under alder is reversed under conifer (Figure 5b). Penicillium spp. were at their peak in April and September, and Mucorales were at their peak in February and June. The mixed alder-conifer plots showed a trend similar to the alder plots, with Penicillium spp. reaching their highest levels in June, and Mucorales reaching their peaks in April and September (Figure 5c).

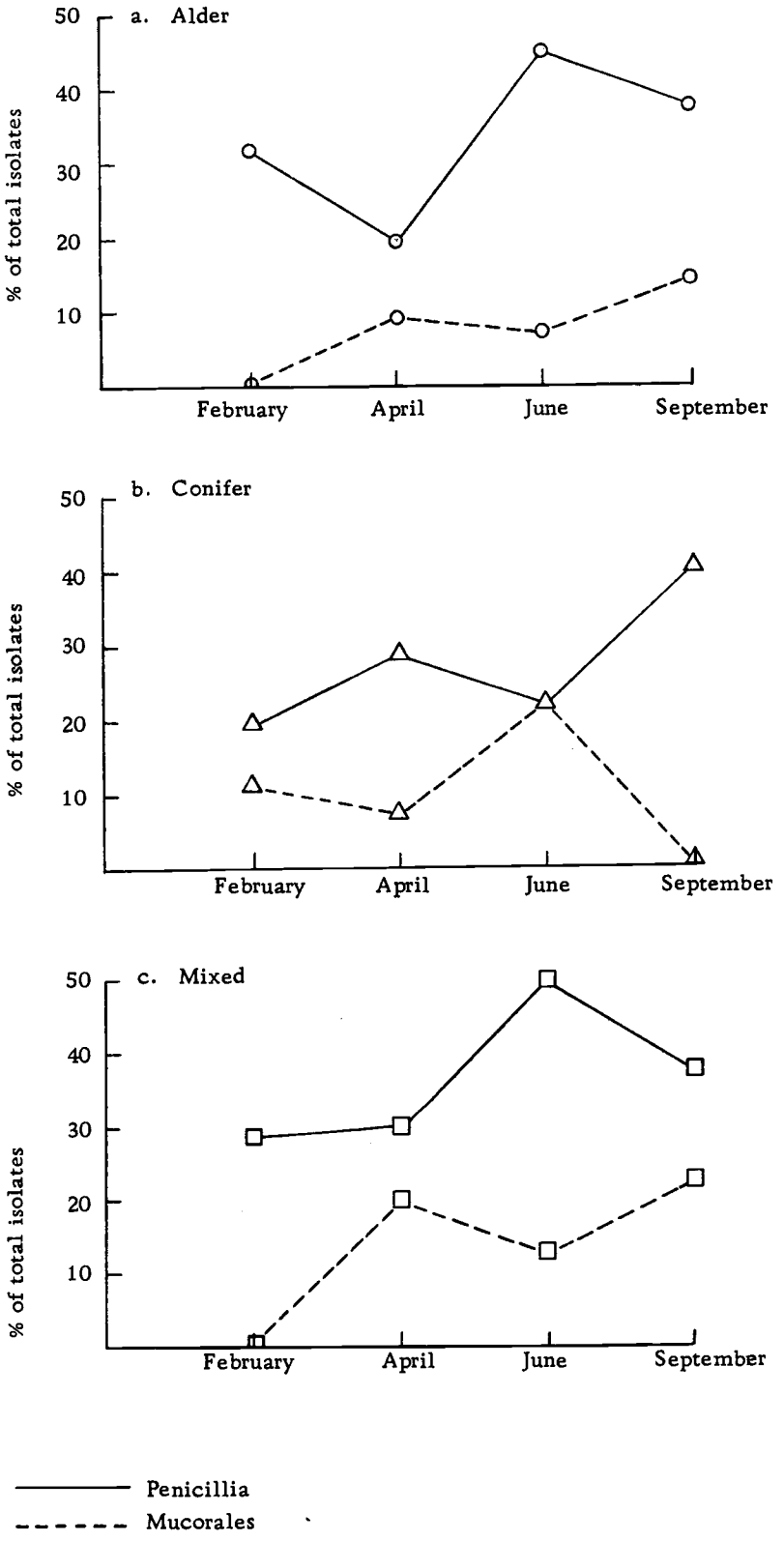

Figure 5. Relative proportion of Mucorales and Penicillia at four intervals during the year.

There also appears to be a seasonal relationship between the two most frequent species, Trichoderma viride and Penicillium nigricans. In alder soils, T. viride appeared with highest frequency in February and April, and with lowest frequencies in June and September, whereas P. nigricans occurred with highest frequency in September, and lowest in April. Under conifers, T. viride was dominant in April and September, and infrequent in February and June. P. nigricans had its highest frequency in February and June, and its lowest in April. Under the mixed alder-conifer, T. viride was isolated infrequently most of the year, except in April when it had a high frequency. The reverse situation is true for P. nigricans, since it remained at a moderately steady level all year, except in June when it became dominant. A mutual relationship between T. viride and Penicillium spp. as a group exists under alder and mixed alder stands. However, under conifers, T. viride and Penicillium spp. have a similar pattern. Hence, under conifers, this relationship can be said to exist only between T. viride and P. nigricans.

Seasonal fluctuations in Penicillium daleae are the reverse of P. nigricans. P. daleae is one of the most frequently isolated of the Penicillia. In all three soils, it was most frequent during April, the same month that P. nigricans was lowest. Penicillium janthinellum appears to fluctuate seasonally only under conifers. Under alder and mixed alder-conifer, it remained at low levels throughout the year.

Under conifers, however, its highest levels were in April and September. This, again, is in contrast to P. nigricans and to most species of Penicillium.

IV. DISCUSSION

Use of the three different isolation methods facilitated isolation of organisms occurring with both high and low frequencies. Martin's rose bengal streptomycin agar was chosen as a primary medium because of its characteristic colony restriction and suppression of bacterial development. Preliminary testing of other media also showed that this medium supported the largest number of colonies without a significant loss in number of species. It should be noted that the total flora within the soil could not be obtained by the use of a single isolation medium, since no medium supports growth of all microfungi. Other media would doubtlessly have yielded a few additional species.

Thornton (1956) observed that "in relatively undisturbed soils a small number of species assume dominance as a result of particular, favorable conditions". This may be consistent with what is occurring on the alder-conifer study plots. As compared with other soil microfungal surveys on hardwood and conifer soils (Tresner et al., 1954; Christensen, M. 1960; Christensen et al., 1962; Christensen and Whittingham, 1965), diversity of species per stand is equivalent. As observed by Lu et al. (1967), by use of dilution plate technique, the three stands support a large number of molds. They reported alder as having 225 thousand/gr. soil in the F horizon and 73 thousand/gr. soil in the A11 horizon; conifer as having 709 thousand/gr. soil in the

F and 195 thousand/gr. soil in the A11; and mixed alder-conifer as 291 thousand/gr. soil in the F and 79 thousand/gr. soil in the A11. However, relatively few species have assumed dominance on the study plots. The alder plot is represented by a greater diversity of dominant species. Interestingly, the alder plot is also represented by a greater diversity of higher vegetation than either of the other two plots (Franklin and Pechanec, 1967).

A prominence of Penicillium species is readily apparent. This genus not only occurred commonly, but its representative species were present with very high frequencies. This very large and diverse genus includes many common soil organisms. Other studies (Bollen and Wright, 1961; Christensen et al., 1962; Reddy and Knowles, 1965; Christensen and Whittingham, 1965) have shown Penicillium to be the most abundant genus in other soil types.

Penicillium nigricans was the most frequent of the Penicillium isolates. According to Raper and Thom (1949) it is a soil mold widely distributed in nature, but is encountered less frequently than other divaricate forms. Its precise role in decomposition processes is not known, but it occurs abundantly upon organic materials undergoing slow decomposition. Tresner et al. (1954) reported high frequencies of occurrence for Penicillium nigricans in the maple-basswood forest. In their study Penicillium nigricans was observed to have a narrow range of environmental tolerance, and served as an indicator of soils

found in climax stands. Hodges (1962) reported Penicillium nigricans from southern forest tree nursery soils, but did not consider it a dominant form. Brandsberg (1969) found Penicillium nigricans on decaying litter of Abies grandis, Pinus ponderosa, and Pinus monticola.

Another closely related organism, Penicillium janthinellum, is even more ubiquitous. Raper and Thom (1949) report this species to be the most abundant Penicillium found in soils. It has been isolated from samples world-wide in origin. It is also commonly isolated from decaying vegetation undergoing final stages of decomposition. It has been reported from deciduous forest soils (Tresner et al., 1954; Christensen et al., 1962) and is an important colonizer of coniferous forest soils (Hodges, 1962; Hayes, 1965). In this study it occurred frequently in all three forest stands, the conifer stand showing the highest frequency (83%), alder next with a frequency of 50%, and the mixed stand last with 33%.

Penicillium daleae, which occurred with a frequency of 83% in alder soil, appears to be a rare fungus elsewhere. Since its original isolation by Zaleski (1927), it has been reported only twice (Al-Doory, Tolba, and Al-Ari, 1959; Christensen and Backus, 1961). It is closely related to P. janthinellum, but is characterized by intense purple-red reverse pigmentation on Czapeks agar, asymmetric-divaricate penicilli, and production of coarsely roughened, banded conidia.

Little is known about this species due to its rare occurrence in nature.

Penicillium thomii was restricted totally to the alder stand, occurring there with a frequency of 50%. It reportedly occurs commonly in soils as well as on lumber and other wood products (Raper and Thom, 1949). Examining the flora of forest soils and litter, Stapp and Bortels (1935) found P. thomii to be abundant and to be especially active in the decomposition of tannin. This species has been isolated commonly from other deciduous forest stands (Tresner et al., 1954; Christensen et al., 1962; Christensen and Whittingham, 1965), and it has also been reported from forest soils of Abies grandis and Pinus monticola (Brandsberg, 1969).

Trichoderma viride was another frequent isolate from both alder and conifer soils with a frequency of 100% in both, but it occurred in the mixed alder-conifer soil with a frequency of only 33%. T. viride is a rapidly growing organism, nutritionally versatile, and strongly cellulytic. It is interesting to note that Bollen and Wright (1961) rarely isolated Trichoderma species from 25 year old Douglas fir forest soils in Oregon. This forest type would be most comparable with the mixed alder-conifer stand at Cascade Head. It would appear that although Trichoderma is widespread in its distribution, its frequency varies considerably from one ecological area to another. Many workers have observed that T. viride is most abundant in moist or waterlogged habitats (Bisby et al., 1935; Pugh, 1962; Warcup, 1951). This may

explain its frequency occurrence in the alder-conifer study plots. The frequency of Trichoderma increased significantly during the wet months (February -April) and generally decreased during the summer months. It was only during the spring months that T. viride was isolated with any regularity from the mixed alder-conifer study plots.

Bisby (1939) wrote a comprehensive review of species concepts within the genus Trichoderma, relating his own and other workers' experiences with variation and intergradation among widely accepted species. He concluded that Trichoderma should be considered a monotypic genus, with all isolates being Trichoderma viride. Some workers disagree with this concept, and use a variety of species to describe the genus. Rifai (1969) adopted the concept of species aggregates, defined as aggregations of morphologically very similar, hardly separable species, for Trichoderma. The author feels that most isolates in the genus were too variable to be split beyond T. viride. There were two isolates which showed consistently different characteristics from T. viride. One organism was identified as Trichoderma album Preuss (Gilman, 1957), the other as closely related to Trichoderma glaucum Abbott or Trichoderma sp. variant #2 (Christensen, 1960). All other isolates were identified as T. viride according to Bisby's concept.

Aureobasidium pullulans was commonly isolated from all three soils and was one of two dark-spored Dematiaceous fungi which

occurred with any frequency. Aureobasidium pullulans is a filamentous, yeast-like fungus which produces spores by budding. It is widespread in occurrence. Kendrick and Burges (1962) found Aureobasidium throughout the year on a high proportion of the L-layer needles of Pinus silvestris. It was considered one of the primary saprophytic colonizers of the needles after the death of the tissues. Smit and Wieringa (1953) isolated A. pullulans from recently dead leaves of various deciduous trees. Reddy and Knowles (1965) reported that Aureobasidium was able to degrade pectin and lipids.

Two other dark-spored species were isolated with more than occasional frequency from alder soil. Trichocladium sp. was isolated with 50% frequency from alder soil. This species of Trichocladium, although very distinct, did not fit the description of any previously described species. Dr. M. B. Ellis reports that this is probably a Trichocladium state of another fungus (Ellis, personal communication). The identification of this fungus is particularly important ecologically, because it was most characteristic of the alder forest soil. Gliomastix murorum var. felina was isolated from all three stands, but most commonly from the alder soil. Brandsberg (1969) reports the isolation of this variety from Abies grandis, Pinus ponderosa, and Pinus monticola decaying litter.

Cladosporium herbarum is a dark-spored species common to many soil types. It was isolated frequently from both alder soil and

mixed-stand soil, but less frequently from conifer soil.

The genus Aspergillus was represented by a variety of species. Alder soil yielded the greatest number of species. Conifer and mixed soils had fewer species, and none with high frequency. Bollen and Wright (1962) also reported Aspergillus species to be rare in Douglas fir soils. Aspergillus subsessilis occurred with a frequency of 50% in alder soils.

Two other fungi were also isolated regularly from the study plots. Cephalosporium curtipes was found frequently in all three stands. Fusidium viride was a frequent isolate in the alder stand, but never isolated from conifer or mixed.

Little can be concluded concerning the unidentified species since their identity is unknown. Forms A11-1, A11-2, C12-1, and M11-5 all represent high frequency isolates unique to one of the three stands. This makes them interesting ecologically.

In relation to dominant tree species and understory vegetation, the mixed stand is intermediate in species composition between pure red alder and pure conifer (Franklin and Pechanec, 1967). Similarly, the mixed stand is intermediate between the alder and the pure conifer in relation to soil microfungal populations. Because mineral soil composition as well as climate and moisture remain constant throughout the stands, the differences in soil microfungal populations between the three stands is most probably related only to differences in the

species composition of the higher vegetation as they affect soil properties.

Higher vegetation can influence the microbial activity of a soil in many ways. These include: effects on soil moisture and temperature as a consequence of shading; effects due to differences in litter fall; and effects due to root exudates or leachates. Since there was little difference in populations between the L and A11 horizons, probably the effect of root exudates was negligible since rhizosphere effects have little influence on activity in the A horizon.

Using dilution plates and plate counts of fungal colonies, Bollen et al. (1969) found the greatest number of fungi in the F horizon. These present results suggest that where the total number of fungi reported by Bollen et al. (1969) is high in the F horizon, the total number of species either remains the same as in the L horizon or decreases. Possibly the organic material available restricts fungal activity to a certain few species.

Bollen et al. (1967) indicated that there was a decrease in the number of molds in the A11 horizon for all three study soils. This report, and the work of the present study, indicate that although the number of molds in the A11 is restricted, the number of species present is not. The A11 horizon appeared to support a great number of species, especially in the alder soils.

This increase in species numbers for the A11 horizon could partially be attributed to a filtering effect of fungal propagules. Spores from species found in the L and/or F horizon could filter through the soil and be isolated in the A11 horizon. These additional spores could cause the enhancement of species numbers as indicated for the A11 horizon.

Increase in Penicillia in the F and A11 horizons of mixed alder-conifer soils was also reported by Bollen et al. (1967). It appears then that the F and A11 horizons of the mixed alder-conifer stand support not only a large number of Penicillia, but also a great diversity of species.

Mucorales are known to be moisture sensitive, and as percent moisture decreases with decreasing soil horizons, it seems logical that the Mucorales would also decrease. Bollen et al. (1967) reported the conifer stand to have a higher moisture content in the A11 than the other soils. This might possibly account for the higher percentage of Mucorales in the A11 horizon of the conifer stand.

Seasonal fluctuations in populations of soil fungi vary in timing and extent with the locality. Cobb (1932), in counting colonies of fungi and bacteria from duff and soil layers under a hemlock stand in New York, found large numbers of fungi on materials collected in April, and smaller numbers during summer and fall months. In contrast, Thornton (1956) found the frequencies of fungal isolates in mixed

oakwood and heath soils in England to be greatest during the summer months. Williams and Parkinson (1964) found no significant seasonal qualitative or quantitative differences in fungal populations of mineral soils in England. Presumably these differences reflect differences in seasonal patterns of soil moisture, soil temperature, and litter fall among the three localities.

Bollen et al. (1967) studied seasonal microbial properties associated with the alder, conifer, and mixed alder-conifer plots at Cascade Head during the course of one year. Fluctuations in mold populations of the F horizons of alder and conifer plots were roughly parallel. Numbers decreased from March to April, increased in July, and decreased again in September. Mold populations in the F layer of the mixed stand changed little from season to season. There were no marked differences among the three plots in numbers of molds in mineral soil. The highest numbers occurred in July and the lowest in September.

There are no dramatic seasonal changes in climate at Cascade Head. Temperatures are moderate throughout the year. There is a high annual rainfall most of which falls in winter. Although there is little precipitation through the summer, the humidity remains high and there are frequent fogs.

Seasonal peaks of populations of soil microfungi (Tables 1, 2, and 3) parallel seasonal changes in soil moisture (Bollen et al., 1967)

in the three stands. There is a decrease in percent moisture in the soil from April to July, a slight increase from July to September, and a steady increase from September to March. The rise in fungal species from June to September may be due to stimulation by increase in temperature. Jensen (1934) observed such a temperature-related stimulation upon soil microbial populations. Daily temperatures increase slightly from June through August at Cascade Head.

V. SUMMARY AND CONCLUSIONS

The alder-conifer study plots at Cascade Head Experimental Forest are a unique natural laboratory. Adjacent stands of pure red alder, pure conifer, and mixed alder-conifer are equivalent with respect to soil type and physical and climatic factors. Since differences in the soil are largely a product of the vascular vegetation, the effects of the dominant higher vegetation on the soil microfungal populations can be studied directly.

The soil microfungi of the three stands can be characterized as follows: The alder plot supports a large number (16) of codominant species. Those most characteristic of this plot are: Penicillium nigricans, Trichoderma viride, Penicillium daleae, Aspergillus subsessilis, Gliomastix murorum var. felina, Penicillium thomii, and Trichocladium sp.. The conifer plot supports a smaller number of dominants (7). Several of these do not occur as dominants under alder. The most characteristic species are: Trichoderma viride, Penicillium janthinellum, and Cephalosporium curtipes. The mixed alder-conifer plot resembles the conifer plot in having few (5) dominant species. There are no species which are dominant in this stand which do not occur as dominants in one of the other plots. Trichoderma viride, which is a dominant in both the alder and the conifer plots, is not a dominant in the mixed plot.

The species composition of the soil microfungal populations in the adjacent stands correlates well with the dominant vascular vegetation. The microfungal population of the mixed alder-conifer plot is intermediate between those of the pure alder and the pure conifer. This correlation is presumably due to either direct or indirect influences of the dominant tree species on the soil, since other factors such as soil type, soil moisture, climate, etc., are consistent throughout the plots.

The following species are reported for the first time from Oregon soils: Aspergillus subsessilis, Eupenicillium pinetorium, Eupenicillium stolkiae, Penicillium daleae, and Trichocladium sp..

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APPENDIX

APPENDIX

List of species deposited with Commonwealth Mycological Institute:

150782 *Eupenicillium pinetorum*

150783 *Eupenicillium stolkiae*

150777 *Penicillium aculeatum*

150775 *Penicillium daleae*

150774 *Penicillium frequentans*

150785 *Penicillium funiculosum*

150770 *Penicillium italicum*

150772 *Penicillium janthinellum*

150773 *Penicillium nigricans*

150768 *Penicillium purpurogenum*

150781 *Penicillium rugulosum*

150766 *Trichocladium* sp.