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SOME FUNGI IN THE FOREST SOILS OF NEWFOUNDLAND

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SUMMARY

A study was made of the fungal population of acidic soils from six coniferous plantations in Newfoundland. Sixty fungi, belonging to 53 species and 25 genera, including 38 Fungi Imperfecti, 13 Phycomycetes, one Ascomycete, one Basidiomycete and seven sterile mycelia, were isolated. This is the first report of soil fungi from Newfoundland and includes several species hitherto not reported from boreal forests of Canada. The genera, in order of their frequency of isolation were *Penicillium*, *Mortierella*, *Oidiodendron*, *Fusarium*, *Aspergillus*, *Mucor*, *Alternaria*, *Chryso-sporium*, *Cladosporium*, *Phialophora*, *Trichoderma*, *Rhizopus*, *Absidia*, *Aureobasidium*, *Cephalosporium*, *Colletotrichum*, *Cylindrocarpon*, *Cryptococcus*, *Monilia*, *Myrothecium*, *Phoma*, *Verticillium*, *Cunninghamella*, *Chaetomium* and *Rhizoctonia*. Soils from well drained to moist areas yielded a higher number of fungal species than those from dry areas.

The fungal populations in forest soils have been investigated by several workers throughout the world (Barron, 1968; Chesters, 1949; Gilman, 1957). However, most studies have been conducted on the soils of hardwood or mixed forests; fewer researchers have investigated soils from coniferous forests. The pioneer studies on the fungi of Canadian soils were carried out by Bisby et al. (1933, 1935) and Timonin (1935). Since then several investigations have been conducted on soil fungi of Canadian forests. Although studies have been carried out to determine the occurrence and distribution of fungi in stands under different sites in boreal forests (Bhatt, 1970; Cavender, 1972; Morral, 1968; Morral and Vanterpool, 1968; Reddy and Knowles, 1965; Widden and Parkinson, 1973), little is known about the soil fungi of these forests in northeastern Canada, particularly in Newfoundland. In 1971, a study was initiated to isolate and identify fungi from the soils of forest stands under different site conditions. This paper lists 60 different fungi along with their relative densities in the soils of six coniferous plantations on the Island.

TABLE I

PLOT LOCATION, HISTORY, NATURAL VEGETATION, PLANTED TREE SPECIES, SOIL CHARACTER

Area no.	Plot location	History	Natural vegetation	Planted tree species
1	Cormack	Burned-cutover	Several species of trees, shrubs and herbs, including pin cherry, <i>Prunus pensylvanica</i> L. f.; red osier dogwood, <i>Cornus stolonifera</i> Michx.; speckled alder, <i>Alnus rugosa</i> (Du Roi) Spreng.; low bush blueberry, <i>Vaccinium angustifolium</i> Ait.; and scattered young trees of aspen, <i>Populus tremuloides</i> Michx.; and white birch, <i>Betula papyrifera</i> Marsh.	Norway spruce, <i>Picea abies</i> L.; white spruce, <i>Picea glauca</i> (Moench) Voss; Sitka spruce, <i>Picea sitchensis</i> (Bong.) Carr.; Japanese larch, <i>Larix leptolepis</i> Sieb. & Zucc.; Douglas fir, <i>Pseudotsuga menziesii</i> (Mirb.) Franco; and Sitka-white spruce hybrid.
2	Serpentine Lake	Cutover	Balsam fir, <i>Abies balsamea</i> (L.) Mill.; scattered black spruce, <i>Picea mariana</i> (Mill.) B.S.P.; and white spruce regeneration; <i>Dryopteris</i> and a few mosses.	Red spruce, <i>Picea rubens</i> Sarg.; and Sitka spruce.
3	Stephenville	Farmland	Regeneration of birch, <i>Betula</i> spp.; black spruce and white spruce; and several types of grasses.	White spruce—28 provenances.
4	Bottom Brook	Cutover	Regeneration of balsam fir and birch; fireweed, <i>Epilobium angustifolium</i> L.; catberry or scarlet elder, <i>Sambucus pubens</i> Michx.; brambles or blackberries, <i>Rubus</i> sp.	Six species of Japanese fir and one balsam fir, including needle fir, <i>Abies holophylla</i> Maxim.; Nikko fir, <i>A. homolepis</i> Sieb. & Zucc.; Maries fir, <i>A. mariësi</i> Mast.; <i>A. mayriana</i> Myiabe & Kudo; Saghalin fir, <i>A. sachalinensis</i> Mast.; Veitch fir, <i>A. Veitchii</i> Lindl.; and balsam fir.
5	Middle Brook	Cutover	Regeneration of balsam fir, white birch and pin cherry, and several types of shrubs, herbs and grasses.	Same as in Bottom Brook
6	Highlands River	Cutover	Regeneration of balsam fir, scattered white birch, several types of shrubs, herbs and grasses.	Same as in Bottom Brook

* After Damman (1964).

MATERIALS AND METHODS

Collection and analyses of soil samples.—Soils were collected from six coniferous plantations for physical and chemical analyses, and for isolation of fungi. These plantations were located at Cormack, Serpentine Lake, Stephenville, Bottom Brook, Middle Brook and Highlands River (latitude 48°05'N to 49°22'N, and longitude 57°19'W to 58°46'W) in western Newfoundland.¹ Their histories, vegetation cover, tree species planted and important soil characteristics are summarized in TABLE I.

¹ The forests of Newfoundland are situated in the Boreal Forest Region of Canada (Rowe, 1972), and consist mostly of balsam fir, *Abies balsamea* (L.) Mill., black spruce, *Picea mariana* (Mill.) B.S.P., and white birch, *Betula papyrifera* Marsh.

ISTICS AND AVERAGE NUMBER OF FUNGAL SPECIES ISOLATED FROM THE SIX PLANTATION AREAS

Moisture regime scale*	Soil characteristics					Average no. of fungal species isolated
	Texture	pH	Organic matter (%)	Available nitrogen (lbs/acre)	Available phosphorus (lbs/acre)	
Well drained:—2	Loam, deep, stone-free overlain with a very thin layer of raw humus.	4.8	12.3	40.6	10	35
Somewhat moist to moist, with patches of wet and poorly drained soil:—3-4	Loam, with variable amounts of sand and clay, overlain with a thick (15-22 cm) layer of raw humus.	4.8	14.8	36.2	6	25
Well drained, with a few patches of wet and poorly drained soil:—3	Loam, deep, stone-free, overlain with a very thin layer of raw humus.	4.7	11.0	33.6	2.0	24
Well drained:—2	Sandy loam, well drained, stone-free, overlain with a thin layer of humus.	4.5	15.4	41.4	8.0	29
Somewhat moist to moist:—3-4	Loam, stone-free, well drained, overlain with a thin layer of raw humus.	4.6	12.9	42.0	4.0	31
Dry:—1	Loam, mostly stone-free, well drained, overlain with a thin layer of raw humus.	4.4	15.1	32.2	8.0	14

Five samples of the top 10-15 cm of soil were collected with a soil auger from widely separated locations in each of the plantations. The surface humus and litter were removed before collecting the soil samples. The collections were made during the month of August and stored for 2-3 wk at 0 C in plastic bags with rubber band seals. A composite sample for each plantation was prepared by mixing the five collections. A small portion of each composite sample was used for making fungal isolations; the remaining larger portion was used for conducting physical and chemical analyses of the soils. The portions of the composite samples used for analysis of available nutrients were air dried at 40 C and sieved through a 10 mesh (2-mm sieve opening) screen. In addition a sample of each was also sieved through a 100 mesh (0.149-mm sieve

opening) screen to obtain the finer soil aggregate required for analysis of the organic matter.

The pH of the soil samples was determined, before drying, in 1:1 soil-water suspension using a Beckman pH meter Model Xeromatic II (Jackson, 1958). Mechanical analysis of the dried soil samples was conducted by the hydrometer method (Bouyoucos, 1934, 1951). The percent organic matter was determined by estimating the organic carbon, using the wet digestion method of Walkley and Black (1934) and multiplying the results with "Van Bemmelen's factor" of 1.724 (Allison, 1965). Available nitrogen (NH_4-N plus NO_3-N) was determined by the steam distillation method (Bremner and Kenney, 1965) and available phosphorus was determined by Truog's method (1930).

Isolation of fungi.—Microfungi from the soil samples were isolated by Warcup's soil plate method (1950), using 0.005 to 0.015 g of soil and Czapek-Dox agar medium (Thom and Raper, 1945) with 0.5% yeast extract added, pH 4.0. Other media, such as malt extract agar, potato-dextrose agar and corn-meal agar, were also tried but Czapek-Dox agar medium proved the best for isolation.

One plate containing Czapek-Dox agar medium and inoculated with sterile distilled water (without inoculum), was always incubated as a control with each isolation attempt. A total of 42 plates (excluding the controls), 7 plates per composite soil sample from each of the 6 sites, was incubated at 25 C and examined periodically. They were kept at least 3 wk in order to allow slow-growing species to develop sufficiently. After isolation, the cultures were purified by single spore or single hyphal tip isolation method (Warcup, 1955). Rose bengal (one part in 15,000 parts of medium) was added to the medium to avoid bacterial contamination during purification of some cultures (Martin, 1950).

After establishment of pure cultures, investigations on the identification of these fungi were conducted by subculturing most of these fungi on potato-dextrose agar or 2% malt extract agar; *Penicillia* and *Aspergilli*, however, were grown on Czapek-Dox agar. The majority of cultures were incubated at 25 C until a stage of development suitable for identification had been reached.

Estimation of fungal populations.—Population counts were based on the appearance of colonies of fungi on the surface of the medium. The developing colonies of each species were counted and the prevalence of a species was expressed in terms of relative density, which is used to compare the relationship of one species to another or to the group.

It was calculated by dividing the number of clones of each species by the total number of clones in a forest population and multiplying by 100 (Cavender and Raper, 1965). In the present investigations the formula was applied as follows:

Average Relative Density (D) of a species in a site

$$= \frac{\text{No. of fungal colonies of a species in 7 plates}}{\text{Total no. of colonies of all species in 7 plates}} \times 100.$$

RESULTS

Two hundred and twenty-five apparently different fungi were isolated from the soils of the six coniferous plantations. Of the fungi, 39 have been identified to species, 14 to genus, and seven have been classified as either dark or white sterile mycelia (TABLE II). The fungi identified include 13 Phycomycetes, one Ascomycete, one Basidiomycete and 38 Fungi Imperfecti.

As could be expected in an investigation of this nature, the majority of species belong to Fungi Imperfecti (Deuteromycetes). Prominent genera were *Penicillium* which had the highest number of species, i.e., eight, followed by *Oidiodendron* and *Fusarium* each having four species, and *Aspergillus* with three species. Among the Phycomycetes, the genus *Mortierella* was the most common with five species, followed by the genus *Mucor* with three species. There was only one Ascomycete, a species of *Chaetomium*, and one Basidiomycete, a species of *Rhizoctonia*.

Aureobasidium pullulans, *Trichoderma polysporum* and *Rhizopus elegans* were the most common species, being found in the soils from all the six areas (TABLE II). However, *Mucor ambiguus*, *M. hiemalis*, *M. silvaticus*, *Penicillium spinulosum*, *Chaetomium* sp., *Chrysosporium pannorum*, *Colletotrichum* sp., *Cylindrocarpon* sp., *Monilia geophila*, *Myrothecium* sp. and *Phoma* sp. were each isolated from only one area.

The results also show that the two *Absidia* spp. were found only in soils in the cutovers at Cormack, Serpentine Lake, Bottom Brook, Middle Brook and Highlands River, and not in that from the farmland at Stephenville. However, *Penicillium spinulosum*, *Chrysosporium pannorum*, *Colletotrichum* sp., *Monilia geophila* and *Myrothecium* sp. were isolated only from the farmland soil. *Mucor* spp. were found only in soils from the burned-cutover area at Cormack, and *Chaetomium* sp. and *Phoma* sp. were isolated only from soils collected from the cutover at Highlands River. The species numbers ranged from 14 to 35 in the

TABLE II
LIST OF FUNGI AND THEIR AVERAGE RELATIVE DENSITIES (D) IN SOILS FROM THE SIX
LOCATIONS/SITES UNDER CONIFEROUS PLANTATIONS

Fungal species	Sites												
	Cormack		Serpentine Lake		Stephenville		Bottom Brook		Middle Brook		Highlands River		
	D	D	D	D	D	D	D	D	D	D	D	D	
<i>Phycomycetes</i>													
Mucoraceae													
<i>Absidia glauca</i> Hagem	36		32		—		38		28		11		
<i>Absidia spinosa</i> Lendner	60		41		—		53		30		19		
<i>Mucor ambigua</i> Vuill.	20		—		—		—		—		—		
<i>Mucor hiemalis</i> Welmer	34		—		—		—		—		—		
<i>Mucor sibiricus</i> Hagem	18		—		—		—		—		—		
<i>Rhizopus elegans</i> Eidam	47		32		28		40		37		28		
<i>Rhizopus nigricans</i> Ehrenberg	20		—		26		—		42		9		
Mortierellaceae													
<i>Mortierella isabellina</i> (Oudemans) Zycha	21		—		—		28		—		6		
<i>Mortierella vana</i> Linnemann	19		—		—		29		—		—		
<i>Mortierella ramanniana</i> (Moeller) Linnemann	72		—		51		73		—		16		
<i>Mortierella vinacea</i> Dixon-Stewart	46		—		39		56		—		9		
<i>Mortierella</i> sp.	28		—		—		—		—		12		
Choanophoraceae													
<i>Cunninghamella echinulata</i> Thaxter	31		27		19		16		14		—		
<i>Ascomycetes</i>													
Chaetomiaceae													
<i>Chaetomium</i> sp.	—		—		—		—		—		18		
Basidiomycetes													
<i>Rhizoctonia</i> sp.	10		8		—		—		—		—		
<i>Fungi Imperfecti</i>													
<i>Alternaria</i> sp.	30		—		70		48		36		—		
<i>Alternaria tenuis</i> Nees	56		64		—		69		52		—		
<i>Aspergillus carneus</i> (v. Tiegh.) Blochwitz	8		22		—		—		30		10		
<i>Aspergillus fumigatus</i> Fresenius	—		37		29		—		46		—		
<i>Aspergillus niger</i> van Tieghem	16		30		21		—		26		—		
<i>Arthrospidium pululans</i> (de Bary) Arnaud	26		30		42		18		24		10		
<i>Cephalosporium</i> sp.	—		—		—		42		38		—		
<i>Chrysosporium pannorum</i> (Link) Hughes	—		—		18		—		—		—		

soils from cutover areas, although the highest number was found in the burned-cutover.

Although the present data are meagre, the results do indicate that the distribution of the fungal species and the number of species varied among soils of different moisture regimes. Species of *Mortierella* were only found in soils from dry sites. However, *Aspergillus fumigatus*, *Oidiodendron griseum*, *Fusarium oxysporum* and *Penicillium rolfsii* were most common in soils from somewhat moist to moist sites; *F. oxysporum* and *P. rolfsii* were not obtained from soils from dry sites. Well-drained to moist soils yielded an average of 24 to 35 different fungi as compared to only 14 fungi isolated from dry soil (TABLE I). These results are also supported by the relative density data on various fungal species from the six sites (TABLE II). The relative density for the species varied from a low of 2% to a high of 94% in soils from well-drained to moist sites at Cormack, Serpentine Lake, Stephenville and Bottom Brook. However, it varied from a low of 2% to a high of 28% in soils from the dry site at Highlands River.

DISCUSSION

This paper is the first attempt to study the soil microflora of the forests of Newfoundland and it records 60 different fungi isolated from acidic soils of six coniferous plantations established on the Island.

Although the list of fungi shown in TABLE II represents only a part of the soil microflora occurring in these plantations, it does include 10 species hitherto not reported from boreal forest regions of Canada. These are *Absidia glauca*, *A. spinosa*, *Rhizopus elegans*, *Cunninghamella echinulata*, *Aspergillus carneus*, *Penicillium raistrickii*, *P. spinulosum*, *Fusarium culmorum*, *F. oxysporum* and *F. poae*.

The data in TABLES I and II show differences in the average number of fungal species isolated, but they do not indicate if such a variation could be attributed only to factors such as stand history, nutrient content, organic matter and pH of the soils. *Fusarium* species were found in low relative densities in all the soil samples. This could be ascribed to acidic nature of the soils with high organic matter (Eiker, 1974; Gordon, 1954; Morral and Vanterpool, 1968; Park 1963; Warcup, 1957); all the present soil samples had a low pH and high organic content (TABLE I). Among the various *Fusarium* species isolated, *F. oxysporum* was the most abundant. The high incidence of *Penicillium* species in the soils from the six areas is not surprising in view of similar reports from organic soils elsewhere. The same is perhaps true of *Trichoderma* spp. Therefore the occurrence of *Trichoderma viride*

in the Newfoundland soils is not surprising. Rao (1970) remarked that this species is generally accepted as characteristic of acid soils. Although Basidiomycetes are frequently found fruiting on soil in the boreal forests of Newfoundland, only one species was isolated during the present investigations. Perhaps the isolation techniques used in the present investigations did not favour the isolation of Basidiomycetes. Similar observations have also been made by several other researchers, including Bhatt (1970), Eicker (1974) and Gochenaur and Whittingham (1967).

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