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Fungal succession on fir needles in Germany

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Summary

The mycoflora on the needles of a German fir, *Abies alba* was investigated. Living, green needles and two types of fallen needles from the L and the F horizons of the leaf litter were examined for their fungal flora. Hyphomycetes were frequently isolated from all types of needles. Zygomycetes were isolated only from the L and F horizons. Patterns of fungal species are analyzed for each needle type. From the correspondence between the fluctuations of the leaf-litter constitution and fungal occurrence, a possible pattern for the fungal succession on the decaying fir needles is described. The present fungal succession is compared with those on Japanese and French fir needles published previously. They all have different features from the generalized scheme based on English pine needles. Effects of climatic conditions which seem to affect fungal succession are discussed.

Introduction

For ecological studies of microfungi, the methods used for isolating fungal species from natural substrata, as well as sampling procedures, are very important (Harley, 1971; Miller, 1974; Parkinson, 1982). Every fungal isolation method is more or less selective for fungal species colonized on/in the substrata (Swift, 1976; Parkinson, 1982), and might affect the floristic data obtained.

Kendrick and Burges (1962) have documented the fungal succession associated with the needle decay of *Pinus sylvestris* with data obtained by three different isolation methods. Hudson (1968) reviewed the phenomenon for the whole pinaceous substrata. The ecology of microfungi in the litter of coniferous forests has been studied in Europe and America by several methods (Brandesberg, 1962; Hayes, 1965a–c; Widden and Parkinson, 1973; Söderström 1975; Lehmann and Hudson, 1977; Mitchell and Miller, 1978a, b,; Mitchell et al., 1978; Gourbière, 1974a, b, 1975, 1979, 1980a, b, 1981, 1982, 1984). Hudson's ideas (Hudson, 1968) have been widely accepted and frequently quoted.

In Japan, where the climate differs from that in Europe, some mycologists have conducted similar ecological works for pinaceous substrata (Tubaki and Saito, 1969; Tokumasu, 1978, 1980, 1981; Aoki et al., 1990). Their results are not directly comparable with those of European or

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American researchers because of differences in methods, climate, substrate specificities, and so on. To compare and estimate the effects of different environmental conditions on fungal succession and fungal distribution, a standardization and qualification of methods and substrata are needed.

In Europe, *Abies alba* Mill. is the most common fir species. In this paper, a fungal succession on the needles of the German fir was investigated by using the same washing method applied by Aoki et al. (1990) for Japanese fir needles. The results are compared with those on the Japanese fir needles and with those of Gourbière (1974a, b, 1975, 1979, 1980a, b, 1981, 1982, 1984) on fungi from French fir needles.

Materials and Methods

Description of the study site The study site was a stand of fir (*Abies alba*) in the Schönbuch near Tübingen, Baden-Württemberg, Germany, which is located at 48° 33′N lat., 9° 3′E long., about 450 m above sea level. The forest consisted of well-developed firs (ca. 50 cm in diam near the base). The ground was mostly open, with a few herbaceous plants and shrubs.

Collection of needle samples Samples of living and fallen needles were collected from October 1988 through August 1989. Living needles were collected four times from one of the lowest branches (about 2 m above the ground) of a given fir tree: on 9 Oct. 1988, and 3 Jan., 4 Apr., and 1 July 1989. Litter samples were collected eight times at plots under the canopy of the tree: on 9 Oct. and 4 Dec. 1988, and 3 Jan., 6 Feb., 4 Apr., 4 June, 1 July, and 5 Aug. 1989. In every collection, a block (ca. 5 × 10 cm, ca. 1 cm thick) was cut from the organic horizon, put into a clean paper bag, and brought back to the laboratory.

Sorting of needles The litter sample was sorted into several types of needles which appeared to correspond to different stages of decay. Sorted needle types were characterized as reported by Aoki et al. (1990), except for F_2 needles. The F_2 needles were fragile with minute soil particles on the surface and sometimes losing the mesophylle. The H layer was indistinct in the forest.

After sorting, the total number of needles of each type was counted and the total weight was recorded. All the needles were then stored in sterile and dried paper envelopes at 4°C until the beginning of the fungal analysis, i.e., within one week.

Washing method The needle types selected for fungal analysis were living needles (V), L (brown), F_1, and F_2. Needles of types V and F_1 were examined every three months, those of type L every two months, starting in October 1988. The mycoflora of F_2 needles was examined in August 1989.

The washing technique of Harley and Waid (1955) as modified by Tokumasu (1978, 1980) was used for the fungal analysis in Tübingen. The procedure for washing was the same as that adopted by Aoki et al. (1990) in studying the fungal succession on fir needles in Japan. Twenty needles from each selected type were divided into four sets. Each set was placed into a sterile test-tube with a sterile metal cap. Ten ml of sterilized 0.005% Aerosol OT solution (di-*iso*-octyl sodium sulfosuccinate) was added the tube as a washing detergent. The tube was shaken vigorously in a Voltex Genie mixer at a constant intensity for one minute. The contents were allowed to settle, then the detergent was changed. Washing with the detergent was repeated ten times. Tokumasu (1978) indicated that dormant propagules adherent to sample needles could be removed more efficiently by this washing procedure than by the original (Harley and Waid, 1955) or a modified (Kendrick and Burges, 1962) method. From the results of dilution plate counts for determining the number of fungal propagules in the waste detergent after washing, a ten-times washing was sufficient for

removal of surface-borne propagules on the needles. Then the needles were rinsed with sterilized distilled water three times in the same manner. The rinsed needles were transferred to sterile filter paper in 9-cm Petri dishes and dried for one day to suppress vigorous bacterial growth after plating (Widden and Parkinson, 1973). Ten sets of two needles were laid down onto diluted (half-strength) cornmeal-agar plates (Difco Laboratories) and incubated. For inducing more species to sporulate, the plates were set on a shelf near the window of the laboratory under the fluctuating light and temperature conditions of night and day.

The incubated plates were observed microscopically four times: after three days, one week, two weeks, and four weeks. This observation schedule enabled us to find most fungi inhabiting the needles (Tokumasu, 1978). Fungi appearing on and around the needles were isolated into pure culture and identified (Aoki et al., 1990). Fungal species were also identified directly by making microscopic preparations from the incubated plates. The occurrence of individual fungi was evaluated by the percentage frequency, calculated by the following equation: The percentage frequency of a given fungus (%)=[number of needles from which the fungus was detected/20 (total number of needles in each examination)] × 100.

Results

Leaf-litter decomposition in appearance The seasonal fluctuation of the calculated leaf-litter constitution is given in Table 1. The proportion of L needles fluctuated between 5% and 17% of the total litter needles. The ratio of L to F_1 needles was roughly 1 : 4 except in December 1988 and February 1989 (nearly 1 : 2), when the L layer showed a peak, indicating a heavier leaf fall in the study forest during the winter. A peak of F_1 needles was recorded in April 1989, four months after the highest peak of L needles. At the same time, the ratio of L to F_1 needles returned to 1 : 4. This suggests that about half of the winter L needles had decomposed, being transformed and added to the F_1 needles. Peaks of F_2 needles were detected in June and August 1989. They occurred two to four months later than the highest peak of F_1 needles. The peaks of F_2 needles were accompanied by reductions of the F_1 needles (to about one half of the proportion in April). It cannot, however, be concluded that the newly-formed F_1 needles were rapidly transformed to F_2 needles during this

Table 1. Percentage constitution of a ten-gram mixture (dry weight) of fir leaf-litter at each collection time.

Needle type	1988		1989				Average
	Oct.	Dec.	Feb.	Apr.	June	Aug.	
L layer	(10)	(17)	(15)	(13)	(5)	(6)	(11)
Lgn (green)	1	1	1	4	0.5	1.5	1.6
Lbr (brown)	8	15	14	9	4	4	9
Lgy (grey)	1	1	0	0	0.5	0.5	0.5
F layer	(90)	(83)	(85)	(87)	(95)	(94)	(89)
F_1	44	27	34	53	23	19	33.3
F_2	46	56	51	34	72	75	55.6
Total no. needles (Ten-gram leaf litter)	3659	3283	2856	3059	3539	3250	
L/F_1 ratio	0.23	0.63	0.44	0.25	0.22	0.32	0.33

Table 2. List of species observed on the needles of *Abies alba*.

	V	L	F_1	F_2
Zygomycotina		++	++	++
Absidia sp.			+	
Mortierella isabellina Oudemans et Koning		++	++	++
M. ramanniana (Möller) Linneman		++	++	++
M. ramanniana var. *augulispora* (Naumov) Linneman			+	
M. humilis Linneman ex Gams		+	++	
M. hyalina (Harz) Gams		++	++	+
M. vinacea Dixon-Stewart		++	++	++
M. verticillata Linneman		+	+	++
Mortierella sp. 7			++	
Mortierella sp. 8		+		
Mucor hiemalis Wehmer		++	++	++
M. racemosus f. *sphaerosporus* (Hagem) Schipper			+	
M. mucedo (Linnaeus) Fresenius		+	+	+
Piptocephalis sp.			+	++
Syncephalis sp.			+	+
unidentified				
Ascomycotina	++	++	++	
unidentified Pyrenomycete	++	++		
unidentified Discomycetes		++	++	
Basidiomycotina		++	+	
unidentified				
Deuteromycotina (*: dematiaceous species)	++	++	++	++
Acremonium sp. 1		+	+	
Acremonium sp. 2			+	
Acremonium sp. 3			+	
**Acrodontium crateriformae*		+	+	
**Alternaria alternata* (Fries) Keissler	++	++	++	
**Anungitea* sp.		++	++	++
**Arthrinium* anamorph of *Apiospora montagnei* Saccardo	+	+		
Aspergillus sp.	+	+		
**Aureobasidium pullulans* (de Bary) Arnaud	++	++	++	++
**Aureobasidium* sp. 1	+			++
**Chalara longipes* (Preuss) Cooke	+	++	++	+
**Cladobotryum* sp.	+			
**Cladosporium cladosporioides* (Fresenius) de Vries	++	++	++	++
**C. herbarum* (Persoon) Link ex S. F. Gray	++	++	++	++
**C. macrocarpum* Preuss		+		
**C. sphaerospermum* Penzig		+	+	
Cylindrocarpon sp.			+	+
**Dactylaria candidula* (Höhnel) Bhatt et Kendrick		++	++	++
**Dactylaria lepida* Minter			+	+
**Endophragmiella boewei* (Crane) Hughes		++	++	

**Epicoccum purpurascens* Ehrenberg ex Schlechtendahl	++	++	++	++
**Exophiala* spp.	++	++	++	++
Fusarium oxysporum Schlechtendahl				
Fusarium poae (Peck) Wollenweber	+	++	++	+
Fusarium solani (Martius) Appel et Wollenweber				
Fusarium spp.				
Gliocladium sp. 1		++	++	++
Gliocladium sp. 2		+	+	
Monacrosporium sp.			+	
**Monodyctis levis* (Wiltshire) Hughes		+		
Oedocephalum sp.	+			
Paecilomyces sp.		+		++
Penicillium brevicompactum Dierckx				
P. janczewskii Zaleski				
P. restrictum Gilman et Abbott	+	++	++	++
P. simplicissimum (Oudemans) Thom				
P. spinulosum Thom				
Penicillium spp.				
Phoma sp.	+	+	+	
Phomopsis sp.	++			
**Polyscytalum fecundissimum* Riess	++	+		
**P. verrucosum* Sutton		++	++	
Sagenomella aff. *alba* Gams et Söderström		++	+	
**Scytalidium lignicola* Pesante	++	++	++	++
**S. thermophilum* (Cooney et Emerson) Austwick				
Selenophoma sp.		+		
**Septonema* sp.		+	+	+
**Thysanophora penicillioides* (Roumeguère) Kendrick		++	++	+
**Trichocladium asperum* Harz			+	
Trichoderma hamatum (Bonorden) Bainier aggr.		++	++	++
T. polysporum (Link ex Persoon) Rifai aggr.		+		
T. pseudokoningii Rifai aggr.	+	++	++	+
T. viride Persoon ex S. F. Gray aggr.		++	++	++
**Tripospermum acerinum* (Sydow) Spegazzini	+			
**T. myrti* (Lind) Hughes	+	+		
**T. camelopardus* Ingold, Dann et McDougall	++	++	+	
Verticillium sp.	+	++	++	++
Volutella sp.		+		
(*)unidentified (17 spp.)				

period. In April, the F_1 needles were apparently composed of F_1 needles remaining from the previous year and new F_1 needles formed in the last winter. The increased portion of F_2 needles in June and August might be largely attributable to F_2 needles derived from old F_1 needles.

Whole fungal flora on the fir needles The species observed on the German fir needles are listed in Table 2. Species of Zygomycotina occurred only on fallen needles. Species of the Deuteromycotina (over 80 spp.), including many dematiaceous species (at least 30 spp., marked with an asterisk in

Table 3. Vertical distribution of selected fungi (with average frequency of 5% or more on at least one needle type).

	Needle type			
	V	L	F_1	$(F_2)^{2)}$
I. Fungi of constant distribution				
Aureobasidium pullulans	58	52	20	25
Scytalidium spp.	55	32	54	50
Chalala longipes	10	67	25	+[1)]
Cladosporium herbarum	17	20	19	15
Cladosporium cladosporioides	14	43	31	30
Exophiala sp.	++	33	70	40
Penicillium spp.	+	56	86	100
Epicoccum purpurascens	++	++	15	10
Fusarium spp.	+	++	10	+
Verticillium sp.	+	13	18	55
II. Fungi of partial distribution				
Phomopsis sp.	18	0	0	0
Cladobotryum sp.	++	0	0	0
Polyscytalum fecundissimum	21	+	0	0
Ascomycete (Pyrenomycete)	++	++	0	0
budding yeast	++	+	+	0
sterile white hyphae	20	++	++	0
Tripospermum camelopardus	80	15	+	0
Alternaria alternata	+	10	+	0
Ascomycetes (Discomycetes)	0	24	++	0
Thysanophora penicillioides	0	77	38	+
Anungitea sp.	0	67	33	10
Endophragmiella boewei	0	42	20	0
Polyscytalum verrucosum	0	36	19	0
Trichoderma pseudokoningii	+	++	11	0
Basidiomycete	0	++	+	0
Sagenomella aff. *alba*	0	++	+	0
Mortierella humilis	0	+	++	0
Gliocladium sp. 1	0	16	18	10
Dactylaria candidula	0	14	29	15
Mucor hiemalis	0	14	18	40
Trichoderma viride	0	35	74	80
Mortierella ramanniana	0	35	71	80
Mortierella isabellina	0	31	70	95
Mortierella vinacea	0	13	30	75
Trichoderma hamatum	0	++	11	10
Mortierella hyalina	0	++	++	25
Mortierella verticillata	0	+	+	50
Paecilomyces sp.	0	+	0	55
Mortierella sp. 7	0	0	++	0
unidentified 1	0	0	++	10
Piptocephalis sp.	0	0	+	20
Aureobasidium sp.	+	0	0	10

[1)] Annual average frequency (%): ++, 9–5; +, 4–1.
[2)] Average frequency of the data of August in 1989.

Table 2), were isolated frequently from both living and fallen needles and were recorded from different needle types. Basidiomycetous hyphae provided with clamp connections were observed on L and F_1 needles.

Vertical distribution of fungal species The vertical distribution of selected fungi is shown in Table 3. Fungi which occurred on at least one needle type with an average annual frequency of 5% or more are listed. They are divided into two groups based on the pattern of vertical distribution, i.e., constant distribution for fungi isolated from every needle type, and partial distribution for fungi isolated from limited needle types. Fungi of partial distribution were, however, mostly isolated from plural needle types, except for *Phomopsis* sp., *Cladobotryum* sp., *Mortierella* sp. 7, unidentified 1, and *Piptocephalis* sp.

Some of the fungi of constant distribution showed peaks in their average frequencies on specific needle types: *Aureobasidium pullulans* on V needles, *Chalara longipes, Cladosporium herbarum*, and *Cladosporium cladosporioides* on L needles, *Exophiala* sp. on F_1 needles, *Penicillium* spp. on F_1 and F_2 needles, and *Verticillium* sp. on F_2 needles.

Among the fungi listed under partial distribution, *Phomopsis* sp. and *Cladobotryum* sp. were restricted to V needles. *Polyscytalum fecundissimum, Tripospermum camelopardus*, and sterile white hyphae were most frequently observed on the same needle type. *Alternaria alternata* and unidentified discomycetes showed the highest frequencies on L needles. *Thysanophora penicillioides, Anungitea* sp., *Endophragmiella boewei*, and *Polyscytalum verrucosum* were isolated most frequently from L needles but also occurred on F_1 needles with relatively high average frequencies. *Gliocladium* sp. 1 and *Dactylaria candidula* were isolated from L, F_1 and F_2 needles, and had their highest average frequencies on F_1 needles. *Mucor hiemalis*, five species of *Mortierella*, and two species of *Trichoderma* were also isolated on all types of needles from L through F_2. Most of them showed higher average frequencies in the later stages of needle decay. *Mortierella* sp. 7, unidentified 1, and *Piptocephalis* sp. were observed only

Table 4. Seasonal fluctuation of selected fungi[1] on V (living) needles.

Fungus	Average frequency (%)	1988	1989		
		Oct.	Jan.	Apr.	July
I. Fungi of continuous occurrence					
Scytalidium spp.	55	65	85	35	35
Tripospermum camelopardus	80	75	65	80	100
Aureobasidium pullulans	58	80	40	+[2]	100
II. Fungi of discontinuous occurrence					
Cladosporium herbarum	16	40	+	−	15
Cladosporium cladosporioides	14	45	+	−	+
Polyscytalum fecundissimum	21	80	−	+	−
Chalara longipes	10	35	−	−	+
Epicoccum purpurascens	7.5	30	−	−	−
Alternaria alternata	5	20	−	−	−
sterile white hyphae	20	−	45	35	−
Phomopsis sp.	18	−	−	−	70

[1] Fungi that occurred at least once with a frequency of 20% or higher are listed.
[2] Percentage frequency: +, 10–5%; −, 0%.

on F needles.

Seasonal fluctuation of fungal flora The seasonal fluctuations of the fungal flora on the respective needle types are shown in Tables 4–6. Fungi observed at least once with a percentage frequency of 20% or higher are listed. The occurrence patterns of fungi could be divided into two: continuous and discontinuous. They are shown separately in the Tables.

V needles (Table 4). Only three species of microfungi occurred continuously. These mostly ap-

Table 5. Seasonal fluctuation of selected fungi[1] on L (brown) needles.

Fungus	Average frequency (%)	1988		1989			
		Oct.[3]	Dec.	Feb.	Apr.	June	Aug.
I. Fungi of continuous occurrence							
Thysanophora penicillioides	77	65	95	75	85	70	70
Chalara longipes	67	55	95	80	80	60	30
Penicillium spp.	56	30	100	40	50	85	30
Anungitea sp.	67	40	90	75	85	95	15
Cladosporium cladosporioides	43	40	75	55	35	35	20
Cladosporium herbarum	20	+[2]	40	+	25	15	20
Exophiala sp.	33	+	85	25	45	20	15
Mortierella ramanniana	35	+	55	45	40	50	15
Mortierella isabellina	31	+	45	60	30	40	+
Alternaria alternata	10	+	15	20	+	+	+
Ascomycete (Discomycete)	24	50	+	35	30	15	+
Aureobasidium pullulans	53	65	30	30	50	55	85
II. Fungi of discontinuous occurrence							
Ascomycete (Pyrenomycete)	9.2	+	45	−	−	−	−
Polyscytalum verrucosum	36	25	65	15	55	55	−
Gliocladium sp. 1	16	−	55	15	+	15	−
Epicoccum purpurascens	9.2	−	15	+	20	15	−
Mortierella vinacea	13	−	15	+	25	30	−
Dactylaria candidula	14	−	25	+	+	40	−
Verticillium sp.	13	−	30	+	+	45	−
Endophragmiella boewei	42	+	55	20	75	90	−
Trichoderma viride	34	−	45	25	40	80	15
Trichoderma pseudokoningii	5.8	−	−	35	−	−	−
sterile white hyphae	5.8	−	−	25	+	−	+
Mucor hiemalis	13	−	+	15	+	40	+
Trichoderma hamatum	8.3	−	−	−	25	20	+
Basidiomycete	8.3	−	+	−	15	25	+
Mortierella hyalina	7.5	−	+	+	+	20	−
Scytalidium spp.	32	65	+	−	40	40	35
Tripospermum camelopardus	15	20	+	−	+	−	50
Tripospermum myrti	3.3	−	−	−	−	−	20

[1], [2]: See Table 4.

[3] From the leaf-litter constitution, the leaf-loss of the fir was presumed to become heavier at the study site during the winter.

peared as dominants of the monthly microflora: *Aureobasidium pullulans* in October 1988 and July 1989, *Scytalidium* spp. in January 1989, and *Tripospermum camelopardus* in April and July 1989. *Polyscytalum fecundissimum*, listed under the discontinuous occurrence, was also one of the dominants in October 1988. These species characterized the seasonal flora of the V needles. Compared with the other types, V needles had remarkably fewer fungi of continuous occurrence. These species became less frequent in the L horizon, except for *Aureobasidium pullulans*. Fungi of discontinuous occurrence have a remarkable feature: most of them could be isolated only in limited periods. Six species were isolated in October, and only a few species were mainly isolated in other months. This was an outstanding characteristic of the seasonal flora of V needles. Several fungi of discontinuous occurrence became more frequent and more stable in the L horizon, e.g., *Cladosporium cladosporioides, C. herbarum*, and *Chalara longipes*.

L needles (Table 5). Compared with the V needles, more species were observed on L needles. Twelve species of microfungi were isolated as fungi of continuous occurrence. Dominants were also mostly included in this group: *Thysanophora penicillioides* in October 1988 and April 1989, *Aureobasidium pullulans* in October 1988 and August 1989, *Penicillium* spp. in December 1988, *Chalara longipes* in February 1989, *Anungitea* sp. in April and June 1989. These fungal species were observed constantly throughout the year, almost all with high frequencies. *Scytalidium* spp. was one of the fungi of discontinuous occurrence which appeared among the dominants in October 1988. Some other constant microfungi, however, were present in equally high frequencies every month. Therefore, differences in the bimonthly mycoflorae of L needles were not obvious for the microfungi of continuous occurrence. Some microfungi which occurred unstably on the living needles became members of continuous occurrence on this needle type, e.g., *Cladosporium cladosporioides, C. herbarum, Chalara longipes* and *Alternaria alternata*. Two species of *Mortierella* were constantly and for the first time isolated from this type of needle.

For the fungi of discontinuous occurrence, the mycoflora of L needles changed throughout the year. The bimonthly mycoflorae showed a contrast between October and December 1988. That in October 1988 was more similar to that in August 1989 than that in December 1988. That in December 1988 was similar to that in February 1989 and the following months. The microfungi of discontinuous occurrence are described from December 1988.

Polyscytalum verrucosum, Gliocladium sp. 1, *Epicoccum purpurascens, Mortierella vinacea, Dactylaria candidula, Verticillium* sp., *Endophragmiella boewei, Trichoderma viride*, and others occurred in December. Most of those species were new entries on fir needles, and were isolated continuously till June 1989. Their percentage frequencies ranged from relatively high to low. *Scytalidium* spp. and *Tripospermum camelopardus*, in contrast, showed declining average frequencies and became unstable. In February 1989, *Trichoderma pseudokoningii* and sterile white hyphae joined the mycoflora. Both showed reduced frequencies or disappeared in April, but *Trichoderma hamatum* and an unidentified basidiomycete joined the microflora. In August, the microflora was much altered. Most species which occurred in the previous months with relatively high to moderate frequencies disappeared or showed reduced frequencies, and two species of *Tripospermum* occurred alternately. *Scytalidium* spp. joined the flora in April 1988 and occurred till August. These fungi had a denotative peak of occurrence in October 1988.

F needles (Table 6). More species were observed as fungi of continuous occurrence on F needles than on L needles. The seasonal alternation of the mycoflora became unclear for this needle type.

Table 6. Seasonal fluctuation of selected fungi[1] on F_1 needles.

Fungus	Average frequency (%)	1988	1989			(F_2)[3]
		Oct.	Jan.	Apr.	July	(Aug.)
I. Fungi of continuous occurrence						
Cladosporium cladosporioides	31	45	20	15	45	(30)
Trichoderma viride	74	90	90	45	70	(80)
Scytalidium spp.	54	65	15	80	55	(50)
Anungitea sp.	33	20	20	60	30	(+)
Mucor hiemalis	18	15	15	25	15	(40)
Thysanophora penicillioides	38	15	25	45	65	(+)
Mortierella vinacea	30	15	20	25	60	(75)
Mortierella ramanniana	71	75	70	60	80	(80)
Mortierella isabellina	70	55	65	65	95	(95)
Exophiala sp.	70	50	80	55	95	(40)
Penicillium spp.	86	85	80	80	100	(100)
Aureobasidium pullulans	20	25	25	20	+[2]	(25)
Chalara longipes	25	25	25	40	+	(+)
Endophragmiella boewei	20	+	20	35	20	
Epicoccum purpurascens	15	+	+	20	20	(+)
Polyscytalum verrucosum	19	+	+	25	35	
Cladosporium herbarum	19	+	+	+	50	(15)
Fusarium spp.	10	+	+	+	15	(+)
II. Fungi of discontinuous occurrence						
Mortierella sp. 7	6.3	−	25	−	−	
Trichoderma pseudokoningii	11	−	40	+	−	(+)
Gliocladium sp. 1	18	−	45	15	+	(+)
Dactylaria candidula	29	−	45	30	40	(15)
Verticillium sp.	18	−	20	+	40	(55)
Trichoderma hamatum	11	−	+	30	+	(+)
Mortierella hyalina	7.5	−	−	25	+	(25)
sterile white hyphae	8.8	−	+	−	30	
Mortierella verticillata	2.5	−	−	+	−	(50)
Piptocephalis sp.	1.8	−	−	−	+	(20)

[1), 2)]: See Table 4.

[3)] Percentage frequency on F_2 needles (in parentheses).

Microfungi such as *Cladosporium cladosporioides, Anungitea* sp., *Thysanophora penicillioides* and *Chalara longipes* declined in frequency on F needles, while *Trichoderma viride*, three species of *Mortierella, Exophiala* sp., and *Penicillium* spp. increased in frequency.

Among the fungi of discontinuous occurrence, two species of *Mortierella* and two species of *Trichoderma* newly occurred or increased in frequency. *Dactylaria candidula* and *Verticillium* sp. also increased in frequency. These species altered gradually through the year.

The mycoflora of F_2 needles in August was similar to that of F_1 needles in July (Table 6).

Discussion

With the same washing method that was performed by Aoki et al. (1990) for Japanese fir needles, mycofloral alternation was investigated for German fir needles (*Abies alba*). During the one-year observation period, more than ninety species were isolated. In this study, many species were observed as fungi of continuous occurrence (or distribution). They formed the main mycofloral components of individual needle types and were followed by many fungi of discontinuous occurrence. These two groups of fungi were termed "main colonizers" and "subordinate colonizers," respectively (Aoki et al., 1990).

By comparing the monthly mycofloral alternation (Tables 4–6) with the changes of the leaf-litter constitution (Table 1), a correspondence was found between the fungal alternation and the decay of the fir needles.

The leaf loss of the fir appeared to become heavier in winter. The mycoflora of L needles was different between October and December. This might be caused by the difference between the mycoflorae of the old L horizon remaining in October and the new L horizon settled in December. In December, many new fungi colonized the L needles, making the mycoflora richer than in the previous months.

Such microfungi as *Tripospermum camelopardus* and *Scytalidium* spp., which were transferred with the V needles, might decline after the leaf fall. *Aureobasidium pullulans* appeared to be equally adapted to both V and L needles because its percentage frequency was nearly the same on both types. This fungus, however, showed a reduced percentage frequency on F needles.

In contrast, a series of fungal colonizations was recognized on L needles: *Thysanophora penicillioides, Chalara longipes*, two species of *Cladosporium, Penicillium* spp., *Anungitea* sp., *Exophiala* sp., *Mortierella ramanniana, M. isabellina* and other fungi of continuous occurrence; and *Gliocladium* sp. 1, *Mortierella vinacea, Trichoderma viride, Dactylaria candidula, Verticillium* sp., *Endophragmiella boewei* and other fungi of discontinuous occurrence. These were newly and/or more frequently isolated from L needles, to which many of them seemed better adapted than to F needles (Table 3). From February through June, two more species of *Trichoderma, Mucor hiemalis, Mortierella hyalina* and an unidentified basidiomycete joined the colonization. Peaks of these new colonizers mostly occurred in December or June. The number of colonizers increased gradually toward the end of this period, which seemed to correspond with the period when a large part of the older L needles were transformed into F_1 needles. The mycoflorae of L needles in August and October might consist mainly of microfungi on the remaining L needles, newly formed by the recent defoliation. These mycoflorae were very similar to those of V needles in July and in October.

The changes in litter constitution suggest that F_1 needles in April were in the initial stage of a new accumulation from decomposed L needles. The mycoflorae of F_1 needles in April and July (Table 6) might reflect such a substrate accumulation. *Thysanophora penicillioides, Epicoccum purpurascens, Polyscytalum verrucosum, Cladosporium herbarum, Anungitea* sp., and other common fungi on the L needles increased in frequency on F needles during this period. In October and January, the mycoflorae of F_1 needles were somewhat different from those in the other months. So-called litter fungi, such as *Thysanophora penicillioides, Chalara longipes*, two species of *Cladosporium, Polyscytalum verrucosum* and *Endophragmiella boewei*, declined in frequency. It was presumed that in April and July F_1 needles contained newly formed F_1 needles derived from the decomposition of part of the L

needles.

The annual dominant altered on L needles. *Tripospermum camelopardus* was the annual domi-

Fig. 1. Fungal succession on decaying fir needles.

1) *: Occurred continuously on at least one needle type.

2) +: Occurred at a lower average frequency (less than 5%).

3) Occurred especially on F_2 needles.

nant on the living, V needles. The species became a member of discontinuous occurrence (subordinate colonizer) on L needles, and dropped further in average frequency on F needles. *Thysanophora penicillioides* was not observed on V needles but became the annual dominant on L needles. On the F needles, however, its average frequency dropped. *Penicillium* spp. was less frequent on V needles, but it became a member of continuous occurrence (main colonizer) on L needles, then the dominant on F needles. In most cases, these dominant fungi were each followed by several other fungi which behaved similarly.

Based on these fungal alternations, a diagram was made for the possible fungal succession on the German fir needles (Fig. 1). Main colonizers, which occurred continuously on a certain needle type, are indicated with asterisks.

The results of the present study are directly comparable with those for Japanese fir needles (*Abies firma* Sieb. et Zucc.) obtained by Aoki et al. (1990), because both studies used the same washing method and the same sampling procedure. Comparison of the present data with the Japanese ones revealed several interesting results. First, many fungi were common to both areas and some of them presented the same or similar distribution patterns in the organic horizon, except for the frequencies of the individual fungi. For example, some species of *Tripospermum* were observed on attached, living needles and L needles in Japan. In the present study, *Tripospermum camelopardus* was isolated frequently from the same needle types. Two species of *Cladosporium, Aureobasidium pullulans* and *Alternaria alternata* occurred on all types of German fir needle. This was in agreement with findings from Japanese fir needles. On both German and Japanese needles, *Thysanophora penicillioides* and *Anungitea* sp. were isolated firstly from L needles, then from F_1 and F_2 needles. Their frequencies were remarkably lower in the Japanese probes. *Mucor hiemalis* and species of *Mortierella*, section *Micromucor*, were also observed on L and F needles in Germany and in Japan. Similarly, species of *Mortierella*, section *Mortierella*, were mainly isolated from F needles.

Second, several fungi were restricted to one area. Such species were especially found on the attached, living needles. Species of *Sporobolomyces*, which were common on Japanese needles, were not observed on German fir needles. On the contrary, *Chalara longipes*, which was not isolated from Japanese needles, was common on German fir needles. On other needle types, *Trichoderma polysporum* occurred only on the German needles, while *Trichoderma harzianum* was isolated only from the Japanese needles. *Codinaea simplex* and *Chaetopsina fulva* were also observed only on Japanese fir needles. Further, in Japan, species of *Trichoderma* and *Penicillium* were isolated frequently from all types of needles, including the attached, green ones. Species of both these genera were, however, isolated from German needles mainly of the litter horizon (L and F needles).

Gourbière (1974a, b, 1975, 1979, 1980a, b, 1981, 1982, 1984) studied on mycoflora of needles of *Abies alba* in France. He also used a washing method. Aoki et al. (1990) compared their Japanese data with Gourbière's and found similarities in the mycoflorae. We used a similar isolation method to Aoki et al. (1990) for the same substrate materials from southwest Germany and obtained data that were comparable to those of Gourbière in France. With neutral PDA medium, Gourbière (1975, 1979, 1980a) found markedly lower average frequencies of several fungi than those in the present study. However, he also used acidic and alkaline medium conditions (Gourbière, 1981, 1984). On the acidic PDA medium (pH 1.5), *Tripospermum camelopardus* and *Penicillium* spp. occurred with high frequencies as found in the present study. Average frequencies of *Cladosporium herbarum, Cladosporium cladosporioides, Alternaria alternata, Mortierella hyalina* and others were also higher on

alkaline media (pH 8.3) than neutral. Using diluted medium conditions, we found similar values for these fungi to those reported by Gourbière (1981, 1984). The dilution of isolation media might reduce the selective effects caused by the strong media (Swift, 1976; Parkinson, 1982).

As discussed by Aoki et al. (1990), fungal successions on the needles of *Abies firma* in Japan and *Abies alba* in France (Gourbière, 1975, 1979, 1980a) seem to deviate in several features from the well-adopted scheme on pine needles, which was given by Kendrick and Burges (1962) and generalized by Hudson (1968). The present results also do not agree well with it. Several saprophytic fungi were isolated frequently from the attached green needles, as also reported by Gourbière. Further, the whole fungal alternation pattern in Germany was intermediate between Japanese data and the given scheme. Gourbière's data were based on fir needles collected in high mountain stands in France. The present study was carried out in a forest in southwest Germany. Data on the *momi* fir needles (*Abies firma*) were obtained from a low mountain forest in central Honshu, Japan. In Germany, Kowalski and Lang (1984), Butin and Wagner (1984) and Butin (1986) also mentioned the occurrence of several saprophytic microfungi on attached, living and senescent needles of Norway spruce [*Picea abies* (L.) Karst.], when reporting on tree diseases. Kendrick and Burges (1962) commented that their own results could not be applied directly to other locations or to other tree species.

In conclusion, we assume that the fungal succession phenomena were not monotonous, but were much affected by the surrounding environments, such as seasonal changes, climatic conditions, geographical locations and so on. Fungal colonization patterns might be modified by these factors, as suggested by Kendrick and Burges (1962).

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摘　　要

ドイツ産モミ属針葉上の菌類遷移

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ドイツ産モミ属針葉樹 *Abies alba* 葉上の菌類相を調査した．緑色生葉及びリターからの L 及び F の分解段階の落葉を材料に用いた．不完全糸状菌類はすべての分解段階の針葉から頻繁に分離された．接合菌類は L および F 層のみから分離された．出現菌類を針葉の各分解段階ごとに分析し，リターの針葉組成割合の変動と菌出現の相関から分解途上のモミ属針葉上での菌類遷移を記載した．本結果での菌類遷移を既に報告されている日本及びフランス産モミ属針葉についての結果と比較した．それら全てが英国産マツ属針葉に基づく一般化された図式とは異なった特徴を示した．菌類遷移に影響すると予想される気候条件の効果について論議した．