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Mycofloral succession on *Pinus densiflora* needles on a moder site

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Mycofloral succession on decaying pine needles in a *Pinus densiflora* forest on a moder site was investigated in Sugadaira, Nagano Pref., central Japan. Dead needles on the tree, fallen needles obtained from two recognizable sub-layers of the L layer and the upper sub-layer of the F_1 layer in the organic horizon were examined for their fungal flora using both washing and surface sterilization techniques. The major interior colonizer in freshly fallen needles varied with the season: *Chaetopsina fulva* in summer and *Selenosporella curvispora* in the other seasons. *Thysanophora penicillioides* was a remarkable external colonizer of freshly fallen needles in summer, while soil fungi were external colonizers of such needles in the other seasons. A possible successional change of major fungi with the needle decay was suggested. The observed seasonal alternation of the species colonizing freshly fallen needles was discussed in relation to climatic conditions.

Key Words——fungal succession; moder site; needle decomposition; *Pinus densiflora*; seasonal fluctuation.

A noticeable characteristic in pine forest litter is that the leaf litter input occurs throughout the year. This feature and the slow decomposition rate of needles often contribute to the formation of a thick accumulation of leaf litter on the forest floor. This organic horizon of pine forest usually can be divided into three or more distinct sub-layers corresponding to progressive stages of the needle decay (Kendrick, 1959).

Using this specific attribute of pine forest litter, Kendrick and Burges (1962) studied monthly the vertical distribution of mycoflora in the organic horizon and analyzed its seasonal fluctuation. From their analyses they described in detail the fungal succession associated with the progressive decay of *Pinus sylvestris* L. needles on a mor site. They found several unique characteristics of the fungal succession on pine needles. A major discovery was that the exterior and interior of pine needles are colonized by different groups of fungi. They also pointed out that many fungi participating in the succession are uncommon or host-specific. The former finding has been confirmed in most subsequent studies (Gremmen, 1957; Kendrick and Burges, 1962; Hayes, 1965a, b; Tubaki and Saitô, 1969; Black and Dix, 1977; Lehmann and Hudson, 1977; Mitchell and Millar, 1978; Soma and Saitô, 1979). However, the latter one has not been always confirmed (Hayes, 1965; Widden and Parkinson, 1973; Tokumasu, 1978; Tokumasu et al., 1994).

Another well-known fact is that climatic differences lead to variations in the form of humus, which is usually classified into three types: mor, moder and mull. One of the immediately apparent differences between a mor and a mull is the great accumulation of plant debris, chiefly leaf litter, often to be found on the former, and its relative absence from the latter (Kendrick, 1959). The mor-type organic horizon can often be seen in coniferous forests, especially in cold regions such as the sub-arctic region, and the mull type is often found in the deciduous broad-leafed forests and grassland that are distributed in temperate regions. However, there are various intermediate forms between mor and mull and they are often described as moder. In Japan, the humus of most forests of two-leafed pines can be categorized as the moder type because the H layer is usually indistinct and the L layer often disappears from the organic horizon in summer. In contrast, the pine forests selected for the study of fungal succession on decaying needles in Europe and Canada developed on mor sites, where the H layer and the L layer are distinct throughout the year.

In Japan, Tubaki and Saitô (1969) and Soma and Saitô (1979) studied the fungal successions in two-leafed pine leaf litter in Miyagi Pref., the Tohoku district, but few works have been conducted on this subject for pine leaf litter on moder sites.

To study the influence of macro climate on the microfungal succession on decaying pine needles, I have investigated fungal successions in the organic horizons of pine forests that develop on moder sites in Japan. This paper describes a succession of microfungi on decaying needles in a *Pinus densiflora* Sieb. & Zucc. forest which developed in a cool temperate area of the central mountainous region of the Honshu. The succession was drawn from the data of the mycoflora on the dead needles on the tree and those in the upper sub-layers of

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the organic horizon, which were examined several times. It will be compared with the successions on moder sites in Japan and those on mor sites in Europe, and its characteristics will be discussed.

Materials and Methods

The study site Investigations were carried out on the campus of the Sugadaira Montane Research Center, University of Tsukuba, which is situated at latitude 36°31′N and longitude 138°21′E, and at an altitude of about 1,330 m above sea level.

The area is dominated by a cool temperate climate. A set of meteorological data for the Center is given in Table 1. Its annual normal temperature is 6.9°C and total annual precipitation is about 1,100 mm. The area is covered with snow from December to March.

The study site was located on a gentle slope facing south-east that was covered with a stand of 11-12 m high *Pinus densiflora* The stand had developed under natural conditions in an area that formerly carried a herbaceous plant community of *Miscanthus sinensis* Anderss. At the site, the canopy was closed and the ground was mostly open except for sparse herbaceous plants and some shrubs and mosses.

Profile of the organic horizon At the site, the L and F layers of the organic horizon were recognizable from the middle of October to early June, but the typical L layer was indistinct in the rest of the year. The H layer was very thin and indistinct throughout the year. The L layer was further divided into two sub-layers. The surface sub-layer consisted of freshly fallen needles and the underlying one of discolored and partly blackened needles. They are referred to here as the L and OL layers. The F layer was also subdivided into two layers, i.e., the F_1 and F_2 layers. The former was also divided into two layers. The upper layer was composed of black needles with relatively high tensile strength and rather hard tissues. The layer below it consisted of black, collapsed needles with softened tissues, low tensile strength and usually high moisture content, but which retained their original form and integrity. They are referred to here as the F_{11} and the F_{12} layers.

Table 1. Monthly means of temperature and precipitation during the main survey period (1977-1979) at the Sugadaira Montane Research Center.

Month	Temperature °C			Precipitation (mm)
	mean	maximum	minimum	
Jan.	−5.7	−0.5	−9.8	62.7
Feb.	−5.3	−0.5	−10.0	78.0
Mar.	−1.6	3.9	−6.6	68.7
Apr.	5.2	11.2	−0.9	99.3
May	10.6	17.0	3.8	73.7
Jun.	16.2	21.1	11.0	143.3
Jul.	19.6	24.7	14.1	129.3
Aug.	19.8	24.5	15.4	80.0
Sep.	15.8	20.6	10.6	93.3
Oct.	10.0	16.0	3.9	107.7
Nov.	5.1	10.3	−1.0	66.7
Dec.	−0.8	4.6	−4.2	86.0

Collection of needle samples Samples of leaf litter were collected from the summer of 1977 through the autumn of 1978: in July, August, October and November of 1977, and January, May, July, August and November of 1978. They are here called as the main samples. In addition, supplementary samples were collected in October 1987 and June 1994.

The needle samples of the L, OL, and F_{11} layers were obtained from a block (ca. 20 × 20 cm, ca. 5 cm thick) cut out of the organic horizon at each sampling time. In October 1977, the sample of the OL layer could not be collected due to the lack of needles. Dead needles on the tree (D-type needles) were collected four times by using litter traps: in October 1977, November 1978, July 1979 and April 1992.

Analysis of fungal flora Two techniques were adopted for analysis of mycoflora: a surface sterilization technique (Kendrick and Burges, 1962) and a washing technique of Harley and Waid (1955) as modified by Tokumasu (1978, 1980). At each sampling, ten needle-bundles were collected from each objective layer. Each bundle was separated into two single needles. One of them was subjected to surface sterilization and the other to serial washing.

For surface sterilization, a 0.1% aqueous solution of mercuric chloride was used, after Kendrick and Burges (1962). Ten single needles from a specified layer were dipped into the solution in a Petri dish for 1.5 min, and then transferred into a sterile test-tube with a sterile plastic cap. Ten ml of sterilized 0.005% Aerozol OT solution (di-iso-octyl sodium sulfosuccinate) was poured into the tube as a washing detergent. The tube was shaken vigorously in a vortical type shaker at a constant intensity for 1 min. The contents were allowed to settle for 30 s, then the old detergent was removed. The washing with the detergent was repeated three times. Then the needles were rinsed with sterilized water two times in the same manner. The rinsed needles were transferred to sterile filter papers in 9-cm Petri dishes and dried for 1 d to suppress vigorous bacterial growth after plating (Widden and Parkinson, 1973). Five sets of two needles were laid down onto the surface of half-strength cornmeal agar (Difco) plates.

In the washing method, ten single needles from one layer were put into a sterile test-tube with a sterile plastic cap. They were washed five times with sterilized 0.005% Aerozol OT solution and rinsed three times with sterilized distilled water. The rinsed needles were treated in the same way as the surface-sterilized needles and plated out on the same agar medium.

To induce many species to sporulate, the plates were put in transparent plastic containers that were set on a bench in the laboratory and kept under the fluctuating room light and temperature conditions of night and day. In January 1978, an additional set of the washed needles was prepared and incubated at 4°C.

Table 2. Average percentage frequencies of occurrence (AFO) of fungi isolated from D-type needles.

Fungi	Surface-sterilized needles	Washed needles
White sterile (E2453)	70.0	*[a]
Aureobasidium pullulans	32.5	72.5
White sterile (E2456)	22.5	*
Alternaria alternata	7.5	25.0
Nigrospora oryzae	5.0	5.0
Cladosporium cladosporioides	2.5	80.0
Acremonium murorum	0.0	2.5
Acrodontium crateriforme	0.0	7.5
Arthrinium anamorph of *Apiospora montagnei*	0.0	5.0
Chaetopsina fulva	0.0	2.5
Colletotrichum sp.	0.0	17.5
Epicoccum nigrum	0.0	27.5
Malbrachea sp.	0.0	2.5
Paecilomyces farinosus	0.0	2.5
Phoma sp.	0.0	2.5
Septonema ochracea	0.0	7.5
Stemphylium botryosum	0.0	7.5
Trichoderma koningii	0.0	2.5
Tripospermum sp.	0.0	10.0
Total number of species	20	18

a) Occurred but could not be counted.

All the treatments were performed within 3 h after the sample collection.

The incubated plates were observed microscopically four times at 1 wk intervals. Fungi appearing on and around the needles were isolated and identified. Several common species were also identified directly by making microscopic preparations from the incubated plates.

Percentage frequency of occurrence of a given species for each layer or needle type at a sampling time (PFO) was calculated as follows: PFO(%)=number of needles bearing a specified fungus/10 (total number of needles examined per each layer at each sampling) $\times 100$. Average percentage frequency of occurrence of a given fungus for each layer or needle type (AFO) was calculated as follows: AFO(%)=summed PFO of a given species in one layer/the number of samples. The number of samples was 11 for the L and F_{11} layers, 10 for the OL layer and 4 for the D-type needle.

Results

Table 2 lists species recorded from the samples of the D-type needles and their AFO values. A sterile fungus (E2453) was the major interior colonizer of the needles with a very high AFO value (70%), followed by *Aureobasidium pullulans* (de Bary) Arnaud (32.5%). Members of "common primary saprophytes" Hudson (1968) were the major surface colonizers.

Table 3 lists all fungi that occurred on the surface-sterilized needles of three sub-layers of the main and the supplementary samples. Their AFO values are shown in the same table. The major interior colonizers of the D-type needles were recorded rather frequently in the L layer, but their AFO values were less than 20% and no other species with an AFO value of 10% or more was recorded. In the OL layer, *Chaetopsina fulva* Rambelli, *Selenosporella curvispora* MacGarvie and a black rhizomorph-forming basidiomycete occurred with AFO values of more than 10%. *Chaetopsina fulva* and the black rhizomorph-forming fungus occurred in all the layers studied and showed the highest AFO values in the OL layer. Unlike these species, *S. curvispora* never occurred in the L layer but had high AFO values in the OL and F_{11} layers of 31% and 64.5%, respectively. *Trichoderma koningii* Oudemans was another interior colonizer in the F_{11} layer. *Verticicladium trifidum* Preuss, a dominant interior colonizer in Europe (Gremmen, 1957; Kendrick and Burges, 1962; Hayes, 1965a, b; Black and Dix, 1977; Lehmann and Hudson, 1977; Mitchell and Millar, 1978; Tokumasu et al., 1994) occurred mainly in the L and OL layers, but its AFO values in these layers were less than 10%.

Table 4 lists all species recorded from the washed needles of three sub-layers of the main and the supplementary samples. Their AFO values are shown in the same table. By using the washing method, both surface and interior colonizers could be recorded. The distribution patterns of the interior colonizers shown in Table 4 were similar to those observed by the surface sterilization method (Table 3) except for that of *C. fulva*. This fun-

Table 3. Average percentage frequencies of occurrence (AFO) of fungi isolated from surface-sterilized needles of different sub-layers.

Fungi	Layer		
	L	OL	F11
White sterile (E2453)	14.0	7.0	5.6
Aureobasidium pullulans	12.5	6.0	3.9
Verticicladium trifidum	7.5	7.0	1.9
Chaetopsina fulva	3.5	18.0	9.3
Cladosporium cladosporioides	3.0	3.0	5.7
Thysanophora penicillioides	2.8	2.0	7.6
Phoma sp.	2.8	0.0	3.8
Black rhizomorph-forming basidiomycete	1.9	11.0	4.8
Stenella variabilis	1.9	0.0	1.9
Trichoderma koningii	1.0	9.0	22.5
Polyscytalum fecundissimum	1.0	2.0	1.0
Nigrospora oryzae	1.0	0.0	1.0
Epicoccum nigrum	1.0	0.0	5.7
Arthrinium anamorph of *Apiospora montagnei*	1.0	0.0	1.0
Colletotrichum sp.	1.9	0.0	0.0
Malbrachea sp.	1.0	0.0	0.0
Selenosporella curvispora	0.0	31.0	64.5
Verticillium psalliotae	0.0	3.0	5.8
Chalara sp.	0.0	3.0	0.0
Alternaria alternata	0.0	1.0	2.9
Cladosporium herbarum	0.0	1.0	0.0
Unidentified (Coelomycete)	0.0	1.0	0.0
Mortierela isabellina	0.0	0.0	5.7
Acremonium sp. 1	0.0	0.0	2.9
Chaetomium globosum	0.0	0.0	2.9
Mortierella ramanniana	0.0	0.0	2.9
Penicillium sp. 1	0.0	0.0	2.9
Acrodontium crateriforme	0.0	0.0	1.0
Anungitea continua	0.0	0.0	1.0
Chloridium virescens var. *chlamydosporum*	0.0	0.0	1.0
Mariannaea elegans	0.0	0.0	1.0
Mortierella sp.	0.0	0.0	1.0
Penicillium thomii	0.0	0.0	1.0
Scolecobasidium humicola	0.0	0.0	1.0
Trichoderma polysporum	0.0	0.0	1.0
Verticillium balanoides	0.0	0.0	1.0
Total number of species	16	15	31

gus had the highest value in the L layer here. All the common primary saprophytes showed decreasing AFO values in the more decomposed layers, but their patterns of decrease were various. *Thysanophora penicillioides* (Roum.) Kendrick and *T. koningii* were first recorded in the L layer with AFO values of 10% or more. The former reached the highest AFO value in the OL layer (27.0 %). The latter showed high AFO values of more than 50% in both the OL and F_{11} layers.

Table 5 shows the results of incubation at 4°C. The total number of species observed was very small, and all the species recorded were also recorded from the needles incubated under the normal conditions. *Mortierella* spp. were the major sporulating fungi after 32 days' incubation.

Table 6 presents the seasonal fluctuations of two remarkable interior colonizers in the upper three sub-layers of the organic horizon during 1977-1978. *Chaetopsina fulva* had a rather high PFO value in the L layer in July, August and October 1977, but did not appear again in the same layer until July of 1978. In the OL layer it showed high PFO values in August and November. Then it appeared in the F_{11} layer in October and had a high PFO value in November of 1977. Having appeared again in

Table 4. Average percentage frequencies of occurrence (AFO) of fungi isolated from washed needles of different sub-layers.

Fungi	Layer		
	L	OL	F11
Cladosporium cladosporioides	70.9	42.0	30.0
Aureobasidium pullulans	42.7	5.0	0.0
Alternaria alternata	33.6	6.0	6.4
Chaetopsina fulva	30.9	16.0	5.5
Epicoccum nigrum	29.1	22.0	8.2
Thysanophora penicillioides	16.4	27.0	13.6
Trichoderma koningii	15.5	54.0	81.8
Phoma sp.	9.1	7.0	1.8
Mortierella isabellina	9.1	18.0	30.0
Acrodontium crateriforme	8.2	5.0	2.7
Penicillium sp. 1	6.4	6.0	9.1
Black rhizomorph-forming basidiomycete	6.4	16.0	10.9
Tripospermum sp.	5.5	0.9	0.0
Septonema ochracea	5.5	3.0	8.2
Arthrinium anamorph of *Apiospora montagnei*	5.5	0.0	0.0
Colletotrichum sp.	4.5	6.0	2.7
Cladosporium herbarum	4.5	0.0	0.9
Acremonium sp. 1	4.5	4.0	3.6
Polyscytalum fecundissimum	3.6	10.0	10.9
Mortierella parvispora	3.6	2.0	7.3
Trichoderma croceum	2.7	4.0	0.9
Mucor spp.	2.7	8.0	15.5
Mortierella alpina	2.7	7.0	17.3
Verticicladium trifidum	1.8	8.0	1.8
Stemphylium botryosum	1.8	0.0	0.0
Pestalotiopsis sp.	1.8	0.0	0.0
Penicillium sp. 3	1.8	0.0	0.0
Myrothecium roridum	1.8	0.0	0.0
Mortierella ramanniana	1.8	11.0	23.6
Fusarium spp.	1.8	9.0	8.2
Cylindrotrichum sp.	1.8	0.0	2.7
Chalara sp.	1.8	4.5	2.7
Verticillium psalliotae	0.9	8.0	36.4
Tripospermum acerinum	0.9	1.0	0.0
Tolypocladium sp.	0.9	1.0	0.9
Stachybotrys sp.	0.9	0.0	0.0
Sporidesmium goidanichii	0.9	0.0	2.7
Phialophora sp.	0.9	0.0	0.0
Penicillium sp. 5	0.9	0.0	5.5
Penicillium brevicompactum	0.9	0.0	1.8
Paecilomyces penicillatus	0.9	1.0	0.0
Nigrospora oryzae	0.9	1.0	0.9
Mortierella verticillata	0.9	11.0	35.5
Malbrachea sp.	0.9	1.0	0.0
Hyalodendron sp.	0.9	0.0	0.9
Acremonium sp. 2	0.9	0.0	0.0
Penicillium thomii Maire	0.8	1.8	5.8
Selenosporella curvispora	0.0	12.7	50.9
Mortierella globulifera	0.0	12.0	30.0
Trichoderma polysporum	0.0	4.0	0.9

Mortierella gamsii	0.0	4.0	3.6
Mariannaea elegans	0.0	3.0	7.3
Unidentified 1 (hyphomycete)	0.0	2.0	5.5
Sympodiella multiseptata	0.0	2.0	2.7
Rhinocladiella atrovirens	0.0	2.0	0.0
Anungitea continua	0.0	2.0	8.2
Chaetopsina sp.	0.0	1.8	0.0
Verticillium balanoides	0.0	1.0	7.3
Sporidesmium omahutaense	0.0	1.0	13.6
Mortierella sp.	0.0	1.0	0.0
Mortierella pulchella	0.0	1.0	0.9
Mortierella nana	0.0	1.0	0.0
Aspergillus fumigatus	0.0	1.0	0.0
Mortierella hyalina	0.0	0.9	3.6
Vollutera cilliata	0.0	0.0	1.8
Tridentaria implicans	0.0	0.0	0.9
Scolecobasidium humicola	0.0	0.0	2.7
Ramichloridium schulzeri	0.0	0.0	0.9
Penicillium sp. 4	0.0	0.0	0.9
Penicillium sp. 2	0.0	0.0	2.5
Mortierella elongata	0.0	0.0	0.9
Monacrosporium sp.	0.0	0.0	0.9
Endophragmia hyalosperma	0.0	0.0	0.9
Dactylaria clavata	0.0	0.0	5.8
Chloridium virescens var. *chlamydosporum*	0.0	0.0	1.8
Total number of species	47	50	57

the L layer in July of 1978, it repeated a similar downward shift of PFO value to the F_{11} layer. On the contrary, *S. curvispora* never occurred in the L layer. This fungus showed rather high PFO values in both the OL and F_{11} layers, but almost disappeared from the OL layer during the months when *C. fulva* was recorded with high PFO values in this layer.

Discussion

Successional changes of interior colonizers The interior colonizers on the D-type needles have apparently changed with the progress of the seasons. The interior colonizers with highest AFO values on the D-type needles were a white sterile fungus (E2453) and *A. pullulans* (Table 2). The white sterile fungus was frequently recorded from the D-type needles in both the summer and autumn, while *A. pullulans* occurred mainly in the summer of 1978. However, it is uncertain whether these fungi were the only major interior colonizers of the dead needles on the tree because the number of samples was only four.

The first interior colonizers in fallen needles also shifted with the progress of the seasons. *Chaetopsina fulva* was a frequent colonizer in freshly fallen needles in the summers of both 1977 and 1978 but not in the other seasons (Table 6). This may have caused the low AFO value (3.5%) for this species as an interior colonizer in the L layer (Table 3). It is noticeable that by the washing

Table 5. Percentage frequency of occurrence (PFO) of fungi recorded from the washed needles collected in January 1978 after incubation for 32 d (bold figures) and 72 d (italic figures) at 4°C.

Fungi	Layer			
	D	L	OD	F11
Cladosporium spp.	90	*60*	50	40
Epicoccum nigrum	*60*	0	*30*	*30*
Mortierella verticillata	0	20	10	50
Mortierella alpina	0	10	50	60
Alternaria alternata	20	*10*	0	0
Black rhizomorph-forming basidiomycete	0	*40*	*10*	0
Mortierella gamsii	0	30	*20*	0
Mortierella globulifera	0	*20*	0	*20*
Phoma sp.	*20*	0	0	0
Paecilomyces farinosus	0	10	0	0
Thysanophora penicillioides	0	0	20	0
Chalara sp.	0	0	*10*	0
Fusarium sp.	0	0	*10*	0
Rhinocladiella atrovirens	0	0	*10*	0
Unidentified 1	0	0	*10*	0
Mortierella parvispora	0	0	0	50
Verticillium psalliotae	0	0	0	*30*
Mortierella pulchella	0	0	0	10
Mortierella sp.	0	0	0	10
Total number of species	4	8	11	9

Table 6. Seasonal changes of percentage frequencies of occurrence (PFO) of two interior colonizers in the upper three layers of the organic horizon.

Fungi	Year	1977				1978				
	Month	Jul.	Aug.	Oct.	Nov.	Jan.	May	Jul.	Aug.	Nov.
Chaetopsina fulva	L layer	40	90	50	0	0	0	70	80	0
	OL layer	0	60	*[a]	70	0	0	0	40	30
	F_{11} layer	20	0	30	70	0	0	0	0	0
Selenosporella curvispora	L layer	0	0	0	0	0	0	0	0	0
	OL layer	60	10	*	0	0	80	90	50	10
	F_{11} layer	100	70	0	100	90	50	100	70	30

a) Not examined.

method the fungus was also recorded frequently in the summer months and had a rather high AFO value (30.9%) in the L layer needles (Table 4). These facts indicate that the fungus is a pioneer colonizer of freshly fallen needles in the warmer months at the study site. Tubaki and Saitô (1969) mentioned that the fungus is an early colonizer of freshly fallen needles of *Pinus densiflora*.

Selenosporella curvispora was the most frequent interior colonizer of the OL and the F_{11} layers (Tables 3, 4). This fungus has not hitherto been reported as an interior colonizer of pine needles. It was completely absent in the L layer. In the F_{11} layer, it showed very high PFO values except for October 1977 (Table 6). As the conidia of this fungus have never germinated on any artificial media adopted, the fungus may arrive at the untrodden needles by means of mycelia. In the L layer, the physical conditions may be unsuited for mycelial growth of the species because humidity and temperature fluctuate considerably.

The seasonal fluctuations of two interior fungi (Table 6) indicate that prior-colonizers did not easily replace competitive species for a considerably long time. It appears that *C. fulva* prevented establishment of *S. curvispora* when the former could quickly establish itself in freshly fallen needles. The reverse appears to be true in the needles colonized by the latter species. Similar phenomena were observed by Bruehl and Lai (1966) among ubiquitous soil fungi and plant pathogens in wheat straw buried in soil.

A possible succession of interior colonizers at the site may be summarized as follows. The interior of the D-type needles is colonized by a white sterile fungus in all seasons. *Aureobasidium pullulans* colonizes abundantly the dead needles on the tree only in summer. *Chaetopsina fulva* quickly invades fallen needles in summer. On the other hand, *S. curvispora* colonizes the needles fallen in the other seasons when the surrounding conditions become more moist and stable due to the continuous accumulation of fallen needles.

Successional changes of surface colonizers On the surface of the D-type needles, the common primary saprophytes were diverse (Table 2). *Cladosporium cladosporioides* (Fresen.) de Vries was very common, followed by *Alternaria alternata* (Fries) Keissler, *A. pullulans* and *Epicoccum nigrum* Link. They were also major surface colonizers in the L layer. These results apparently disagree with the view of Hudson (1968) that the paucity of the common primary saprophytes is a major difference distinguishing the fungal succession on pine needles from that on other kinds of leaves. Similar results to the present study have been obtained by Widden and Parkinson (1973), Tokumasu (1978) and Tokumasu et al. (1994).

The "secondary saprophytes" (Hudson, 1968) on the needle surface also changed seasonally at the study site. *Thysanophora penicillioides* colonized freshly fallen needles frequently in summer, but infrequently in other seasons. Consequently, the AFO value of this fungus was only 16.4% in the L layer and 27.0% in the OL layer (Table 4). In the other seasons, members of soil fungi commonly colonized such needles (Table 4). For example, *T. koningii* had an AFO value of 15.5% in the L layer, and members of *Mortierella* were recorded constantly from the OL layer and abundantly from the F_{11} layer (Table 4). It is well known that most species of soil *Mortierella* prefer humid and cool conditions. Carreiro and Koske (1992) found that *Mortierella* sp. were dominant in the microfungal communities of milled, deciduous leaf litter in microcosms incubated at 0°C and 10°C for long periods. In the present study, a similar result was obtained when the washed needles were incubated at 4°C for relatively long periods (Table 5). As shown in Table 1, the organic horizon at the study site is exposed to humid and cold conditions for a long time. Consequently, many *Mortierella* species may occur abundantly in the OL and F_{11} layers.

Tokumasu (1978) reported that *Anungitea continua* Matsushima was a major surface colonizer of the F_1 layer needles in Sugadaira and may be one of the species changing needle color to black. In this study the fungus was also recorded but very infrequently from the F_{11} layer. However, supplemental surveys showed that the fungus was somewhat abundant in the F_{12} layer (Tokumasu, unpublished data).

A possible succession of the surface colonizers may be summarized as follows. The well-known common primary saprophytes in the temperate regions first colonize the surface of dead needles on the tree. They decline gradually after the needle falls. In summer, *T.*

penicillioides establishes itself on the L layer needles, while *T. koningii* invades such needles throughout the year. Some members of soil fungi such as *Mortierella* sp. are very common in the F_{11} layer owing to their tolerance for lower temperature and higher moisture conditions.

Climatic influence on fungal succession The climatic characteristics of Sugadaira appear to be the main cause of the observed seasonal changes of the major earlier colonizers on/in freshly fallen needles. From the middle of November to the end of April, it is very cold in Sugadaira (Table 1) and the organic horizon is daily exposed to low temperatures of about 0°C. In particular, the temperature in the organic horizon is constantly below 0°C during the four snow-clad months. Most of the fungi inhabiting the organic horizon may be inactive during this period. The fallen needles during the cold season may remain in a situation that suppresses would-be colonizers. After snow-melt, the species tolerant of lower temperature may start to colonize stocked needles and some species may succeed in establishing their colonies. In summer, freshly fallen needles may be quickly colonized by the species that prefer warmer conditions to cooler.

As repeatedly mentioned, in Sugadaira, the first colonizers on/in freshly fallen needles in summer were different from those in other seasons. Such differences have not been observed in the fungal successions on decaying pine needles studied on mor sites in Europe and Canada. In Japan, Tubaki and Saitô (1969) found in a *Pinus densiflora* forest on a moder site in a suburb of the city of Sendai that two interior colonizers, *C. fulva* and *V. trifidum* occurred abundantly on/in decaying needles. They noted that *C. fulva* is a relatively earlier colonizer on fallen needles and occurred throughout the year. They also reported that *V. trifidum* occurred throughout the year and that *Endophragmia alternata* Tubaki & Saitô (=*Sporidesmium goidanichii* (Rambelli) Hughes) was the only important external secondary saprophyte at the study site. On the other hand, Soma and Saitô (1979) obtained a different result in a coastal *Pinus thunbergii* Parl. plantation near the city of Sendai. At this site, *V. trifidum* was the only major interior colonizer, and three species, *E. alternata*, *Kriegeriella mirabilis* Höhn. and an unidentified dematiaceous fungus, were the major external secondary saprophytes. However, neither report mentioned the seasonal fluctuations of the early invaders of freshly fallen needles. Therefore, more information on the fungal successions on moder sites in Japan is needed in order to conclude that the seasonal distribution of the first colonizers on/in freshly fallen needles is one of the important characteristics of fungal succession in pine forests on moder sites in Japan.

The species composition of the common primary saprophytes at the study site was very similar to that in Tübingen, Germany (Tokumasu et al., 1994), although *Cladosporium herbarum* (Persoon) Link replaced *C. cladosporioides* there. On the other hand, Tokumasu (1991) found that *C. herbarum* was often one of the common *Cladosporium* species on freshly fallen pine needles in Hokkaido, the northernmost region of Japan, which is dominated by a cool temperate climate. Thus, the replacement of *Cladosporium* species may be a phenomenon reflecting climatic differences between Sugadaira in Japan and Tübingen in Germany.

Except for two species, *Mariannaea elegans* (Corda) Samson and *Sympodiella multiseptata* Tubaki & Yokoyama, all fungi isolated from the supplementary samples were also recorded from the main samples. In addition, the composition of the species with high AFO values was not largely different from that of the main samples. These facts suggest that the species composition of the fungal community in the organic horizon at the study site had been stable for at least 15 yr. Probably, the macro climate has largely contributed to the stabilization of mycoflora in a given area.

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