Mycoscience 35: 29–37, 1994

Fungal succession on pine needles in Germany

Seiji Tokumasu[1], Takayuki Aoki[2]* and Franz Oberwinkler[2]

[1] Sugadaira Montane Research Center, University of Tsukuba, Sanada, Nagano 386-22, Japan
[2] Lehrstuhl Spezielle Botanik/Mykologie und Botanischer Garten, Universität Tübingen, Auf der Morgenstelle 1, Tübingen 1, D-72076 F. R. Germany

Accepted for publication 8 November 1993

The mycofloral succession on the needles of *Pinus sylvestris* was investigated in Tübingen, southwest Germany. Dead needles attached to the branches (D-type), those caught on branches (C-type) and three types of fallen needles, i.e., freshly fallen (L-type), slightly discolored (OL-type) and almost black needles (F-type) were examined for their fungal flora. Common primary saprophytes were rich on the dead needles on the tree, and on the L-type needles. They were replaced by successive species that contained the well-known species preferring pine needles as their substratum, such as *Verticicladium trifidum* or *Sympodiella acicola*. Their ecological niches in pine leaf litter and their distribution patterns from a biogeographical viewpoint were discussed.

Key Words——fungal succession; pine needle decomposition; *Pinus sylvestris*; southwest Germany; washing method.

Introduction

A well-documented substratum succession of fungi is that on decaying pine needles. Pine leaf litter has several characteristics that are advantageous in studying substratum succession of fungi (Kendrick, 1959). The whole fungal succession with the decay of pine needles at a mor site was first demonstrated by Kendrick and Burges (1962) in England. Since this work, there have been many studies of the fungal succession on pine needles in the British Isles. Several experimental works have been performed to elucidate the effects of biological or abiological factors on colonization patterns of fungi on decaying pine needles (Black and Dix, 1976a, b, 1977; Hayes, 1965b; Lehmann and Hudson; 1977; Mitchell and Millar, 1978a, b; Mitchell et al., 1978; Parkinson and Balasooria, 1967). Similar studies on the fungal succession in pine leaf litter have been carried out in Canada (Widden and Parkinson, 1972) and Japan (Tubaki and Saitô, 1969; Soma and Saitô, 1979). In addition, the mycofloras of pine leaf litter have been examined in various regions of the world (Brandesberg, 1969; Kendrick, 1963; Manoch et al., 1986; Tokumasu, 1978, 1985, 1987, 1988, 1990; Tokumasu et al., 1988).

In this paper, fungal succession on decaying needles of *Pinus sylvestris* (Scots pine) are described at two sites in Tübingen, southwest Germany. The pine is the commonest tree in Western Europe. Gremmen (1957, 1959) studied the mycoflora on various substrata derived from this pine in the Netherlands and found many interesting facts concerning fungal succession on the needles. Few works have been conducted on this subject for pine leaf litter in this area.

Materials and Methods

Descriptions of the study sites Two pine stands (A and B) were selected in the city of Tübingen, Baden-Württemberg, Germany, which is located at 48°33′N lat., 9°3′E long., and about 450 m above sea level. Site A is in the Schönbuch, and site B is in the Morgenstelle Campus of the University. Both stands consisted of young pines (ca. 20 cm diam near the base). The ground was mostly open except for poor herbaceous plants and some shrubs at both sites.

Collection of needle samples Dead needles on the tree and on the ground were collected bimonthly from November 1988 through September 1989. From the lower branches of a given tree, two kinds of dead needles were collected: those recently dead and still attached to the branches (D-type needles) and those detached but caught by the branches (C-type needles). Fallen needles were obtained from a block (ca. 20×20 cm, ca. 2 cm thick) cut out of the organic horizon at each sampling time. The needles were divided into three categories based on their appearance and the degree of decay. These were freshly fallen, brown needles (L-type needles), partly discolored needles (OL-type needles) and dark brown to black needles (F-type needles). The L-type needles were obtained at all sampling times. In both April and September, the OL-type needles were rare and were not obtained in sufficient numbers from the sampled blocks at either site. For the same reason, the F-type needles were not obtained in January and July at

* Present address: National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries, Kannondai, Tsukuba 305, Japan.

Table 1. List of species observed on the needles of *Pinus sylvestris* at sites A and B.

	Site A	Site B	Species common to both sites
Zygomycotina			
Absidia galuca Hagem		*	
Mortierella isabellina Oudemans	*	*	*
Mortierella parvispora Linnemann	*	*	*
Mortierella ramanniana (Möller) Linnemann	*	*	*
Mortierella verticillata Linnemann		*	
Mortierella vinacea Dixon-Stewart	*	*	*
Mucor hiemalis Wehmer	*	*	*
Mucor mucedo (L.) Fresen.	*	*	*
Mucor sp.	*		
Ascomycotina			
Lophodermium sp.	*		
Basidiomycotina			
Unidentified		*	
Deuteromycotina			
Acremonium larvarum (Petch) W. Gams	*		
Acremonium sp. 1	*	*	*
Acremonium sp. 2		*	
Acrodictys sp.	*		
Acrodontium crateriforme (van Beyma) de Hoog	*	*	*
Alternaria alternata (Fries) Keissler	*	*	*
Anungitea continua Matsushima		*	
Anungitea fragilis Sutton	*	*	*
Arthrinium anamorph of *Apiospora montagnei* Saccardo		*	
Arthrobotrys sp.	*		
Aureobasidium pullulans (de Bary) Arnaud	*	*	*
Bahusakala sp.	*	*	*
Botrytis cinerea Persoon	*	*	*
Chalara longipes (Preuss) Cooke	*	*	*
Chalara sp. 1	*	*	*
Chalara sp. 2		*	
Cladosporium herbarum (Persoon) Link	*	*	*
Cladosporium macrocarpum Preuss	*	*	*
Cladosporium cladosporioides (Fresen.) de Vries	*	*	*
Clonostachys compactiuscula (Sacc.) Hawksaworth		*	
Dactylaria sp.	*		
Epicoccum nigrum Link	*	*	*
Fusarium stilboides Wollenw.	*	*	*
Fusarium sp.			
Fusidium griseum Link	*		
Hansfordia pulvinata (Berk. & Curt.) Hughes	*		
Heteroconium sp.	*	*	*
Hormiactella asetosa Hol.-Jech.	*		
Hyalodendron sp.	*	*	*
Mariannaea elegans (Corda) Samson	*		
Monacrosporium sp.	*	*	*
Monocillium tenue W. Gams	*	*	*
Oidiodendron tenuissimum (Peck) Hughes		*	
Penicillium thomii Maire	*		
Penicillium sp. 1	*	*	*
Penicillium sp. 2	*	*	*
Penicillium sp. 3	*	*	*

Penicillium sp. 4		*	
Penicillium sp. 5	*	*	*
Phoma glomerata (Corda) Wollenw. & Hochapfel	*		
Pithomyces chartarum (Berk. & Curt.) M. B. Ellis	*		
Polyscytalum fecundissimum Riess	*	*	*
Polyscytalum verrucosum Sutton		*	
Ramichloridium anceps (Sacc. & Ellis) de Hoog	*		
Scolecobasidium humicola Barron & Busch		*	
Septonema sp.	*		
Sesquicillium candelabrum (Bonord.) W. Gams		*	
Sporidesmium sp.	*		
Sporothrix sp.	*		
Sympodiella acicola Kendrick		*	
Thysanophora penicillioides (Roum.) Kendrick	*	*	*
Trichoderma viride Persoon	*	*	*
Trichoderma virens (Miller, Giddens & Foster) von Arx		*	
Trichoderma sp.	*	*	*
Tripospermum sp.	*	*	*
Troposporella monospora (Kendrick) M. B. Ellis	*		
Ulocladium botrytis Preuss		*	
Verticicladium trifidum Preuss	*	*	*
Verticillium lecanii (Zimm.) Viegas		*	
Unidentified 1	*		
Unidentified 2	*		
Unidentified 3	*		

site A, or in January, May and July at site B.

Fungal analysis The washing technique of Harley and Waid (1955) as modified by Tokumasu (1978, 1980) was adopted for the analysis of the mycoflora. For every needle type, ten needle bundles were chosen, and ten single needles derived from different bundles were collected. They were put into a sterile test-tube with a sterile metal 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 one minute. The contents were allowed to settle for 30 sec., then the old detergent was removed. Washing with the detergent was repeated five times. Then the needles were rinsed with sterilized water three times in the same manner. Repeated preliminary experiments showed that this procedure was sufficient to remove inactive fungal propagules adhering to the surface of needles. The rinsed needles were transferred to sterile filter papers in 9-cm Petri dishes and dried for one day 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 Lab.) plates. For inducing more species to sporulate, the plates were set on a shelf near a window of the laboratory and kept under the fluctuating light and temperature conditions of night and day.

The incubated plates were observed microscopically four times at one week intervals. Fungi appearing on and around the needles were isolated and identified. Several common 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: Percentage frequency = [number of needles from which the fungus was detected/10 (total number of needles in each examination)] × 100.

Results

Whole fungal flora on the pine needles The list of species observed on the Scots pine needles at the study sites is shown in Table 1. Total number of species was 73. From site A 54 species were recorded and from site B 52. Thirty-three species were common to both sites.

Vertical distribution of fungi Table 2 shows the vertical distribution of selected fungi at site A. Four species were recorded from all the needle types. They had their own maximum frequency values on either type of needle on the tree and declined with the progress of decomposition on the ground. All the species of partial distribution are recorded from plural needle types but no such fungi occurred on the D-type needles. Three species occurred on the C-type needle but attained only very low frequencies. The fungi of partial distribution reached their maximum frequencies on the F-type needle except for *Mortierella isabellina*. *Verticicladium trifidum* was apparently the most prevalent fungus at this site. It appeared to colonize quickly freshly fallen needles and its frequency increased with the progress of needle decay and reached

Table 2. Vertical distribution of selected fungi (with average frequency of 10% or more on at least one needle type) at site A.

	Needle type[1]				
	D	C	L	OL	F[2]
I. Fungi of constant distribution					
Aureobasidium pullulans	66.7	70	58.4	30	*[3]
Cladosporium herbarum	66.7	71.7	53.3	52.5	10
Alternaria alternata	21.7	18.3	18.3	10	*
Epicoccum nigrum	20	31.7	15	17.5	*
Cladosporium cladosporioides	10	*	*	*	0
II. Fungi of partial distribution					
Mortierella isabellina	0	*	*	12.5	*
Mucor hiemalis	0	*	11.7	25	27.5
Trichoderma viride	0	*	0	*	20
Verticicladium trifidum	0	0	38.3	47.5	90
Mortierella ramanniana	0	0	*	12.5	10
Polyscytalum fecundissimum	0	0	0	*	15
Anungitea fragilis	0	0	0	15	27.5

[1] D: dead needles still attached to branches, C: detached needles caught in the branches, L: freshly fallen needles, OL: partly discolored needles, F: dark brown to black needles.
[2] Total number of needles examined was 60 of the D-, C- and L-type needles and 40 of the OL- and F- type needles.
[3] Average frequency is less than 10%.

Table 3. Vertical distribution of selected fungi (with average frequency of 10% or more on at least one needle type) at site B.

	Needle type[1]				
	D	C	L	OL	F[2]
I. Fungi of constant distribution					
Aureobasidium pullulans	88	58.3	53.3	*	*[3]
Cladosporium herbarum	45	41.7	43.3	37.5	13.3
Alternaria alternata	20	18.3	*	*	*
II. Fungi of partial disribution					
Epicoccum nigrum	20	11.7	0	0	0
Mucor hiemalis	0	*	13.3	20	43.3
Trichoderma viride	0	*	*	*	23.3
Verticicladium trifidum	0	0	18.3	42.5	46.7
Polyscytalum fecundissimum	0	0	3.3	10	10
Mortierella ramanniana	0	0	*	17.5	23.3
Penicillium sp. 5	0	0	*	15	*
Mortierella parvispora	0	0	*	0	10
Sesquicillium candelabrum	0	0	16.7	0	0
Chalara sp. 2	0	0	10	0	0
Mortierella isabellina	0	0	0	17.5	10
Mortierella verticillata	0	0	0	10	16.7
Penicillium sp. 1	0	0	0	*	20
A basidiomycete	0	0	0	40	0
Sympodiella acicola	0	0	0	22.5	0
Anungitea fragilis	0	0	0	0	30
Mucor mucedo	0	0	0	0	10

[1] D: dead needles still attached to branches, C: detached needles caught in the branches, L: freshly fallen needles, OL: partly discolored needles, F: dark brown to black needles.
[2] Total number of needles examined was 60 of the D-, C- and L- type needles, 40 of the OL-type and 30 of the F-type needles.
[3] Average frequency is less than 10%.

Table 4. Seasonal fluctuation of selected fungi[1] on D-type needles.

Species	Average frequency	1988 Nov.	1988 Jan.	1988 Apr.	1989 May	1989 Jul.	1989 Aug.
Site A							
I. Fungi of continuous occurrence							
Aureobasidium pullulans	66.7	100	80	40	40	90	50
Cladosporium herbarum	66.7	90	100	70	10	60	70
II. Fungi of discontinuous occurrence							
Alternaria alternata	21.7	20	10	30	0	70	0
Epicoccum nigrum	20	40	0	50	0	0	30
Site B							
I. Fungi of continuous occurrence							
Aureobasidium pullulans	88	100	90	90	70	80	100
Cladosporium herbarum	45	10	100	80	50	20	10
II. Fungi of discontinuous occurrence							
Epicoccum nigrum	20	0	40	40	30	10	0
Alternaria alternata	20	0	10	30	30	40	10

[1] Fungi with average frequency of 20% or more.

Table 5. Seasonal fluctuation of selected fungi[1] on C-type needles.

	Average frequency	1988 Nov.	1988 Jan.	1988 Apr.	1989 May	1989 Jul.	1989 Aug.
Site A							
I. Fungi of continuous occurrence							
Cladosporium herbarum	71.7	100	100	60	80	50	40
Aureobasidium pullulans	70	50	50	50	70	100	100
II. Fungi of discontinuous occurrence							
Epicoccum nigrum	31.7	70	0	10	30	50	30
Site B							
I. Fungi of continuous occurrence							
Aureobasidium pullulans	58.3	100	70	40	50	10	80
II. Fungi of discontinuous occurrence							
Cladosporium herbarum	41.7	70	70	20	70	0	20

[1] Fungi with an average frequency of 20% or more.

its highest value, 90%, on the F-type needle. *Anungitea fragilis* may be a surface colonizer of needles: it was first recorded from the OL-type needles and from 27.5% of the F-type needles. *Polyscytalum fecundissimum* showed a similar distribution pattern to *A. fragilis* but was rather infrequent. Three other species were ubiquitous soil fungi.

Table 3 shows the vertical distribution of prominent fungi at site B. Fungi of continuous distribution also decreased their frequencies with the progress of needle decay on the ground. *Epicoccum nigrum*, a species of continuous distribution at site A, was restricted to the needles on the trees at this site. Selected species of partial distribution reached 17 in number. Only two soil fungi, *Mucor hiemalis* and *Trichoderma viride*, were recorded from the C-type needles with low frequencies. Some species of partial distribution tend to be limited to one needle type. *Sesquicillium candelabrum* and *Chalara* sp. were restricted to the L-type needles. An unidentified basidiomycete and *Sympodiella acicola* were limited to the OL-type needles, and *A. fragilis* and *Mucor mucedo* were limited to the F-type needles. *Verticicladium trifidum* was again the fungus with the highest frequency, but its value was only 46.7% on the F-type needles.

Seasonal fluctuation of fungus flora The seasonal fluctuations of the fungus flora on the individual needle types are shown in Tables 4–8, which list the fungi with average frequency of 20% or more. The occurrence patterns of fungi could be divided into two: continuous and discontinuous. They are shown separately in the tables.

On the D-type needles, the same two species, *Aureobasdium pullulans* and *Cladosporium herbarum*, occurred continuously at both sites (Table 4). At site A, both spe-

Table 6. Seasonal fluctuation of selected species[1] on L-type needles.

	Average frequency	1988 Nov.	1988 Jan.	1988 Apr.	1989 May	1989 Jul.	1989 Aug.
Site A							
I. Fungi of continuous occurrence							
Aureobasidium pullulans	53.3	70	20	40	50	80	90
Cladosporium herbarum	53.3	70	90	40	50	50	20
Site B							
I. Fungi of continuous occurrence							
Aureobasidium pullulans	53.3	70	30	20	60	40	100
II. Fungi of discontinuous occurrence							
Cladosporium herbarum	43.3	70	40	40	60	0	50

[1] Fungi with an average frequency of 20% or more.

Table 7. Seasonal fluctuation of selected species[1] on OL-type needles.

	Average frequency	1988 Nov.	1988 Jan.	1988 Apr.	1989 May	1989 Jul.	1989 Aug.
Site A							
Cladosporium herbarum	52.5	60	100	—[2]	40	10	—
Verticicladium trifidum	47.5	30	30	—	60	70	—
Aureobasidium pulluans	30	40	30	—	50	0	—
Mucor hiemalis	25	30	40	—	0	30	—
Site B							
Verticicladium trifidum	42.5	40	10	—	90	30	—
A basidiomycete	40	0	50	—	50	60	—
Cladosporium herbarum	37.5	70	50	—	30	0	—
Sympodiella acicola	22.5	10	0	—	80	0	—
Mucor hiemalis	20	0	40	—	40	0	—

[1] Fungi with an average frequency of 20% or more.
[2] Not examined.

Table 8. Seasonal fluctuation of selected fungi[1] on F-type needles.

	Average frequency	1988 Nov.	1988 Jan.	1988 Apr.	1989 May	1989 Jul.	1989 Aug.
Site A							
Verticicladium trifidum	90	70	—[2]	90	100	—	100
Mucor hiemalis	27.5	70	—	30	0	—	10
Anungitea fragilis	27.5	0	—	80	30	—	0
Trichoderma viride	20	0	—	30	0	—	50
Site B							
Verticicladium trifidum	46.7	20	—	20	—	—	100
Mucor hiemalis	43.3	40	—	40	—	—	50
Anungitea fragilis	30	20	—	50	—	—	20
Mortierella ramanniana	23.3	20	—	0	—	—	50
Trichoderma viride	23.3	0	—	30	—	—	40
Penicillium sp.	20	10	—	30	—	—	20

[1] Fungi with an average frequency of 20% or more.
[2] Not examined.

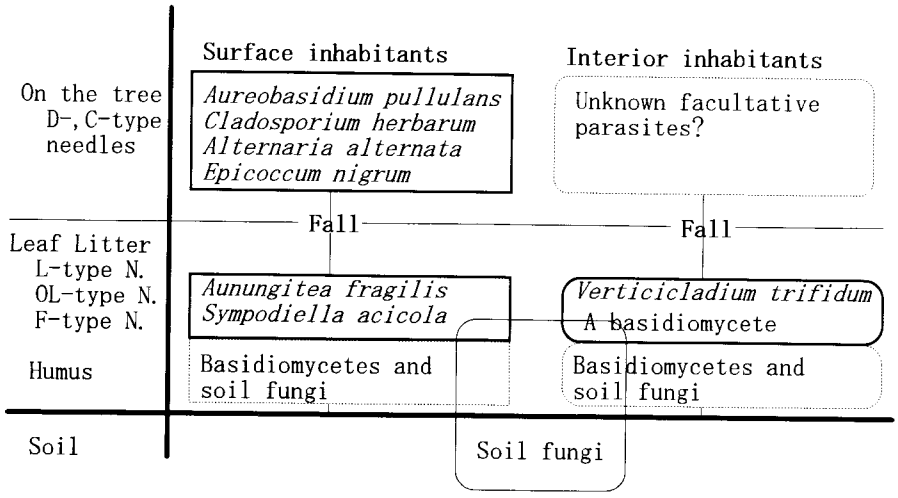

Fig. 1. Fungal succession on decaying pine needles in Tübingen. Facts are enclosed by solid lines and probabilities by dotted lines.

cies showed a similar seasonal fluctuation pattern. They reached the lowest frequency in May. In that month, two discontinuous fungi, *Alternaria alternata* and *E. nigrum* disappeared at site A. At site B, *A. pullulans* maintained high frequency throughout the year, but *C. herbarum* was rather infrequent from summer to early winter. The fluctuation pattern of discontinuous species at this site roughly agreed with that of *C. herbarum*.

On the C-type needles, fungi of continuous occurrence at site A were the same as those of the D-type needles (Table 5). On the C-type needles of this site, they remained high in frequency in May. *Aureobasidium pullulans* was the only species of continuous occurrence at the site. *Cladosporium herbarum* did not occur in July on this type of needle.

On the L-type needles, *A. pullulans* and *C. herbarum* were dominant fungi and *A. alternata* and *E. nigrum* became less prominent than on the D- and C-type needles (Table 6).

On the OL-type needles, *V. trifidum* occurred constantly and its average frequency reached 47.5% at site A and 42.5% at site B (Table 7). At the former site, it occurred more frequently in May and July than in November and January. At the latter site, its frequency was the highest, 90%, in May but less than 50% in other months. At site B, an unidentified basidiomycete occurred with 50% or more frequency at each sampling time except November. *Sympodiella acicola* was a high frequency fungus in May but negligible or absent in other months. *Mucor hiemalis*, a common soil fungus, was recorded as a selected species at both sites.

On the F-type needles at site A, *V. trifidum* was prevalent at every sampling time. *Anungitea fragilis* showed the highest frequency of occurrence in April (Table 8). At site B, *V. trifidum* and *A. fragilis* appeared to be a prominent fungi, though this needle type was examined only 3 times. At both sites, some soil fungi had an average frequency of 20% or more.

Discussion

On the basis of the results, a diagram was made for the possible fungal succession on *P. sylvestris* needles in Tübingen (Fig. 1). As expected, the pattern of succession is very similar to that on the needles of the same pine at Delamere Forest in England (Kendrick and Burges, 1962). Furthermore, this pattern essentially agrees with a general scheme of fungal succession on tree leaves above the soil shown by Hudson (1968).

It is certain that *V. trifidum* is the first colonizer of freshly fallen needles from the organic horizon at both sites. Gremmen (1957) first reported a prevailing occurrence of the species on pine needles in the Netherlands. Kendrick and Burges (1962) showed that it was an early colonizer of freshly fallen needles and that it could quickly penetrate inner tissues of needles. Furthermore they found it to persist for about two years there. The same habit of this species has been reported on decaying needles of *P. sylvestris* (Hayes, 1965a, b; Lehmann and Hudson, 1977; Black and Dix, 1977; Mitchell et al., 1978) and *Pinus nigra* Arnold var. *maritima* (Mitchell and Millar, 1978) in the British Isles. Tubaki and Saitô (1969) and Soma and Saitô (1979) described the species as a prevalent interior dweller of fallen needles of two native pines in Japan. These reports suggest that *V. trifidum* may be an interior colonizer of needles in Tübingen, though this was not confirmed in the present work. No other interior colonizer may occur except for *V. trifidum* at site A. One basidiomycetous species occurred only on the OL-type needles at site B and formed prominent rhizomorphs on the substrata (Table 7). A rhizomorph-forming basidiomycete, *Marasmius androsaceus* (L.: Fr.) Fr., has been reported as an early interior colonizer of fallen needles in the British Isles (Lehmann and Hudson, 1977; Mitchell and Millar, 1978a. b) and in Japan (Soma and Saitô, 1979). It can infect fallen needles within one or two months later after defoliation (Lehmann and Hudson, 1977; Mitchell and Millar, 1978a). It is probable that the basidiomycete found at the site may occupy the

same ecological niche as *M. androsaceus* in other localities. In addition, the seasonal distribution pattern of the fungus suggests that it and *V. trifidum* may be antagonistic (Table 7) and the latter could not succeed in establishing an overwhelming dominance on fallen needles at site B unlike at site A.

On the surface of the needles, the main common primary saprophytes (Hudson, 1968) were the same on the dead leaves on the tree at both sites. Thus *A. pullulans* and *C. herbarum* were very common and followed by *A. alternata* and *E. nigrum*. They were still common on the L-type needles and gradually decreased with the progress of decay. Kendrick and Burges (1962) have described that, with the exception of *A. pullulans*, the sugar fungi are conspicuously absent from the early stages of the breakdown of pine needles. Hudson (1968) has noted from the results of Kendrick and Burges (1962) that a major difference distinguishing the fungal succession on pine needles from that of other leaf types is the paucity of the common primary saprophytes. However, *C. herbarum* with *A. pullulans* was very common and abundant and other common primary saprophytes were recorded uncommonly in Tübingen. The same tendency has been found in the British Isles (Hayes, 1965a), Canada (Widden and Parkinson, 1973) and in Japan (Tokumasu, 1985). Furthermore, Kendrick (1963) showed that some other common primary saprophytes were not always infrequent at the study site in the Delamere Forest. These results suggest that the species richness and abundance of common primary saprophytes on dead pine needles may largely depend on the conditions of the study site or locality. Thus the paucity of common primary saprophytes may not be a characteristic of the fungal succession on pine needles.

Kendrick and Burges (1962) pointed out that the species that replaced the primary saprophytes as surface inhabitants were also uncommon on other substrata. At their study site, *Sympodiella acicola* and *Troposporella monospora* (as *Helicoma monospora* Kendrick) were very common and formed a dematiaceous hyphal network on the needle surface that darkened the needles. However, there are a few reports that both or one of them was prevalent on the blackish and black needles (Lehmann and Hudson, 1977; Parkinson and Balasooria, 1967). The former species was recorded on the OL-type needle with an average frequency of 22.5% at site B. *Anungitea fragilis* occurred with 30% frequency on the F-type needle of the same site (Table 3). This species was also recorded on the OL- and F-type needles of site A. Both species may be major surface colonizers forming dematiaceous hyphal networks on decaying needles in Tübingen.

Except for ubiquitous soil fungi, the secondary saprophytes of freshly fallen pine needles may be restricted to a few fungal species having high preferences for the pine needles, as pointed out by Kendrick and Burges (1962). The rich records of *V. trifidum* in various localities strongly suggest that the fungus has a wide distribution and possesses a high preference for fallen pine needles. There are infrequent records of it from other substrata (Hayes, 1965a; Tubaki and Saitô, 1969). The records of *S. acicola* are also mostly restricted to fallen pine needles (Ellis and Ellis, 1985).

In a similar study of fungal succession in Canada, Widden and Parkinson (1973) did not find any characteristic secondary saprophytes like those noted by Kendrick and Burges (1962) on the fallen needles of different pine species. In Japan, a different species, *Sporidesdmium goidanichii* apparently forms dematiaceous hyphal networks on the needle surface and darkened the needles, while the major interior colonizer was *V. trifidum*, the same as in Europe (Tubaki and Saitô, 1969; Soma and Saitô, 1979). In addition, characteristic inhabitants of the temperate pine needles have not been recorded at localities distributed in subtropical or tropical regions (Tokumasu, 1985; Manoch et al., 1986). It is probable that clear differences exist in the composition of secondary saprophytes specific to pine needles between various climatic zones or regions.

Between the two sites studied, there were some differences in the composition of secondary saprophytes and in their frequencies. In the clearest case, *V. trifidum* was predominant on the F-type needle at site A while its average frequency reached only 47% on the same needle type at site B. The differences observed may be partly attributable to the degree of disturbance of the sites. Site A is located in a well-established wood and the organic horizon was almost natural. On the other hand, the horizon at site B may be affected more strongly by human disturbances than site A because it is at a corner of the campus of the university and is often traversed.

Aoki et al. (1992) have studied the fungal succession on fir needles in Tübingen by adopting the same washing method and a similar sampling procedure. It and the present work were done during the same period and the sampling site of the former work was a fir stand in Schönbuch adjacent to site A. Consequently the results of both studies can be compared directly. A peculiar feature of fungal successions on fir needles is the abundance of saprophytic species on living needles on the tree (Gourbière, 1980; Aoki et al., 1990; Aoki et al., 1992). This is supported by the fact that we recorded only 20 species from dead pine needles on the tree at site A, while Aoki et al. (1992) found 26 species on living fir needles. This tendency was maintained in the organic horizon, where a larger number of species was recorded from fir needles than from pine needles in a comparable degree of decay. In the succession on fir needles, many species that may highly prefer fir needles showed maximum frequencies on the L-type needles, and many soil fungi were more abundant and frequent on the F-type needle of fir than pine. These facts suggest that fir needles are richer in the species involved in their decay, at least in the earlier stages.

Some prominent species on fir needles appear to prefer fir needles to pine needles. These are *Chalara longipes, Endophragmiella boewei* (J.L. Crane) S.J. Hughes, *Polyscytalum verrucosum* and *Thysanophora penicillioides*. They were not or rarely found on fallen pine needles at site A (Table 1). On the other hand, the representative species preferring pine needles was *V. trifidum*

which did not occur on the fir needles collected at an adjacent site to site A (Aoki et al., 1992). This may largely result from differences in the chemical nature between fir and pine needles. Kendrick and Burges (1962) noted that the chemical nature of the pine needles determines the fungi involved in the succession. Black and Dix (1976a, b) found that *T. penicillioides* can grow under high concentrations of ferulic acid and decompose the compound. They pointed out that this ability of the fungus may give it a competitive advantage in the colonization of substrata including this compound or similar substances, such as conifer needles. The fungus also occurred on pine needles at site A but with rather low frequency. This means that the fungus may prefer fir needles to pine needles. Thus comparisons between mycofloral successions of different substrata distributed within a small area may be one of the effective approaches for searching for species with a high preference for a special substratum. This may contribute to a advance in analysis of substratum successions of fungi.

Acknowledgements——This work was carried out in the Botanical Institute, Lehrstuhl Spezielle Botanik/Mykologie und Botanischer Garten, Universität Tübingen. We are grateful to all the collaborating members of the institute. The study was financially supported by DAAD (Deutscher Akademischer Austauschdienst) of the Federal Republic of Germany.

Literature cited

Aoki, T., Tokumasu, S. and Tubaki, K. 1990. Fungal succession on momi fir needles. Trans. Mycol. Soc. Japan **31**: 355–374.

Aoki, T., Tokumasu, S. and Oberwinkler, F. 1992. Fungal succession on fir needles in Germany. Trans. Mycol. Soc. Japan **33**: 359–374.

Black, R. L. B. and Dix, N. J. 1976a. Spore germination and germ hyphal growth of microfungi from litter and soil in the presence of ferulic acid. Trans. Br. Mycol. Soc. **66**: 305–311.

Black, R. L. B. and Dix, N. J. 1976b. Utilization of ferulic acid by microfungi from litter and soil. Trans. Br. Mycol. Soc. **66**: 313–317.

Black, R. L. B. and Dix, N. J. 1977. Colonization of Scots pine litter by soil fungi. Trans. Br. Mycol. Soc. **68**: 284–287.

Brandesberg, J. W. 1969. Fungi isolated from decomposing conifer litter. Mycologia **61**: 373–381.

Ellis, M. B. and Ellis, J. P. 1985. "Microfungi on Land Plants An Identification Handbook," 125 p. Croom Helm, London.

Gourbière, F. 1980. Les champignons microscopiques liés aux aiguilles de sapin (*Abies alba* Mill.). 5. Synthèse des études précédentes. Bull. Soc. Mycol. Fr. **96**: 35–42.

Gremmen, J. 1957. Microfungi decomposing organic remains of pines. Fungus **27**: 34–42.

Gremmen, J. 1959. A contribution to the mycoflora of pine forest in the Netherlands. Nov. Hedw. **1**: 251–285.

Harley, J. L. and Waid, J. S. 1955. A method of studying active mycelia on living roots and other surfaces in the soil. Trans. Br. Mycol. Soc. **38**: 104–118.

Hayes, A. J. 1965a. Some microfungi from Scots pine litter. Trans. Br. Mycol. Soc. **48**: 179–185.

Hayes, A. J. 1965b. Studies on the decomposition of coniferous leaf litter II. Changes in external features and succession of fungi. J. Soil Sci. **16**: 242–257.

Hudson, H. J. 1968. The ecology of fungi on plant remains above the soil. New Phytol. **67**: 837–874.

Kendrick, W. B. 1959. The time factor in the decomposition of conifer leaf litter. Can. J. Bot. **37**: 907–912.

Kendrick, W. B. and Burges, A. 1962. Biological aspects of the decay of *Pinus sylvestris* leaf litter. Nov. Hedw. **4**: 313–342.

Kendrick, W. B. 1963. Fungi associated with breakdown of pine leaf litter in the organic horizon. Mycopath. Mycol. Appl. **19**: 241–245.

Lehmann, P. F. and Hudson, H. J. 1977. The fungal succession on normal and urea-treated pine needles. Trans. Br. Mycol. Soc. **68**: 221–228.

Manoch, L., Tokumasu, S. and Tubaki, K. 1986. A preliminary survey of microfungal flora of pine leaf litter in Thailand. Trans. Mycol. Soc. Japan **27**: 159–165.

Mitchell, C. P. and Millar, C. S. 1978a. Mycofloral succession on Corsican pine needles colonized on the tree by three different fungi. Trans. Br. Mycol. Soc. **71**: 303–317.

Mitchell, C. P. and Millar, C. S. 1978b. Effect of lime and urea decomposition of senescent Corsican pine needles colonized by *Lophodermium pinastri*. Trans. Br. Mycol. Soc. **71**: 375–381.

Mitchell, C. P., Millar, C. S. and Minter, D. W. 1978. Studies on decomposition of Scots pine needles. Trans. Br. Mycol. Soc. **71**: 343–348.

Parkinson, D. and Balasooria, I. 1967. Studies on fungi in a pine wood soil. I. Nature and distribution of fungi in the different soil horizons. Rev. Ecol. Biol. Sol. **4**: 463–478.

Soma, K. and Saitô, T. 1979. Ecological studies of soil organisms with references to the decomposition of pine needles I. Soil macrofaunal and mycofloral surveys in coastal pine plantations. Rev. Ecol. Biol. Sol. **16**: 337–354.

Tokumasu, S. 1978. Leaf litter fungi of the forests of *Pinus densiflora* and four introduced pines at Sugadaira, central Japan. Trans. Mycol. Soc. Japan **19**: 383–390. (in Japanese).

Tokumasu, S. 1980. Observations on the fungal flora on the pine leaf litter. In: "Biseibutu no Seitai," Vol. 7, pp. 129–144. Gakkai Shuppan Center, Tokyo. (in Japanese).

Tokumasu, S. 1985. Microfungal flora on decaying pine needles collected from Kitaiôjima, a subtropical island in the Pacific. Trans. Mycol. Soc. Japan **26**: 481–486.

Tokumasu, S. 1987. Microfungi on decaying pine needles in Peru. In: "Studies on Cryptogams in Southern Peru," (ed. by Inoue, H.), pp. 169–188. Tokai University Press, Tokyo.

Tokumasu, S. 1988. Hyphomycetes from pine leaf litter of Nepal. In: "Cryptogams of the Himalayas Vol. 1. The Kathmandu Valley," (ed. by Watanabe, M. and Malla, S. B.), pp. 147–154. Department of Botany, National Science Museum, Tsukuba.

Tokumasu, S. 1990. Microfungi from coniferous leaf litter of Chile. Bull. Natn. Sci. Mus., Tokyo, Ser B, **16**: 135–145.

Tokumasu, S., Tubaki, K. and Tan, T. K. 1988. Microfungal flora on freshly fallen pine needles in Singapore. Trans. Mycol. Soc. Japan **29**: 427–430.

Tubaki, K. and Saitô, T. 1969. *Endophragmia alternata* sp. nov. and other Hyphomycetes on *Pinus* leaves in Japan. Trans. Br. Mycol. Soc. **52**: 477–482.

Widden, P. and Parkinson, D. 1973. Fungi from Canadian coniferous forest soils. Can. J. Bot. **51**: 2275–2290.