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Fungal successions on pine needles fallen at different seasons: the succession of interior colonizers*

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Experimental studies were carried out to investigate seasonal effects on the fungal succession in the interior of decaying pine needles. At different seasons, the needles fallen for a short period were collected and marked, then placed on the surface of the O horizon in a pine forest. The needles were removed at intervals and their interior fungal communities were examined by using a surface sterilization technique. The successions of interior colonizers observed on the fallen needles at four different times are roughly divided into three groups based on the composition of species colonizing from litter. Seasonal shifts in the species combination were discussed with climatic and biotic factors. As a result, temperature at the surface of litter appeared to be a cardinal factor contributing to these seasonal changes in the succession of interior colonizers.

Key Words——first invader; fungal succession; interior colonizers; needle decomposition; seasonal change.

In the Temperate Zone, pines usually shed their needles throughout the year, and leaf litter in pine forests is composed of various needles that have fallen in the four seasons. Individual seasons have different climatic conditions, which may greatly influence the succession of fungi associated with the decay of fallen needles.

To study the influences of climatic conditions on fungal succession, it is necessary to follow the sequence of fungal colonization on/in needles fallen at different times. However, it is almost impossible to pursue a cluster of dead needles fallen for a very short time in the O horizon over a long time without marking individual needles. Various techniques have been applied to label a group of fallen needles for such work. For example, Hayes (1965a) attached individual coniferous needles to lengths of nylon fishing line with glue. Lehmann and Hudson (1977) tagged individual needles by bending a small piece of aluminum embossing tape around the fascicle. Mitchell and Millar (1978a) tied a bundle of five needle pairs with a length of braided nylon fishing line attached to a metal peg.

Lehmann and Hudson (1977) compared fungal successions on needle groups fallen at different seasons in Britain. They studied the fungal successions on the needles fallen in early and late autumn and found little difference between them.

In an earlier paper (Tokumasu, 1996), I described a fungal succession on decaying pine needles on a moder site in Japan. This was inferred from repeated observations of the vertical distribution of mycoflora in the upper sub-layers of the O horizon. Unlike from the observation in Britain (Lehmann and Hudson, 1977), the species colonizing freshly fallen needles in the summer were found to be distinct from those in other seasons at the site.

The purpose of this investigation is to describe early stages of fungal successions on the pine needles that fell at different seasons and to study the seasonal influence on the first colonizers on/in freshly fallen needles. Field experiments described were carried out at the same site as the earlier work (Tokumasu, 1996). Because Kendrick and Burges (1962) found that the surface and interior of pine needles are colonized by different groups of fungi, the surface and interior colonizers were separately recorded by combining a surface sterilization method with a washing one. This paper describes and discusses the successions of interior colonizers on needles that were surface-sterilized with a 0.1% aqueous solution of mercuric chloride. The successions of surface colonizers are described elsewhere in this journal (Tokumasu, 1998).

Materials and Methods

Descriptions of 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. This site has been described in detail in Tokumasu (1996), and climatic data for the experimental period are shown in Figs. 1, 2.

The study site was located on a gentle slope that faces southeast and was covered with a stand of 11–12 m height *Pinus densiflora* Sieb. et Zucc. The canopy at the site was closed. The ground was mostly

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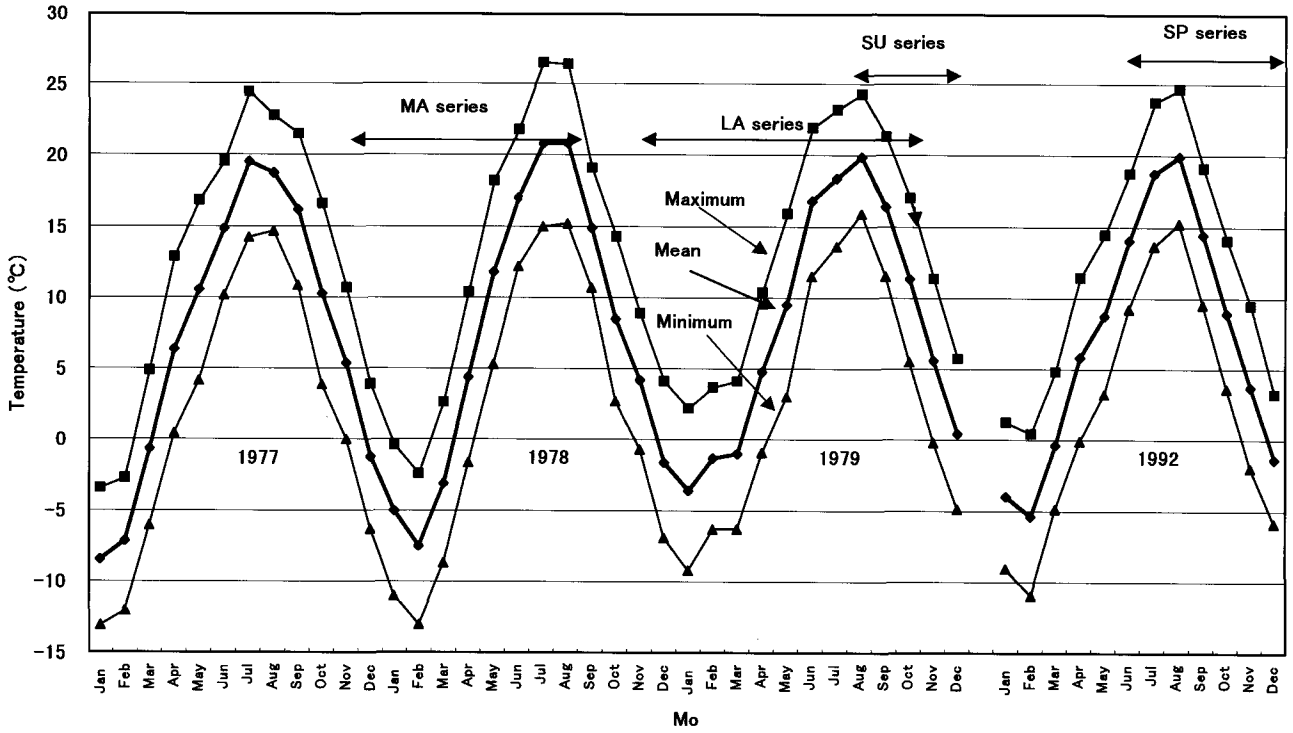

Fig. 1. Monthly mean temperatures, monthly means of daily maximum and minimum temperatures from 1977 to 1979 and 1992. Each bar with arrowheads indicates an experimental period. Names of series are given in the text.

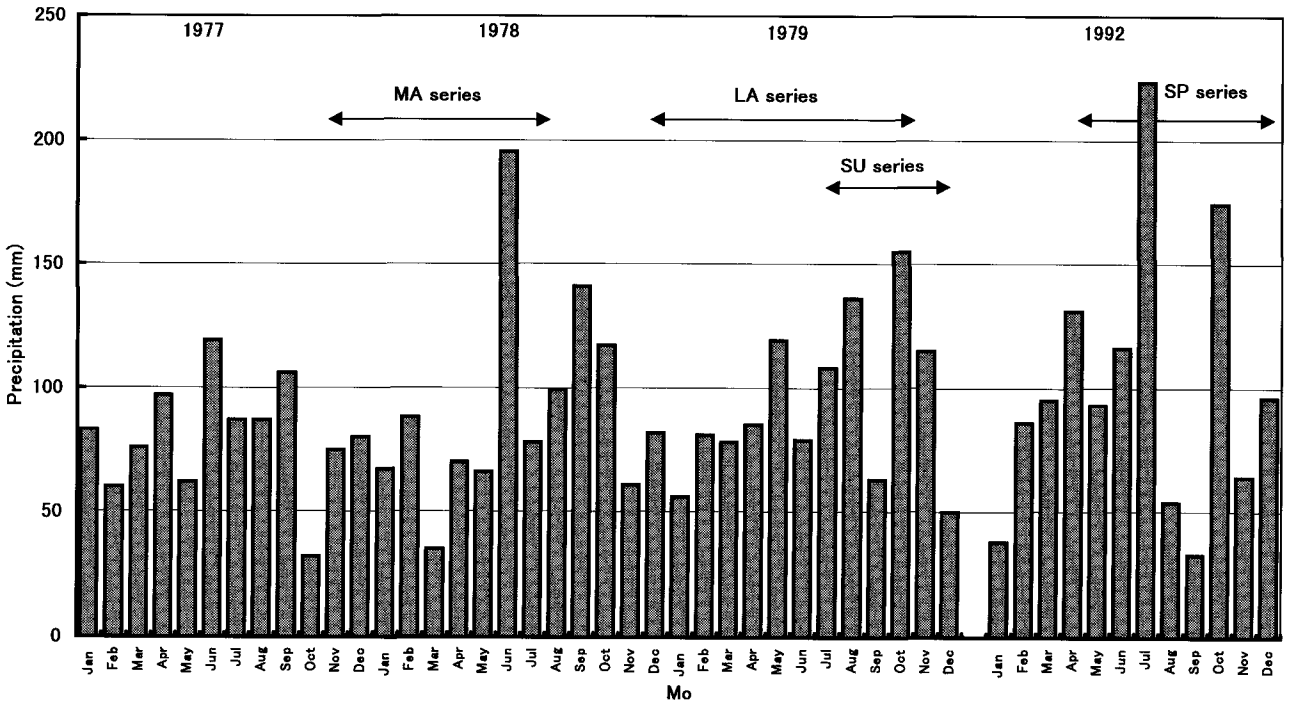

Fig. 2. Monthly precipitation from 1977 to 1979 and 1992.
Each bar with arrowheads indicates an experimental period. Names of series are given in the text.

open except for sparse herbaceous plants and some shrubs and mosses.

Seasonal fluctuation of needle fall Monthly production of leaf litter at the study stand from October 1977 to December 1979 is shown in Fig. 3. About 40% of the annual leaf fall at the stand was recorded from October to December.

Profiles of the O horizon The L and F layers of the O

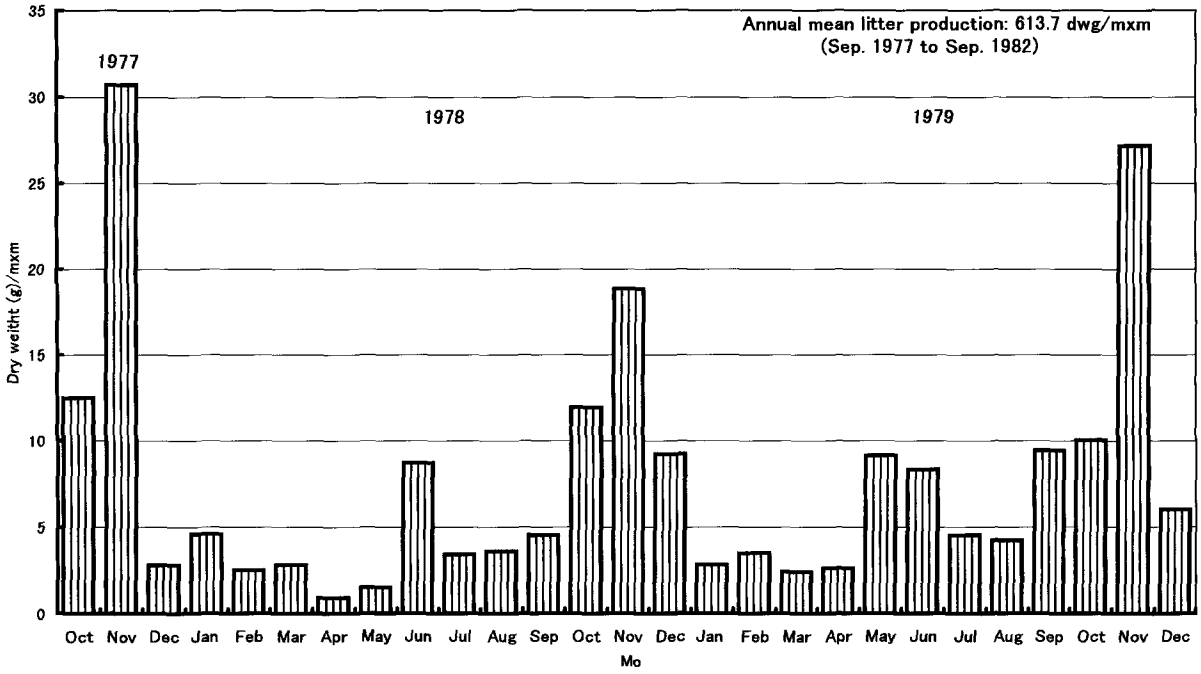

Fig. 3. Monthly amounts of fallen needles from Oct. 1977 to Dec. 1979.

horizon at the site were recognizable from the second half of October to the first half of June, but the typical L layer became indistinct in the rest of the year. The H layer was very thin and indistinct throughout the year. The organic horizon is described in detail in Tokumasu (1996).

Field experiments The periods of field experiments are indicated in Figs. 1, 2. The starting times were decided by taking account of the seasonal pattern of both air temperature and leaf fall. The middle autumn (MA) series was started on 15 October 1977, just before the autumn peak of leaf fall; the late autumn (LA) series on 22 November 1978, just after that peak; the spring (SP) series, a supplementary experiment, on 25 April 1992, just after the thaw; the summer (SU) series on 15 July 1978, just after the Bai-u rainy season.

At the start of the MA, LA and SU series, sufficient needles for subsequent sampling during the experimental period were collected from a several-day accumulation of fallen needles in litter traps of 50 cm diam made of butterfly nets. In the case of the SP series the uppermost needles of the L layer were collected. After sorting to remove any obviously damaged needles, twelve needle pairs were attached to a very fine, 1.5 m long polypropylene thread at 10-cm intervals by tying the part of the short branch to the thread. Twenty such threads were prepared for the MA and LA series and 15 for the SP and SU series. One thread was immediately used to study the mycoflora at the starting point.

For each series, a relatively undisturbed area of 2 m × 2 m was selected in the study site. The threads were placed in parallel at right angles to the slope on the surface of litter at 15-cm intervals and each end was fastened to the base of a thin iron rod 1.5 m high that had been driven into the ground in advance. In this way the needles attached to the threads lay on the litter surface.

In the MA series, samples were collected at short intervals until 2 December 1977, then at irregular longer intervals until August 1978. In the LA series, samples were collected at monthly intervals except when the ground was covered with root snow. In the SP and SU series, the first two samples were collected at 2-wk intervals and the remainder monthly.

At each collection, a thread was chosen arbitrarily and removed. The material was transported to the laboratory, and 10 of the 12 needle pairs were selected for analysis of fungal community.

Analysis of fungal community To examine fungal colonizers of inside needles, a surface sterilization technique (Kendrick and Burges, 1962) was adopted. Each of the 10 needle pairs was separated into two single needles. One was subjected to surface sterilization and the other was exclusively washed. The results obtained from the washed needles will be discussed in another article.

Ten single needles from a thread were immersed in a 0.1% aqueous solution of mercuric chloride 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 the sterile filter paper in a 9-cm Petri dish

and dried for 1 d to suppress vigorous bacterial growth after plating (Widden and Parkinson, 1973). Five sets of two needles were placed on the surface of half-strength cornmeal agar (Difco) plates.

All the treatments were performed within 3 h after the removal of sample needles.

The plates were put in transparent plastic containers on a laboratory bench and kept under the fluctuating light and temperature conditions of night and day. The incubated plates were observed microscopically at least four times at 1-wk intervals. All colonies which grew into medium were picked from plates onto 2.5% malt extract agar slants. After incubation for 1 mo, the slants were observed by light microscopy to check sporulation and then roughly divided into fruiting and non-fruiting groups. For the former, identification was attempted to species level. The latter were sorted into presumptive species on the basis of cultural characteristics. They are provisionally described as E3304 or a rhizomorph-forming fungus in this text.

Percentage frequency of occurrence for each species at a sampling time was calculated as follows: number of needles bearing a specified fungus/10 × 100.

A correlation of association between two major species in one series was measured by using a contingency table and a χ^2 estimate (Mueller-Dombois and Ellenberg, 1974; Dighton, 1984).

Results

MP series As shown in Table 1, a sterile fungus (E2453) was a predominant species in the pioneer community developed in the needles on the tree. It colonized 90% of intact needles. The fungus continued to occur on the needles for a long time but decreased its frequency and disappeared from the sample of August 1978.

This series was set out in an earlier stage of the autumn peak of needle fall. Consequently, the air temperature was still high and leaf litter had not yet frozen up yet (Fig. 1). Because there were few freshly fallen needles on the litter surface at the setting, most sample needles contacted with partly decomposed discolored needles. The latter had fallen in summer and appeared to be colonized by *Verticicladium trifidum* Preuss (the anamorph of *Desmazierella acicola* Lib.) and *Chaetopsina fulva* Rambelli (Tokumasu, 1996).

Verticicladium trifidum apparently succeeded in invading the sample needles quickly. It colonized 20% of the needles of the sample 17 d after placement and maintained a moderate frequency for about 2 mo, while *C. fulva* occurred only infrequently. However, *V. trifidum* was not recorded from over-wintered needles. *Selenosporella curvispora* MacGarvie became a dominant interior colonizer of over-wintered needles after the thaw, though it never occurred before winter. Conidia of both *V. trifidum* and *S. curvispora* did not germinate on

Table 1. Percentage frequencies of the fungi with a frequency of 50% or more at some sampling time in MA series.

Yr	1977							1978			
Sampling mo	Oct	Oct	Oct	Nov	Nov	Nov	Dec	Jan	May	Jul	Aug
Time after exposure of litter (d)	0	10	17	24	31	38	52	89	215	268	307
Fungal species											
White sterile fungus (E2453)	90	90	60	60	80	40	50	0	50	40	0
Verticicladium trifidum	0	0	20	60	40	50	10	70	0	0	0
Selenosporella curvispora	0	0	0	0	0	0	0	0	90	90	90
Total no. of species isolated	3	6	4	4	7	6	7	3	5	3	4

Table 2. Percentage frequencies of the fungi with a frequency of 50% or more at some sampling time in LA series.

Yr	1978		1979							
Sampling mo	Nov	Dec	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Time after exposure of litter (mo)	0	1	5	6	7	8	9	10	11	12
Fungal species										
White sterile (E2453)	90	80	90	90	30	40	0	0	0	0
White sterile (E2456)	10	50	0	0	40	30	10	20	30	0
Aureobasidium pullulans	10	30	20	0	90	10	10	0	0	0
Rhizomoph-forming fungus	0	0	0	0	30	40	60	40	30	40
Selenosporella curvispora	0	0	0	0	30	50	50	80	80	30
Trichoderma koningii	0	0	0	0	0	0	20	10	50	60
Total no. of species isolated	4	4	6	5	8	7	5	6	8	9

any common medium tested.

LA series Table 2 shows the results of the LA series. The sterile fungus E2453 again colonized 90% of the intact needles. The species continued for a long time to survive on the needles in high frequencies and disappeared in August 1979. *Aureobasidium pullulans* (de Bary) Arnaud and E2456 were also recorded from the intact needles. The former occurred on 90% of the needles removed in June, then quickly decreased in frequency.

Selenosporella curvispora and a rhizomorph-forming fungus were the first invaders of sample needles from the O horizon in this series. The latter may be a species of leaf litter decomposing agaric. Both species invaded 30% of the needles removed in June. The former species continued increasing in frequency until October, then declined. The latter occurred constantly with moderate frequencies until 12 mo later. *Trichoderma koningii* Oudem. was first recorded from the sample 9 mo after setting, and thereafter gradually increased and to a frequency of 60% in November 1979.

SP series Table 3 shows results of the SP series. This series was set almost 15 yr after the SU series had been started. Major fungi recorded in this series were similar to those in the LA series.

The dominant interior colonizer of intact needles in this series was also E2453 (Table 3). *Aureobasidium pullulans* followed, but its frequency did not reach 50%.

Ceuthospora sp. occurred on 70% of the needles removed after 1 mo but never appeared again. As in the MA series, the rhizomorph-forming fungus was first recorded from the sample needles of June and increased in frequency until November, then declined. *Selenosporella curvispora* was first recorded from the sample of September in this series. It occurred on 60% of the sample needles and then gradually decreased. Unlike the MA series, the rhizomorph-forming fungus colonized earlier and more frequently than *S. curvispora* in this series.

SU series As shown in Table 4, the major interior colonizer of the needles on the tree was *A. pullulans*. The species was quickly succeeded by *C. fulva* and *V. trifidum* after the sample needles were placed on the litter surface. The former occurred on 90% of the needles removed after 2 wk and maintained a high frequency until December. *Verticicladium trifidum* also occurred first on the needles removed after 2 wk, then continued to occur until December. The highest frequency for this fungus was 60%.

A characteristic future of this series was the rapid colonization of *C. fulva*. This fungus produces a mass of wet philaoconidia on a setiform conidiophore. Conidia of this species germinate easily on common media.

Correlation analysis Tables 5–7 show the results of the correlation analysis between major secondary colonizers in the LA, SP and SU series. In the LA and SP series, *S. curvispora* and the rhizomorph-forming fungus were recorded together six sampling times (Tables 2, 3).

Table 3. Percentage frequencies of the fungi with a frequency of 50% or more at some sampling time in SP series.

Yr	1992									
Sampling mo	Apr	May	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Time after exposure of litter (mo)	0	0.5	1	2	3	4	5	6	7	8
Fungal species										
White sterile (E2453)	100	80	80	40	20	0	0	0	0	0
White sterile (E2456)	30	30	60	50	40	20	20	10	20	0
Ceuthospora sp.	0	0	70	0	0	0	0	0	0	0
Rhizomorph-forming fungus	0	0	0	10	40	30	50	80	70	30
Selenosporella curvispora	0	0	0	0	0	0	60	40	20	10
Total no. of species isolated	4	7	4	6	8	7	13	11	7	4

Table 4. Percentage frequencies of the fungi with a frequency of 50% or more at some sampling time in SU series.

Yr	1979						
Sampling mo	Jul	Jul	Aug	Sep	Oct	Nov	Dec
Time after exposure of litter (mo)	0	0.5	1	2	3	4	5
Fungal species							
Aureobasidium pullulans	80	30	20	10	10	10	30
Chaetopsina fulva	0	90	70	70	80	40	60
Verticicladium trifidum	0	20	60	20	30	60	40
Total no. of species isolated	3	5	4	7	8	7	9

Table 5. Contingency table (2×2) for *Selenosporella curvispora* – rhizomorph-forming fungus correlation in LA series (data period is from 7 to 12 mo later).

		Selenosporella curvispora		
		Present	Absent	Total
Rhizomorph-forming fungus	Present	12	12	24
	Absent	20	16	36
	Total	32	28	60

$\chi^2=0.0251$.

Table 6. Contingency table (2×2) for *Selenosporella curvispora* – rhizomorph-forming fungus correlation in SP series (data period is from 3 to 8 mo later).

		Selenosporella curvispora		
		Present	Absent	Total
Rhizomorph-forming fungus	Present	9	21	30
	Absent	9	21	30
	Total	18	42	60

$\chi^2=0.0793$.

Table 7. Contingency table (2×2) for *Chaetopsina fulva* – *Verticicladium trifidum* correlation in SU series (data period is from 3 to 8 mo later).

		Verticicladium trifidum		
		Present	Absent	Total
Chaetopsina fulva	Present	10	31	41
	Absent	13	6	19
	Total	23	37	60

$\chi^2=8.866$.

Statistical analysis shows that the relation of the two species is clearly exclusive. In contrast, it also shows that *V. trifidum* and *C. fulva* in the SU series (Table 4) can coexist. In the MA series the relation between *V. trifidum* and *S. curvispora* was not estimated because they were never recorded from the same samples, as shown in Table 1.

Based on the above results, fungal successions observed in pine needles fallen at different seasons are summarized in Table 8. The monthly means of air temperature that the experiments were started and the 10 d means of air temperature after the setting are also indicated.

Discussion

A sterile form (E2453) and *A. pullulans* were major interior colonizers of senescent and dead needles on the tree in the study site. The former was recorded in all seasons and appeared predominant except in summer. The identity of this sterile form was unclear. There are many reports of *Lophodermium pinastri* (Schrad.) Chev. in Europe (Kendrick and Burges, 1962; Hayes 1965a; Lehmann and Hudson 1977; Mitchell and Millar, 1978a, b; Mitchell et al., 1978) and in Japan (Soma and Saitô, 1979). The species colonizes most naturally senescent needles on the tree and can survive for several mo in the O horizon after needle fall and reproduce there. It can grow on common agar media but not fruit on them. E2453 is a probably fungus like *L. pinastri*, because no needles of abnormal appearance were chosen for experiments. *Aureobasidium pullulans* frequently colonized the needles fallen in summer, where E2453 also occurred but rather infrequently. The fungus has been recorded as an external colonizer of senescent or freshly fallen needles (Kendrick and Burges, 1962; Tokumasu, 1978; Soma and Saitô, 1979), but in this study, it was also recorded as an internal colonizer of dead needles on the tree in summer. Furthermore, it colonized 90% of the needles removed after 7 mo in the LA series (Table 2).

Table 8. Fungal successions observed in pine needles fallen at different seasons.

Season of needle fall	Mid-autumn	Late autumn	Spring	Summer
On the tree				
Primary colonizers	White sterile fungus (E2453)	White sterile fungus (E2453)	White sterile fungus (E2453)	*A. pullulans*
On litter				
Secondary colonizers (First wave)	*Verticicladium trifidum*	*Aureobasidium pullulans*	*Ceuthospora* sp.	*Chaetopsina fulva* *V. trifidum*
In litter				
Secondary colonizers (Second and third waves)	*Selenosporella curvispora*	*S. curvispora* Rhizomorph-forming fungus *Trichoderma koningii*	*S. curvispora* Rhizomorph-forming fungus	
Monthly mean air temperature of the staring mo (°C)	9.4	3.0	5.5	18.1
Mean daily air temperature during 10 d after setting (°C)	7.8	1.3	8.0	16.8

These facts suggest that this fungus can invade inner tissues of the needles on the tree and sometimes expand to freshly fallen needles on the ground.

Chaetopsina fulva, *V. trifidum*, *S. curvispora* and the rhizomorph-forming fungus were litter-inhabiting fungi that first invaded freshly fallen needles (Table 8). Mitchell and Millar (1978a) listed internal colonizers of pine needles along with their distribution within the needle, decomposer role, if known, and the needle type colonized. Among the five species mentioned above, only *V. trifidum* appears on the list as a cellulolytic internal colonizer invading after needle fall. The species has been recorded as a very common and often prevailing interior colonizer of fallen pine needles in Europe (Gremmen, 1957; Kendrick and Burges, 1962; Hayes, 1965a, b; Black and Dix, 1977; Lehmann and Hudosn, 1977; Mitchell and Millar, 1978a; Tokumasu et al., 1994), and also Japan (Tubaki and Saitô, 1969; Soma and Saitô, 1979).

The other four species do not appear on the list, but a *Ceuthospora* species (C. *pinastri* (Fr.) Höhnel) and a *Trichoderma* species (*T. viride* Pers. ex Gray) are included. *Ceuthospora* sp. and *T. koningii* recorded in this study may correspond to those species, respectively. In addition, the listed *Marasmius androsaceus* (L.: Fr.) Fr. is a rhizomorph-forming fungus that is cellulolytic and lignolitic. The rhizomorph-forming fungus found in this study probably plays a similar part in the decomposition of pine needles in litter to that of *M. androsaceus*. *Ceuthospora* sp. occurred once with a very high frequency in the SP series (Table 3). There are some doubts that it invaded the sample needles after needle fall because it was found on falling needles (Mitchell and Millar, 1978a).

Selenosporella curvispora is not listed as an interior colonizer by Mitchell and Millar (1978a). The species was the commonest internal colonizer found in this site in the previous work (Tokumasu, 1996), and this finding was confirmed in the present work. *Chaetopsina fulva* is not also included in the list. The species has been recorded from freshly fallen needles of *P. densiflora* in Japan (Tubaki and Saitô, 1969; Tokumasu, 1978, 1980, 1996), but never in other regions of the world as an interior needle colonizer. The roles of these species in the decomposition process of pine needles have not been examined.

The successions of interior colonizers observed on the fallen needles laid on the surface of litter at four different times can be roughly divided into three groups based on the composition of the interior colonizers invading from litter (Tables 1–4) and their correlation (Tables 5–7). Thus, in the MA series, the periods of appearance of the major interior colonizers, *V. trifidum* and *S. curvispora*, did not overlap. In the LA and SP series, the appearance of *S. curvispora* overlapped with that of the rhizomorph-forming fungus and they were mutually exclusive (Tables 5, 6). By contrast the dominants in the SU series, *C. fulva* and *V. trifidum*, coexist (Table 7). These shifts in the species combination are interesting, as there is no reported work on the fungal succession on pine needles showing such seasonal differences in major interior colonizers.

Although this phenomenon reflects the overall influence of many climatic elements, air temperature appeared to be one of the cardinal factors contributing to these seasonal changes. Temperature at the needle fall appears to be particularly important. As shown in Table 8, *S. curvispora* became dominant or nearly so in the series starting in late autumn and spring, when monthly mean air temperature was low, 3.0 and 5.5°C respectively. In the SU series starting in July, when the monthly mean was about 18°C (Table 8), *C. fulva* and *V. trifidum* became dominant. However, it is natural to consider that most fungi respond to the mean temperature of a shorter period than 1 mo in becoming active. In fact, the low mean air temperature during the 10 d after setting in the LA series (1.3°C) may not allow would-be colonizers in the O horizon to invade freshly fallen needles. Furthermore, the temperature at the surface of the O horizon under snow cover remains around the freezing point. This means that most needles that fall at the autumn peak of leaf fall would remain largely intact until the following spring.

The temperature ranges for growth of major interior colonizers may be another important factor contributing to the shifts in the species composition. This is suggests by the fact that, like *S. curvispora* (Tokumasu, 1996), the abundantly produced conidia of *V. trifidum* failed to germinate on any artificial medium tested and that direct field observations confirmed that the rhizomorph-forming fungus used rhizomorphs as an effective means of expansion in litter. Thus, the major interior colonizers found at the study site appear to arrive at the needles by means of mycelia, and only *C. fulva* appears to reach the needles by conidia. Consequently, fungi with a relatively rapid mycelial growth rate under the temperature conditions of needle fall may often succeed in invasion of the freshly fallen needles.

From this viewpoint, the steep temperature drop in late autumn may contribute to the characteristic features of the fungal succession at the study site. In early autumn, most fungi inhabiting the surface layer of the litter may not be psychrophilic because they can colonize freshly fallen needles as dominants in rather warmer period (Tokumasu, 1996). The low temperature may hinder the rapid hyphal growth of these fungi in late autumn, and the freshly fallen needles that they can colonize may be limited to those contiguous to their dwelling needles. At this site, the annual peak of leaf fall occurs in the second half of the autumn (Fig. 3). As a result, most autumn-fallen needles may remain largely intact until the following spring, as mentioned above. It is certain that the fungi colonizing these over-wintered needles after the thaw are psychrotolerant (Dix and Webster, 1995) and endure the low temperature and humid conditions under snow and just after the snow melt, and probably they grow slowly under such conditions. Such fungi may become dominant interior colonizers of the site in the succession estimated from a comparative study of mycoflora among sub-layers of the O horizon. At the study site, the dominant interior colonizer is *S. curvispora*

(Tokumasu, 1996).

Precipitation is thought to be a limiting factor of fungal activity as well as temperature. In this area, however, there is enough rainfall in all seasons that this is probably not a major factor (Fig. 2).

Some workers have pointed out that the first invader of freshly fallen needles from litter is sometimes affected by the species that previously colonized needles on the tree, especially those of parasites (Lehmann and Hudson, 1977; Mitchell and Millar, 1978a). For example, Mitchell and Minter (1978a) observed the fungal successions on three types of needles colonized on the tree by three different fungi. They collected such needles at the end of September and successively laid them out on the litter surface after marking. They found that needles differed in internal colonizers but not external ones during the successions. In this study, to obtain data on more natural fungal succession, intact needles fallen just before the beginning of experiments were used after elimination of the needles apparently infected by parasites. The major interior colonizers of intact needles fallen at four different seasons were a sterile form E2453 and *A. pullulans* (Tables 1–4), of which the former may be intrinsic because the latter was recorded as a dominant surface colonizer of pine needles, as already mentioned above. However, no clear correlation was found between the dominant species of intact needles and those of secondary colonizer (Table 8). Therefore, it is difficult to estimate the influences of previous residents on the successors from the O horizon.

As mentioned above, the relationship between two interior colonizers recorded from the same samples is divided into two types. From the χ^2 values in Tables 5–7, the correlation between *S. curvispora* and the rhizomorph-forming fungus is mutually exclusive. On the other hand, that between *C. fulva* and *V. trifidum* is one of coexistence. *Verticicladium trifidum* is apparently a major needle decomposer (Mitchell and Millar, 1978a), but the role of *C. fulva* in the decay process is not clear. Therefore, there are several possible relationships between these two species. One possibility is that *C. fulva* is a parasite of *V. trifidum*. A second is that it is a secondary saprophytic fungus associated with the cellulolytic partner (Garrett, 1963). Another possibility is that both species inhabit different parts within a needle and do not interact substantially. The data obtained in this study, however, cannot explain the type of relationship between these two species.

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