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Fungal succession in the early decomposition process of pine cones on the floor of *Pinus densiflora* forests

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The fungal succession on pine cones on the floor of *Pinus densiflora* forest was investigated in the early decomposition process (within ca. 30% decrease in dry weight). The fungal flora was examined by both washing and surface-sterilization methods on artificially placed cones and naturally fallen cones. The decomposition rates of artificially placed cones were 0.081-0.082 yr^{-1}. On withered cones still attached to the tree, *Pestalotiopsis* spp. were dominant. These fungi also occurred with higher frequencies after cones had lain on the floor and on cones in the L and FH horizons. *Xylaria* sp. and *Phomopsis* sp., which seem to colonize the interior of the tissue, occurred with higher frequencies on the cones on the tree, but their occurrence frequencies decreased after cones had lain on the forest floor. Conversely, *Mortierella* spp. and *Trichoderma* spp. newly occurred or their occurrence frequencies increased on lying cones. Of these, *Trichoderma koningii* increased rapidly and showed high occurrence frequencies. *Thysanophora penicillioides*, which prefers coniferous substrates, showed higher occurrence frequencies in the early stages of lying on the forest floor. On cones lying on the floor, the fungal flora did not significantly change during the investigation period.

Key Words——decomposition of pine cones; fungal succession; *Pinus densiflora*.

Many studies have focused on the successional changes in fungal flora during the decomposition processes in a variety of plant remains (e.g., Saitô, 1966; Frankland, 1966; Chapela et al., 1988). As regards coniferous litter, fungal successions on the decaying needles of a variety of species have been investigated: e.g., *Pinus sylvestris* L. in England (Kendrick and Burges, 1962), *P. strobus* L. and four other pine species in Canada (Widden and Parkinson, 1973), *P. densiflora* Sieb. et Zucc. and four introduced pines in Japan (Tokumasu, 1978), *P. thunbergii* Parl. in Japan (Soma and Saitô, 1979) and *Abies firma* Sieb. et Zucc. in Japan (Aoki et al., 1990). However, little is known of the successional changes of fungus flora during the decomposition of pine cones, which are one component of coniferous litter.

Cones are more resistant to microbial attacks than leaves, and remain for a long time in the A_0 horizon. Certain characteristic basidiomycetous fungi, e.g., *Strobilurus stephanocystis* (Hora) Sing. and *Baeospora myosura* (Fr.: Fr.) Sing., can be found on buried cones. It is suspected that the fungal flora on decaying cones differs from that on needles. The purpose of this study is to examine changes in fungal flora in the early decomposition process of pine cones on the floor of forests of Japanese red pine, *Pinus densiflora* Sieb. et Zucc.

Materials and Methods

Study sites Two study sites, A and B, were selected in Japanese red pine forests at Kushima (34°23′N lat., 132°13′E long.), Saeki-chô in Hiroshima Prefecture, western Japan. This region has a warm-temperate monsoon climate. The averages of annual mean air temperature and precipitation during the last five years have been 12.6°C and 1962 mm, respectively. Some characteristics of the study sites are given in Table 1. Site A was located on the middle of a mountain slope, with an elevation of 570 m and a southwesterly aspect. Site B was located on ridge of a mountain, with an elevation of 380 m and a southwesterly aspect. The dominant understories at site A were *Eurya japonica* Thunb., *Magnolia salicifolia* Maxim. and *Parabenzoin trilobum* (Sieb. et Zucc.) Nakai in the shrub layer, and *Ardisia japonica* Bl. in the herbaceous layer. Those at site B were *Rhododendron reticulatum* D. Don in the shrub layer, and *Ilex crenata* Thunb. in the herbaceous layer. The temperature of the boundary between the A_0 and A horizons at site A was slightly lower than that at site B. The A_0 horizon at site A was thicker and more humid than that at site B during the investigation period (from November 1991 to October 1992). The amounts (kg dry weight ha^{-1} yr^{-1}) of cone fall and the percentages of the amount of cone fall to that of litter fall during the period were 28.1 and 0.6 at site A, and 11.1 and 0.4 at site B, respectively.

Table 1. Description of the study sites.

	Site A	Site B
Elevation (m)	570	380
Position on slope	middle	ridge
Slope aspect	sw	sw
Slope angle (°)	22–34	15–30
Temperature of the boundary between A_0 and A horizons* (°C)		
Min.	0.3	0.7
Max.	23.3	25.0
Thickness of A_0 horizon (cm)	5	2
Moisture content of A_0 horizon* (%)	85–243	11–192
Amount of cone fall* (kg dry weight $ha^{-1}yr^{-1}$)	28.1	11.1
Percent of cone fall to litter fall	0.6	0.4
Dominant vegetation		
Tree layer	*Pinus densiflora* Sieb. et Zucc.	*P. densiflora*
Shrub layer	*Eurya japonica* Thunb., *Magnolia salicifolia* Maxim., *Parabenzoin trilobum* (Sieb. et Zucc.) Nakai	*Rhododendron reticulatum* D. Don
Herbaceous layer	*Ardisia japonica* Bl.	*Ilex crenata* Thunb.

*Records from Nov. 1991 to Oct. 1992.

Seasonal fluctuation in cone fall was not observed.

Experiment using artificially placed cones and naturally fallen cones It is difficult to judge objectively the decomposition stages of pine cones in the field by the position in the A_0 horizon and the color of cones, because cone litter does not stratify clearly, unlike needle litter, and cone color differs slightly from one parent tree to another. So we used artificially placed cones on the forest floor. Cones which had dispersed seeds at least one year earlier were gathered from Japanese red pine trees on each site on 16 June 1992. Five of these were used for the investigation of the fungal flora of cones on the tree. Other cones were bored with a small hole at the base, air-dried and weighed. Eight dried cones were tied on a fishing line at intervals of 20 cm. Five such cone-strings were prepared for each site. Subsamples were dried at 80°C to constant weight and the factors for air-dried weights were determined. The cone-strings were placed on the forest floor at each site on 13 July 1992 at intervals of 2 m. Five cones from each site, consisting of one cone from each string, were recovered after 4, 12, 24, 40 and 65 weeks and brought back to the laboratory for cooling with ice. Three cones in each string were left on the forest floor for a longer-term experiment.

The fungal flora of naturally fallen cones was examined at site A. Five cones each in the early stages of decomposition, whose shape was intact, were collected from the surface or the upper part of A_0 horizon (L layer) and from the lower part of A_0 horizon (FH layer) on 4 September 1993 and March 24 1994.

Investigation of the fungal flora of cones The fungal flora was investigated by both washing (WA) and surface-sterilization (SS) methods. The former method was used to remove the attached propagules on the surface of cone scales and to detect fungi growing on or near the surface of the scales, and the latter method was used to detect fungi growing within scale tissues. Twenty scales were randomly picked off of each cone in the experiment using the artificially placed cones. After rinsing thoroughly with sterile water to eliminate soil and plant debris, 10 were used for the WA method and another 10 for the SS method. In the case of the naturally fallen cones, 24 scales were used, 12 for the WA method and 12 for the SS method. Dry weights of the remainder were determined. Five scales of these were used for the determination of C/N ratio.

Total carbon and nitrogen contents of the scales were determined with a CN corder MT-500 (Yanagimoto Mfg. Co., Ltd., Kyoto).

The WA procedure was conducted according to Tokumasu (1978) with slight modification. The scales were washed 20 times with 5-min sonication in 0.005% Aerosol OT (Katayama Chem. Indus. Co. Ltd. Osaka) solution, followed by 3 times in sterilized water, changing the solution for each washing. The number of washings with detergent solution was determined after examining the decrease in the number of propagules in the washing solution by the plate counting technique. The number of propagules reached a minimum after 20 washings in cones in various stages of decay.

In the SS method, the scales were soaked in 70% ethanol for 2 min, then rinsed with sterilized water 5 times.

The scales after each treatment were aseptically blotted-dried on filter paper for one day. The scales were then divided vertically into two parts. One half was placed on a malt extract agar (MEA) medium, containing 2% malt extract (Difco Lab., Detroit), 2% glucose (Katayama Chem. Indus. Co., Ltd., Osaka), 0.1% peptone (Kyokutô Pharm. Indus. Co., Ltd., Tokyo) and 1.5%

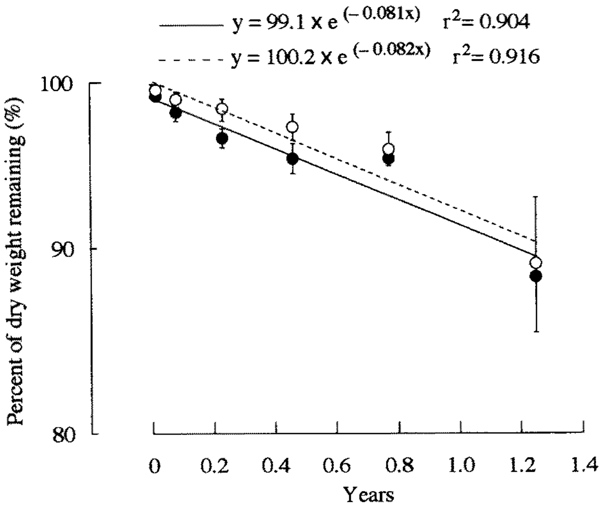

Fig. 1. Decrease in dry weight of cones with time after positioning.
●, Site A; ○, Site B. Vertical bars indicate S.D.

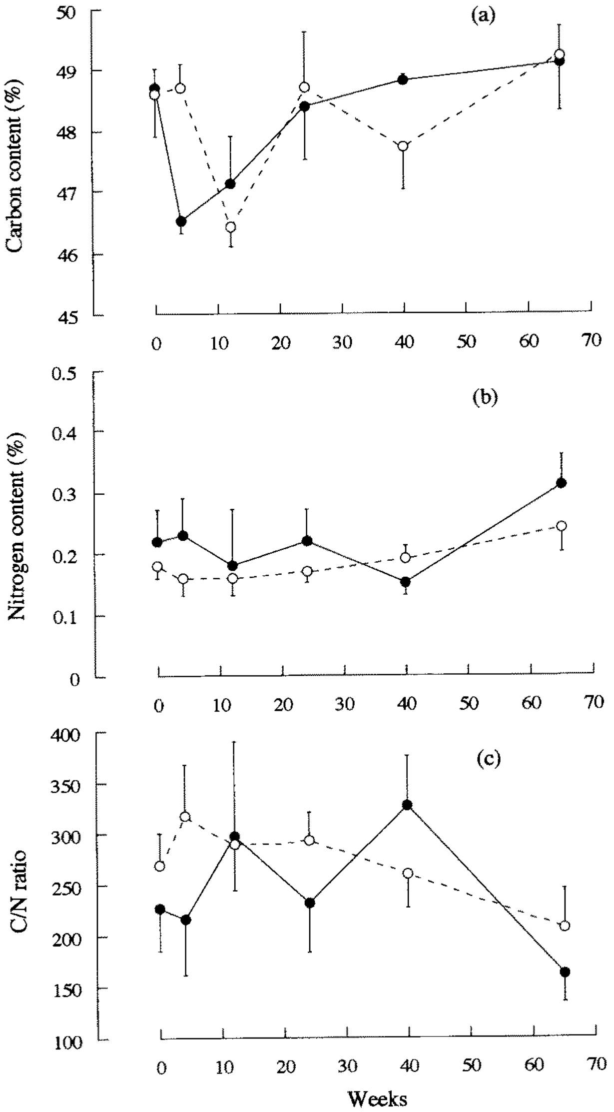

Fig. 2. Changes in carbon (a) and nitrogen (b) contents and C/N ratio (c) of cones after positioning.
—●—, Site A; --○--, Site B. Vertical bars indicate S.D.

agar, pH 6.0, in Petri dishes; the other half was used for the determination of dry weight. On the naturally fallen cones, half-strength cornmeal agar medium (1/2 CMA), which was used by Tokumasu (1978) on account of its convenience for the observation and isolation of fungi, was also used to get further information. The 1/2 CMA contained 0.85% cornmeal agar (Nissui Pharm. Co., Ltd., Tokyo) and 0.75% agar. In this case 6 of 12 scales from each treatment were put on the MEA medium and the other 6 on the 1/2 CMA medium. The plates were incubated at room temperature for 4 weeks, and fungi occurring on and around the scales were subcultured and identified.

The frequency of occurrence of fungi The occurrence frequency of fungal species on the artificially placed and naturally fallen cones was expressed as a percentage calculated by the following equation: Occurrence frequency (OF) (%)=[(number of the scales with the fungus)/(number of the scales tested)]×100. The results are shown for fungi with frequency of 10% and over in each treatment at one sampling time.

Results

Changes in dry weight and C/N ratio in the early decomposition process As seen in Fig. 1, the dry weight of cones gradually decreased with the lapse of time. The rates of decomposition at sites A and B were estimated by applying a negative exponential (Swift et al., 1979). The rates at sites A and B calculated from the gradients of the regression equations were 0.081 yr^{-1} and 0.082 yr^{-1}, respectively.

Figure 2 shows the changes in carbon and nitrogen

Table 2. Contents of carbon and nitrogen, and C/N ratios of pine cones on the tree and the ground.

	On the tree		L horizon		FH horizon	
	Sept. '93	Mar. '94	Sept. '93	Mar. '94	Sept. '93	Mar. '94
C%	49.3 ± 0.4^{a}	50.8 ± 1.1^{a}	48.1 ± 0.2^{b}	49.5 ± 1.2^{a}	49.9 ± 0.8^{a}	50.3 ± 0.8^{a}
N%	0.22 ± 0.03^{a}	0.19 ± 0.04^{a}	0.28 ± 0.07^{ab}	0.38 ± 0.11^{bc}	0.35 ± 0.10^{bc}	0.45 ± 0.07^{c}
C/N	223 ± 29^{ab}	274 ± 49^{a}	179 ± 44^{bc}	139 ± 37^{cd}	150 ± 37^{cd}	114 ± 19^{d}

Values are the averages±S.D. of 5 samples. Values with different letters in each row are significantly different at $P<0.05$ (Mann-Whitney *U*-test).

contents and C/N ratio of artificially placed cones. The carbon content of cones decreased by about 2% soon after placement, then increased to reach the initial level (48-49%) 24 weeks after. Thereafter it maintained the initial level. The nitrogen contents were around 0.2% up to 40 weeks after placement and increased slightly thereafter. The C/N ratios were in the range of 200-300 up to 40 weeks, but showing a large variation. Thereafter the C/N ratios decreased slightly, corresponding to the increase of nitrogen. Table 2 shows the contents of carbon and nitrogen and the C/N ratios of naturally fallen cones in the L and FH horizons at site A. The carbon contents (48-50%) of cones in the L and FH horizons were similar values to those of cones on the tree. The nitrogen contents of cones on the tree and in the L and FH horizons increased in this order, corresponding to the decrease of the C/N ratio. This result shows that the C/N ratio decreased with the progress of decomposition. The C/N ratios of cones in the L and FH horizons were at the same or slightly lower levels than those of artificially placed cones after 65 weeks (162±26).

Changes of fungal flora on the artificially placed cones The fungal flora of the cones just before positioning was checked for contamination by other fungi during the preparation of the samples. It was found to be almost the same as that on the tree, showing no contamination.

Figure 3 (white bars) shows the changes of fungal flora on washed cones at the site A. From the cones on the tree, *Pestalotiopsis* spp. were found on all scales tested. These fungi continued to occur with higher OFs until 65 weeks. *Penicillium coalescens* Quintanilla, which was first reported from soil under conifers in Spain (Quintanilla, 1983/1984), also showed relatively high OF (>30%). This species showed the highest OF at 4 weeks, but its OF decreased rapidly thereafter. After positioning of cones, *Mortierella* spp. and *Trichoderma koningii* Oudem. aggr. newly occurred and their OFs increased from 12 or 24 weeks after positioning. *Thysanophora penicillioides* (Roum.) Kendrick showed higher OF only in the period between 24 and 40 weeks. *Cylindrocladium* sp., *Gliocephalotrichum bulbilium* J. J. Ellis et Hesseltine and *Trichoderma harzianum* Rifai aggr. tended to occur with higher OF in the WA method than the SS method. *G. bulbilium* and *T. harzianum* showed

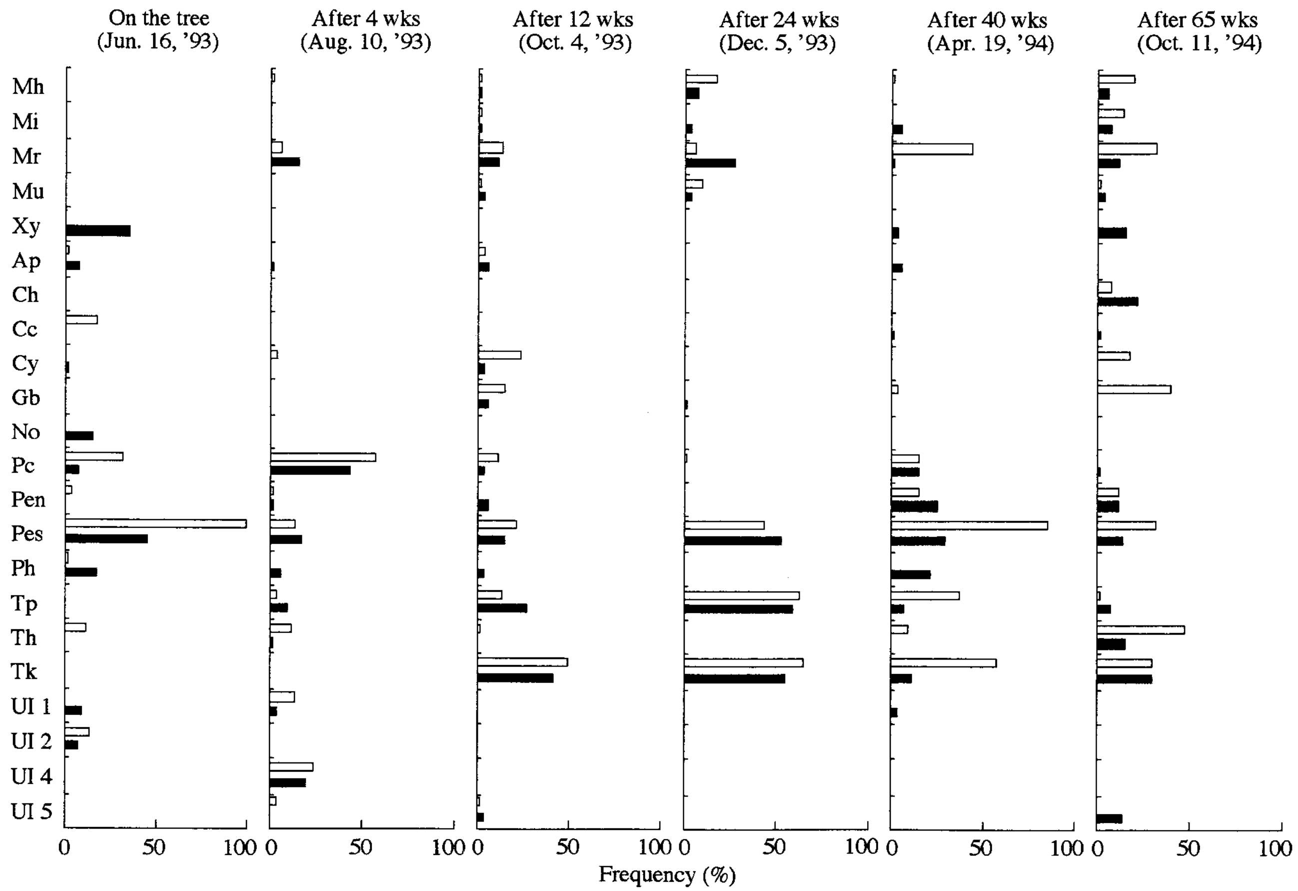

Fig. 3. Occurrence frequency of fungi on pine cones positioned on the forest floor at site A.
□, Washed; ■, Surface-sterilized. Mh, *Mortierella hyalina;* Mi, *M. isabellina.;* Mr, *M. ramanniana.;* Mu, *Mucor* sp.; Xy, *Xylaria* sp. (imperfect state); Ap, *Aureobasidium pullulans;* Ch, *Chloridium* spp.; Cc, *Cladosporium cladosporioides;* Cy, *Cylindrocladium* sp.; Gb, *Gliocephalotrichum bulbilium;* No, *Nodulisporium* sp.; Pc, *Penicillium coalescens;* Pen, *Penicillium* spp.; Pes, *Pestalotiopsis* spp.; Ph, *Phomopsis* sp.; Tp, *Thysanophora penicillioides;* Th, *Trichoderma harzianum* aggr.: Tk, *T. koningii.* aggr.; and Ul, unidentified species.

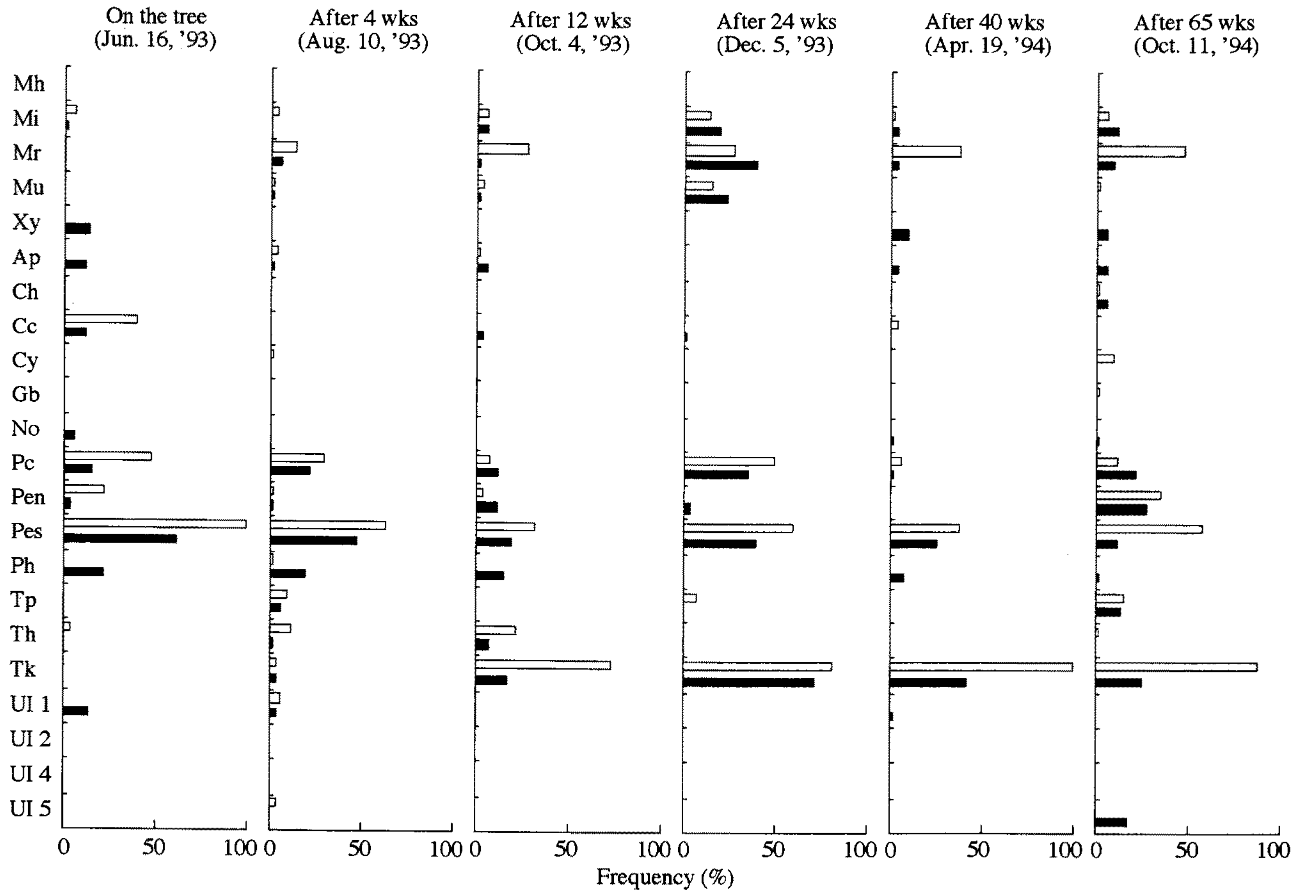

Fig. 4. Occurrence frequency of fungi on pine cones positioned on the forest floor at site B. ▭, Washed; ■, Surface-sterilized. See the legend of Fig. 3 for abbreviations of species names.

higher OFs at 65 weeks.

The occurrence patterns of *P. coalescens, Pestalotiopsis* spp., *T. penicillioides* and *T. koningii* in the SS method were similar to those in the WA method (black bars in Fig. 3). Fungi which characteristically occurred in the SS method were *Nodulisporium* sp., *Phomopsis* sp. and *Xylaria* sp. (imperfect state). *Nodulisporium* sp. occurred only on the cones on the tree. *Xylaria* sp. and *Phomopsis* sp. occurred irregularly during the investigation period.

The occurrence patterns of fungi at site B were essentially the same as those at site A (Fig. 4). These results show that differences in enviromental conditions such as moisture between sites A and B did not noticeably affect the fungal flora. The notable characteristics of fungal occurrence at site B were as follows: *Mortierella hyalina* (Harz) W. Gams did not occur. OFs of *Cylindrocladium* sp., *G. bulbilium* and *T. penicillioides* were much lower. OF of *P. coalescens* was highest at 24 weeks. These results show that differences in environmental conditions such as moisture between sites A and B did not noticeably affect the fungal flora.

Fungal flora on naturally fallen cones in the L and FH horizons As shown in Fig. 5, in September 1993 the results by the WA method for the L and FH horizons were fundamentally the same as those by the SS method, except that in the WA method *Cylindrocladium* sp. was found and the OF of *Gliocladium virens* Miller et al. was higher. Notable differences between the L and FH horizons were as follows: *G. bulbilium* occurred with higher OF in the L horizon in both the WA and SS methods. Conversely, *Mortierella ramanniana* (Möller) Linnem. and *G. virens* occurred with higher OFs in the FH horizon in the WA method.

The fungal flora in the L and FH horizons in March 1994 was essentially the same as that in September 1993, except that *Cylindrocladium* sp. occurred only in September in the WA method, and *P. coalescens* also occurred in September with higher OF in both the WA and SS methods. On the other hand, *M. hyalina* and *T. penicillioides* occurred only in March.

The fungal flora of cones in the L and FH horizons closely resembled that of the artificially placed cones at 65 weeks. Remarkable exceptions were as follows: *G. virens* was absent in the positioned cones, and *Xylaria* sp. was absent in the naturally fallen cones.

Fungal flora found with 1/2 CMA medium Occurrence of fungi on naturally fallen cones in the L and FH horizons at site A was investigated in September 1993 and March 1994 by using 1/2 CMA medium (Fig. 6). The number of taxa with OF of 10% and over in at least one test was 32 in tests with 1/2 CMA medium, and 23 in tests with

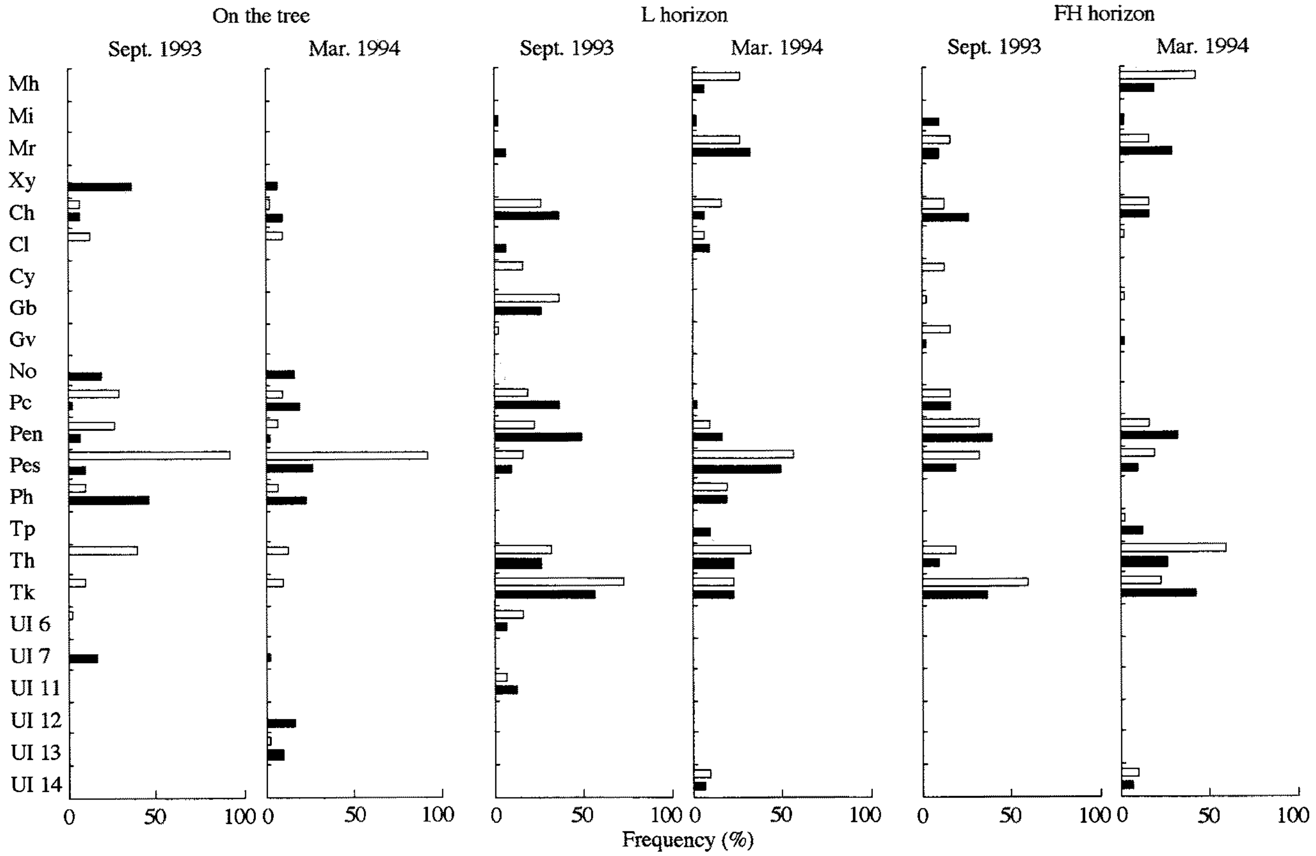

Fig. 5. Occurrence frequency of fungi on naturally fallen cones in the L and FH horizons at site A on Sept. 4, 1993 and Mar. 24, 1994. □, Washed; ■, Surface-sterilized. See the legend of Fig. 3 for abbreviations of species names other than: Cl, *Cladosporium* spp.; and Gv, *Gliocladium virens* Miller et al.

MEA medium. The number of taxa common to both media was 16 (Figs. 5, 6). The number of fungal taxa increased in the cones in the L and FH horizons in March, which had lower C/N ratios, representing a more advanced stage of decay. Characteristic fungal species occurring only in the 1/2 CMA medium were Discomycete 1, *Fusidium* sp., *Gliocladium* sp., *Geniculosporium* sp. and *Monacrosporium* sp. *Geniculosporium* sp. occurred on the tree only in the SS test. On the other hand, *Cladosporium* spp. and *Nodulisporium* sp. were found only in the MEA medium. The latter fungus occurred only on the cones on the tree. Only one basidiomycetous fungus was isolated by using the 1/2 CMA medium in the the present investigation.

Discussion

Nakane et al. (1984) reported that the decomposition rate of the litter in a *P. densiflora* forest in Hiroshima Prefecture was 0.251 yr^{-1}. The decomposition rates of the needles and stems of *P. densiflora* in Saitama Prefecture were 0.474 yr^{-1} and 0.07-0.20 yr^{-1}, depending on the diameter of stems (Kawahara and Sato, 1977). The decomposition rates of pine cones in the present study 0.081-0.082 yr^{-1}, were lower than that of needle litter and the same level as those of stems with diameters of 9-10 cm. The difference in moisture content in the A_0 horizon between site A and B seems hardly to affect the decomposition rate of cones.

It seems that the temporary fall in carbon content in the early decomposition stage was caused by biological breakdown and leaching of simple organic carbon materials. After that, the carbon content recovered to the initial level, although the dry weight of cones decreased. It is known that the C/N ratio decreases with the progress of decomposition (Swift et al., 1979), suggesting that the C/N ratio could be used as an index of decompositional stage. Although the values of C/N ratio of samples from L and FH horizons overlapped each other, the average C/N ratios of cones were related to their position (L and FH horizons) in the A_0 horizon, This suggests that the stage of decay of cones might be judged by the position of the cones. The decrease in the C/N ratio of cones in the present study had a time lag, 40 weeks at site A and 24 weeks at site B. Swift et al. (1979) stated that nitrogen in organic materials is mineralized before being converted to cell substances of a microbial body. Nitrogen in cones may be released slightly in a soluble form in the early decompositional phases, while thereafter, microbial biomass is built up to maintain nitrogen in the cones. As a result, the nitrogen content apparently increased and the C/N ratio decreased. It has been sug-

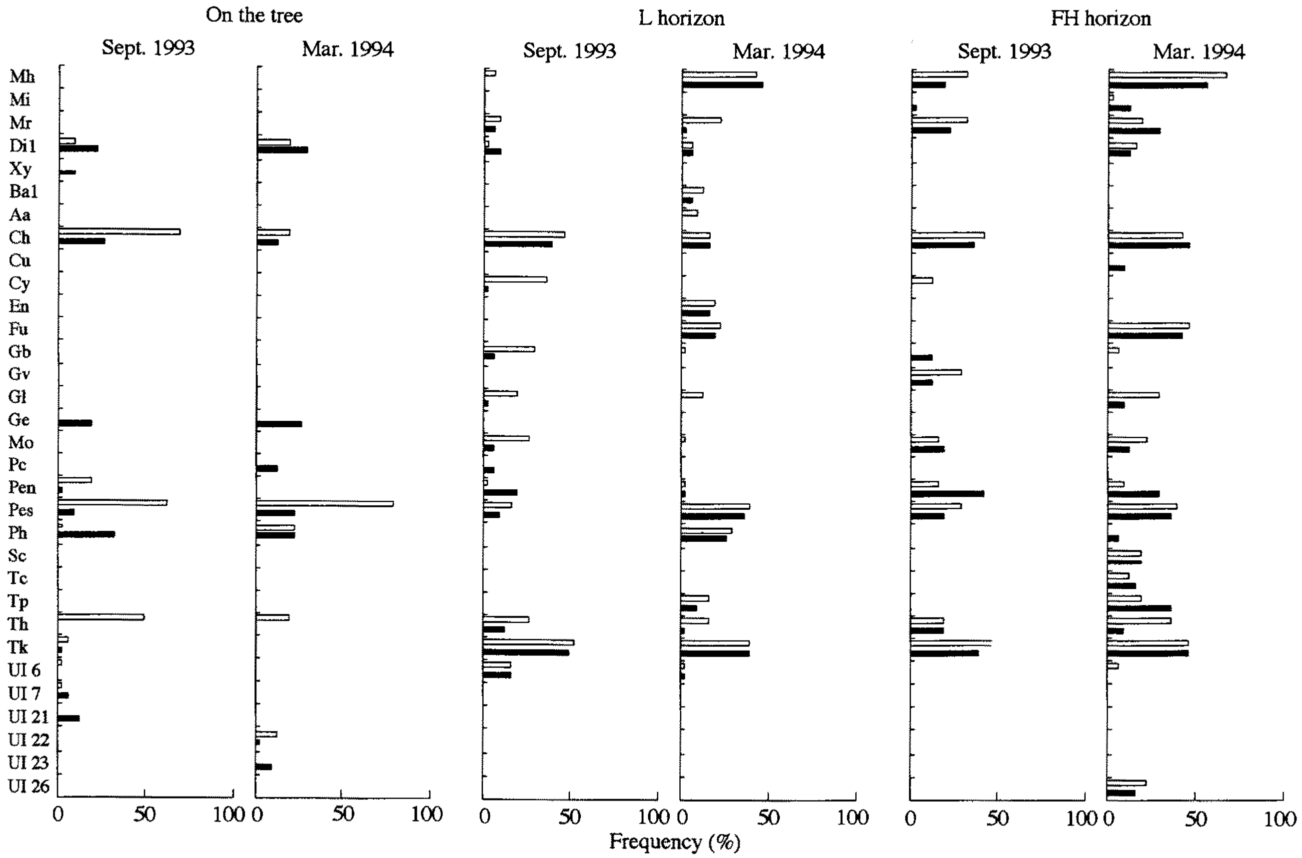

Fig. 6. Occurrence frequency of fungi on naturally fallen cones in the L and FH horizons at site A on Sept. 4, 1993 and Mar. 24, 1994, using the 1/2 CMA medium.

▭, Washed; ■, Surface-sterilized. See the legend of Figs. 3 and 5 for abbreviations of species names other than: Di1, Discomycete 1; Ba1, Basidiomycete 1; Aa, *Alternaria alternata* (Fr.) Keissler; Cu, *Cryptophiale udagawae* Pirozinski et Ichinoe; En, *Endophragmia* sp.; Fu, *Fusidium* sp.; Gl, *Gliocladium* sp.; Ge, *Geniculosporium* sp.; Mo, *Monacrosporium* sp.; Sc, *Selenosporella curvispora* MacGarvie; Tc, *Thozetalla cristata* Pirozynski et Hodges.

gested that the initial nitrogen content of substrates correlates with their decomposition rates (Swift et al., 1979; Neely et al., 1991). The initial nitrogen contents of pine cones were low (0.22±0.05% at site A and 0.18±0.02% at site B), so the decomposition rates were also low.

The dry weights of the naturally fallen cones were estimated at the same time as those attached to the branches, by applying their size [length × (diameter)^2] to the regression equation showing the relation between size and dry weight of cones gathered from the tree at site A. The remaining dry weights were calculated as the percents of weights of decaying cones to the estimated weights. The mean values±standard deviations in the L and FH horizons in September 1993 were 92.8±5.6% and 74.9±9.1%, and those in March 1994 were 80.9±13.8% and 73.5±11.1%, respectively. Although these values might be underestimates because the weight of the naturally fallen cones was also decreased due to physical destruction, the decomposition stages of the naturally fallen cones could be objectively compared with those of the artificially placed cones by using the weight decrease and the C/N ratio. It was concluded that the naturally fallen cones in the L and FH horizons in the present study represent the early stages of decomposition.

Figure 7 shows the patterns of the successional change of typical fungi which have an OF of 30% or more in at least one test, during the decomposition process of pine cones at site A. Data on naturally fallen cones in the L and FH horizons were arranged in progressive stages of decomposition, based on the values of the C/N ratio. External species in the figure are those whose average OF by the WA method was more than two-fold that by the SS method; and internal species are those whose average OF by the SS method was more than twice the OF by the WA method.

The fungal flora of the cones on the tree was greatly changed by their transfer to the forest floor. However, the subsequent change of species composition on the floor was not so great. This phenomenon seems to be related to the low decomposition rate of cones.

In the present study, *Pestalotiopsis* spp. occurred on the scales with the highest OF in a wide range of the decaying process, from the cones on the tree to those in the FH layer. Aoki et al. (1990) also reported that a spe-

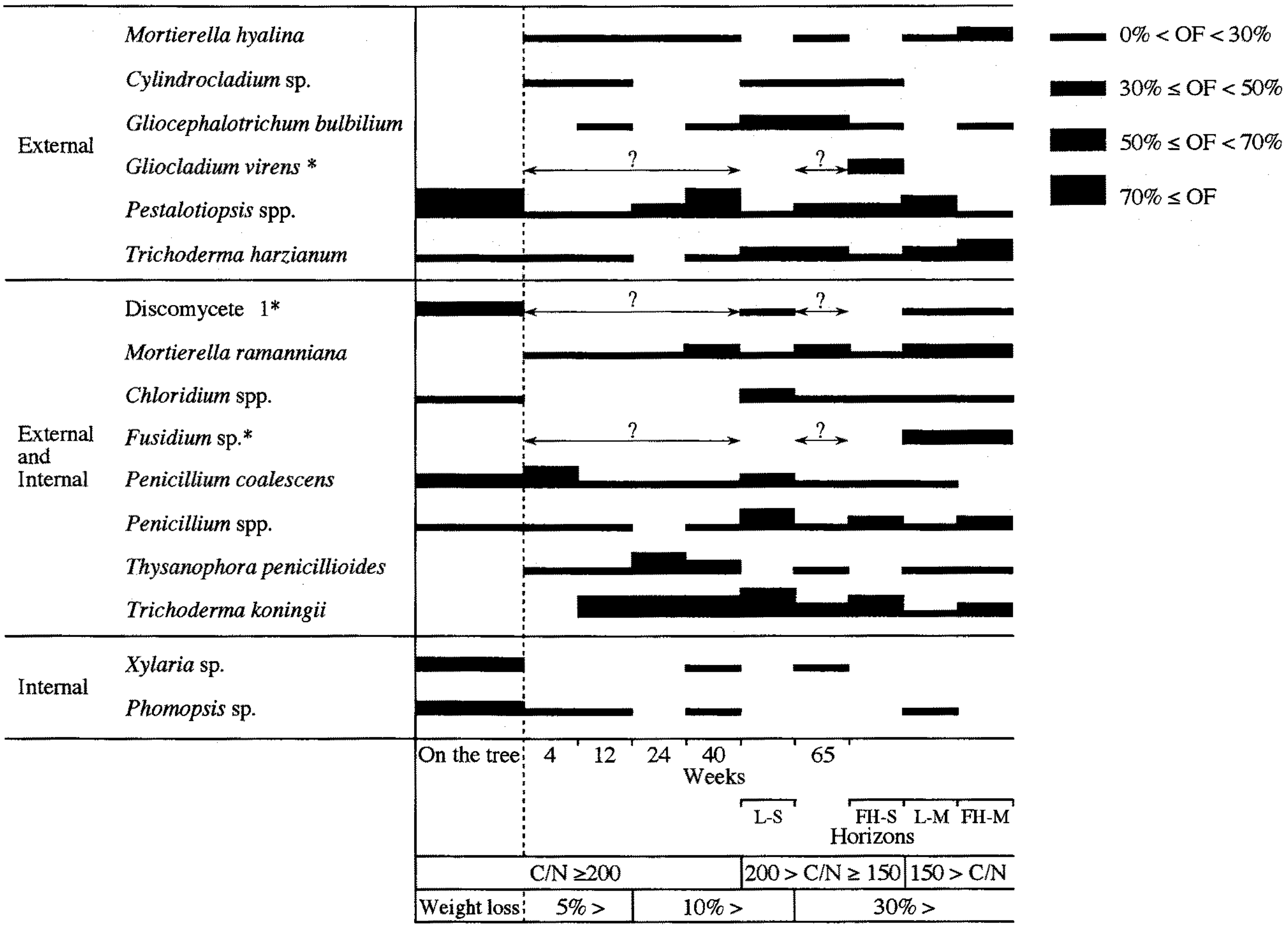

Fig. 7. Fungal succession on decaying pine cones in the *Pinus densiflora* forest.
Species with an OF of 30% or above at at least one sampling time are shown. *, Species found on the naturally fallen cones using the 1/2 CMA medium. External and internal species were determined by their average OFs showing more than two-fold differences between the WA and SS tests. Naturally fallen cones were positioned based on the C/N ratio. L-S, L horizon in Sept. 1993; FH-S FH horizon in Sept. 1993; L-M, L horizon in Mar. 1994; FH-M, FH horizon in Mar. 1994.

cies of *Pestalotiopsis* occurred in *Abies firma* needles in a wide range of the decaying process, from living needles to F_2-type black to grayish brown, collapsed and fragile needles with minute soil particles on the surface. However, higher OF of *Pestalotiopsis* was not reported from other coniferous needles. The dominance of this fungus seems to be the characteristic of the mycoflora of the cones. In the successional pattern of fungal flora during the decomposition processes of plant remains reported by Hudson (1968), the initial colonizers of decaying plant materials are weak parasites and also primary saprophytes. It is well known that *Pestalotiopsis* has parasitic ability (Suto and Kobayashi, 1993). In this study species belonging to this genus may be dominant first colonizers in decaying cones. Pine cones which remain on the branches after dispersal of seeds are exposed to severe environmental conditions, such as dryness and ultraviolet radiation. Some species of *Pestalotiopsis* appear to survive such conditions and also to flourish on the forest floor, coexisting with other species.

Cladosporium spp., including *C. cladosporioides* (Fres.) de Vries, which was also reported to be a primary saprophyte in pine needle litter (Hudson, 1968; Tokumasu, 1978), occurred mainly on the surface of the cones on the tree, but their OFs were not high. Similarly, *Aureobasidium pullulans* (de Bary) Arnaud, which is also included in the common primary saprophytes (Hudson, 1968), scarcely occurred in the present study. These phenomena might be related to the limitation of subsutrates such as sugars, which these fungi can utilize in natural conditions.

Xylaria sp., *Nodulisporium* sp., *Phomopsis* sp. and *Geniculosporium* sp. were internal initial colonizers on the cones on the tree. They occurred only when competitive epiphytic fungi were killed by sterilization, and they disappeared or only occasionally occurred on the cones on the forest floor, in contrast to *Pestalotiopsis* spp. They are probably not adapted to the environmental conditions (more humid, organic matter rich, etc.) of the forest floor, and their competitive ability is inferior to that of species which grow vigorously in the A_0 horizon. Some species of these four genera were reported to be almost ubiquitous endophytic fungi in woody plants (Petrini, 1986), including pine species (Carroll et al., 1977; Carroll and Carroll, 1978; Hata and Futai, 1993). However, *Nodulisporium* has been rarely reported from *Pinus*. The above four species might also be present in living cones.

Xylariaceous fungi were frequently reported to occur on woody materials (e.g., Chapela et al., 1988; Griffith and Boddy, 1990). Fungi belonging to this family, *Xylaria* sp. and *Nodulisporium* sp. (anamorph of xylariaceous fungi), were found on the cones. *Chloridium*, which is commonly isolated from advanced decaying wood (Gams and Holubovà-Jechovà, 1976), occurred with higher OFs in the cones in the later stage of decay. From these facts and the fact that the cones had the larger C/N ratio, it is suggested that the cones are more similar to wood than needles.

Soil fungi, *Mortierella* spp., *Penicillium* spp. (except for *P. coalescens*) and *Trichoderma* spp., occurred only on the forest floor, or their OFs gradually increased with the progress of decomposition. These fungi are also regarded as secondary saprophytes (Hudson, 1968). *Trichoderma koningii* increased markedly, showing that it has advantageous characteristics in competition with other fungi in pine cones in the A_0 horizon. *Penicillium coalescens* occurred with higher OFs in the relatively early stages of the decomposition.

Thysanophora penicillioides occurred with high OFs in the early stages of decomposition at site A. This tendency was not observed at site B. In the naturally fallen cones, this fungus occurred in both the L and FH horizons in March 1994, but not in September 1993. Tokumasu et al. (1994) mentioned that this species prefers the needle litter of fir species to that of pine species. This species occurred with relatively high frequencies in fir needles in Europe, especially in the relatively earlier stages of decay (Aoki et al., 1992; Sieber-Canavesti and Sieber, 1993). Aoki et al. (1990) also reported that *T. penicillioides* was isolated more frequently from the L-type needles of *A. firma* and seemed to prefer winter and spring seasons in Japan, although the frequency of occurrence was not high. The affinity of this fungus for lower temperature might be responsible for its higher occurrence at site A and abundance in March.

No particular consideration was paid to the time of starting the experiment using artificially placed cones, because 1) no seasonal fluctuation in cone fall was observed, and 2) little difference in fungal flora was found between September and March, suggesting that the time of beginning the experiment would not greatly affect the results. Only species which appears under particular environmental conditions, such as lower temperature, during a limited period, might be affected by the beginning time.

The occurrence patterns of fungi were different to some extent between MEA and 1/2 CMA. Fast-growing fungi spread over the surface of scales and medium in MEA. Tokumasu (1978) has pointed out that this may be one reason for the smaller number of species detected in nutrient-rich media. Also, species which could not sporulate in MEA were probably present. On the other hand, it is seems that *P. coalescens, Xylaria* sp., etc. found it difficult to sporulate or to form distinctive colonies in 1/2 CMA. So it is useful to use nutrient-rich media and nutrient-poor media simultaneously.

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