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FULL PAPER

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Colonization and lignin decomposition of *Camellia japonica* leaf litter by endophytic fungi

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Abstract Endophytic fungi occur on various types of leaf litter, but few studies have been done on their roles as saprophytes in decomposition. This study examined the succession of fungi in live, newly shed, and decomposing leaves at 2 months of decomposition of *Camellia japonica* and chemical changes in decomposing leaves colonized by endophytes. *Coccomyces nipponicum*, *Lophodermium* sp., *Geniculosporium* sp. 1, and *Colletotrichum gloeosporioides* were isolated from living leaves. *Coccomyces nipponicum* and *Lophodermium* sp. were also isolated frequently from newly shed and decomposing leaves. These two fungi caused a decrease of lignin content and bleaching in decomposing leaves under field and laboratory conditions. Total hyphal length in decomposing leaves was higher in bleached portions than in surrounding nonbleached portions, which probably reflected the early onset of hyphal growth of endophytes inside leaf tissue at leaf senescence or death. Incubation of newly shed leaves that were sterilized to exclude previously established endophytes resulted in no occurrence of bleached portions in decomposing leaves on the forest floor. This result indicated that these endophytes were incapable of colonizing leaves directly after litterfall and that the persistence of endophytes from live leaves was crucial for their colonization in decomposing leaves.

Key words Bleach · Decomposition · Endophyte · Lignin · Rhytismataceae

Introduction

Endophytes include those fungi that can colonize internal plant tissues at some time in their life without causing apparent harm to their host (Petrini 1991). These fungi have been studied intensively in terms of their ecological relationship with live leaves as parasites (Clay 1987; Andrews and Hirano 1991; Carroll 1995). In contrast, the ecology of endophytes on leaf litter has received little attention even though they often occur in dead leaves as saprophytes in the initial stages of decomposition (Hudson 1968; Müller et al. 2001; Kumaresan and Suryanarayanan 2002; Osono 2002; Osono et al. 2004). The growth of most endophytes depends on readily available compounds such as soluble sugars (Carroll and Petrini 1983; Sieber-Canavesi et al. 1991; Petrini et al. 1991, 1992), but xylariaceous endophytes are reported to possess the ability to decompose lignin and polymer carbohydrates such as cellulose. In *Fagus crenata* leaves, for example, the colonization of xylariaceous endophytes resulted in lignin decomposition and whitening, or bleaching, of leaf litter under both field and laboratory conditions (Osono 2002; Osono and Takeda 2001a, 2002). There have been few other reports on the role of endophytes in chemical changes of decomposing leaf litter, and further studies are necessary to clarify the ecology of endophytes as saprophytes on leaf litter (Andrews 1991).

This study examined (i) the succession of fungi in live, newly shed, and decomposing leaves, (ii) the fungal biomass and chemical changes in decomposing leaves colonized by endophytes under field and laboratory conditions, and (iii) the occurrence of these fungi in sterilized leaves. *Camellia japonica* L., an evergreen tree, was chosen for the study because endophytes possibly persisted in dead leaves from live leaves and took part in lignin decomposition in the initial stage of decomposition. Circumstantial evidence for this is that (i) *Coccomyces nipponicum* Nagao and an unidentified species of *Lophodermium* were responsible for lignin decomposition in bleached portions of fallen leaves (Fig. 1; Koide and Osono 2003) and (ii) species in *Coccomyces* and *Lophodermium* are known as endophytes of tree leaves (Cabral 1985; Osorio and Stephan 1991; Hata and Futai 1996; Okane et al. 1997). The occurrence of bleaching in sterilized leaves from which previously established endophytes were excluded was then compared with

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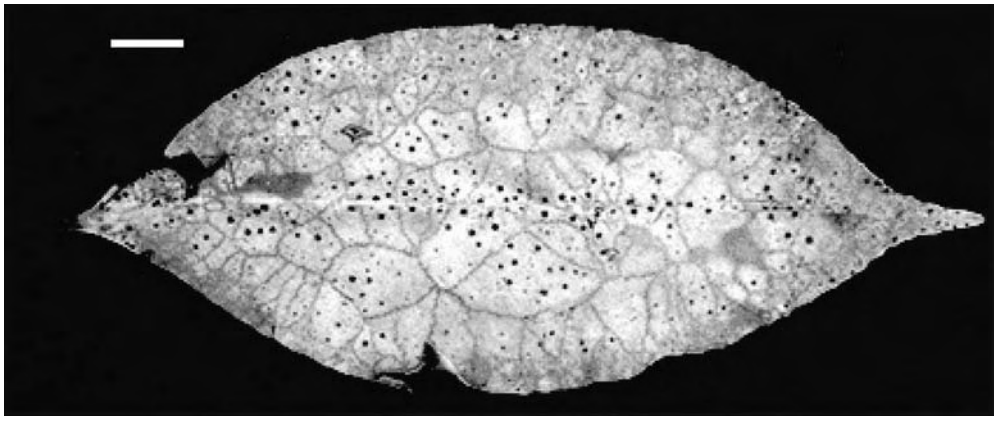


Fig. 1. A bleached leaf of litterfall of *Camellia japonica*. *Black dots* are fruiting bodies of rhytismataceous fungi. *Bar* 1 cm

nonsterilized leaves to test the ability of these endophytes to infect litter directly after litterfall.

Materials and methods

Study site

The study site was located in Oharano Forest Park of Kyoto City (34°57′N, 135°37′E), Kyoto, Japan. The mean annual temperature is 15.3°C and annual precipitation is 1581.1 mm. The study plot, $20 \times 10\,m^2$ in area, was laid out within a temperate secondary forest where *Quercus serrata* Murr., *C. japonica*, and *Pinus densiflora* Sieb. et Zucc. dominated in number and basal area. The plot was divided into ten subplots of $4 \times 5\,m^2$. There was no replication by plot.

Collection of leaves

A total of 30 live leaves of *C. japonica* were collected from six randomly selected trees within the study plot in November 2003. A total of 30 newly shed leaves were collected by means of traps of nylon netting at 1.0 to 1.5 m height in May 2003, a peak period of leaf fall of this tree species. These leaves were placed in paper bags and taken to the laboratory. One leaf disc was punched out from the edge of each single leaf with a sterile cork borer (5.5 mm in diameter). A total of 30 discs were prepared for each of live and newly shed leaves.

Litterbag experiments

Decomposition of *C. japonica* leaves was studied by means of a litterbag method (Osono and Takeda 2001b). The experiment was carried out twice.

In May 2002, newly shed leaves were collected from the forest floor in the study plot and taken back to the laboratory. The leaves were air-dried at room temperature (~15°C) for 1 week. The leaves (3 g) were enclosed in a litterbag ($15 \times 15\,cm^2$) made of polypropylene shade cloth with a mesh size of approximately 2 mm. A total of 20 bags were prepared. About 10 g of other shed leaves was used for the determination of oven-dry mass at 40°C and initial chemical composition. Litterbags were placed on the surface of the litter layer in late May, 2 bags per subplot. The bags were attached to the forest floor with metal pins to prevent movement and loss and to ensure good contact between the bags and the litter layer. The bags were retrieved in late July at 2 months after placement. The bags were put into paper bags and taken to the laboratory. Ten of 20 bags were used for fungal isolation and hyphal length estimation. Bleached portions were evident on the surface of leaves inside of these litterbags (denoted as decomposing leaves) (see Fig. 1). A total of 30 leaf discs were punched out with a sterile cork borer (5.5 mm in diameter) from bleached portions of decomposing leaves, and another 30 leaf discs were prepared from surrounding, nonbleached portions of the same leaves. Thus, a total of 60 discs were punched out and used for fungal isolation. The remaining parts of bleached and nonbleached portions were used for hyphal length estimation. Another 10 bags were used for the determination of oven-dry mass at 40°C, leaf area, and chemical composition.

In May 2003, newly shed leaves were collected and enclosed in litterbags according to the same procedure as the previous year. A total of 20 bags was prepared, and 10 of them were sterilized by exposure to ethylene oxide gas at 60°C for 3 h (denoted as sterilized leaves) and another 10 were not exposed to ethylene oxide (denoted as nonsterilized leaves). Litterbags were then placed on the forest floor in early June, one sterilized and one nonsterilized bag per subplot, according to the same procedure as the previous year. The bags were retrieved in early August at 2 months after placement. The bags were put into paper bags and taken to the laboratory. They were used for the determination of oven-dry mass at 40°C, leaf area, and chemical composition. Before the experiment, the sterilized leaves (1.0 g) were placed on 2% malt extract agar [MA; malt extract 2%, agar 2% (w/w)]. After 8 weeks of incubation at 20°C in darkness, no microbial colonies had developed on the plates. Thus, the effectiveness of the sterilization used in this study was confirmed.

Fungal isolation

Fungi were isolated from leaf discs with the surface sterilization method (Kinkel and Andrews 1988) according to Osono et al. (2004) but with several modifications. First, leaf disks were submerged in a solution of 15% hydrogen peroxide (v/v) for 30 s. Second, the surface-sterilized disks were plated on petri dishes (9 cm in diameter) containing 2% MA, one disc per plate. Third, the plates were incubated at 20°C in darkness and observed at 3 days and at 2 and 4 weeks after the surface sterilization. The plates were then incubated at room temperature under natural light condition for another 8 weeks. Any fungal hyphae or spores appearing on the plates were subcultured onto fresh MA plates, incubated, and identified. Preparations for fungal isolation were carried out within 6 h of sampling.

Coccomyces nipponicum and *Lophodermium* sp. were similar in their colony features and micromorphology on nutrient agar plates, so that they were difficult to distinguish based on microscopic observation of isolated strains. Thus, in the present study, these two species were not distinguished from each other and are referred to simply as rhytismataceous fungi.

Hyphal length estimation

Bleached and nonbleached portions of decomposing leaves in ten litterbags retrieved were cut separately and combined to make three sets for each portion. Hyphal lengths of these samples were estimated using the agar film method of Jones and Mollison (1948) but with several modifications (Osono et al. 2003b). The samples were processed within 24 h of sampling. Samples of 1.0 g or 2.5 g (fresh weight) were homogenized in a blender at 10000 rpm in 49.0 or 47.5 ml, respectively, of distilled water for 3 min. The suspension (20 ml) was diluted with 20 ml molten agar solution [final concentration, 1.5% (w/v)] and mixed at low speed on a magnetic stirring plate. Three agar films were prepared for each suspension in a haemocytometer (0.1 mm depth), transferred to glass slides, and dried for 24 h. The films were stained with fluorescent brightener (FB) for 1 h. FB binds to chitin in the fungal cell wall (West 1988) and enables visualization of all hyaline hyphae that are live or ghost (empty).

The stained films were mounted between slides and coverslips with one drop of immersion oil (type DF; Cargille Laboratories, Cedar Grove, NJ, USA) and examined with a Nikon Microphot-SA epifluorescent microscope equipped with a high-intensity mercury light source (Nikon, Tokyo, Japan). A Nikon UV-1A filter cube was used for examination of FB-stained hyphae. Darkly pigmented hyphae that were not stained with FB were observed by bright-field microscopy. Microscope fields were selected randomly, and 25 fields were observed for each slide at 1000× magnification. Hyphal lengths were estimated using an eyepiece grid and the grid intersection method (Olson 1950). Total hyphal length was calculated as the sum of FB-stained hyphal length and darkly pigmented hyphal length. Separate litter samples were dried at 40°C for 1 week to convert fresh weight to dry weight.

Leaf area measurement

The leaves in litterbags and the preserved initial leaves were pressed between board papers, oven-dried at 40°C for 1 week, and weighed. Mass loss of the leaves was determined as a percentage of the original mass. Leaves in litterbags were then photocopied and scanned with a photoscanner (EPSON GT-8000; Seiko Epson, Nagano, Japan). By the image analysis performed on a Macintosh computer using public domain NIH image software (written by U.S. National Institutes of Health), area of bleached portions and total leaf area were measured (Osono and Takeda 1998). Proportion of bleached area to total leaf area was expressed as a percentage, and mean values of the proportion were calculated. Bleached and nonbleached portions were then cut separately and combined to make one sample for each portion. The samples were ground in a laboratory mill to pass through a 0.5-mm screen and used for chemical analyses.

Chemical analyses

Lignin content in the samples was estimated by gravimetry according to a standardized method using hot sulfuric acid digestion (King and Heath 1967). Total carbohydrate content was estimated by the phenol-sulfuric acid method (Dubois et al. 1956). Soluble carbohydrates were extracted with 50% methanol (v/v), and their content was estimated by the phenol-sulfuric acid method. Holocellulose fraction was not determined by direct analysis but was calculated as a difference between total carbohydrate and soluble carbohydrates. The details of the methods are described in Osono and Takeda (2005).

Pure culture decomposition test

Six strains of four taxa (three strains of Rhytismataceae, one of coelomycete sp. 1, one of *Colletotrichum gloeosporioides*, and one of *Geniculosporium* sp. 1) isolated from *C. japonica* leaves were used for the pure culture decomposition test (Osono and Takeda 2002; Osono et al. 2003a). Newly shed leaves of *C. japonica* were collected from the forest floor of the study plot in May 2002 and oven-dried at 40°C for 1 week. The leaves were preserved in a vinyl bag until the experiment began.

Leaf discs including the primary vein were punched out with a cork borer (10 mm in diameter) and sterilized by exposure to ethylene oxide gas at 60°C for 3 h. Leaf discs (0.6 g) were placed on the surface of petri dishes (9 cm in diameter) containing 20 ml 2% agar. Inocula for each assessment were cut out of the margin of the previously inoculated petri dishes on 2% MA with a sterile cork borer (6 mm in diameter) and placed on the agar, one inoculum per plate. The plates were incubated for 8 weeks at 20°C in

darkness. The disks were then retrieved, oven-dried at 40°C for 1 week, and weighed. Initial litter was also sterilized, oven-dried at 40°C for 1 week, and weighed to determine the original mass. Mass loss of decomposed leaves was determined as a percentage of the original mass. Five plates were prepared for each strain, and five uninoculated plates served as a control. Chemical analyses were performed for strains that caused mass loss of more than 5.0%. The discs from five plates were combined to make one sample for each strain and used for analyses of lignin and holocellulose as described previously.

Statistical analysis

The frequency of a single species was calculated as the number of discs from which the species grew compared to the total number of discs tested in each leaf type, expressed as a percentage. A species was regarded as frequent when the frequency of the species was greater than or equal to 10%. Only the results of the frequent species are presented in this study. Fisher's exact probability test was used to evaluate the difference in frequencies between bleached and nonbleached portions of decomposing leaves. A paired *t* test was used to evaluate the difference in hyphal length between bleached and nonbleached portions of decomposing leaves.

Results

Frequency and biomass of fungi in leaves

Geniculosporium sp. 1, *Colletotrichum gloeosporioides*, and rhytismataceous fungi (*C. nipponicum* and *Lophodermium* sp.) were isolated from living leaves (Table 1). Rhytismataceous fungi were also isolated from newly shed leaves, but *Geniculosporium* sp. 1 and *C. gloeosporioides* decreased in frequency. When rhytismataceous fungi were isolated from bleached portions of decomposing leaves, their fruiting bodies were formed on the portions. However, these fungi were not isolated from nonbleached portions; *Geniculosporium* sp. 1 and *C. gloeosporioides* were not frequent in these portions. An unidentified coelomycete sp. 1, which had darkly pigmented hyphae, was the most frequent fungus in decomposing leaves. The frequency of coelomycete sp. 1 was significantly lower in bleached portions than in nonbleached portions.

Total hyphal length in decomposing leaves was significantly higher in bleached portions than in nonbleached portions (see Table 1). In contrast, the hyphal length of darkly pigmented hyphae and its proportion to total length were significantly lower in bleached portions than in nonbleached portions.

Chemical properties of leaves colonized by fungi

Mass loss of decomposing leaves was 23% in 2002; this loss was 29% in nonsterilized leaves and 31% in sterilized leaves in 2003 (Table 2). Mass loss was not significantly different between nonsterilized and sterilized leaves in 2003. Bleached portions accounted for 17% of total leaf area of decomposing leaves in 2002 and for 2% in nonsterilized leaves in 2003, but no bleached portions occurred in sterilized leaves. In 2002 and 2003, lignin content was lower in bleached portions than in nonbleached portions of nonsterilized leaves. In 2003, lignin content was higher and holocellulose content was lower in sterilized leaves than in nonsterilized leaves.

Mass loss of leaves colonized by fungi under pure culture condition ranged from 1.4% to 14.8% (Table 3). Three strains of Rhytismataceae caused the bleaching of leaves, decrease of lignin content, and increase of holocellulose content as compared to control leaves. In contrast, coelomycete sp. 1 caused increase of lignin con-tent and decrease of holocellulose content as compared to control leaves. *Colletotrichum gloeosporioides* and *Geniculo-*

Table 1. Frequency (%) of fungi and hyphal length (m/g dry litter) in live, newly shed, and bleached and nonbleached portions of decomposing leaves

	Live leaves	Newly shed leaves	Decomposing leaves		
			Bleached	Nonbleached	*P*
Frequency of fungi					
Rhytismataceae	7	20	13	0	**
Geniculosporium sp. 1	23	3	7	10	ns
Colletotrichum gloeosporioides	13	0	0	3	ns
Coelomycete sp. 1	0	0	40	67	*
Hyphal length					
Total hyphae	nd	nd	6786 ± 378	3875 ± 432	***
Darkly-pigmented hyphae	nd	nd	213 ± 52	697 ± 113	**
Proportion (%) of darkly pigmented hyphae to total length	nd	nd	3 ± 1	18 ± 1	*

Values indicate means ± SE ($n = 3$)
Fisher's exact probability test and paired *t* test were used to evaluate the difference in frequencies and hyphal lengths, respectively, between bleached and nonbleached portions
*** $P < 0.001$; * $P < 0.05$; ** $P < 0.10$; ns, nonsignificant; nd, not determined

Table 2. Mass loss (% original mass), proportion (%) of bleached area to total leaf area, and lignin and holocellulose contents (mg/g dry litter) in bleached and nonbleached portions of decomposing leaves

Type of leaves	Time (months)	Mass loss	Proportion of bleached area	Lignin		Holocellulose	
				Bleached	Nonbleached	Bleached	Nonbleached
2002							
Initial	0	0.0 ± 0.0	0.0 ± 0.0	nd	212	nd	191
Nonsterilized	2	22.9 ± 0.9	16.8 ± 1.8	221	270	296	265
2003							
Initial	0	0.0 ± 0.0	0.0 ± 0.0	nd	212	nd	170
Nonsterilized	2	29.3 ± 0.8	1.6 ± 0.7	194	229	166	174
Sterilized	2	31.2 ± 1.5^{ns}	0.0 ± 0.0	nd	313	nd	143

Values indicate means ± SE (n = 10)
Paired t test was used to evaluate the difference in mass loss between nonsterilized and sterilized leaves

Table 3. Mass loss (% original mass) and lignin and holocellulose contents (mg/g dry litter) of uninoculated (control) and decomposed leaves by fungus under pure culture condition

Fungus	Mass loss	Lignin	Holocellulose
Control, uninoculated	0.0 ± 0.0	261	177
Rhytismataceae[a]	14.8 ± 0.0	227	193
Rhytismataceae[a]	8.6 ± 0.0	233	186
Rhytismataceae[a]	7.1 ± 0.0	248	186
Coelomycete sp. 1	9.5 ± 0.0	330	153
Colletotrichum gloeosporioides	1.9 ± 0.0	nd	nd
Geniculosporium sp. 1	1.4 ± 0.0	nd	nd

Values indicate means ± SE (n = 5)
[a]Three strains of rhytismataceous fungi (*Coccomyces nipponicum* or *Lophodermium* sp.) were used in the test

sporium sp. 1 caused negligible mass loss as compared to control leaves.

Discussion

Occurrence of endophytes in decomposing leaves

Two rhytismataceous fungi (*C. nipponicum*, *Lophodermium* sp.), *Geniculosporium* sp. 1, and *C. gloeosporioides* are endophytic fungi of *C. japonica* leaves. Species in *Coccomyces*, *Lophodermium*, *Geniculosporium*, and *Colletotrichum* have been reported as foliar endophytes of other temperate trees (Cabral 1985; Boddy and Griffith 1989; Legault et al. 1989; Osorio and Stephan 1991; Hata and Futai 1996; Okane et al. 1997; Osono 2002; Osono et al. 2004). The rhytismataceous fungi occurred frequently in newly shed and decomposing leaves. Koide and Osono (2003) reported previously that these fungi occurred in fallen leaves and were responsible for the bleaching. Frequency of *Geniculosporium* sp. 1 was low in decomposing leaves, but Koide et al. (2005) indicated that this fungus increased its frequency in the later stages of decomposition. Such a temporal decrease of *Geniculosporium* in the initial stages was also reported in *Fagus crenata* leaves (Osono 2002) and was likely attributable to competition with other species for readily available energy sources such as soluble carbohydrates that were abundant in the mesophyll of newly shed leaves. This result, that *C. gloeosporioides* disappeared at leaf death, was also reported in *Swida controversa* leaves (Osono et al. 2004) and suggests that the fungus is a parasite of live leaves.

The greater total hyphal length in the bleached portion of decomposing leaves probably reflected the early onset of ingrowth of rhytismataceous fungi inside the leaf tissue at leaf senescence or death. A similar situation had been already found for *Rhabdocline parkeri*, an endophyte latent in healthy-looking needles of *Pseudotsuga mensiesii*, which started hyphal growth as soon as senescence of leaves began (Stone 1987). These endophytes seem to be successful in colonization of dead tissues before fungi that colonize after litterfall. In fact, the darkly pigmented hyphae of coelomycete sp. 1 were reduced in bleached portions as compared to nonbleached portions, suggesting that its colonization of bleached portions was eliminated by the prior colonization by endophytes.

The observation that bleached portions did not occur on sterilized, endophyte-excluded leaves indicated that the endophytes causing bleach, probably the rhytismataceous fungi, were incapable of colonizing leaves directly after litterfall and that persistence from live leaves was crucial for colonization. Similarly, Osono (2002) reported that *Ascochyta* sp., endophytic on *F. crenata* leaves, failed to infect the inside of sterilized freshly fallen leaves on the forest floor. One possible explanation for the disappearance of these fungi in sterilized leaves seems to be found in their life history characteristics, which were typically demonstrated in *Lophodermium piceae,* an endophyte in *Picea abies* needles (Osorio and Stephan 1991). This fungus invades latently inside healthy needles, produces ascospores soon after leaf death, and releases ascospores to infect live needles again.

Role of endophytes in chemical changes

The field and laboratory experiments showed that rhytismataceous fungi caused the decrease of lignin content and bleaching of leaves. Xylariaceous endophytes have been found to be the only group of foliar endophytes that are ligninolytic and are able to decompose the lignocellulose matrix in leaves (Carroll and Petrini 1983; Osono and

Takeda 2001a,b, 2002). The present study is thus the first to demonstrate that rhytismataceous endophytes also have ligninolytic activity and, acting as pioneer decomposers, take part in delignification in the initial stage of decomposition. On the other hand, most foliar endophytes have limited ability to decompose lignocellulose and depend on readily available carbohydrates (Carroll and Petrini 1983; Sieber-Canavesi et al. 1991; Petrini et al. 1991, 1992). *Colletotrichum gloeosporioides* and *Geniculosporium* sp. 1 can be included in this group. *Geniculosporium* species also showed a limited ability to decompose larch needle litter (Osono et al. 2003a) but had ligninolytic activity in beech leaf litter (Osono and Takeda 2002), suggesting that the decomposing ability of this species depends on litter types used as substrata.

There have been few reports of such selective loss of lignin in the initial stages of leaf litter decomposition in temperate forests (Berg and McClaugherty 2003; Osono and Takeda 2005). In most litter types, conversely, polymer carbohydrates such as cellulose were selectively removed in the initial stages, as was shown for nonbleached portions of *C. japonica* leaves. As far as we know, the occurrence of bleached portions within 2 months of decomposition is the earliest record. For example, xylariaceous fungi bleached *F. crenata* leaves in at least 6 months of decomposition (Osono and Takeda 2001a; Osono 2002). Saito (1965) and Hintikka (1970) reported bleaching caused by Basidiomycetes on forest floor materials beneath the litter layer, i.e., in later stages. The delignification caused by rhytismataceous fungi in the initial stage of decomposition thus provides a unique situation for subsequent decomposition processes of *C. japonica* leaves. Their colonization produced the within-leaf heterogeneity of chemical properties in leaf litter, which may alter the pattern of substrate utilization by succeeding fungi (Osono 2003) and decomposition processes. Further studies are thus needed to follow long-term patterns of decomposition and fungal succession in bleached and nonbleached portions of *C. japonica* leaves.

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References

Andrews JH (1991) Future research directions in phyllosphere ecology. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. Springer, New York, pp 467–479

Andrews JH, Hirano SS (eds) (1991) Microbial ecology of leaves. Springer, New York

Berg B, McClaugherty C (2003) Plant litter, decomposition, humus formation, carbon sequestration. Springer, Berlin

Boddy L, Griffith GS (1989) Role of endophytes and latent invasion in the development of decay communities in sapwood of angiospermous trees. Sydowia 41:41–73

Cabral D (1985) Phyllosphere of *Eucalyptus viminalis*: dynamics of fungal populations. Trans Br Mycol Soc 85:501–511

Carroll G (1995) Forest endophytes: pattern and process. Can J Bot 73(suppl 1):S1316–S1324

Carroll GC, Petrini O (1983) Patterns of substrate utilization by some fungal endophytes from coniferous foliage. Mycologia 75:53–63

Clay K (1987) The effect of fungi on the interaction between host plants and their herbivores. Can J Plant Pathol 9:380–388

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Hata K, Futai K (1996) Variation in fungal endophyte populations in needles of the genus *Pinus*. Can J Bot 74:103–114

Hintikka V (1970) Studies on white-rot humus formed by higher fungi in forest soils. Commun Inst For Fenn 69:1–68

Hudson HJ (1968) The ecology of fungi on plant remains above the soil. New Phytol 67:837–874

Jones PCT, Mollison JE (1948) A technique for the quantitative estimation of soil microorganisms. J Gen Microbiol 2:54–69

King HGC, Heath GW (1967) The chemical analysis of small samples of leaf material and the relationship between the disappearance and composition of leaves. Pedobiologia 7:192–197

Kinkel LL, Andrews JH (1988) Disinfestation of living leaves by hydrogen peroxide. Trans Br Mycol Soc 91:523–528

Koide K, Osono T (2003) Chemical composition and mycobiota of bleached portion of *Camellia japonica* leaf litter at two stands with the different nitrogen status. J Jpn For Soc 85:359–363

Koide K, Osono T, Takeda H (2005) Fungal succession and decomposition of *Camellia japonica* leaf litter. Ecol Res 20:in press

Kumaresan V, Suryanarayanan TS (2002) Endophyte assemblages in young, mature and senescent leaves of *Rhizophora apiculata*: evidence for the role of endophytes in mangrove litter degradation. Fungal Divers 9:81–91

Legault D, Dessureault M, Laflamme G (1989) Mycoflore des aiguilles de *Pinus banksiana* et *Pinus resinosa*. I. Campignons endophytes. Can J Bot 67:2052–2060

Müller MM, Valjakka R, Suokko A, Hantula J (2001) Diversity of endophytic fungi of single Norway spruce needles and their role as pioneer decomposers. Mol Ecol 10:1801–1810

Okane I, Nakagiri A, Ito T (1997) Preliminary study of endophytic fungi in evergreen plants from Ishigaki and Iriomote islands. IFO Res Commun 18:45–51

Olson FCW (1950) Quantitative estimates of filamentous algae. Trans Am Microsc Soc 69:272–279

Osono T (2002) Phyllosphere fungi on leaf litter of *Fagus crenata*: occurrence, colonization, and succession. Can J Bot 80:460–469

Osono T (2003) Effects of prior decomposition of beech leaf litter by phyllosphere fungi on substrate utilization by fungal decomposers. Mycoscience 44:41–45

Osono T, Takeda H (1998) Fragmentation process of leaf litter in Mull-type organic soil. J Jpn For Soc 80:196–200

Osono T, Takeda H (2001a) Effects of organic chemical quality and mineral nitrogen addition on lignin and holocellulose decomposition of beech leaf litter by *Xylaria* sp. Eur J Soil Biol 37:17–23

Osono T, Takeda H (2001b) Organic chemical and nutrient dynamics in decomposing beech leaf litter in relation to fungal ingrowth and succession during three year decomposition processes in a cool temperate deciduous forest in Japan. Ecol Res 16:649–670

Osono T, Takeda H (2002) Comparison of litter decomposing ability among diverse fungi in a cool temperate deciduous forest in Japan. Mycologia 94:421–427

Osono T, Takeda H (2005) Decomposition of lignin, holocellulose, polyphenol and soluble carbohydrate in leaf litter of 14 tree species in a cool temperate forest. Ecol Res 20:41–49

Osono T, Fukasawa Y, Takeda H (2003a) Roles of diverse fungi in larch needle-litter decomposition. Mycologia 95:820–826

Osono T, Ono Y, Takeda H (2003b) Fungal ingrowth on forest floor and decomposing needle litter of *Chamaecyparis obtusa* in relation to resource availability and moisture condition. Soil Biol Biochem 35:1423–1431

Osono T, Bhatta BK, Takeda H (2004) Phyllosphere fungi on living and decomposing leaves of giant dogwood. Mycoscience 45:35–41

Osorio M, Stephan BR (1991) Life cycle of *Lophodermium piceae* in Norway spruce needles. Eur J For Pathol 21:152–163

Petrini O (1991) Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. Springer, New York, pp 179–197

Petrini LE, Petrini O, Leuchtmann A, Carroll GC (1991) Conifer inhabiting species of *Phyllosticta*. Sydowia 43:148–169

Petrini O, Sieber TN, Toti L, Viret O (1992) Ecology, metabolite production, and substrate utilization in endophytic fungi. Nat Toxins 1:185–196

Saito T (1965) Coaction between litter-decomposing hymenomycetes and their associated microorganisms during decomposition of beech litter. Sci Rep Tohoku Univ Ser IV (Biol) 31:255–273

Sieber-Canavesi F, Petrini O, Sieber TN (1991) Endophytic *Leptostroma* species on *Picea abies*, *Abies alba*, and *Abies balsamea*: a cultural, biochemical, and numerical study. Mycologia 83:89–96

Stone JK (1987) Initiation and development of latent infections by *Rhabdocline parkeri* on Douglas-fir. Can J Bot 65:2614–2621

West AW (1988) Specimen preparation, stain type, and extraction and observation procedures as factors in the estimation of soil mycelial lengths and volumes by light microscopy. Biol Fertil Soils 7:88–94