

FULL PAPER

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## Phyllosphere fungi on living and decomposing leaves of giant dogwood

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**Abstract** Phyllosphere fungi on living leaves and their succession on decomposing leaves were studied on giant dogwood (*Swida controversa*). A total of 12 and 34 fungal species were isolated from the interior and surface, respectively, of living leaves, and 15 frequent species were considered as phyllosphere fungi. Six of these 15 species were also frequent on decomposing litter. Characteristic successional trends were observed in the 6 phyllosphere fungi during decomposition. The sum of frequencies of endophytes decreased as decomposition progressed, and no endophytes were isolated from the litter at the 11th month of decomposition. The sum of frequencies of epiphytes increased as decomposition progressed. Endophytes and epiphytes showed different responses to litter mass loss and concentrations of nitrogen, lignin, and total carbohydrates during the decomposition process. These results suggested that epiphytes may survive on decomposing leaves as primary decomposers on the ground, thereby excluding endophytes by competition for available energy sources, and that epiphytes may have a greater contribution to decomposition than endophytes in dogwood leaves.

**Key words** Decomposition · Dogwood · Endophyte · Epiphyte · Succession

### Introduction

The phyllosphere, the living leaf as a whole, includes both the interior and the surface (Carroll et al. 1977) and is colonized by a variety of microorganisms (Fokkema and van den Heuvel 1986; Andrews and Hirano 1991).

Phyllosphere fungi include endophytes and epiphytes that colonize the interior and surface of the phyllosphere, respectively, thereby occupying two distinct habitats of the leaf (Petrini 1991). Phyllosphere fungi occur not only on living leaves but also on decomposing leaves at the initial stages of decomposition, and their succession during decomposition has been demonstrated in several litter types (Hudson 1968). The ecology of phyllosphere fungi on decomposing leaves should be studied to evaluate the role of phyllosphere fungi in energy flow and nutrient dynamics in ecosystems (Andrews 1991).

Several papers have reported the ecology of phyllosphere fungi in litter decomposition of Japanese beech (*Fagus crenata* Blume); these included the colonization of litter and the succession of phyllosphere fungi (Osono and Takeda 2001; Osono 2002), their litter decay potentialities (Osono and Takeda 2002), and the effect of prior decomposition of litter by phyllosphere fungi on substrate utilization by fungal decomposers (Osono 2003). These studies indicated that the changes in chemical properties of decomposing litter influenced the succession of phyllosphere fungi, and that the survival of phyllosphere fungi until the latter stages of decomposition was related to their ability to use structural lignocellulose in beech litter. Further studies are needed to examine the succession of phyllosphere fungi on other litter types that have different chemical composition and decay patterns from beech litter.

In contrast to beech litter, litter of giant dogwood *Swida controversa* (Hemsley) Sojak (Cornaceae) was characterized by a low lignin content, rapid mass losses, and the net release of nitrogen without net immobilization into the litter (Osono and Takeda, unpublished data). It is hypothesized that the successional patterns of phyllosphere fungi on dogwood litter were different from those of beech litter due to the difference in chemical property and litter decomposition processes. Thus, we investigated (i) the phyllosphere fungi on living leaves of dogwood and (ii) succession of phyllosphere fungi in the interior of decomposing leaves to examine the successional patterns of phyllosphere fungi in decomposing leaves of dogwood.

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## Materials and methods

### Study area

Samples were collected in a cool temperate deciduous forest dominated by *F. crenata* in Ashiu Experimental Forest of Kyoto University (35°18' N and 135°43' E) about 40 km north of Kyoto, Japan. Details of the study site are described in Osono and Takeda (2001).

### Collection of living leaves

A total of 80 living leaves of giant dogwood (*S. controversa*) were collected at the study site at monthly intervals from May to December 1999 (10 leaves each month). On each sampling occasion, the leaves were cut from two branches at about 8 m height from each of two randomly selected trees (5 leaves from each tree). The leaves were placed in paper bags and taken to the laboratory.

Two leaf disks were punched from each single leaf from the central part of leaves, avoiding the primary vein, with a sterile cork borer (5.5 mm in diameter). One disk was used for a surface sterilization method and the other for a washing method (described below). Fungal isolation was carried out within 6 h of sampling.

### Litter bag method

Decomposition of dogwood leaf litter was studied by the litter bag method (Crossley and Hoglund 1962). Freshly abscised leaves were collected in November 2000 by shaking the trees over traps of nylon netting. Leaves were oven-dried at 40°C for 1 week. The litter (2 g) was enclosed in a litter bag (15 × 15 cm) made of polypropylene shade cloth with a mesh size of approximately 2 mm. Initial samples (10 g) were preserved for chemical analyses.

A study plot 20 × 8 m in area was laid out in the study site and divided into ten subplots 4 × 4 m for the experiment. There was no replication by site. The decomposition study covered a 11-month period from December 2000 to November 2001. Litter bags were placed on the litter layer in each of ten subplots in December 2000 (four bags per subplot). Litter bags were attached to the forest floor with metal pins to prevent movement or loss and to ensure good contact between the bags and the litter layer. Sampling of the bags took place four times, at 5 (May), 7 (July), 9 (September), and 11 months (November 2001) after the placement. On each sampling occasion, a total of ten bags were collected from ten subplots (one bag per subplot). The bags were placed in paper bags and taken to the laboratory.

Foreign plant remains attached to the outside of the bags were carefully removed with forceps. Two litter pieces (approximately 5 × 5 mm) of dogwood were taken from each bag, weighed, and used for surface sterilization method (described below). Fungal isolation was carried out within 24 h of sampling. The remaining litter was weighed and used for mass determination and chemical analyses.

### Fungal isolation

For the isolation of fungi, the surface sterilization method (Kinkel and Andrews 1988) and modified washing method (Harley and Waid 1955) were used according to Osono (2002).

For surface sterilization, leaf disks were submerged in 70% ethanol (v/v) for 1 min to wet the surface, then surface sterilized for 1 min (living leaves) or 15 s (decomposing leaves) in a solution of 15% hydrogen peroxide (v/v), and then submerged again for 1 min in 70% ethanol. The disks were rinsed with sterile, distilled water, transferred to sterile filter paper in Petri dishes (9 cm in diameter), and dried for 24 h to suppress vigorous bacterial growth after plating (Widden and Parkinson 1973). The surface-sterilized disks were then placed on 9-cm Petri dishes containing LCA (Miura and Kudo 1970), one disk per plate. LCA contains glucose 0.1%, KH<sub>2</sub>PO<sub>4</sub> 0.1%, MgSO<sub>4</sub> · 7H<sub>2</sub>O 0.02%, KCl 0.02%, NaNO<sub>3</sub> 0.2%, yeast extract 0.02%, and agar 1.3% (w/v). LCA was used because its low glucose content suppresses overgrowth of fast-growing species and because LCA effectively induces sporulation and is useful for identification (Osono and Takeda 1999).

For modified washing, leaf disks were washed in a sterile test tube using a vertical shaker for 1.5 min to isolate fungi growing actively on the surface. The disks were washed serially in two (living leaves) or five (decomposing leaves) changes of 0.005% Aerosol-OT (di-2-ethylhexyl sodium sulfosuccinate) solution (w/v) and rinsed with sterile distilled water four times (living leaves) or five times (decomposing leaves). The washed disks were treated in the same manner as used in the plating-out procedure of the surface-sterilized leaves.

Plates were incubated at 20°C in darkness and observed at 3 days and at 2, 4, and 8 weeks after sterilization or washing (Osono and Takeda 1999). Any fungal hyphae or spores appearing on the plates were subcultured onto fresh LCA plates, incubated, and identified.

### Chemical analyses

Mass losses of litter in the litter bags were determined after drying samples to a constant weight at 40°C taking mass of leaf pieces used for fungal isolation into account. Mean values of mass loss were calculated for each sampling. The samples from ten replicates were then combined, ground in a laboratory mill to pass a 0.5-mm screen, and used for chemical analyses. Total nitrogen content was measured by automatic gas chromatography (NC analyzer Sumigraph NC-900; Sumitomo, Osaka, Japan).

The amount of lignin in the samples was estimated by gravimetry according to a standardized method using hot sulfuric acid digestion (King and Heath 1967). Each sample was extracted with alcohol-benzene at room temperature and the residue treated with 72% sulfuric acid (v/v) for 2 h at room temperature with occasional stirring. The mixture was then diluted with distilled water to make 2.5% sulfuric acid solution and autoclaved at 120°C for 60 min. After

cooling, the residue was filtered and washed with water through a porous crucible (G4), dried at 105°C, and weighed as acid-insoluble residue. The filtrate (autoclaved sulfuric acid solution) was used for total carbohydrate analysis as described next.

Total carbohydrate content was estimated by the phenol-sulfuric acid method (Dubois et al. 1956) according to Fukui (1969). The sulfuric acid solution derived from the lignin analysis was used for the total carbohydrate analysis. Five percent phenol (v/v) and 98% sulfuric acid (v/v) were added to the solution. The optical density of the solution was then measured by a spectrophotometer at 490nm using known concentrations of D-glucose as standards.

### Definitions and statistical analyses

The frequency of each species was calculated as a percentage of the number of disks from which the species grew compared to the total number of disks tested in each living or decomposing leaf. When the frequency of a species on any leaf type was significantly higher ( $P < 0.05$ ) than zero by Fisher's exact probability test, the species was regarded as frequent. When the correlation coefficient was determined for the linear relationship between the frequency of fungi and litter mass and concentrations of nitrogen, lignin, and total carbohydrates, the arcsin transformation was used for the frequency of fungi because the data were in the form of proportions.

In this study, "phyllosphere" denotes the interior and surface of living leaves. Fungi isolated from living leaves were categorized into endophytes, epiphytes, and infrequent species. Endophytes were frequent species isolated from the phyllosphere by the surface sterilization method. Epiphytes were frequent species isolated from the phyllosphere by the washing method. Fungi isolated from decomposing leaves were categorized into endophytes, epiphytes, litter inhabitants, and infrequent species. Litter inhabitants were frequent species isolated from decomposing leaves. The relative frequency of endophytes, epiphytes, litter inhabitants, and infrequent species was calculated as a percentage of the sum of frequencies of fungi in the category to the total frequency of all fungi in each leaf type.

### Results

#### Phyllosphere fungi and their occurrence on decomposing leaves

Twelve fungal species were isolated from the interior and 34 from the surface of the phyllosphere of dogwood (Appendix A). Fifteen species were regarded as phyllosphere fungi (Table 1). Five species frequent in the interior were regarded as endophytes and 10 species frequent on the surface were regarded as epiphytes. These phyllosphere fungi were divided into two groups according

**Table 1.** Frequency (%) of phyllosphere fungi on leaves of *Swida controversa*

Fungus	Group	Living		Litter				
		Surface	Interior	Interior				
		Total (80)	Total (80)	Total (80)	May (20)	July (20)	Sept. (20)	Nov. (20)
<b>Endophytes</b>								
<i>Xylaria</i> sp. (anamorph)	I	0	9	15	40	10	10	0
<i>Phomopsis</i> sp.	II	3	14	1	5	0	0	0
<i>Colletotrichum gloeosporioides</i>	II	16	41	0	0	0	0	0
Ascomycete sp. 1	II	4	15	0	0	0	0	0
Coelomycete sp. 1	II	3	8	0	0	0	0	0
<b>Epiphytes</b>								
<i>Phoma</i> sp. 1	I	75	8	20	10	25	30	15
<i>Cladosporium cladosporioides</i>	I	59	0	20	5	20	20	35
<i>Pestalotiopsis</i> sp. 1	I	36	0	14	0	0	0	55
<i>Alternaria alternata</i>	I	19	0	8	5	15	5	5
<i>Clonostachys rosea</i>	I	13	0	6	0	0	10	15
<i>Trichoderma viride</i>	II	18	0	5	0	5	5	10
<i>Epicoccum nigrum</i>	II	9	0	3	5	5	0	0
<i>Pestalotiopsis</i> sp. 3	II	33	0	0	0	0	0	0
Coelomycete sp. 2	II	16	0	0	0	0	0	0
<i>Pestalotiopsis</i> sp. 2	II	10	0	0	0	0	0	0
<b>Litter inhabitants</b>								
<i>Arthrinium</i> sp.		3	3	29	0	15	65	35
<i>Trichoderma polysporum</i>		0	0	11	0	20	5	20
<i>Trichoderma koningii</i>		0	0	10	0	5	5	30
<i>Geniculosporium</i> sp. 1		1	0	8	15	15	0	0
<i>Chaetomium globosum</i>		0	0	8	5	5	5	15

Phyllosphere fungi were divided into two groups according to the frequency on decomposing leaves (see text) Numbers in parentheses indicate the number of leaf disks examined

to their frequency on decomposing leaves. Group I included 6 species (*Xylaria* sp. (anamorph), *Phoma* sp. 1, *Cladosporium cladosporioides*, *Pestalotiopsis* sp. 1, *Alternaria alternata*, and *Clonostachys rosea*) that were frequent on the decomposing leaves; group II included the other 9 species that were not frequent on the decomposing leaves.

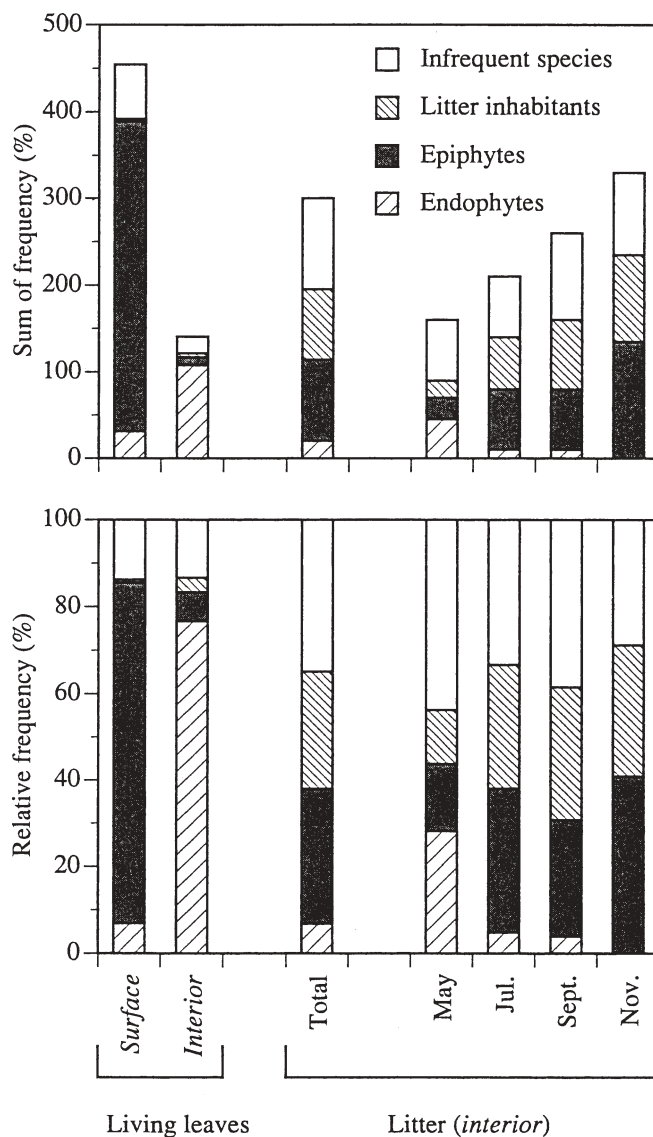
#### Succession of fungi on decomposing leaves

Forty-seven fungal species were isolated from the interior of decomposing leaves (see Appendix A). Characteristic successional trends were observed in phyllosphere fungi in Group I and litter inhabitants during decomposition (see Table 1). The frequency of *Xylaria* sp. (anamorph) decreased as decomposition progressed. The frequencies of *Phoma* sp. 1 and *A. alternata* increased initially and then decreased. The frequencies of *C. cladosporioides*, *Pestalotiopsis* sp. 1, and *C. rosea* increased as decomposition progressed. *Arthrinium* sp. (anamorph of *Apiospora montagnei*), *Trichoderma polysporum*, *T. koningii*, *Geniculosporium* sp. 1, and *Chaetomium globosum* were frequent species on decomposing leaves classified as litter inhabitants. The frequency of *Geniculosporium* sp. 1 decreased as decomposition progressed, that of *Arthrinium* sp. increased initially and then decreased, that of *T. polysporum* was variable, and those of *T. koningii* and *C. globosum* increased as decomposition progressed.

The sum of frequencies and the relative frequency of endophytes, epiphytes, litter inhabitants, and infrequent species are shown in Fig. 1. Endophytes as a group accounted for 7%, 77%, and 7% of the total frequency in the surface of living leaves, the interior of living leaves, and the interior of decomposing leaves, respectively. The sum of frequencies and the relative frequency of endophytes decreased as decomposition progressed. In November, no endophytes were isolated from the decomposing leaves. Epiphytes as a group accounted for 79%, 7%, and 31% of the total frequency in the surface of living leaves, the interior of living leaves, and the interior of decomposing leaves, respectively. The sum of frequencies and the relative frequency of epiphytes increased as decomposition progressed. In November, epiphytes accounted for 41% of all fungi in the decomposing leaves. Litter inhabitants as a group accounted for 27% of the total frequency in the interior of decomposing leaves. The sum of frequencies of litter inhabitants increased as decomposition progressed, and their relative frequency increased from May to July and was relatively constant at about 30% thereafter.

#### Correlation of fungi with litter decomposition

The changes in the remaining mass of dogwood litter and concentrations of nitrogen, lignin, and total carbohydrates are shown in Fig. 2. About 6% of the original litter mass remained at the end of the study period. The decay constant as Olson's  $k$  (Olson 1963) was 1.54 ( $n = 5$ ,  $R^2 = 0.92$ ). Concentrations of nitrogen and lignin increased whereas the concentration of total carbohydrates decreased as de-



**Fig. 1.** Sum of frequencies (*upper*) and relative frequencies (*lower*) of endophytes, epiphytes, litter inhabitants, and infrequent species in the interior and on the surface of living and decomposing leaves of *Swida controversa*

composition progressed. The mass of nitrogen, lignin, and total carbohydrates decreased as the decomposition progressed. About 6%, 2%, and 7% of the original mass of nitrogen, lignin, and total carbohydrates remained at the end of the study period.

Endophytes and epiphytes showed different responses to litter decomposition (Table 2). The frequency of the endophytic fungus *Xylaria* sp. (anamorph) was significantly and positively correlated to litter mass. The frequency of some epiphytes was significantly and negatively correlated to litter mass (*C. cladosporioides* and *C. rosea*) or positively correlated to lignin concentration (*Phoma* sp. 1) or to total carbohydrate concentrations (*A. alternata*). The frequency of *Pestalotiopsis* sp. 1 was not significantly correlated to the changes in litter mass and litter quality during decomposition. The frequencies of *Arthrinium* sp. and *T. koningii* were

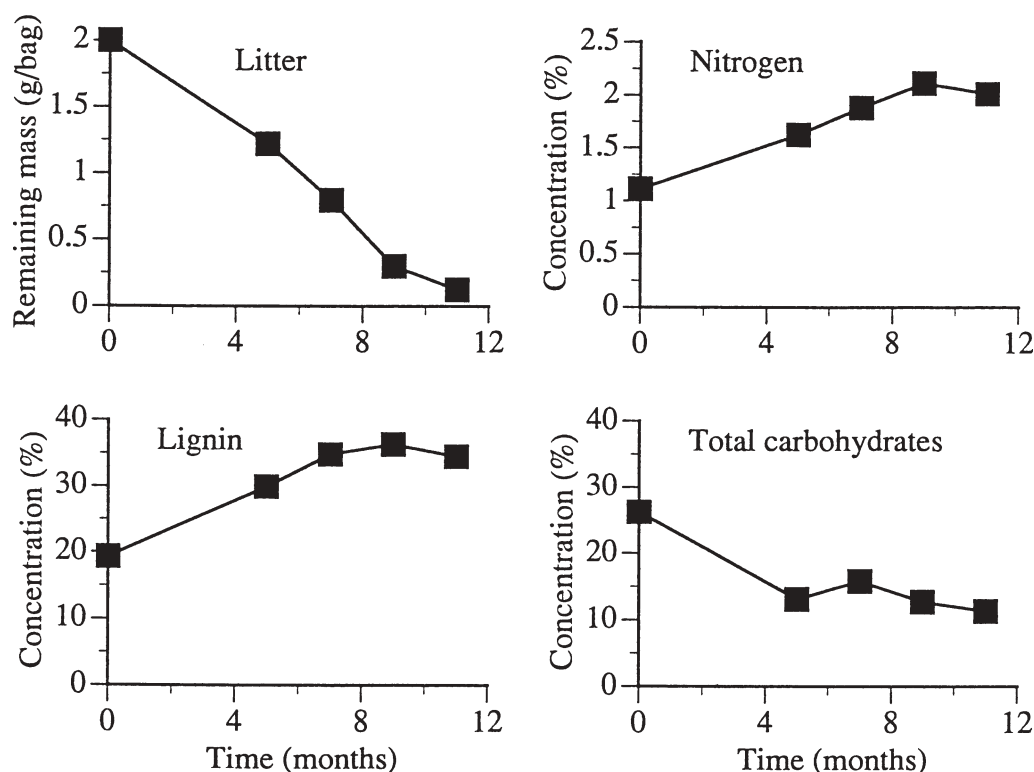
**Table 2.** Correlation coefficients for linear relationship between frequency of fungi and litter mass and concentrations of nitrogen, lignin, and total carbohydrates

Fungus	Litter mass	Nitrogen, %	Lignin, %	Total carbohydrates, %
Endophytes				
<i>Xylaria</i> sp. (anamorph)	0.906*	-0.770	-0.712	0.361
Epiphytes				
<i>Phoma</i> sp. 1	-0.454	0.747	0.881*	0.352
<i>Cladosporium cladosporioides</i>	-0.912*	0.803	0.761	-0.311
<i>Pestalotiopsis</i> sp. 1	-0.652	0.330	0.156	-0.676
<i>Alternaria alternata</i>	0.251	-0.081	0.223	0.915*
<i>Clonostachys rosea</i>	-0.937**	0.807	0.593	-0.790
Litter inhabitants				
<i>Arthrinium</i> sp.	-0.891*	0.926**	-0.314	0.989**
<i>Trichoderma polysporum</i>	-0.630	0.689	0.149	0.586
<i>Trichoderma koningii</i>	-0.868*	0.567	-0.480	0.665
<i>Geniculosporium</i> sp. 1	0.926**	-0.639	0.755	-0.845*
<i>Chaetomium globosum</i>	-0.652	0.156	-0.676	0.330

$n = 4$

\*  $P < 0.05$ , \*\*  $P < 0.01$

**Fig. 2.** Changes in litter mass and concentrations of nitrogen, lignin, and total carbohydrates during the decomposition of *Swida controversa* leaves



significantly and negatively correlated to litter mass, and the frequency of *Arthrinium* sp. was also significantly and positively correlated to concentrations of nitrogen and total carbohydrates. The frequency of *Geniculosporium* sp. 1 was significantly and positively correlated to litter mass and significantly and negatively to total carbohydrate concentrations. The frequencies of *T. polysporum* and *C. globosum* were not significantly correlated to the changes in litter mass and litter quality during decomposition.

## Discussion

Of the 15 species recorded as phyllosphere fungi, 6 in Group I occurred frequently on decomposing leaves. Species in *Xylaria*, *Phoma*, *Cladosporium*, *Pestalotiopsis*, and *Alternaria* have been reported to occur on both the phyllosphere and litter of several litter types (Ruscoe 1971; Wildman and Parkinson 1979; Mishra and Dickinson 1981; Cabral 1985; Kuter 1986; Aoki et al. 1990, 1992; Nakagiri et al. 1997; Okane et al. 1998; Osono 2002). Nine species classified in Group II were frequent on living leaves but not on decomposing leaves. This group is equivalent to “group III”

of beech leaves (Osono 2002). These fungi may fail to develop mycelia and be excluded at leaf death or may persist as surface colonizers of decomposing leaves.

Analyses of the sum of frequencies and the relative frequency of endophytes, epiphytes, litter inhabitants, and infrequent species revealed successional trends during litter decomposition. The correlation between the frequency of phyllosphere fungi, and litter decomposition indicated that changes in the litter quality influenced the succession of phyllosphere fungi. Dogwood leaves were characterized by relatively low lignin content and high availability of carbohydrate energy sources for rapid litter decomposition (Osono and Takeda, unpublished data). The frequency of endophytes decreased while that of epiphytes increased during decomposition. One of the reasons for this may be that epiphytes survived on decomposing leaves of dogwood as primary decomposers of the readily available carbohydrates, thereby excluding endophytes by competition for available energy sources. This finding suggests that epiphytes may have a greater contribution to decomposition than endophytes in dogwood litter.

The increase of epiphytes during decomposition of dogwood litter was in contrast to the schematic pattern of fungal succession reviewed by Hudson (1968) on the litter of several tree species and also to beech litter that had relatively high lignin content (Osono and Takeda 2001; Osono 2002). In beech litter, the frequency of epiphytes decreased as decomposition progressed due to the exhaustion of readily available resources of plant origin, while the frequencies of endophytes increased because of their ability to decompose the residual lignocellulose matrix (Osono and Takeda 2002). This comparison of successional patterns between dogwood and beech suggested that the relative importance of endophytic and epiphytic phyllosphere fungi in decomposition may be variable depending on the availability of readily available resources in the litter.

Further studies are needed to provide a more detailed picture of the succession of phyllosphere fungi and other litter inhabitants and to quantify the biomass and activity of the fungal community during the decomposition of dogwood litter. In addition, the litter decomposition potentials of these fungi should be investigated to evaluate the functional roles of phyllosphere fungi in litter decomposition.

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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, Berlin Heidelberg New York, pp 467–479
- Andrews JH, Hirano SS (1991) *Microbial ecology of leaves*. Springer, Berlin Heidelberg New York
- Aoki T, Tokumasu S, Tubaki K (1990) Fungal succession on momi fir needles. *Trans Mycol Soc Jpn* 31:355–374
- Aoki T, Tokumasu S, Oberwinkler F (1992) Fungal succession on fir needles in Germany. *Trans Mycol Soc Jpn* 33:359–374
- Cabral D (1985) Phyllosphere of *Eucalyptus viminalis*: dynamics of fungal populations. *Trans Br Mycol Soc* 85:501–511
- Carroll G, Müller EM, Sutton BC (1977) Preliminary studies on the incidence of needle endophytes in some European conifers. *Sydowia* 29:87–103
- Crossley DAJ, Hoglund MP (1962) A litter bag method for the study of microarthropods inhabiting leaf litter. *Ecology* 43:571–573
- 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
- Fokkema NJ, van den Heuvel J (1986) *Microbiology of the phyllosphere*. Springer, Berlin Heidelberg New York
- Fukui S (1969) *Analysis of reducing sugars*. Tokyo University Press, Tokyo
- Harley JL, Waid JS (1955) A method of studying active mycelia on living roots and other surfaces in the soil. *Trans Br Mycol Soc* 38:104–118
- Hudson HJ (1968) The ecology of fungi on plant remains above the soil. *New Phytol* 67:837–874
- 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
- Kuter GA (1986) Microfungal populations associated with the decomposition of sugar maple leaf litter. *Mycologia* 78:114–126
- Mishra RR, Dickinson CH (1981) Phylloplane and litter fungi of *Ilex aquifolium*. *Trans Br Mycol Soc* 77:329–337
- Miura K, Kudo M (1970) An agar-medium for aquatic hyphomycetes (in Japanese). *Trans Mycol Soc Jpn* 11:116–118
- Nakagiri A, Okane I, Ito T, Katumoto K (1997) *Lanceispora amphibia* gen. et sp. nov., a new amphispheariacean ascomycete inhabiting senescent and fallen leaves of mangrove. *Mycoscience* 38:207–213
- Okane I, Nakagiri A, Ito T (1998) Endophytic fungi in leaves of ericaceous plants. *Can J Bot* 76:657–663
- Olson J (1963) Energy storage and the balance of producers and decomposers in ecological systems. *Ecology* 44:322–331
- 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 (1999) A methodological survey on incubation of fungi on leaf litter of *Fagus crenata* (in Japanese with English summary). *Appl For Sci Kansai* 8:103–108
- Osono T, Takeda H (2001) Organic chemical and nutrient dynamics in decomposing beech leaf litter in relation to fungal ingrowth and succession during 3-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
- Petrini O (1991) Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS (eds) *Microbial ecology of leaves*. Springer, Berlin Heidelberg New York, pp 179–197
- Ruscoe QW (1971) Mycoflora of living and dead leaves of *Nothofagus truncata*. *Trans Br Mycol Soc* 56:463–474
- Widden P, Parkinson D (1973) Fungi from Canadian coniferous forest soils. *Can J Bot* 51:2275–2290
- Wildman HG, Parkinson D (1979) Microfungal succession on living leaves of *Populus tremuloides*. *Can J Bot* 57:2800–2811

**Appendix A.** Frequency (%) of fungi isolated from living and decomposing leaves of *Swida controversa*

Fungus	Living leaves		Decomposing leaves, interior
	Surface	Interior	
<i>Xylaria</i> sp. (anamorph)	0	9	15
<i>Colletotrichum gloeosporioides</i>	16	41	0
Ascomycete sp. 1	4	15	0
<i>Phomopsis</i> sp.	3	14	1
Coelomycete sp. 1	3	8	0
<i>Phoma</i> sp. 1	75	8	20
<i>Cladosporium cladosporioides</i>	59	0	20
<i>Pestalotiopsis</i> sp. 1	36	0	14
<i>Alternaria alternata</i>	19	0	8
<i>Pestalotiopsis</i> sp. 3	33	0	0
<i>Trichoderma viride</i>	18	0	5
<i>Clonostachys rosea</i>	13	0	6
Coelomycete sp. 2	16	0	0
<i>Pestalotiopsis</i> sp. 2	10	0	0
<i>Epicoccum nigrum</i>	9	0	3
<i>Arthrinium</i> sp.	3	3	29
<i>Trichoderma polysporum</i>	0	0	11
<i>Trichoderma koningii</i>	0	0	10
<i>Geniculosporium</i> sp. 1	0	1	8
<i>Chaetomium globosum</i>	0	0	8
<i>Fusarium graminearum</i>	5	0	4
Dark sterile mycelia	4	0	1
<i>Penicillium thomii</i>	3	0	4
<i>Cladosporium herbarum</i>	3	0	1
<i>Geniculosporium</i> sp. 2	0	3	0
<i>Geniculosporium</i> sp. 3	0	3	0
<i>Aureobasidium</i> sp.	0	1	0
Hyphomycete spp.	0	1	8
<i>Cladosporium sphaerospermum</i>	6	0	0
<i>Monochaetia</i> sp.	6	0	0
Ascomycete spp.	4	0	8
<i>Cladosporium oxysporum</i>	3	0	0
<i>Phoma</i> spp.	3	0	0
<i>Trichoderma</i> spp.	3	0	0
<i>Curvularia</i> sp.	1	0	0
<i>Mucor hiemalis</i>	1	0	0
<i>Nigrospora oryzae</i>	1	0	0
<i>Paecilomyces</i> spp.	1	0	0
Synemmatous fungus	1	0	0
<i>Trichoderma atroviride</i>	1	0	0
<i>Trichoderma longibrachiatum</i>	1	0	0
<i>Trichoderma pseudokoningii</i>	1	0	0
<i>Lecanicillium lecanii</i>	1	0	0
<i>Chaetomium</i> sp. 1	0	0	5
<i>Fusarium</i> spp.	0	0	4
<i>Nigrospora oryzae</i>	0	0	4
<i>Acremonium kiliense</i>	0	0	3
Coelomycete spp.	0	0	3
<i>Mariannaea elegans</i>	0	0	3
<i>Umbelopsis ramanniana</i>	0	0	3
<i>Mortierella</i> sp.	0	0	3
<i>Acremonium</i> sp.	0	0	1
<i>Arthrinium phaeospermum</i>	0	0	1
<i>Cladosporium tenuissimum</i>	0	0	1
<i>Cylindrocarpon magnusianum</i>	0	0	1
<i>Trichoderma virens</i>	0	0	1
<i>Idriella lunata</i>	0	0	1
<i>Mortierella alpina</i>	0	0	1
<i>Mucor racemosus</i>	0	0	1
<i>Mucor</i> sp.	0	0	1
<i>Nodulisporium</i> sp.	0	0	1
<i>Penicillium citrinum</i>	0	0	1
<i>Penicillium janthinellum</i>	0	0	1
<i>Penicillium waksmanii</i>	0	0	1
<i>Trichoderma hamatum</i>	0	0	1
<i>Trichoderma harzianum</i>	0	0	1
<i>Pochonia suchlasporia</i>	0	0	1
White sterile mycelia	1	8	14
Number of species	12	34	47