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FULL PAPER

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Distribution of phyllosphere fungi within the canopy of giant dogwood

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Abstract Distribution of phyllosphere fungi within the canopy of giant dogwood (*Swida controversa*) was examined. Canopies of two dogwood trees (about 8 m height) were divided into five parts in relation to the order of shoots within the current-year shoot, the height of leaf layers, and the distance from the main trunk, and leaves were collected from the five positions. A total of 13 and 33 species were isolated from the interior and surface of leaves by surface sterilization and washing methods, respectively. Species composition of fungi was different markedly between interior and surface of an individual leaf, whereas it was similar among five canopy positions in the interior or on the surface. Of 13 frequent species regarded as phyllosphere fungi, 6 species showed no difference in frequencies among five positions within the canopy. The other 7 species showed significant preference within the current-year shoot, between the leaf layer, and/or at the distance from the trunk. The probable effect of leaf properties was detected on 2 of the 7 species, while the frequencies of the other 5 species were not related to the leaf properties but were affected by the order of shoots (leaf age), the height of leaf layer, and/or the distance from the trunk (sunlight intensity).

Key words Current-year shoot · Distribution · Dogwood · Endophyte · Epiphyte

Introduction

The phyllosphere is the living leaf as a whole, which includes the interior and 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, occupying two distinct habitats in the leaf (Petrini 1991). Spatial distribution of phyllosphere fungi is an important aspect of phyllosphere ecology and has been investigated in various scales, which include geographical distribution (Carroll and Carroll 1978; Rollinger and Langenheim 1993), within-canopy distribution (reviewed in Petrini 1991), and within-leaf distribution (Bertoni and Cabral 1988; Wilson and Carroll 1994; Dobranic et al. 1995; Hata and Futai 1995; Lodge et al. 1996; Sahashi et al. 1999).

Distribution of phyllosphere fungi within the tree canopy is affected by the microenvironmental conditions on phyllosphere such as sunlight intensity and moisture and by the physical and chemical properties of individual leaves (Dix and Webster 1995). The within-canopy distribution of phyllosphere fungi has been investigated in relation to height (Bernstein and Carroll 1977; Carroll 1979; Wildman and Parkinson 1979; Andrews et al. 1980; Johnson and Whitney 1989), distance from the trunk (Andrews et al. 1980; Petrini and Carroll 1981), sun and shade leaves from different parts of the canopy (Wilson et al. 1997; Osono and Mori 2003), and compass direction (Johnson and Whitney 1989; Petrini and Fisher 1990). In these studies, however, these effects were examined separately, making it difficult to distinguish the relative importance of the microenvironments and the leaf properties on the colonization of phyllosphere fungi. More detailed studies are necessary to evaluate the spatial pattern of occurrence of fungi within the canopy that is heterogeneous in microenvironments and leaf properties.

The present study investigated the distribution of phyllosphere fungi within the canopy of giant dogwood *Swida controversa* (Hemsley) Sojak (Cornaceae) with special reference to the order of shoots within current-year shoot, the height of leaf layers, and the distance from the main trunk. The canopy of dogwood in open sites was characterized by a multilayered distribution of leaves, that is, the distribution of leaves is discrete in relation to height. Leaves from upper and lower layers have different physical and chemical properties (Kodani and Togashi 1995). Within

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Table 1. Property of canopy position and individual leaf

	Position					Position effect[d]			
Shoot order Layer Openness Code	First Upper Open FUO	Highest Upper Open HUO	First Lower Open FLO	Highest Lower Open HLO	First Lower Suppress FLS	Shoot order	Layer	Shoot order × layer	Openness
Position property									
Flush order[a]	1	3.3 ± 0.1	1	3.0 ± 0.2	1				
Height (m)[b]	8.2	8.2	4.2	4.2	3.2				
Openness (%)[b]	30.5	30.5	28.8	28.8	5.2				
Leaf property									
LMA (mg/cm^2)[c]	9.5 ± 0.4	11.6 ± 0.4	7.8 ± 0.3	9.0 ± 0.3	4.8 ± 0.1	**	**	ns	**
Nitrogen concentration (%)[c]	1.6 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	2.2 ± 0.1	1.8 ± 0.1	**	*	ns	ns
Polyphenol concentration (%)[c]	10.5 ± 0.5	11.5 ± 0.3	9.1 ± 0.7	10.6 ± 0.8	7.5 ± 0.6	*	ns	ns	ns

Values are mean ± SE
LMA, leaf mass area
[a] $n = 40$
[b] Height and openness were measured for individual #1 only
[c] $n = 8$
[d] $*P < 0.05$, $**P < 0.01$; ns, nonsignificant

the lower leaf layer, sunlight was less intense around the main trunk than at the periphery of the layer because of self-shading by the upper layer. Dogwood shows the flush + succeeding type of leaf emergence (Kikuzawa 1983) in which winter buds develop to first-order shoots, whose axillary buds sometimes elongate second-order shoots. Such a shoot elongation pattern can form higher-order shoots under open conditions (Kodani and Togashi 1992). Leaves in the higher-order shoots have different physical and chemical properties from leaves in the lower-order shoots, such as the first-order ones (Kodani and Togashi 1995). Therefore, dogwood can be a suitable material to examine the pattern of occurrence of fungi within the canopy as the effect of the microenvironmental conditions such as sunlight intensity can be separated from that of the physical and chemical properties of individual leaves.

Materials and methods

Study area

Samples were collected in a cool temperate deciduous forest dominated by *Fagus crenata* Blume, 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 leaves

Two individual trees of giant dogwood were chosen along a forestry road at 710 to 730 m a.s.l. in the study site. Height and diameter at breast height (DBH) for individual #1 were 8.6 m and 15.5 cm, respectively, and 7.9 m and 14.5 cm for individual #2. These trees were not suppressed by neighbor trees and had two leaf layers (upper layer at 8.2 m and lower layer at 3.2–4.2 m; Table 1) within their canopies (Fig. 1). The two trees were located at least 2 km apart.

On September 2000, after the growth of current-year shoots had finished, current-year shoots were sampled from two trees. We found from preliminary observation that current-year shoots at the upper layer and at the periphery of the lower layer consisted of multiple-ordered shoots. The order of shoots ranged from two to five depending on current-year shoots. As current-year shoots at the lower layer and around the main trunk consisted only of the first-order shoot, these patterns of shoot elongation reflected the light availability for each shoot within the canopy (Kodani and Togashi 1992). Thus, we need to quantify the light environment within the canopy as openness. The openness is a summary of the spatial distribution of canopy openings, calculated by hemisphaerical photograph (Chazdon and Field 1987). The hemispherical photographs were taken in overcast sky conditions of these positions of individual #1, using a Coolpix 910 digital camera with a FC-E8 fish-eye lens (Nikon, Tokyo, Japan). The current-year shoots from the upper layer were under open condition (openness 30.5%), while those from the lower layer were under either open (28.8% at the periphery) or suppressed (5.2% near the trunk) conditions, depending on the distance from the trunk (see Table 1). Therefore, the canopies of dogwood were first divided into three positions, i.e., upper+open (UO), lower+open (LO), and lower+suppress (LS) (see Fig. 1). Four current-year shoots were then collected from each of three positions of each of the two trees, and a total of 24 current-year shoots were sampled. The shoots were cut from the north side of the canopy of individual #1 and from the northeast side of the canopy of individual #2. The shoots were placed in paper bags and taken to the laboratory.

The current-year shoots were cut into each order parts. Leaves were sampled from the first-order shoot and the highest-order (2–5, mean 3.3) shoot for UO, from the first-order shoot and the highest-order (2–5, mean 3.0) shoot for LO, and from the first-order shoot of LS (see Table 1,

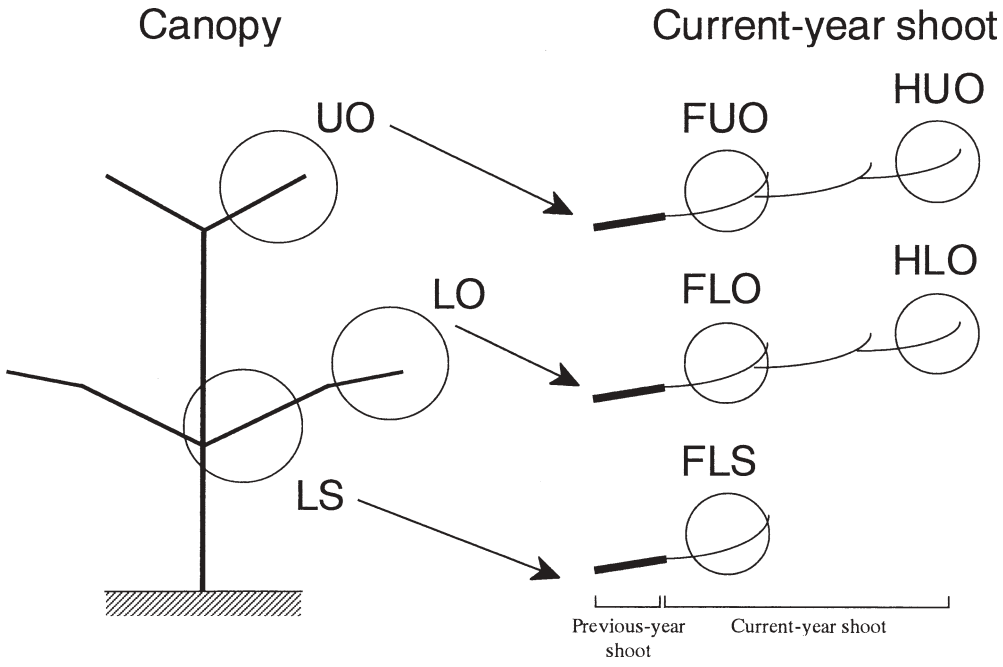

Fig. 1. Schematic diagram of canopy (*left*) and current-year (*right*) shoots of *Swida controversa*. Two trees examined had two (upper and lower) leaf layers. The upper layer was under open condition, while the lower layer was under either open (at periphery) or suppressed (around the main trunk) conditions depending on the distance from the trunk. Therefore, the canopy was first divided into upper+open (*UO*), lower+open (*LO*), and lower+suppress (*LS*). Current-year shoots from UO and LO were divided into the first-order shoots (*FUO* and *FLO*) and the highest-order shoot (mean, third-order, HUO and HLO), whereas current-year shoots from LS consisted of the first-order shoot (*FLS*) only. Therefore, leaves from the canopy fell into the following five categories: first-order+upper+open (*FUO*), highest-order+upper+open (*HUO*), first-order+lower+open (*FLO*), highest-order+lower+open (*HLO*), and first-order+lower+suppress (*FLS*)

Fig. 1). Therefore, leaves from the canopies fell into the following five categories: first-order+upper+open (FUO), highest-order+upper+open (HUO), first-order+lower+open (FLO), highest-order+lower+open (HLO), and first-order+lower+suppress (FLS). Five leaves were sampled from each of 40 shoots, and a total of 200 leaves were used for the investigation.

Two leaf disks were punched from each single leaf with a sterile cork borer (5.5 mm in diameter) from the central part of the leaves, avoiding the primary vein. The disks were used for fungal isolation, one disk for a surface sterilization method and the other for a washing method as described below. Fungal isolation was carried out within 6 h of sampling. The remaining part of the leaves was used for the measurement of leaf mass area (LMA) and concentrations of nitrogen and total polyphenol.

Fungal isolation

A surface sterilization method (Kinkel and Andrews 1988) and a modified washing method (Harley and Waid 1955) were used according to the methods described in 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 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_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.02%, KCl 0.02%, $NaNO_3$ 0.2%, yeast extract 0.02%, and agar 1.3% (w/v).

For modified washing, leaf disks were washed in a sterile test tube using a vertical type shaker at 2000 rpm for 1.5 min to isolate fungi growing actively on the surface. The disks were washed serially in two changes of 0.005% Aerosol-OT (di-2-etylhexyl sodium sulfosuccinate) solution (w/v) and rinsed with sterile distilled water four times. The washed disks were treated in the same manner as used in the plating-out procedure of the surface-sterilized leaves.

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

Measurement of LMA and chemical analyses

Leaf mass area (LMA) is a physical property that reflects the structural strength of leaves, and concentrations of nitrogen and polyphenol are chemical properties that reflect the physiological status of an individual leaf (Waterman and

Mole 1994; Kodani and Togashi 1995). Leaves were dried to a constant mass at 40°C. Area and mass of leaves were measured, and LMA (mg/cm^2) was calculated for each leaf. The leaves were then combined for each shoot, 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). Total polyphenol content was estimated by the Folin–Ciocalteau method (Waterman and Mole 1994). Polyphenol was extracted from the sample with 50% methanol (v/v) at 75°C for 60 min, and the extract was added with Folin–Ciocalteau reagent (Nacalai tesque, Kyoto, Japan) and aqueous sodium carbonate. The optical density of the solution was then measured at 760 nm using the known concentrations of tannin acid as standards.

Definition and data reduction

The frequency of all species was calculated as a percentage of the number of disks with the species of the total number of disks tested in each position. When the frequency of a species on any position was significantly ($P < 0.05$) higher than zero by Fisher's exact probability test, the species was regarded as frequent.

In this study, phyllosphere denotes the interior and surface of leaves. Fungi isolated from leaves were categorized into three groups: endophytes, epiphytes, and others. 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.

Occurrence patterns of fungal species within the habitats of single leaf (i.e., interior or surface) and within the five canopy positions were classified using cluster analysis. Cluster analysis results in a hierarchical dendrogram showing species linkages in a criterion similarity (Pearson's correlation coefficient). In the present study, the group average method was used.

The difference in leaf properties (LMA and concentrations of nitrogen and polyphenol) was evaluated in terms of the order of shoot, the height of leaf layer, and the distance from the trunk. A two-way ANOVA was performed to evaluate the difference in the leaf properties (LMA and concentrations of nitrogen and polyphenol) of FUO, HUO, FLO, and HLO using the order of shoot (first-order and highest-order) and height (upper and lower layer) as independent variables. A t test was performed to evaluate the effect of openness (i.e., the distance from the trunk) by comparing the leaf properties between FLO and FLS. The distribution of phyllosphere fungi within the canopy was analyzed in terms of the order of shoot, the height of leaf layer, and the distance from the trunk. A chi-square test was performed for frequencies of fungi because data were in the form of proportions. The effect of the order of shoot was determined comparing the frequency of fungi between FUO+FLO and HUO+HLO. The effect of the height of leaf layer was determined comparing the frequency of fungi between FUO+HUO and FLO+HLO. The effect of openness was determined comparing the frequency of fungi between FLO and FLS.

Results

Property of individual leaf

Leaf mass area (LMA) and concentrations of nitrogen and polyphenol of leaves are shown in Table 1. Leaf mass area was significantly higher at highest-order shoot than at first-order shoot, significantly higher at upper layer than at lower layer, and significantly higher at open position than at suppressed position. Nitrogen concentration was significantly higher at highest-order shoot than at first-order shoot and significantly lower at upper layer than at lower layer. Polyphenol concentration was significantly higher at highest-order shoot than at first-order shoot.

Mycobiota on leaves

Fungi were isolated from 154 of 200 (77%) leaf disks examined, 0 to 3 species (mean, 1 species) per disk, with the surface sterilization method and from 199 of 200 (99.5%) leaf disks, 0 to 6 species (mean, 3 species) per disk, with the washing method. Fifteen fungal species were isolated from the interior and 33 from the surface of the phyllosphere of dogwood (see Appendix). Thirteen species were recorded as phyllosphere fungi (Table 2). Four species frequent in the interior were regarded as endophytes and 10 species frequent on the surface were regarded as epiphytes. *Colletotrichum gloeosporioides* was frequent on both habitats.

Eight to 13 species were isolated from leaf interior of the five canopy positions and 18 to 25 species from leaf surface (Fig. 2). Twenty-seven to 41 isolates were obtained from leaf interior of five canopy positions and 100 to 141 isolates from leaf surface (Fig. 2). Number of species and number of isolates were higher on the surface than in the interior. Species composition was markedly different between the interior and surface of an individual leaf, whereas that value was relatively similar among five canopy positions in the interior or on the surface (Fig. 2).

Effect of position on phyllosphere fungi

Frequencies of *Alternaria alternata*, *Epicoccum nigrum*, *Phoma* sp. 1, and *Clonostachys rosea* were significantly higher at first-order shoot than at highest-order shoot (see Table 2). Frequency of *Aureobasidium* sp. 1 was significantly higher at highest-order shoot than at first-order shoot. Frequencies of *Aureobasidium* sp. 1 and *E. nigrum* were significantly higher at upper layer than at lower layer. Frequencies of *Phoma* sp. 1 and *C. rosea* were significantly higher at lower layer than at upper layer. Frequency of *Aureobasidium* sp. 2 was significantly higher at open position than at suppressed position. Frequency of

Table 2. Frequency (%) of phyllosphere fungi within the canopy of *Swida controversa* and the effects of position

	Position					Position effect[a]		
Shoot order	First	Highest	First	Highest	First	Shoot order	Layer	Openness
Layer	Upper	Upper	Lower	Lower	Lower			
Openness	Open	Open	Open	Open	Suppress			
Code	FUO	HUO	FLO	HLO	FLS			
Endophyte								
Geniculosporium sp. 1	25	18	33	15	45	–	–	–
Xylaria sp. (anamorph)	30	10	13	13	13	–	–	–
Colletotrichum gloeosporioides	0	15	18	10	15	–	–	–
Aureobasidium sp. 2	15	10	13	15	0	–	–	Open
Epiphyte								
Alternaria alternata	60	25	40	28	28	First	–	–
Aureobasidium sp. 1	0	23	3	3	0	Highest	Upper	–
Epicoccum nigrum	23	3	8	0	10	First	Upper	–
Phoma sp. 1	30	25	60	25	48	First	Lower	–
Clonostachys rosea	3	0	23	0	23	First	Lower	–
Colletotrichum gloeosporioides	15	8	10	18	40	–	–	Suppress
Cladosporium tenuissimum	5	8	15	15	5	–	–	–
Cladosporium cladosporioides	90	83	93	90	83	–	–	–
Pestalotiopsis sp. 1	45	25	45	43	43	–	–	–
Phomopsis sp.	15	15	18	3	10	–	–	–

Fungi were isolated from 40 leaf disks in each category

[a] Significant difference ($P < 0.05$) in the order of shoot order, height of leaf layer, and openness (i.e., the distance from the trunk) was described as "First," "Upper," and so on; –, no significant preference

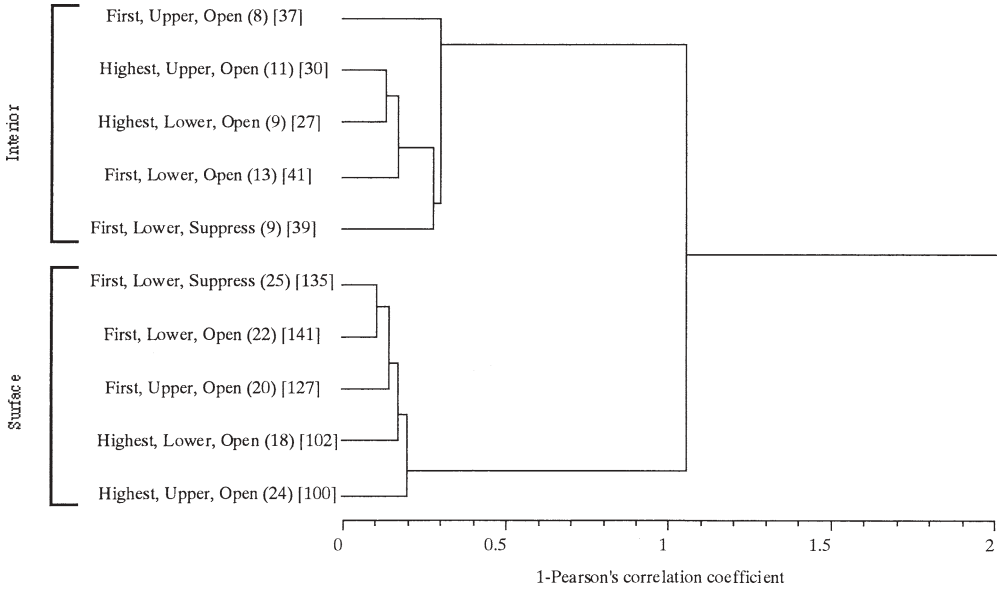

Fig. 2. Dendrogram of mycobiota from five canopy positions and two habitats by the group-average method with Pearson's correlation coefficient based on frequency of each species. *Numbers in parentheses and square brackets* indicate the number of species and the number of isolates, respectively

Colletotrichum gloeosporioides on the surface was significantly higher at suppressed position than at open position. *Geniculosporium* sp. 1, *Xylaria* sp. (anamorph), and *Colletotrichum gloeosporioides* in the interior and *Cladosporium tenuissimum*, *C. cladosporioides*, *Pestalotiopsis* sp. 1, and *Phomopsis* sp. on the surface showed no significant preference within the canopy in relation to the order of shoot, the height of leaf layer, and the distance from the trunk.

Effect of leaf property on phyllosphere fungi

A correlation coefficient was calculated for the linear relationship between leaf properties and the frequencies of seven fungi that showed significant position effect (Table 3). The frequency of *E. nigrum* was significantly and negatively correlated to nitrogen content. The frequency of *C. rosea* was significantly and negatively correlated to LMA and polyphenol content. The frequency of *Colletotrichum*

gloeosporioides was significantly and negatively correlated to LMA. The frequencies of the other four species were not significantly correlated to the leaf properties.

Discussion

Thirteen species were recorded as phyllosphere fungi of dogwood. Nine of the 13 species were common to the previous investigation by Osono et al. (2004) in which 15 phyllosphere fungi (5 endophytes and 10 epiphytes) were found on dogwood leaves in the same study site. In the present study, we added 2 endophytes (*Aureobasidium* sp. 2, *Geniculosporium* sp. 1) and 2 epiphytes (*Aureobasidium* sp. 1, *Cladosporium tenuissimum*) as phyllosphere fungi of dogwood. We also found *Colletotrichum gloeosporioides* was not only endophytic but also epiphytic on dogwood leaves. *Phomopsis* sp. was endophytic in Osono et al. (2004), and in the present study the fungus was also recorded as an epiphyte.

The result that number of species was higher on the surface than in the interior of dogwood leaves was consistent with most previous reports (reviewed in Table 4). Species composition was different between the surface and interior, nine species being common in both habitats. Similarity index (as Sørensen's quotient of similarity) was 0.375. This value was intermediate of the range of previous studies (Table 4). On the contrary, the result of the cluster analysis (see Fig. 2) indicated species composition was relatively similar within the five canopy positions, indicating the canopy position had relatively minor effects on the frequencies of phyllosphere fungi compared to the habitats within a single leaf (i.e., interior vs. surface). Most epiphytic species are commonly found on the surface of a large number of higher plants, whereas endophytes are allowed by the host plant to penetrate into the host tissues without activating the defense barriers erected by the host against pathogens (Petrini 1991). This fungus–plant interaction may influence the colonization of phyllosphere fungi more than the microenvironments of phyllosphere and the leaf properties of dogwood.

The order of shoot affected the frequencies of five epiphytes (*Alternaria alternata*, *Aureobasidium* sp. 1, *Epicoccum nigrum*, *Phoma* sp. 1, and *Clonostachys rosea*). Leaves in the first-order and the highest-order shoots were under the same sunlight intensity but differed in LMA and concentrations of nitrogen and polyphenol. *Alternaria alternata* showed significant preference to the first flush but no preference to the height of leaf layer and the distance from the trunk, suggesting the possible effect of polyphenol content of leaves (see Table 1). However, the frequency of *A. alternata* was not significantly correlated to polyphenol concentration (see Table 3), indicating the frequency of this fungus was influenced by neither the light intensity nor the measured properties of leaves. Rather, the difference in the frequency within the current-year shoots may be due to the difference in leaf age. This difference occurs because the leaves of dogwood emerge almost simultaneously on the first-order shoot in early April, and after that the new leaves emerge successively on higher-order shoots until May to June (Kodani and Togashi 1992), and hence the leaves of the highest-order shoot were younger than the leaves of the first-order shoot. Density of inoculum of *A. alternata* may be higher and colonization may be more intensive in April than later months.

In addition to the order of shoot, the height of leaf layer affected the frequencies of *Aureobasidium* sp. 1, *Epicoccum nigrum*, *Phoma* sp. 1, and *Clonostachys rosea*. Both the order of shoots and the height of leaf layer affected LMA and nitrogen concentration of leaves under the same sunlight intensity. This result indicates the relative importance of the order of shoot and height of leaf layer and the leaf

Table 3. Correlation coefficients[a] for linear relation between leaf properties and frequency of fungi that showed position effect

	LMA	Nitrogen	Polyphenol
Endophyte			
Aureobasidium sp. 2	0.777	0.259	0.788
Epiphyte			
Alternaria alternata	0.016	−0.645	0.082
Aureobasidium sp. 1	0.717	0.353	0.652
Epicoccum nigrum	−0.364	−0.964**	−0.313
Phoma sp. 1	−0.740	−0.407	−0.775
Clonostachys rosea	−0.886*	−0.521	−0.904*
Colletotrichum gleosporioides	−0.825*	−0.108	−0.795

[a] $n = 5$
* $P < 0.05$, ** $P < 0.01$

Table 4. Comparison of interior and surface mycobiota on phyllosphere of tree species

Tree species	Region	Number of species				Sørensen's *QS*	Reference
		Total	Interior	Surface	Common		
Nothofagus truncata	Wellington, New Zealand	20	14	19	13	0.788	Ruscoe (1971)
Eucalyptus viminalis	Buenos Aires, Argentina	37	16	32	11	0.458	Cabral (1985)
Populus tremuloides	Alberta, Canada	28	22	20	14	0.400	Wildman and Parkinson (1979)
Swida controversa	Kyoto, Japan	39	15	33	9	0.375	This study
Swida controversa	Kyoto, Japan	40	13	33	6	0.261	Osono et al. (2004)
Fagus crenata	Kyoto, Japan	60	18	47	5	0.154	Osono (2002)
Pinus banksiana	Québec, Canada	31	8	25	2	0.121	Legault et al. (1989a,b)
Pinus resinosa	Québec, Canada	47	13	37	3	0.120	Legault et al. (1989a,b)

Sørensen's quotient of similarity (*QS*) was calculated by the following equation: $QS = 2a/(2a + b + c)$, where a was the number of common species and b and c were the numbers of species specific to the interior and the surface, respectively

properties was difficult to measure for these four epiphytes. Therefore, the correlation coefficient was calculated for the relationship between the leaf properties and the frequencies of these epiphytes to evaluate the effect of LMA and nitrogen concentration. The frequency of *E. nigrum* was significantly and negatively correlated to nitrogen concentration, and the frequency of *C. rosea* was significantly and negatively correlated to LMA (see Table 3). These results indicate that both the order of shoots and the height of leaf layer and the leaf properties probably influenced the frequencies of *E. nigrum* and *C. rosea*. The higher frequency of *C. rosea* in lower canopy than in upper canopy can be partly ascribed to the high density of airborne inoculum near the ground because litter was another major habitat of this fungus (Osono et al. 2004). On the other hand, the frequencies of *Aureobasidium* sp. 1 and *Phoma* sp. 1 were not significantly correlated to LMA or nitrogen concentration. This result suggests that the order of shoot and the height of leaf layer were more important factors affecting the colonization of leaves by these fungi than the measured leaf properties.

Openness affected the frequencies of *Aureobasidium* sp. 2 in the interior and *Colletotrichum gloeosporioides* on the surface. These fungi showed no preference to the order of shoot and the height of leaf layer, suggesting the effect of the leaf properties including LMA was negligible on their occurrence on dogwood leaves. On the contrary, low LMA in FLS leaves reflected marked decline in sunlight intensity near the main trunk, which may result in reduced desiccation on the surface of leaves under suppressed conditions compared to that under open conditions. *Aureobasidium* sp. 2 may be resistant, and *C. gloeosporioides* may be sensitive, to high sunlight intensity and severe desiccation, affecting their competitiveness relative to other fungi on leaves and hence frequencies of these species. On the other hand, the frequency of *C. gloeosporioides* in the interior of FLS leaves was not different from that in the interior of the other leaves. It is suggested that interior colonization by this fungus was not affected by the success of surface colonization.

In summary, the present study demonstrated the distribution of phyllosphere fungi within the canopy of *Swida controversa*. The six species that showed no colonization preference within the canopy probably are successful colonizers of the phyllosphere under various microenvironments and leaf properties. Seven species showed significant preference within the canopy. The probable effect of leaf properties was detected on only two of seven species, whereas in the other five species the microenvironments influenced the colonization of phyllosphere fungi. The frequencies of these species were affected by the order of shoots (the leaf age), the height of leaf layer, and/or the distance from the trunk (sunlight intensity) rather than the leaf properties. To our knowledge, the present study is the first to show the relative importance of the effect of microenvironments of phyllosphere and the leaf properties and to demonstrate the within-current-year shoot distribution of phyllosphere fungi in deciduous tree species. As the order of shoots reflects the age of the leaf, the within-current-year shoot distribution of phyllosphere fungi may be due to seasonal change of colonization of leaves. Further studies are thus required to follow the phenological relationship between patterns of successive leaf emergence and colonization of phyllosphere fungi over the growing season that influence the distribution of phyllosphere fungi within current-year shoots.

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Appendix. Frequency (%) of fungi isolated from leaves of *Swida controversa*

Fungus	Interior	Surface
Geniculosporium sp. 1	27	0
Xylaria sp. (anamorph)	16	0
Aureobasidium sp. 2	11	0
Colletotrichum gloeosporioides	12	18
Cladosporium cladosporioides	0	88
Pestalotiopsis sp. 1	0	40
Phoma sp. 1	1	38
Alternaria alternata	1	36
Phomopsis sp.	3	12
Cladosporium tenuissimum	0	10
Clonostachys rosea	0	10
Epicoccum nigrum	0	9
Aureobasidium sp. 1	2	6
Sterile mycelia	8	5
Ascomycete spp.	5	2
Coelomycete spp.	2	3
Arthrinium sp.	0	4
Hyphomycete spp.	0	4
Trichoderma viride	0	4
Fusarium spp.	0	3
Acremonium spp.	0	2
Geniculosporium spp.	1	1
Pithomyces chartarum	0	2
Coniothyrium sp.	0	2
Pestalotiopsis sp. 3	0	2
Nodulisporium sp.	1	0
Acremonium kiliense	1	0
Nigrospora sp.	1	0
Cladosporium shpaerospermum	0	1
Flagellospora sp.	0	1
Penicillium sclerotiorum	0	1
Trichoderma hamatum	0	1
Monochaetia sp.	0	1
Paecilomyces carneus	0	1
Penicillium citrinum	0	1
Penicillium melinii	0	1
Penicillium spinulosum	0	1
Penicillium thomii	0	1
Trichoderma sp.	0	1