Diversity of endophytic mycobiota in leaves and twigs of pubescent birch (*Betula pubescens*)

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The endophytic mycobiota of leaves and twigs of *Betula pubescens* were studied. Healthy-looking leaves and twigs were collected from five individual trees at each of five sites in Switzerland in spring 1998. Tissue pieces were excised from twig segments and leaves after surface-sterilisation and incubated on malt extract agar to isolate endophytic fungi. 94% of the 1-yr-old and all of the 4-yr-old twig segments as well as 25% of the leaves were colonised by endophytes. Fifteen species were present in leaves and 19 in twigs. The anamorph of *Venturia ditricha* was most frequently isolated from leaves and that of *Ophiovalsa betulae* from twigs. *Trimmatostroma betulinum*, the anamorph of *Pseudovalsa lanciformis*, *Fusicoccum betulae* and *Phomopsis* sp. were also frequently isolated from twigs. *O. betulae*, *P. lanciformis* and *T. betulinum* preferentially colonised 4-yr-old twig segments whereas *Fusicoccum betulae* and *Phomopsis* sp. occurred mainly in 1-yr-old segments. Species diversity was highest at sites in the upper montane zone (900-1,000 m a. s. l.) and lowest in the sub-alpine zone (1,600 m). The frequency of colonisation by *O. betulae* and *T. betulinum* was, however, highest in the sub-alpine zone. *Fusicoccum betulae* was common in sites with rather high soil humidity. Among-site and among-tree variation of endophyte assemblages was high. Each tree hosted a unique endophyte assemblage. This could result from differences in inoculum availability and concentration, as well as from the unique genetic and environmental predisposition of each of the examined trees. The frequency of the endophyte species or the diversity indices was not influenced by any of the covariates studied. The only statistically significant correlation ($p = 0.013$, $r = -0.952$) existed between the frequency of twig segments colonised by *O. betulae* and the mean tropospheric NO_2 concentration. The frequency of colonisation by *O. betulae* decreased with increasing NO_2 concentration.

Keywords: endophytic fungi, *Betula pubescens*, biodiversity, immission, air pollution.

Communities of endophytic fungi are an inconspicuous but ecologically important component of woody plant species and contribute significantly to the diversity in forest ecosystems (Stone &

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al., 1996). Endophytic fungi of deciduous trees have been investigated in various studies (Petrini & al., 1992). Kowalski & Kehr (1992) studied the endophytic mycobiota of branches of *Betula pendula* Roth from Germany and Poland and Helander & al. (1993) that of *B. pubescens* ssp. *tortuosa* (Ledeb.) Nyman in Finland. Pubescent birch (*B. pubescens* Ehrh.) is the main tree species of the Pino-Betuletum pubescentis, a characteristic plant community of wetlands in Central Europe (Keller & al., 1998). The contribution by pubescent birch to community structure and processes in wetlands is unusually strong (Atkinson, 1992). If such a keystone species is lost vulnerability of its dependants, e.g. host specific endophytes, is high and a cascade of extinction is likely (Tickell, 1997). Unfortunately, pubescent birch has disappeared or has become rare in Central Europe as a consequence of lowering the ground water level by the construction of drainage systems to improve wetlands for agriculture. Birches are, however, not only sensitive to habitat changes but also to air pollutants (Maurer & al., 1998). Air pollutants were shown to reduce the frequency of some endophytic fungi in leaves and twigs of *B. pubescens* ssp. *tortuosa* and *B. pendula* (Helander & al., 1993; Kowalski & Gajosek, 1998). Thus, species composition and frequency of endophytes may be used as indicators of air pollution.

The knowledge of the ecology of pubscent birch is still very limited (Atkinson, 1992). In particular, the diversity and frequency of symbiotic organisms such as endophytic fungi which depend on this pioneer plant species and the environmental factors by which these organisms are influenced are not very well known. Fungal endophytes of *B. pubescens* have thus far only been studied in Finland (Elamo & al., 1999; Helander & al., 1993; Lappalainen & Yli-Mattila, 1999).

This study was designed to examine species composition, species frequency and diversity of the endophytic mycobiota of *B. pubescens* in Central Europe (mainly Switzerland) and to find relationships between endophyte frequency and diversity and site specific parameters (climate, topography, tree dimensions and age, air pollutants). Twigs of two age classes were studied in addition to leaves in this study. An additional aim of this investigation was to add to the overall knowledge of fungal diversity (Hawksworth & Rossman, 1997).

Materials and methods

Sample collection and isolation of fungi

In spring 1998, three healthy-looking twigs were collected at about 2 m above ground from each of five trees (*Betula pubescens*) at

each of five sites (Tab. 1). The twigs were stored at 7–8 C and processed within 48 h after collection. One 1-yr-old and one 4-yr-old shoot segment as well as four leaves were randomly cut from each twig and surface-sterilised for 1 min in 96% (v/v) ethanol, 5 min (3 min for leaves) in 8.6% sodium hypochlorite, and 0.5 min in 96% (v/v) ethanol. Three approximately 5 mm high cylinders (including wood and bark) of twig tissue were excised from the middle part of each shoot segment by sectioning the segment six times transversely with a sterilised hand pruner and two 3×3 mm tissue pieces (the first approximately 5 mm proximally to the tip and the second 3 mm distally to the base; both pieces included the mid rib) were aseptically cut with a scalpel from each leaf. All tissue pieces were plated on 2% (w/v) malt extract agar [MEA; 20 g l^{-1} malt extract (Malzin trocken, Diamalt, Hefe Schweiz AG, Stettfurt) and 15 g l^{-1} agar agar (Typ 2521, Behrens & Co., Hamburg)] amended with 50 mg l^{-1} Terramycine® (Pfizer) in 90 mm diameter Petri dishes. The dishes were incubated at 20 C in the dark for 4–8 weeks. Each individual mycelium growing from the tissue pieces was transferred to fresh MEA and cultivated in pure culture at 20 C until sporulation occurred. Cultures were identified to the genus and, if possible, to the species according to morphological characteristics.

Diversity indices

Hill's diversity numbers were used to measure species diversity and the modified Hill's ratio to determine species evenness of the endophyte assemblages in the twigs at each site (Ludwig & Reynolds, 1988).

Statistical analyses of the results

Statistical analyses were performed only for the twig data. A matrix consisting of the frequencies of fungal colonisation with the sample units (SU) in rows and the most frequently isolated fungi (frequency >5%) in columns was subjected to community ordination using correspondence analysis (Greenacre, 1993; Sieber & al., 1998). A sample unit (SU) was defined as the twig segments of a given tree (4- and 1-yr-old shoot segments combined). With correspondence analysis, SU and fungal species ordinations are obtained simultaneously, thus allowing examination of the ecological relationships between SUs and species in a single analysis.

The variables 'tree height', 'tree age', 'DBH (diameter at breast height)', 'elevation', 'precipitation', 'average temperature in January', 'average temperature in July' and 'tropospheric NO_2 and O_3 concentration' (Tab. 1) were used as independent variables and fre-

Tab. 1. – Site characteristics.

Site	Sampling date	Trees[1]			Grid reference[2]	Soil moisture[3]	Elevation [m a.s.l.]	Exposure	Inclination [°]	Precipitation[4] [mmy^{-1}]	Average Jan. temp.[5] [°C]	Average Jul. temp.[5] [°C]	NO_2[6] [µgm^{-3}]	O_3[6] [µgm^{-3}]
		Height [m]	Age [yr]	DBH [cm]										
Pfäffikon ZH	1998-05-28	5-12 (8.1)	15-40 (22)	8-21 (14)	702.158 E / 244.986 N	moist	540	Flat	0	1120	1.3	21.3	33	39
La Tourbière	1998-05-28	4-10 (4.9)	15-40 (21)	6-18 (11)	570.350 E / 230.500 N	wet to soggy	975	Flat	0	1567	1.8	15.9	20	38
Lindau	1998-05-31	4-5 (8.6)	15-80 (38)	6-41 (20)	644.700 E/ 286.500 N	wet to soggy	940	South	0-2	1717	-0.8	13	25	38
Sâles	1998-06-07	4-10 (5.6)	15-40 (23)	5-13 (7)	566.750 E / 166.800 N	soggy	895	North-west	0-3	1059	-0.8	17.5	50	27
Bödmerenwald	1998-06-21	3.5-7 (5.3)	30-80 (51)	5.5-15 (10)	707.300 E / 204.000 N	dry	1600	North-west	10-30	1755	-4.3	12.5	10	77

[1] Range and mean values in brackets; DBH, diameter at breast height.
[2] Coordinates according to Landeskarte der Schweiz, Wabern, Bern, Switzerland.
[3] Evaluated during sample collection.
[4] Annual mean of the years 1994-1998 (Schweizerische Meteorologische Anstalt SMA, Zürich).
[5] Average of the years 1994-1998 (Schweizerische Meteorologische Anstalt SMA, Zürich).
[6] Mean of the years 1994-1997 of the closest air pollution monitoring station: Dübendorf (Pfäffikon), Delémont (La Tourbière), Sisseln (Lindau), Lausanne (Sâles), Rigi (Bödmerenwald) (BUWAL, 1998).

quencies of colonisation by the most frequently isolated endophytes or diversity indices as dependent variables in linear regression analyses (Sokal & Rohlf, 1981).

Results

Ninety-four percent of the 1-yr-old and all 4-yr-old twig segments as well as 25% of the leaves were colonised by endophytic fungi. One species could be isolated from 24%, two from 40%, three from 18.7%, four from 11.3% and five from 2% of the twig segments. Leaves usually were colonised by only one and only 1.3% by two species. Endophytic thalli were detected in 13% of the leaf tips and 87% of the basal parts of the colonised leaves. Fifteen species were present in leaves and 19 in twigs (Tab. 2). Seven taxa were isolated from more than 5% of either 1- or 4-yr-old shoot segments or both. *Venturia ditricha* (Fr.) P. Karsten (anamorph: *Fusicladium betulae* (Rob. & Desm.) Aderh.) was the most abundant endophyte in leaves. It was isolated most frequently at Sâles (12% of the leaves) and Bödmerenwald (13%) and was detected in only one leaf at Pfäffikon and Lindau. *V. ditricha* did not occur at all at La Tourbière.

Ophiovalsa betulae (Tul.) Petrak (anamorph: *Disculina betulina* (Sacc.) Höhn.) dominated the mycobiota in twigs and was isolated from all except one tree at Sâles (Tabs. 2, 3). It preferentially colonised 4-yr-old twig segments. Among-site variation of the frequency of colonisation was between 80 and 100% for 4-yr-old and between 27 and 87% for 1-yr-old shoots. *O. betulae* was most abundant at Bödmerenwald regardless of shoot age.

Trimmatostroma betulinum (Corda) Hughes was the second most frequently isolated endophyte and was detected in all except two trees, one each at Pfäffikon and Bödmerenwald (Tabs. 2, 3). *T. betulinum* preferentially colonised 4-yr-old twig segments. Among-site variation of the frequency of colonisation ranged from 27 to 87% for 4-yr-old and from 7 to 40% for 1-yr-old shoots. Four-yr-old shoots were most intensely colonised by this fungus at Lindau and 1-yr-old ones at Pfäffikon and Sâles.

Pseudovalsa lanciformis (Fr.) Ces. & De Not. (anamorph: *Coryneum brachyurum* Link), *Phomopsis* sp. and *Fusicoccum betulae* Cooke, listed in descending order of abundance, were also quite frequently isolated. In addition, a dematiaceous, sterile mycelium (sterile mycelium 1) and an *Aposphaeria* species were frequently detected at some sites (Tab. 3). No site had all the endophytes isolated in this study, and the within-site variation of the number of colonised trees was high. *P. lanciformis* preferentially colonised 4-yr-old twig segments, *F. betulae* and *Phomopsis* sp. occurred mainly in

Tab. 2. – Frequency of twig and leaf segments colonized by endophytic fungi.[1]

Taxon[2]	State formed in culture	Frequency (%) of colonization		
		Twigs		Leaves (n=300)
		4-yr-old shoots (n=75)	1-yr-old shoots (n=75)	
Aposphaeria sp. 1		7	4	0
Aposphaeria sp. 2		3	0	0
Asteromella sp.		0	0	2
Cytospora sp.		0	0	1
Epicoccum nigrum Link		1	1	0
Fusicoccum betulae		5	23	0
Geniculosporium sp.		5	1	2
Melanconis stilbostoma (*Melanconium betulium*)	anamorph	3	5	0
Nodulisporium sp. 1		4	0	0
Ophiovalsa betulae (*Disculina betulina*)	anamorph	89	61	0
Pezicula sp. (*Cryptosporiopsis* sp.)	anamorph	1	0	1
Phomopsis sp.		9	21	2
Prosthemium astersporum		3	1	0
Pseudovalsa lanciformis (*Coryneum brachyurum*)	anamorph	24	11	0
Taeniolella exilis (P. Karsten) Hughes		0	0	1
Trimmatosroma betulium		53	31	0
Venturia ditricha (*Fusicladium betulae*)	anamorph and teleomorph	0	0	6
Xylaria sp.	anamorph	4	4	1
Sterile mycelium 1		8	9	0
Other sterile mycelia		11	19	5

[1] Species and genera isolated from less than 1% of the twig segments (T) or the leaves (L): *Cladobotryum* sp. (T), *Libertella betulina* (L), *Myxocyclus polycistis* (T), *Nodulisporium* sp. 2 (L), *Phlyctaeniella* sp. (T), *Pithoascus* sp. (L), *Rhizoctonia* sp. (T), *Rosellinia* sp. (*Nodulisporium* sp. 3) (L), *Sirodothis* sp. (L), *Trichosporidiella* sp. 1 and sp. 2 (L), *Volutella* sp. (L).
[2] Name of anamorphic state in brackets.

1-yr-old segments. No clear preference was demonstrated by *Aposphaeria* sp. 1 and the sterile mycelium 1 (Tab. 3).

Species diversity and evenness indices were higher in 1-yr-old than in 4-yr-old shoots at La Tourbière, Lindau and Sâles (except for the number of abundant species at Sâles) (Tab. 4). The indices did

Tab. 3. – Number of trees colonised and frequency of colonisation (%) by the seven most frequently isolated endophytes (>5%) at each site (P, Pfäffikon ZH; T, La Tourbière; L, Lindau; S, Sâles; B, Bödmerenwald).

Taxon	Number of trees colonised (n=5 for each site)					Frequency of colonised twig segments (%)									
						4-yr-old shoots (n=15 for each site)					1-yr-old shoots (n=15 for each site)				
	P	T	L	S	B	P	T	L	S	B	P	T	L	S	B
Aposphaeria sp. 1	3	1	1	1	0	20	7	0	7	0	7	0	13	0	0
Fusicoccum betulae	0	2	5	3	1	0	0	13	13	0	0	20	60	27	7
Ophiovalsa betulae	5	5	5	4	5	87	93	87	80	100	73	27	73	47	87
Phomopsis sp.	4	3	5	3	0	20	13	7	7	0	27	7	40	33	0
Pseudovalsa lanciformis	5	0	1	4	0	80	0	20	20	0	20	0	0	33	0
Trimmatostroma betulinum	4	5	5	5	4	27	53	87	53	53	40	27	33	40	7
Sterile Mycelium 1	1	0	4	4	0	7	0	7	27	0	0	0	33	13	0

Tab. 4. – Endophyte species diversity and evenness indices for the twig samples at each site.

Site	Number of species		Number of very abundant species[1]		Number of abundant species[2]		Evenness[3]	
	1-yr-old	4-yr-old	1-yr-old	4-yr-old	1-yr-old	4-yr-old	4-yr-old	1-yr-old
Pfäffikon ZH	7	7	3.92	3.94	4.83	4.92	0.76	0.75
La Tourbière	8	6	3.13	4.45	4.36	5.01	0.63	0.86
Lindau	11	10	4.40	6.18	6.27	7.38	0.64	0.81
Sâles	11	8	4.76	6.02	7.12	6.65	0.61	0.89
Bödmerenwald	5	5	2.32	1.67	2.87	2.39	0.70	0.48

[1] Corresponds to Hill's diversity number N2 (Ludwig & Reynolds, 1988).
[2] Corresponds to Hill's diversity number N1 (Ludwig & Reynolds, 1988).
[3] Corresponds to the modified Hill's ratio (Ludwig & Reynolds, 1988).

not depend on shoot age at Pfäffikon and were slightly higher in older shoots at Bödmerenwald. Diversity was highest at Lindau and Sâles and lowest at Bödmerenwald.

Correspondence analysis was performed to visualise, in a symmetric two-dimensional map (Fig. 1), the combined effect of tree individuals and sites on colonisation by the most frequently isolated endophytes. The first two principal axes accounted for almost 60% of the inertia (axis 1, 33.7%; axis 2, 24.0%), explaining 60% of the variability in the data matrix. The points furthest apart in Fig. 1 are *O. betulae* (OB) and *P. lanciformis* (PL) along the 1st principal axis as well as *Fusicoccum betulae* (FB) and *P. lanciformis* (PL) along the 2nd. These three endophytes had the greatest influence on the positions of the points in the graph. Looking only at the 1st principal axis the groups furthest apart are the trees at Bödmerenwald and some trees at La Tourbière on the right-hand side, as opposed to one tree each at Sâles and Pfäffikon on the left-hand side (Fig. 1) – hence the greatest differences in the colonisation by *O. betulae* and *P. lanciformis* are between these extremes (i. e. a high incidence of *O. betulae* is combined with a low incidence of *P. lanciformis* and vice-versa). The 2nd principal axis pulls apart the trees heavily colonised by *F. betulae* and *P. lanciformis* (Fig. 1). The groups furthest apart are the trees at Pfäffikon at the bottom, opposed to some trees at Lindau and Sâles at the top. In fact, the trees at Pfäffikon were more frequently colonised by *P. lanciformis* than those growing at Lindau or Sâles. Conversely, *F. betulae* was never observed at Pfäffikon but was quite abundant at Lindau and Sâles.

The frequency of neither one of the endophyte species nor of the diversity indices correlated with any of the variables 'tree height', 'tree age', 'DBH', 'elevation', 'precipitation', 'average temperature in January', 'average temperature in July' or 'ozone concentration'. The slope of the regression line was statistically significantly different from zero ($p = 0.013$, $r = -0.952$) only for the frequency of twig segments colonised by *O. betulae* versus the mean tropospheric NO_2 concentration (Fig. 2). The frequency of colonisation by *O. betulae* decreased with increasing NO_2 concentration. The frequency of colonisation by *O. betulae* was highest at Bödmerenwald and La Tourbière, the two sites with the smallest input of NO_2, whereas it was lowest at Sâles with the greatest input of NO_2.

Discussion

Only four of the 30 species found during this study occurred in both leaves and twigs indicating that the endophyte assemblages

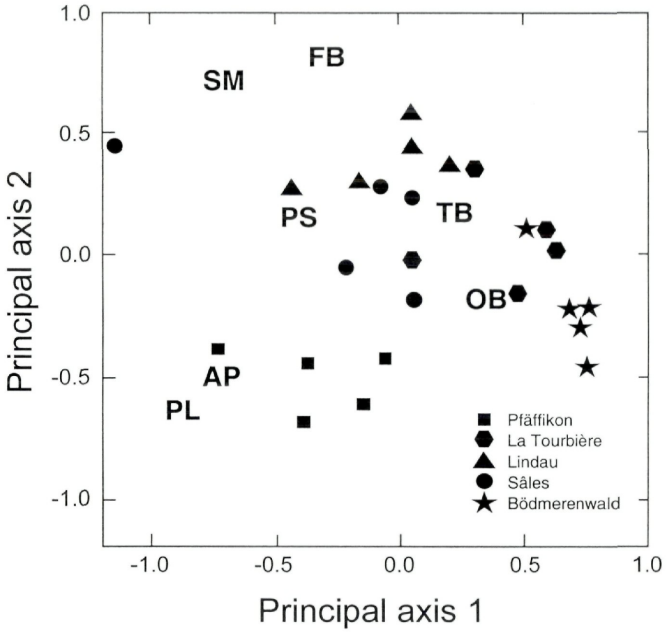

Fig. 1. – Symmetric map, result of correspondence analysis, showing the profiles of the seven most frequently isolated fungi and each tree (symbols) with respect to the first two principal axes. AP = *Aposphaeria* sp. 1; FB = *Fusicoccum betulae*; OB = *Ophiovalsa betulae*; PL = *Pseudovalsa lanciformis*; PS = *Phomopsis* sp.; SM = the sterile mycelium 1; TB = *Trimmatostroma betulinum*.

were organ specific. Organ specificity is a common feature of endophytes (Fisher & al., 1994; Petrini, 1991; Sieber & al., 1991; Sieber & al., 1988).

Helander & al. (1993) isolated seven different species from leaves compared to 15 species in this study. The reason for this difference may be the extreme climatic conditions (mean annual temperature: –2 C; minimum: below –40 C) at the sampling site in the far north of Finland (69°45′ N) in the study of Helander & al. (1993). Similarly, only four species were detected in the leaves at the site (Bödmerenwald) with the most extreme climatic conditions in this study. *Venturia ditricha*, the most frequently isolated leaf endophyte in this study, is probably not conspecific with the *Venturia* sp. found in the

Finnish study (Helander & al., 1993), since the "Finnish" *Fusicladium*-anamorph was morphologically different from *F. betulae*, the anamorph of *V. ditricha* (T. N. Sieber, unpublished). A *Melanconium* species was quite often observed in the leaves from northern Finland but never in leaves collected in this study. *Gnomonia setacea* (Pers.) Ces. & De Not., an endophyte commonly isolated from leaves of *B. pubescens* and *B. pendula* in SW Finland (Lappalainen & Yli-Mattila, 1999) was not detected by Helander & al. (1993) or in this study. The collection of the leaves at the onset of the vegetation period probably was the reason for the absence of this fungus in this study. Apparently healthy leaves collected in August 1999 during another study at Bödmerenwald were frequently colonised by *G. setacea* (N. Barengo, unpublished). In addition, many places are known in Switzerland where perithecia of this fungus were observed in leaf litter (O. Holdenrieder & T. N. Sieber, unpublished).

Another factor affecting species composition and frequency of colonisation by endophytes is host specificity (Petrini & Fisher, 1990; Sieber, 1989). The endophytes assemblages of *B. pubescens* were composed of a number of species which are considered specific to the genus *Betula* such as *Melanconis stilbostoma* (Fr.) Tul. (anamorph: *Melanconium betulinum* Schm. & Kunze), *Pseudovalsa lanciformis, Venturia ditricha, Fusicoccum betulae, Prosthemium asterosporum* T. Kowalski & O. Holdenrieder, *Trimmatostroma betulinum, Myxocyclus polycistis* (Berk. & Br.) Sacc. and *Libertella betulina* Desm. Xylariaceous fungi such as *Xylaria* sp., *Geniculosporium* sp., and *Nodulisporium* spp., known to live endophytically in a large number of hosts but to fruit only on a few of them were also present (Petrini & Petrini, 1985; Whalley, 1996).

Conidial width (n = 95; measured in concentrated lactic acid) of the *O. betulae* isolates found in this study was on average greater (4.3 µm) than that given by Ellis & Ellis (1985) (range: 3–4 µm) or Grove (1937) (range: 3.5–4.0 µm). Thus, the the *O. betulae* isolates from this study would rather fit the description of *O. suffusa* (Fr.) Petrak [width: 4.0–4.5 µm (Ellis & Ellis, 1985); 4–6 µm (Grove, 1937)]. The name *O. betulae* was, however, used in this study firstly because our measurements are based on structures measured in culture, which may, therefore, deviate from the measurements given in the literature, and secondly because *O. suffusa* is described to occur on *Alnus* spp. and not on *Betula* spp.

Approximately 74% of the bark samples of *Betula pendula* were colonised in the study of Kowalski & Gajosek (1998), as compared to almost 100% of the twig segments of *B. pubescens*. Nineteen species were isolated from twigs in this study whereas Kowalski & Gajosek (1998) were able to detect 52 species in bark tissues. Fourteen species

occurred in more than 1% of the tissue samples in our study compared to only 11 species found by Kowalski & Gajosek (1998). Many of the most abundant endophytes were isolated from both *Betula* species. *Godronia cassandrae* Peck, *Pezicula cinnamomea* (DC) Sacc., *Phialocephala* cf. *dimorphospora* Kendrick and *Prosthemium betulinum* Kunze ex Schlecht. were not found in this study. By contrast, Kowalski & Gajosek (1998) did not isolate *Prosthemium asterosporum* and only sporadically found members of the Xylariaceae.

In general, species diversity decreases with increasing altitude and latitude (Pianka, 1983; Whittaker, 1977; Sieber & al., 1999). This, however, only partly applies to the results of this study. Species richness was lowest at the site with the highest elevation (Bödmerenwald, 1,600 m), low at the site with the lowest elevation (540 m) and highest at sites in the upper montane zone between 895 and 975 m. Interestingly, the frequency of colonisation by *O. betulae* and *V. ditricha*, the overall most frequently isolated endophytes from twigs and leaves, was highest at the high-elevation site Bödmerenwald. It was not possible to decide whether the abundance of these two endophytes was due to the absence of other endophyte species or to a competitive advantage of *O. betulae* and *V. ditricha* at this site.

The frequency of *O. betulae* was negatively correlated with the tropospheric NO_2 concentration (Fig. 2). The correlation was statistically significant but more sites will have to be studied to confirm this finding. NO_2 is known as a potentially phytotoxic compound (Wellburn, 1990) which directly or indirectly also may affect endophytic fungi. Effects of air pollutants on the frequency of endophytes or on the species composition of endophyte assemblages have been examined only in a few studies. Helander & al. (1993) observed a decrease of colonisation by a *Venturia* species in leaves of *B. pubescens* var. *tortuosa* in northern Finland after irrigation with water (artificial rain), the pH of which had been adjusted to 3 by adding sulfuric and nitric acid. In another experiment at the same site, acidification (pH 3) of irrigation water with nitric acid alone had, however, no effect on the endophyte colonisation of Scots pine (*Pinus sylvestris* L.) needles (Helander & al., 1994). The NO_2 concentration was correlated with the O_3 concentration in this experiment ($r = 0.876$). However, the relationship was statistically not significant. Reports about the influence of O_3 on fungal activity are contradictory (Manning & Tiedemann, 1995; Scagel & Anderson, 1997). Endophytic colonisation of Sitka spruce needles by *Rhizosphaera kalkhoffii* Bubak was found to be increased on two occasions by exposure in a study of Magan & al. (1995).

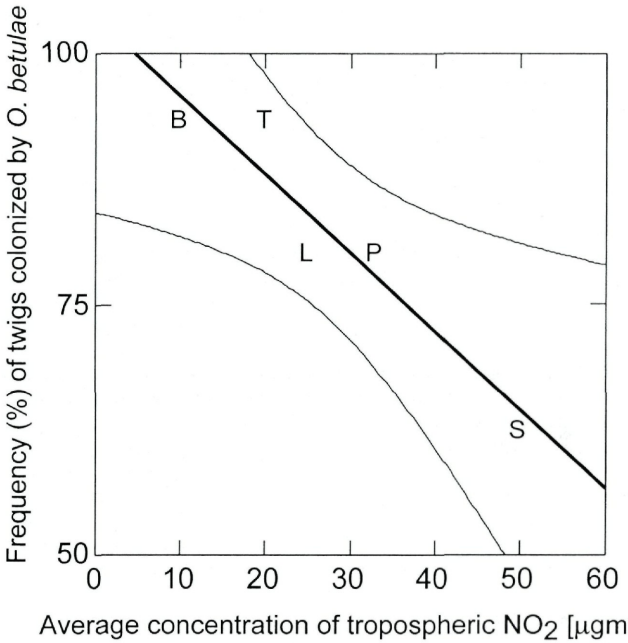

Fig. 2. – Scatterplot showing the relationship between the mean tropospheric NO_2 concentration and the frequency of twigs colonised by *Ophiovalsa betulae* at each site (B = Bödmerenwald, L = Lindau, P = Pfäffikon ZH, S = Sâles, T = La Tourbière). The curve and the 95% confidence interval were fitted by linear regression ($p = 0.013$, $r = -0.952$).

The age of tissues clearly had a great influence on endophyte frequencies in shoots. *O. betulae*, *P. lanciformis* and *T. betulinum* preferentially occurred in older shoots whereas *Fusicoccum betulae* and *Phomopsis* sp. mainly colonised young twig segments. Concomitantly with increasing shoot age, *F. betulae* and *Phomopsis* sp. probably got increasingly more frequently displaced or inhibited by *O. betulae*, *P. lanciformis* and/or *T. betulinum* which were more competitive in older shoots. Alternatively, the observed patterns of colonisation reflect differences in inoculum availability in different years. The influence of the age of tissues on the frequency and species composition of endophyte assemblages has been reported in several studies (Espinosa-Garcia & Langenheim, 1990; Sieber-Canavesi & Sieber, 1993; Sieber & al., 1988).

Among-site and among-tree variability of endophyte assemblages was high. The endophyte assemblages at some sites were unique suggesting a site-specific pattern. Some trees at certain sites hosted, however, endophyte assemblages which clearly differed from those of trees at the same site but were similar to those of trees at other sites. For example, three trees at La Tourbière hosted endophyte assemblages which were similar to those of trees at Bödmerenwald, i. e. they were almost exclusively colonised by *O. betulae* and *T. betulinum* (Fig. 1). In contrast, the other two trees at La Tourbière were more similar to trees at Lindau or Sâles because they were colonised by *F. betulae* and *Phomopsis* sp. in addition to *O. betulae* and *T. betulinum*. Nevertheless, each tree in this study was colonised by a unique endophyte assemblage at least in respect to species frequencies. Individuality of trees seems to be a common feature of endophyte colonisation (Petrini & al., 1992; Sieber, 1988; Sieber & al., 1999). Individuality is probably based on a combination of both genetic disposition and predisposition by microclimatic, edaphic and other environmental conditions.

In the montane zone, *Fusicoccum betulae* preferentially colonised twigs at sites with rather wet to soggy soil conditions. Conversely, *P. lanciformis* occurred most frequently at the driest site in the montane zone. This demonstrates that a change in the composition of the endophyte assemblage and/or frequency of endophyte species may be one of the first effects of a habitat change.

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