Endophytic fungal colonization of branch bases in several forest tree species

T. Kowalski[1] & R.D. Kehr[2]

[1] Department of Forest Pathology, Faculty of Forestry, 31–425 Cracow, Poland
[2] Institut für Pflanzenschutz im Forst, Biologische Bundesanstalt für Land- und Forstwirtschaft, D-3300 Braunschweig, Germany

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The fungal flora of the basal part of living branches was investigated in eleven deciduous and coniferous European tree species. Almost all living branches were colonized. The fungi were located mostly in the dead outer bark and to a lesser extent in living bark and wood. Each tree species was colonized by 41 to 67 taxa in the branch bases and some of them could be considered highly specific fungal endophytes. In general, most of the common branch pruning fungi found in earlier investigations are already present in living branches, giving them an advantage in colonization of dying tissue. The term "endophyte" is discussed in relation to the type of tissue colonized, and the term "phellophyte" is proposed for those fungi typically colonizing only dead outer bark.

Keywords: endophytes, phellophytes, branch pruning, periderm.

Natural pruning of branches is of paramount importance for the production of a clean bole. This process relies mainly on the succession of various fungi which colonize dead branches still attached to the trunk. To obtain more information on the ecology of the fungi involved in natural pruning, the basal parts of living, symptomless branches of 11 tree species whose dead branches had previously been examined in respect to natural pruning (Butin & Kowalski, 1983a; 1983b; 1986; 1990; Kowalski & Butin, 1989) were investigated. The foremost question was whether the most frequent branch pruning fungi are already present in living branches, as various symptomless parts of the tree are often colonized by endophytes (Fisher & Petrini, 1990; Petrini & Fisher, 1988; 1990; Sieber, 1988; 1989; Butin, 1986; Petrini & Müller, 1979).

Material and methods

Collection of branches

Living branches of the same 11 tree species previously studied for natural pruning (Butin & Kowalski, 1983a; 1983b; 1986; 1990; Kowalski & Butin, 1989) were used. First order branches of the same diameter range as in the above studies were collected in pure and

mixed stands of various ages in the vicinity of Hannoversch Münden, Braunschweig and Regensburg (Germany) as well as near Krakow (Poland) mainly in 1990, and in 1986 and 1988. Collecting was usually done in summer and autumn, and in few cases *(Fagus, Abies, Pinus)* additionally in spring. In young stands branches were located in lower and middle parts of the crown up to 2–3 m, whereas in older stands they were taken from the lower part of the crown up to a height of 7 m. Branch diameter at the base was 0,5–6 cm. One branch per tree was pruned randomly using hand cutters; for higher parts of the crown a branch cutter on telescopic poles was employed. In total, 1095 branches were examined, ranging from 50 *(Acer, Alnus)* to 160 *(Fagus)* per tree species (Tab. 3).

Isolation and identification of fungal taxa

A 6 cm long segment was cut from the base of each branch and isolations were carried out within 24 hrs after collection using the surface sterilization technique described by Sieber (1989). Segments were scrubbed under tap water to remove loose particles and sterilized by washing in 96% ethanol (1 min), followed by immersion in sodium hypochlorite with 4% available chlorine (5 min) and finally washing in 96% ethanol (30 sec). After drying with filter paper, 12 pieces of approx. 5 mm length per branch were laid out on petri dishes containing 2% malt agar supplemented with 100 mg/l Streptomycin. Six pieces were derived from various parts of the superficial, dead bark layer, three were cut from living, green portions of the bark and three from the outer parts of the wood. In this paper the dead bark layer is termed "peridermal" and the green, living layer "subperidermal". In all, 13,140 pieces were plated out and incubated at room temperature for several weeks. Subcultures of growing mycelia were inoculated on 2% malt agar on slants or petri dishes. To induce sporulation and/or production of the teleomorph, the cultures were kept for several weeks at 4 C or placed under ultraviolet light (Sylvania F36WBLB) at a 12 h light/darkness cycle at 15 C.

Regardless of whether anamorph or teleomorph were produced in culture, the fungal names cited in the above mentioned publications are used in this paper to enable comparison.

Overall frequency of fungal colonization was defined as the number of pieces of a given tissue type yielding at least one species in relation to the total number of pieces taken from this tissue type.

Results

Colonization frequency

Almost all basal segments of the living branches investigated were colonized by fungi. Of 1,095 branches examined, only 23 did not

Tab. 1.– Frequency of fungal colonization of living branch bases.

tree species	% of branches colonized			% of pieces colonized		
	bark peridermal	bark sub-epidermal	wood	bark peridermal	bark sub-peridermal	wood
			coniferous hosts			
Abies alba	97,0	14,0	6,0	80,0	5,0	2,3
Larix decidua	100,0	18,2	5,5	89,7	6,1	1,8
Picea abies	100,0	43,2	6,5	98,4	14,9	2,7
Pinus sylvestris	99,3	27,6	4,1	92,1	10,6	1,4
			deciduous hosts			
Acer pseudoplatanus	100,0	6,0	2,0	93,7	2,0	0,7
Alnus glutinosa	100,0	28,0	18,0	94,3	9,3	6,0
Betula pendula	100,0	13,0	22,0	92,2	4,7	9,0
Carpinus betulus	100,0	6,3	7,5	89,8	2,1	3,3
Fagus sylvatica	88,1	10,6	7,5	38,0	4,2	3,1
Fraxinus excelsior	100,0	11,4	18,6	78,1	3,8	6,2
Quercus robur	100,0	16,9	9,2	97,9	6,2	3,1
total average	97,9	19,5	9,1	83,7	7,0	3,5

yield any culture. However, there were marked differences in the colonization rate of the various tissue types. The peridermal bark layer was colonized by at least one species in 98% of all branches. Subperidermal bark tissues yielded cultures in 20%, and wood was colonized in only 9% of the branches (Tab. 1). The subperidermal bark tissue was colonized more than twice as frequently in coniferous than in deciduous trees. In contrast, wood of deciduous trees contained twice as many fungi as that of coniferous species (Fig. 1). Some differences in colonization rates were also noticed between individual tree species. Wood was most heavily colonized in *Betula, Fraxinus* and *Alnus,* and least in *Acer.* The high colonization rate from the subperidermal bark layer in *Picea* is very conspicuous. In contrast, fungi were absent from the dead peridermal bark layer only in few branches of *Abies* (3%), *Pinus* (0.7%) and *Fagus* (19.1%), most of which had been collected in early spring (Tab. 1).

In addition to frequency of overall branch colonization (each branch represented by six pieces), there were also extreme differences in the density of colonization by mycelia within various branch tissues. While 84% of pieces from dead bark tissue yielded fungi, living bark tissue was colonized to 7%, wood only to 3.5% (Fig. 1, Tab. 1).

From dead bark, up to seven different fungal species per branch were isolated (Tab. 2). Individual tree species showed differences in this respect. For instance, *Abies* and *Fagus* most commonly yielded only one species, *Carpinus* and *Betula* two species. However, these data are based on the six fragments taken from each branch and it is possible that investigation of a larger number of pieces would result in more fungal species being present within the same branch segment.

Branch diameter

The number of fungi colonizing dead bark of a given branch was partly dependent on branch diameter (Tab. 3). The occurrence index indicates how many fungal species were found on average in the dead bark of one branch. This dependency on branch diameter was stronger in deciduous trees than in conifers. Branches thicker than 2 cm usually contained less species than thin branches, with the exception of *Fagus* and *Quercus.*

Frequency of branch colonization by the most common genera was also influenced by branch diameter (Tab. 4). *Diaporthe carpini, Mollisia cinerea, Sclerophoma pithyophila, Colpoma quercinum* and most *Phomopsis* species were isolated mostly from thin branches. *Sirodothis* spp., *Asterosporium asterospermum* and *Fusicoccum macrosporum* became more frequent with increasing branch diameter. *Petrakia irregularis, Pezicula cinnamomea* (on *Alnus)* and *Neo-*

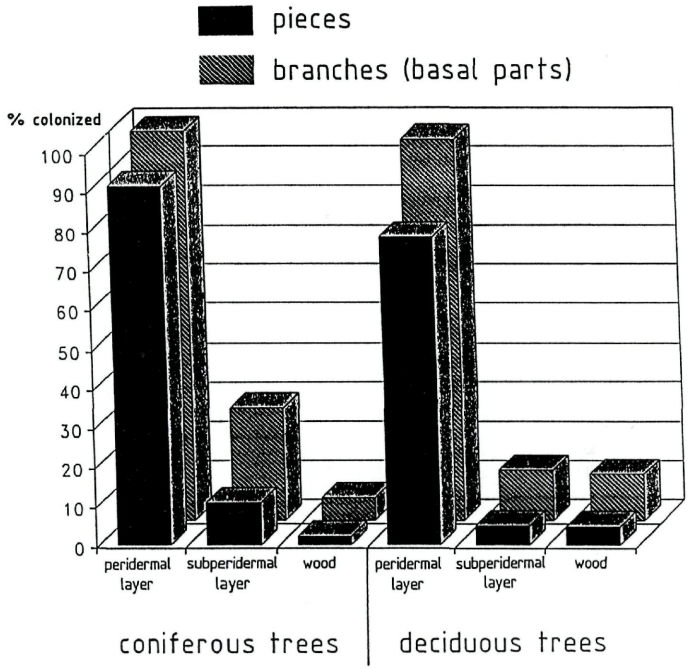

Fig. 1.– Frequency of fungal colonization at the base of living branches.

hendersonia kickxii most commonly colonized branches of 1–2 cm diameter. There were also differences within the same fungal species occurring on different hosts. For instance, *Phialocephala* cf. *dimorphospora* was less frequent on *Alnus* at diameters of more than 2 cm, but on *Quercus* the opposite was true.

Species diversity

Details on fungal distribution according to tree species, frequency in different tissues and the forms produced in culture can be seen in Table 6. The number of isolated taxa per tree species ranged from 41 *(Acer pseudoplatanus)* to 67 *(Fagus sylvatica)*. Some taxonomical problems were encountered. For instance, some genera have not been monographed satisfactorily, and fungal taxa are often keyed out only according to the host. In addition, many fungi produce only the anamorph in culture, and on this basis the species

Tab. 2.– Percentage of branches colonized by a given number of fungal species in peridermal bark.

	number of fungal taxa							
tree species	0	1	2	3	4	5	6	7
				coniferous hosts				
Abies alba	3,0	30,0	20,0	27,0	12,0	5,0	2,0	1,0
Larix decidua	0	7,3	20,0	36,4	23,6	7,3	5,4	0
Picea abies	0	5,2	21,9	40,0	24,5	7,1	1,3	0
Pinus sylvestris	0,7	9,0	27,6	42,7	15,9	3,4	0,7	0
average	0,9	12,1	23,1	37,6	18,9	5,5	1,7	0,2
				deciduous host				
Acer pseudoplatanus	0	8,0	8,0	28,0	28,0	20,0	8,0	0
Alnus glutinosa	0	6,0	32,0	32,0	22,0	4,0	4,0	0
Betula pendula	0	17,0	29,0	22,0	21,0	9,0	2,0	0
Carpinus betulus	0	16,3	38,7	27,5	15,0	2,5	0	0
Fagus sylvatica	11,9	28,1	26,3	21,3	8,1	3,7	0,6	0
Fraxinus excelsior	0	10,0	34,3	31,4	14,3	2,9	5,7	1,4
Quercus robur	0	12,3	16,9	31,5	20,8	13,9	4,6	0
average	3,0	16,4	26,2	26,7	16,9	7,6	3,0	0,2

Tab. 3.– Mutual occurrence* of fungal species in relation to branch diameter (n = number of examined branches).

	branch diameter							
	≤1,0 cm		1,1–2.0 cm		>2,0 cm		total	
tree species	n	O*	n	O*	n	O*	n	O*
				coniferous hosts				
Abies alba	46	2,6	33	2,7	21	1,7	100	2,4
Larix decidua	24	3,4	15	3,5	16	2,6	55	3,2
Picea abies	68	3,2	41	3,1	46	3,0	155	3,1
Pinus sylvestris	44	3,0	37	3,1	64	2,4	145	2,8
				deciduous hosts				
Acer pseudoplatanus	19	3,1	17	4,5	14	3,5	50	3,7
Alnus glutinosa	19	2,9	18	3,3	13	2,6	50	3,0
Betula pendula	34	3,4	25	2,5	41	2,5	100	2,8
Carpinus betulus	33	2,5	29	2,7	18	2,3	80	2,5
Fagus sylvatica	42	1,7	42	1,9	76	2,2	160	2,0
Fraxinus excelsior	25	3,3	21	2,4	24	2,9	70	2,9
Quercus robur	42	2,5	45	3,2	43	3,8	130	3,2
Total	396		323		376		1095	

* Occurrence = $\frac{\Sigma(n_f \cdot n_b)}{\Sigma n_b}$ with n_f = number of fungal species (0, 1, 2 ... 7) per branch and n_b = number of branches with 0, 1, 2 ... 7 species

Tab. 4.– Most common fungal taxa in relation to branch diameter.

tree species	fungal taxa	% of branches colonized (diameter)		
		≤ 1 cm	1,1–2 cm	>2 cm
Abies alba	*Grovesiella abieticola*	19.6	27.3	28.6
	Pezicula spp.	65.2	69.7	33.3
	Phomopsis spp.	73.9	48.5	0
Acer pseudoplatanus	*Petrakia irregularis*	21.1	82.4	42.8
	Phomopsis spp.	78.9	29.4	7.1
Alnus glutinosa	*Cryptospora suffusa*	84.2	77.8	69.2
	Pezicula cinnamomea	15.8	50.0	15.4
	Phialocephala cf. *dimorphospora*	26.3	33.3	23.1
Betula pendula	*Cryptospora betulae*	85.3	92.0	87.8
	Pseudovalsa lanciformis	26.5	40.0	48.8
Carpinus betulus	*Diaporthe carpini*	51.5	17.2	11.1
	Pezicula spp.	60.6	69.0	83.3
Fagus sylvatica	*Asterosporium asterospermum*	26.2	33.3	48.7
	Neohendersonia kickxii	11.9	30.9	10.5
	Pezicula spp.	11.9	21.4	27.6
	Fusicoccum macrosporum	7.1	4.8	25.0
Fraxinus excelsior	*Phomopsis* spp.	48.0	52.4	54.2
	Pezicula cinnamomea	16.0	38.1	29.2
Larix decidua	*Sirodothis* spp.	29.2	53.3	87.5
	Phialocephala cf. *dimorphospora*	41.7	46.7	12.5
Picea abies	*Mollisia cinerea*	77.9	61.9	54.3
	Pezicula livida	47.1	46.5	58.7
	Pezicula cinnamomea	20.6	39.0	54.3
Pinus sylvestris	*Pezicula livida*	65.9	75.7	71.9
	Sclerophoma pityophila	54.5	21.6	14.1
	Sirodothis spp.	13.6	18.9	28.9
Quercus robur	*Amphiporthe leiphaemia*	45.2	48.9	46.5
	Colpoma quercinum	95.2	62.2	60.5
	Pezicula cinnamomea	11.9	20.0	60.5
	Phialocephala cf. *dimorphospora*	14.3	15.6	27.9

identification is almost impossible (e.g. Xylariaceae, *Phialophora, Lecytophora).* Other fungi only produce microconidial or spermatial forms in culture, although on natural substrates macroconidia are readily found (e.g., *Cryptosporium betulinum, Fusicoccum macrosporum, Dothiorella advena, Cryptosporiopsis* spp., *Durandiella gallica).* Finally, in other species (e.g. *Phialocephala* cf. *dimorphospora),* colony characteristics were in agreement with known species but sporulation was not.

Considerations on species diversity apply mainly to fungi from dead bark tissue, the quantity of species in living bark being much smaller (Tab. 6a). In *Quercus robur* dead bark tissue yielded 60 species, living bark tissue 10 and wood only 4. The fungi colonizing living bark tissue and wood are often present also in dead bark tissue, their frequency in the latter being usually much higher than in both living bark tissue and wood combined. Species of *Aposphaeria* are an exception in this respect. These fungi were sometimes more frequent in wood than in living bark tissue (e.g. *Betula, Fraxinus, Quercus).*

In spite of the great species diversity in dead bark, only few fungi per tree species were dominant (Tab. 4). Over 30% of branches were colonized by only one to three fungal species. Approximately half of all fungal taxa (taking into account the sterile mycelia) were present in only one or two branches of each tree species.

Host range

There were marked differences in the spectrum of trees colonized by a given fungal species. Each tree is colonized by a few host-specific fungi (Tab. 5). Some of these were isolated occasionally from other trees, but only when these grew in the vicinity of the main host. Examples are *Amphiporthe leiphaemia,* isolated from *Carpinus betulus* in a stand of *Quercus robur; Anthostomella pedemontana,* isolated from *Fagus sylvatica* under *Pinus sylvestris; Tubakia dryina,* isolated from *Larix decidua* mixed with *Quercus robur; Prosthemium betulinum* and *Pseudovalsa lanciformis* (Anamorph: *Coryneum brachyurum),* isolated from *Fraxinus excelsior* in a stand of *Betula pendula.*

For some fungi, on the other hand, the host range was rather broad. *Alternaria alternata* occurred on nine tree species, but usually only on few branches, *Fraxinus excelsior* being an exception (Tab. 6a). *Cladosporium cladosporioides* was present on all tree species, but was not frequent with the exception of *Fraxinus excelsior. Epicoccum nigrum* was infrequently isolated from nine tree species, the highest percentage being on *Larix decidua. Lecytophora hoffmannii* was present on ten tree species but was comparatively frequent only on coniferous hosts.

Tab. 5.– Host specific fungal endophytes isolated from living branch bases.

tree species	fungal species
Abies alba	*Durandiella gallica, Grovesiella abieticola*
Acer pseudoplatanus	*Diplodina acerina, Myxosporium carneum, Pezicula acericola, Splanchnonema pupula, Petrakia irregularis*
Alnus glutinosa	*Cryptospora suffusa, Melanconis thelebola, Tympanis alnea*
Betula pendula	*Cryptospora betulae, Melanconis stilbostoma, Trimmatostroma betulinum*
Carpinus betulus	*Diaporthe carpini, Melanconiella spodiaea*
Fagus sylvatica	*Asterosporium asterospermum, Fusicoccum macrosporum, Neohendersonia kickxii*
Fraxinus excelsior	*Coniothyrium fraxini*
Larix decidua	*Sirodothis* sp.
Picea abies	*Tryblidiopsis pinastri*
Pinus sylvestris	*Crumenolopsis pinicola, Therrya* spp.
Quercus robur	*Amphiporthe leiphaemia, Colpoma quercinum, Pseudovalsa longipes*

Seven species of *Mollisia* were isolated. Only *M. cinerea* showed a large host range with a frequency of 2.5% *(Carpinus)* to 65.8% *(Picea). M. cinerea* was also one of the few Discomycetes which produced apothecia with ripe ascospores in cultures kept at low temperatures and high humidity. The colonies were variable, even if these were derived from the same inoculum.

The genus *Pezicula* was represented by five species and at least six further culture types. Colonies produced macroconidia and microconidia of the *Cryptosporiopsis*-type and in some cases apothecia with ripe ascospores in culture. Species and culture types were identified by growing several cultures from each tree species simultaneously under the same conditions, with special consideration being given to colony morphology (i.e. growth rate, colour and structure, production of additional structures, pigmentation of media). Only *P. cinnamomea* occurred on all tree species. Its frequency ranged from 3.4% on *Pinus* to 46% on *Abies* (Tab. 6a). *P. livida* was the second most frequent species and was isolated mainly from conifers but also from deciduous trees. *Pezicula carpinea* (and *Pezicula* cf. *carpinea)* occurred on six deciduous tree species, and the other *Pezicula* species were isolated only from one or two hosts.

Five species of *Phialocephala* were isolated. *Ph.* cf. *dimorphospora* was the most frequent one and occurred on all tree species examined with a frequency ranging from 2.1% *(Pinus)* to 40% *(Acer;* Tab. 6a). Only on *Picea abies* was a different species of *Phialocephala* more frequent. A comparison between our isolates of *Phialocephala* cf. *dimorphospora* and an isolate from CBS (Baarn) showed practically no differences in microscopic features. However, there were large differences in growth type and colony structures. In addition, our isolates often produced red crystals absent in the CBS cultures. Further comparison demonstrated that the fungus isolated here as a frequent endophyte is identical to the one isolated from dead branches and termed *Phialocephala* sp. (Butin & Kowalski 1983a, 1983b, 1986) and *Phialocephala dimorphospora* (Butin & Kowalski 1990; Kowalski & Butin 1989) respectively.

Species of *Phomopsis* were isolated from all tree species except *Betula,* but their frequency was very variable, ranging from 2.1% *(Pinus)* to 51.4% *(Fraxinus).* On *Fraxinus, Phomopsis* spp. were the most common taxa, followed by *Pezicula* species (Tab. 6a). Two as yet undeterminable culture types of *Phomopsis* were isolated from several different hosts.

Several genera of Xylariaceae were isolated. All except *Anthostomella* produced only the anamorph or remained sterile, in which case the genus was determined by specific structures arising in culture. The *Geniculosporium*-anamorph of *Hypoxylon serpens* was the only Xylariaceae found on all tree species, with frequencies

Tab. 6a, b.– Endophytes at the base of branches of coniferous and deciduous trees (tree species in alphabetical order).

a. species isolated from more than two branches

Taxon	% of living branches colonized			density index in peridermal layer ***	% dead branches colonized ****
	peridermal bark	subperidermal bark	wood		
		Abies alba			
Coniothyrium fuckelii Sacc.	3.0			1.3	
Didymosphaeria igniaria Booth*	4.0			1.3	
Durandiella gallica Morelet*	6.0			1.8	++
Geniculosporium serpens Chesters & Greenhalgh	12.0			1.6	
Godronia cassandrae Peck*	5.0			1.4	
Grovesiella abieticola (Zell. & Goodd.) Morelet & Gremmen*	24.0			1.5	++
Lecytophora hoffmannii (van Beyma) W. Gams & McGinnis	7.0	2.0		1.1	++
Mollisia cinerea (Batsch:Fr.) Karst.	12.0			1.3	++
Pezicula cinnamomea (DC.) Sacc.	46.0				
Pezicula livida (Berk. & Br.) Rehm*	14.0			2.8	+++
Phialocephala cf. *dimorphospora* Kendrick	7.0			1.1	++
Phialocephala fortinii Wang & Wilcox	6.0			1.3	++++
Phialocephala sp.	3.0			1.3	+++
Phomopsis spp.	50.0	4.0		3.7	++
Rosellinia sp.*	3.0			1.3	
Sclerophoma pithyophila (Corda) Höhn.	3.0			1,7	+
Torula sp.	5.0			2.4	
sterile mycelia	6.0	1.0	1.0		
others**	33.0	1.0	6.0		

		Acer pseudoplatanus			
Aposphaeria spp.	10.0	2.0	2.0	1.8	+++
Diplodina acerina (Pass.) Sutton	22.0	2.0		1.3	
Geniculosporium serpens Chesters & Greenhalgh	8.0			3.0	
Godronia urceolus (Alb. ex Schw.) Karst.	6.0			2.0	
Mollisia cinerea (Batsch ex Merat) Karst.	18.0			2.1	
Mollisia sp.	12.0			2.2	
Myxosporium carneum Lib.	6.0			1.3	
Petrakia irregularis van der Aa	48.0			2.7	
Pezicula acericola (Peck) Sacc.	6.0			2.0	++
Pezicula carpinea (Pers.) Tul.	6.0			1.0	
Pezicula cinnamomea (DC.) Sacc.	30.0	2.0		2.6	
Phialocephala cf. *dimorphospora* Kendrick	40.0			2.0	+++
Phomopsis pustulata Died.	42.0			4.0	++
Splanchnonema pupula (Fr.) Kuntze*	36.0			1.7	+++
Torula sp.	16.0			3.0	
sterile mycelia	8.0				
others**	56.0				
		Alnus glutinosa			
Aposphaeria spp.	12.0	6.0	6.0	1.2	+++
Coniochaeta velutina (Fuck.) Munk				1.0	
Cryptospora suffusa (Fr.) Tul.	78.0	6.0	2.0	3.9	+++
Melanconis thelebola (Fr.) Sacc.*	6.0			1.0	
Melanconium apiocarpum Link	8.0	2.0	2.0	1.8	
Mollisia cinerea (Batsch ex Merat) Karst.	6.0			1.0	++
Pezicula alni Rehm*	6.0			2.3	
Pezicula cinnamomea (DC.) Sacc.	28.0	4.0		2.1	+
Pezicula cf. *carpinea* (Pers.) Tul.*	6.0			2.0	
Phialocephala cf. *dimorphospora* Kendrick	24.0			2.2	++++
Phialocephala sp.	6.0			1.7	
Phialophora spp.	8.0		2.0	1.5	+
Phoma sp.	8.0			1.0	

Taxon	% of living branches colonized			density index in peridermal layer ***	% dead branches colonized ****
	peridermal bark	subperidermal bark	wood		
Phomopsis alnea Höhn.	10.0			2.2	
Prosthemium stellare Riess	6.0			1.7	
Tympanis alnea (Pers.) Fr.*	8.0	4.0	2.0	1.5	+
Verticicladium trifidum Preuss	6.0			1.0	
sterile mycelia	18.0				
others**	48.0	6.0	4.0		
			Betula pendula		
Aposphaeria spp.	14.0	4.0	10.0	1.4	++
Aureobasidium pullulans (de Bary) Arn.	3.0	2.0	4.0	1.0	
Coryneum depressum Schmidt ex Steudel	3.0			1.5	
Cryptospora betulae Tul.	88.0	3.0	2.0	3.7	++
Geniculosporium serpens Chesters & Greenhalgh	4.0			1.8	
Gnomonia sp.	4.0			1.0	
Melanconis stilbostoma (Fr.) Tul.*	16.0			1.3	+
Mollisia cinerea (Batsch ex Merat) Karst.	9.0			1.6	
Petrakia irregularis van der Aa	4.0			1.2	
Pezicula cinnamomea (DC.) Sacc.	23.0			2.1	++
Phialocephala cf. *dimorphospora* Kendrick	14.0			1.1	++++
Pleomassaria siparia (Berk. & Br.) Sacc.	7.0	1.0		1.7	
Pseudovalsa lanciformis (Fr.) Ces. & de Not.	39.0	3.0		2.2	++
Trimmatostroma betulae (Corda) Hughes	19.0	2.0	1.0	1.5	++++
sterile mycelia	13.0		2.0		
others**	22.0		7.0		

			Carpinus betulus		
Amphiporthe leiphaemia (Fr.) Butin*	3.8			1.3	
Aposphaeria spp.	12.5		1.3	2.0	+++
Cryptospora suffusa (Fr.) Tul.*	6.3			2.4	
Daldinia sp.*	5.0		2.5	5.8	
Diaporthe carpini (Fr.) Fuck.*	30.0	2.5		3.9	++++
Geniculosporium serpens Chesters & Greenhalgh	13.8			1.8	
Hypoxylon cf. *fragiforme* (Pers.:Fr.) Kickx*	3.8			1.3	
Melanconiella spodiaea (Tul.) Sacc.*	13.8			2.7	++
Pezicula carpinea (Pers.) Tul.	51.3	2.5		4.0	++
Pezicula cinnamomea (DC.) Sacc.	5.0			4.2	
Pezicula livida (Berk. & Br.) Rehm*	3.8			1.0	
Pezicula sp.*	8.8			4.3	
Phialocephala cf. *dimorphospora* Kendrick	10.0			1.1	+++
Phomopsis sordida (Sacc.) Höhn.	7.5			2.3	
Pseudovalsa lanciformis (Fr.) Ces. & de Not.*	5.0			1.2	
Verticicladium trifidum Preuss	5.0			1.5	
Xylaria spp.*	6.3			1.2	
sterile mycelia	20.0				
others**	35.0	1.3	3.8		
			Fagus sylvatica		
Anthostomella pedemontana Ferr. & Sacc.	1.9		1.0		
Apiognomonia errabunda (Rob. ex Desm.) Höhn.	3.8	1.3	1.3	2.0	
Aposphaeria spp.	6.9	0.6	0.6	1.2	++
Aspergillus sp.	3.1	0.6		1.0	
Asterosporium asterospermum (Pers. ex Gray) Hughes	38.8	1.3		2.9	++++
Coryneum cf. *brachyurum* Link	2.5			1.0	
Cryptospora betulae Tul.*	3.1			1.6	
Diaporthe eres Nitschke	2.5			2.2	

Taxon	% of living branches colonized			density index in peridermal layer ***	% dead branches colonized ****
	peridermal bark	subperidermal bark	wood		
Fusicoccum galericulatum Sacc.	2.5			1.8	++
Fusicoccum macrosporum Sacc.	15.0	0.6		2.9	++
Geniculosporium serpens Chesters & Greenhalgh	11.9			1.8	
Hypoxylon deustum (Hoffm.:Fr.) Grev.*	1.9			2.3	
Hypoxylon fragiforme (Pers.:Fr.) Kickx*	2.5			1.5	
Lecytophora hoffmannii (van Beyma) W. Gams & McGinnis	5.0			1.0	
Mollisia cinerea (Batsch ex Merat) Karst.	3.8	0.6		1.5	
Neohendersonia kickxii (Westd.) Sutton & Pollak	16.3			1.7	+++
Pezicula carpinea (Pers.) Tul.	6.9			1.9	+++
Pezicula cinnamomea (DC.) Sacc.	15.0			1.8	
Pezicula sp.*	3.1			1.8	
Phialocephala cf. *dimorphospora* Kendrick	5.0			2.2	+++
Trimmatostroma betulinum (Corda) Hughes	1.9	1.3		1.3	
Verticicladium trifidum Preuss	10.6			1.7	
Xylaria spp.*	10.6	1.3		1.6	
sterile mycelia	7.5		1.3		
others**	17.5	3.1	4.4		
		Fraxinus excelsior			
Alternaria alternata (Fr.) Keissl	22.9			1.4	+
Cladosporium cladosporioides (Fresen.) de Vries	4.3			3.0	
Coniothyrium fraxini (Died.) Petr. & Syd.	7.1			1.4	
Coniothyrium fuckelii Sacc.	4.3			1.0	+++

Aposphaeria sp.	1.4		5.7	1.0	+++
Cyclothyrium juglandis (Schum. ex Rabenh.) Sutton	7.1	2.9	2.9	1.0	
Fusarium spp.	5.7			1.8	+++
Gelatinosporium cf. *betulinum* Peck	10.0			2.1	
Geniculosporium serpens Chesters & Greenhalgh	7.1			1.0	
Mollisia cinerea (Batsch ex Merat) Karst.	11.4		1.4	1.4	
Phialocephala cf. *dimorphospora* Kendrick	18.6			1.4	+
Phialophora sp.	4.3			1.3	
Pezicula cf. *carpinea* (Pers.) Tul.	5.7			2.0	
Pezicula cinnamomea (DC.) Sacc.	27.1	1.4		1.8	
Phomopsis spp.	51.4			3.5	++++
Pseudovalsa lanciformis (Fr.) Ces. & de Not.*	8.6	1.4		1.5	
Ulocladium cf. *consortiale* (Thüm.) Simmons	5.7			1.2	
Xylohypha sp.	25.7	2.9	1.4	2.8	
sterile mycelia	17.1				
others**	42.9	2.9	7.1		
		Larix decidua			
Alternaria alternata (Fr.) Keissl.	5.5			1.7	
Coniothyrium fuckelii Sacc.	5.5		1.8	1.5	+
Epicoccum nigrum Link	5.5			1.0	+
Gelatinosporium cf. *betulinum* Peck	14.5	1.8		2.4	
Gelatinosporium spp.	12.7			2.1	+
Geniculosporium serpens Chesters & Greenhalgh	29.1	1.8		1.1	
Hypoxylon fragiforme (Pers:Fr.) Kickx*	5.5			1.1	
Lecytophora hoffmannii (van Beyma) W. Gams & McGinnis	5.5			1.3	++
Mollisia cinerea (Batsch ex Merat) Karst.	25.5			2.1	
Pezicula cinnamomea (DC.) Sacc.	12.7	1.8		3.0	
Pezicula livida (Berk. & Br.) Rehm*	7.3			1.0	++++

Taxon	% of living branches colonized			density index in peridermal layer ***	% dead branches colonized ****
	peridermal bark	subperidermal bark	wood		
Phialocephala cf. *dimorphospora* Kendrick	34.5			1.5	+++
Phomopsis occulta Trav.	12.7			1.7	+
Sirodothis spp.	52.7	10.9	1.8	3.1	+
Torula sp.	7.3			1.8	
Trimmatostroma scutellare (Berk. &. Br.) M. B. Ellis	9.1			1.2	+
Tubakia dryina (Sacc.) Sutton	9.1			2.4	
sterile mycelia	16.4				
others**	47.3	1.8	1.8		
		Picea abies			
Alternaria alternata (Fr.) Keissl.	1.9			1.3	+
Aposphaeria sp.	3.2	1.3	0.6	1.0	+
Aspergillus sp.	3.2			1.0	
Aureobasidium pullulans (de Bary) Arn.	1.3		1.9	1.0	
Cystodendron sp.	2.6	1.3		1.0	
Epicoccum nigrum Link	3.9			2.2	+
Geniculosporium serpens Chesters & Greenhalgh	23.9			1.8	
Lecytophora hoffmannii (van Beyma) W. Gams & McGinnis	15.5	1.9	0.6	1.2	+++
Mollisia cinerea (Batsch ex Merat) Karst.	65.8	13.5		2.3	++
Pezicula cinnamomea (DC.) Sacc.	35.5	2.6		3.2	
Pezicula livida (Berk. & Br.) Rehm*	50.3	10.3	1.9	3.0	
Phialocephala cf. *dimorphospora* Kendrick	11.0	1.9		1.6	++++
Phialocephala sp.	25.1	3.2		3.1	++++

Phialophora fastigiata (Lagerb. & Melin) Conant	1.9			1.3	+
Phomopsis occulta Trav.	12.3			1.7	++
Phomopsis sp.	6.5			2.4	
Rhizoctonia sp.	2.6			1.0	
Rosellinia sp.*	1.9			1.3	
Sirodothis sp.	7.1	1.9	1.3	2.0	++
Tryblidiopsis pinastri (Pers.) Karst*	7.1	1.9		2.0	++
Xylaria sp.*	1.9	0.6		1.0	
sterile mycelia	11.0	1.3			
others**	1.8	0.6			
		Pinus sylvestris			
Anthostomella formosa Kirschst.	2.1			2.0	
Cladosporium cladosporioides (Fresen.) de Vries	3.4			1.6	+
Coniochaeta velutina (Fuck.) Munk	2.1			1.0	
Coniothyrium fuckelii Sacc.	16.6			1.4	++
Coniothyrium pithyophilum (Höhn.) Petr. & Syd.	2.1			1.7	
Crumenulopsis pinicola (Rebent.) Groves*	12.4	1.4		1.8	++
Epicoccum nigrum Link	2.1			1.0	+
Lecytophora hoffmannii (van Beyma) W. Gams & McGinnis	10.3			1.7	++++
Mollisia cinerea (Batsch ex Merat) Karst.	18.6			1.9	++
Pezicula cinnamomea (DC.) Sacc.	3.4			1.6	
Pezicula livida (Berk. & Br.) Rehm*	71.0	4.8	0.7	4.1	++++
Phialocephala cf. *dimorphospora* Kendrick	2.1			1.0	+
Phomopsis occulta Trav.	2.1			1.3	
Sclerophoma pithyophila (Corda) Höhn.	28.3	3.4		2.3	++
Sirodothis spp	36.5	7.6	2.1	2.4	+++
Sphaeropsis sapinea (Fr.) Dyko & Sutton	2.1			1.3	+
Therrya spp.	19.3	4.8		1.6	++

Taxon	% of living branches colonized			density index in peridermal layer ***	% dead branches colonized ****
	peridermal bark	subperidermal bark	wood		
Verticicladium trifidum Preuss	8.3			1.7	
sterile mycelia	14.5	2.8	0.7		
others**	20.0	2.8	0.7		
		Quercus robur			
Alternaria alternata (Fr.) Keissl.	4.6			1.0	
Amphiporthe leiphaemia (Fr.) Butin	46.9	3.1		2.8	++
Apiognomonia errabunda (Rob. ex Desm.) Höhn.	1.5			1.0	
Aposphaeria spp.	15.4	1.5	6.9	1.8	
Colpoma quercinum (Pers. ex St. Am.) Wallr.*	71.5	3.8		3.0	++++
Coryneum sp.	2.3			1.3	
Cystodendron sp.	3.1			1.5	
Epicoccum nigrum Link	3.1			1.0	+
Geniculosporium serpens Chesters & Greenhalgh	13.1			1.6	
Lecytophora hoffmannii (van Beyma) W. Gams & McGinnis	4.6			1.0	
Mollisia cinerea (Batsch ex Mèrat) Karst.	16.2			1.5	
Monodictys sp.	2.3			1.0	
Nodulisporium sp.	3.8			1.0	
Pezicula carpinea (Pers.) Tul.	3.1			2.8	
Pezicula cinnamomea (DC.) Sacc.	30.8	2.3		2.2	++
Pezicula spp.*	3.8				
Phialocephala cf. *dimorphospora* Kendrick	19.2			2.1	+++

Phomopsis quercella Died.	3.8			3.4	
Pseudovalsa longipes (Tul.) Sacc.	18.5	0.8	0.8	2.0	++
Rosellinia sp.*	3.1	0.8		2.5	
Ulocladium chartarum (Preuss) Simmons	4.6			1.3	
Verticicladium trifidum Preuss	6.9			1.7	
Xylaria spp.*	5.4			1.1	
sterile mycelia	7.7	0.8			
others**	20.0	1.5	1.5		

Explanations:

* only anamorph observed

** isolated only from one or two branches (see Tab 6b)

** density index = $\frac{\text{number of cultures produced by one species}}{\text{number of branches in which the species was present}}$

**** see Butin and Kowalski (1983a; 1983b; 1986. 1990); Kowalski and Butin (1989), according to the following scale:

\+ up to 5 % of dead branches
++ 6–20 % of dead branches
+++ 21–40 % of dead branches
++++ over 40 % of dead branches

6b.– Fungi isolated only from one or two branches of a given host. Aa: *Abies alba*; Ap: *Acer pseudoplatanus*; Ag: *Alnus glutinosa*; Bp: *Betula pendula*; Cb: *Carpinus betulus*; Fs: *Fagus sylvatica*; Fe: *Fraxinus excelsior*; Ld: *larix decidua*; Pa: *Picea abies*; Ps: *Pinus sylvestris*; Qr: *Quercus robur*.

	Aa	Ap	Ag	Bp	Cb	Fs	Fe	Ld	Pa	Ps	Qr
Basidiomycotina	x							x			
Ascomycetes and Deuteromycetes											
Acremonium sp.				x							
Alternaria alternata (Fr.) Keissler	x	x			x	x				x	
Alternaria tenuissima (Kunze ex. Pers.) Wiltshire				x			x		x		
Aposphaeria epileuca Sacc.										x	
Aspergillus sp.	x		x		x		x	x		x	
Asteroma alneum (Pers.: Fr.) Sutton			x								
Aureobasidium pullulans (de Bary) Arn.			x			x	x	x		x	x
Biscogniauxia nummularia (Bull.) O. Kuntze					x						
Botryosphaeria advena Sacc.											x
Botrytis cinerea Pers.						x		x			
Camarosporium ambiens Grove						x					x
Candida sp.							x			x	x
Cenangium ferruginosum Fr.										x	
Ceuthospora pinastri (Fr.) Höhn.										x	
Chaetomium elatum (Kunze) Fr.						x					
Chaetomium sp.	x	x	x	x						x	x
Chromelosporium sp.						x		x	x	x	
Cladosporium cladosporioides (Fresen) de Vries	x	x	x	x	x	x		x	x		x
Cladosporium herbarum (Pers.) Link			x			x	x				
Cladosporium macrocarpum Preuss							x				
Coleophoma empetri (Rostr.) Petr.							x				
Coniochaeta subcorticalis (Fuckel) Munk											x
Coniochaeta tetraspora Cain	x										
Coniochaeta velutina (Fuckel) Munk	x			x	x	x		x	x		
Coniosporium olivaceum Link: Fr.		x									
Coniothyrium conorum Sacc.	x										

Coniothyrium fuckeli Sacc.				x		x			x		x
Coniothyrium sp.			x		x						x
Coryneum sp.				x							
Cryptocline sp.										x	
Cryptospora suffusa (Fr.) Tul.											x
Cyclothyrium juglandis (Schum. ex Rabenh.) Sutton		x									
Cystodendron sp.		x	x				x	x		x	
Cytoplaea cf. *platani* (Sacc.) Pet. & Syd.			x								
Cytospora abietis Sacc.	x										
Cytospora ambiens Sacc.		x			x	x					
Cytospora intermedia Sacc.											x
Daldinia sp.								x	x		x
Dermea piceina Groves								x	x		
Dermea tulasnei Groves							x				
Diplodina spp.											x
Discula betulina (Westend.) v. Arx				x							
Discula sp.	x			x							
Durella commutata Fuckel		x				x					
Epicoccum nigrum Link			x	x	x	x	x				
Epithyrium resinae (Sacc. & Berl.) Sacc.									x	x	
Exophiala sp.		x									x
Foveostroma sp.			x								
Fusarium sp.	x										x
Gelatinosporium cf. *betulinum* Peck	x			x	x					x	
Gelatinosporium sp.	x									x	
Geniculosporium serpens Chesters & Greenhalgh			x							x	
Geniculosporium spp.			x						x		
Geotrichum candidum Link ex Leman						x					
Gnomonia sp.		x	x			x					x
Godronia cassandrae Peck				x					x		
Harknesia sp.						x					
Hercospora taleola (Fr.) E. Müller											x
Hormonema cf. *dematioides* Lagerberg & Melin				x			x				
Hormonema sp.	x										

	Aa	Ap	Ag	Bp	Cb	Fs	Fe	Ld	Pa	Ps	Qr
Humicola sp.					x						
Hypoxylon deustum (Hoffm.:Fr.) Grev.	x			x			x		x		
Hypoxylon fragiforme (Pers.:Fr.) Kickx	x	x		x					x	x	x
Hypoxylon unitum (Fr.) Nitschke					x						
Lecytophora hoffmannii (van Beyma) W. Gams & McGinnis		x		x	x		x				
Libertella faginea Desm.					x	x					
Melanconium atrum Link						x					
Melanconium cf. *apiocarpum* Link		x									
Microsphaeropsis olivacea (Bonard.) Höhn.							x			x	
Mollisia cinerea (Batsch ex Merat) Karst.					x						
Mollisia spp.	x			x		x		x		x	x
Monocillium sp.								x			
Myxocyclus polycistis (Berk. & Br.) Sacc.				x							
Naemospora sp.							x				
Nectria coccinea (Pers.:Fr.) Fr.						x					
Nectria fuckeliana Booth									x		
Nodulisporium sp.			x			x					
Oidiodendron griseum Robak	x										
Oidiodendron sp.								x			
Ophiostoma piceae (Münch) H. & P. Syd.	x										
Pezicula livida (Berk. & Br.) Rehm						x	x				
Pezicula sp.		x						x			
Phialocephala sp.			x	x							x
Phialophora melini (Nannf.) Conant											x
Phialophora spp.	x	x				x	x				
Phoma divergens Oudem.							x				
Phoma spp.	x	x		x			x				
Phomopsis sp.								x			
Pithomyces chartarum (Berk. & Curt.) M. B. Ellis			x				x				
Pleomassaria siparia (Berk. & Br.) Rehm		x									
Pleurophomopsis sp.						x					

Prosthecium innesii (Currey) Wehm.		x									
Prosthemium betulinum Kunze ex Schlecht.							x				
Pseudaegerita viridis (Bayliss Elliot) Abdullah & Webster			x								
Pseudovalsa longipes (Tul.) Sacc.							x				
Pycnidiella resinae (Ehrenb.:Fr.) Höhn.								x		x	
Pyrenochaeta cf. *quercicola* Bubak & Kabat		x									
Rhinocladiella atrovirens Nannf.										x	
Rhizoctonia sp.	x					x	x	x		x	x
Rosellinia sp.					x	x	x				x
Sclerophoma pithyophila (Corda) Höhn.								x	x		
Scoleconectria cucurbitula (Tode:Fr.) Booth									x	x	
Sirodothis cf. *inversa* (Fr.) Sutton & Funk					x						
Sirodothis sp.	x			x							
Sordaria fimicola (Rob.) Ces. & de Not.								x	x	x	
Sordaria macrospora Auerswald.			x								x
Sporormiella intermedia (Auersw.) Ahmed & Cain					x	x			x	x	
Sporotrichum sp.				x							
Stegonsporium pyriforme (Hoffm.:Fr.) Corda							x				
Stemphylium botryosum Wallr.	x										
Stemphylium sp.							x				
Stigmina sp.									x		
Torula sp.					x	x					
Trimmatostroma betulinum (Corda) Hughes		x	x				x				
Trimmatostroma sp.	x				x					x	
Troposporella fumosa Karst.	x										
Tubakia dryina (Sacc.) Sutton.											x
Tubercularia vulgaris Tode		x			x	x					
Ulocladium chartarum (Preuss) Simmons					x		x	x			
Verticicladium trifidum Preuss.							x		x		
Xylaria spp.	x	x	x								
Xylohypha sp.						x					

of 1.4% *(Pinus)* to 29.9% *(Larix)* of all branches (Tab. 6a). A broad host spectrum was also established for the *Nodulisporium*-anamorph of *Hypoxylon fragiforme*.

Among the other species found on several different hosts, *Verticicladium trifidum* occurred on five deciduous and two coniferous hosts. It was especially common on *Fagus* and *Pinus* (10.6% and 8.3% of all branches, respectively).

The density index (Tab. 6a) points to differences in the distribution of fungi within the branch base. Since six pieces were taken from the dead peridermal layer, this index can range from one to six, indicating the degree of colonization by a given fungus. High density indices were observed for *Pezicula* spp., *Phomopsis* spp., *Cryptospora suffusa, C. betulae, Diaporthe carpini, Sirodothis* sp. and *Colpoma quercinum*. Fungi with large overall branch colonization frequency but with a low density index within branches include *Coniothyrium fuckelii, Crumenulopsis pinicola, Lecytophora hoffmannii, Mollisia cinerea, Neohendersonia kickxii, Phialocephala* cf. *dimorphospora, Pseudovalsa longipes, Therrya* spp. and *Trimmatostroma betulinum*.

Discussion

The aim of this paper was to list descriptively the fungi associated with branch bases of living trees, without attempting any statistical analysis of the data. However, a number of points of interest can be seen even without statistics.

Almost all living branches of forest trees are colonized in their basal parts by endophytic fungi. In this respect, the branch base does not differ from younger parts of the branches or from other organs of the tree.

The fungi that colonize living basal parts of branches are almost exclusively Ascomycetes and Deuteromycetes, and only in few cases Basidiomycetes, as already described for other plant species (Sieber, 1988; Butin, 1986; Petrini, 1986; Widler & Müller, 1984; Luginbühl & Müller, 1980). Although living branches are colonized by many fungal species, only few species per host are dominant and in general only one to three are present in more than 30% of all branches. These results are consistent with those presented by other authors (Fisher & Petrini, 1990; Sieber, 1989; Petrini & Fisher, 1988; 1990; Sieber & Hugentobler, 1987).

Common endophytic taxa

Several fungal taxa are particularly frequent in living plant organs. Commonly occurring species include *Phomopsis* and *Pezicula* (and its *Cryptosporiopsis* anamorph) as well as some ubi-

quitous species of *Alternaria, Cladosporium, Epicoccum,* and several representatives of the Xylariaceae summarized in Petrini (1986) and Carroll (1988). In addition, at least some species of *Mollisia* and *Phialocephala* are very likely common endophytes. *Phialocephala* species were so far not reported to live endophytically. They may have been isolated by other authors but not recognized as such. Most *Phialocephala* isolates sporulate only after an incubation period of several weeks at high humidity and low temperature, as described by Kendrick (1961) for *Phialocephala dimorphospora. Mollisia* species were so far only rarely recorded as endophytes (Sieber, 1989). *M. cinerea* was found as a common endophyte in needles and twigs of *Juniperus* at one location in Switzerland (Petrini & Müller, 1979). In our investigation *M. cinerea* was one of the most common endophytes and occurred on all tree species, although conifers, particularly *Picea abies,* were preferred.

Host range

In relation to the host spectrum, two groups of endophytes can be distinguished. Some taxa are very specific and occur almost exclusively on one host, others are present on many hosts at varying degrees of frequency.

Each tree species has few highly specific endophytes. Examples are cited in Tab. 5. In several cases host specific fungi were also isolated, if only rarely, from other tree species growing in the vicinity of the main host. This supports the hypothesis that some endophytes are able to colonize morphologically similar hosts growing at the same site (Petrini & Fisher, 1988; Petrini, 1986). All the above mentioned fungi are known saprobes, and determination keys show that in their saprobic phase they show some kind of host specificity (Gremmen & Morelet, 1971; Sutton, 1980; Dennis, 1978; van der Aa, 1968; Munk, 1957; Grove, 1935; 1937).

A second group of endophytes demonstrated little or no host specificity and occurred on all or most tree species. *Mollisia cinerea, Phialocephala* cf. *dimorphospora, Pezicula cinnamomea, P. livida,* the *Geniculosporium* anamorph of *Hypoxylon serpens* and several *Phomopsis* species were the most common. Their saprobic character as reported in the literature is variable and not always in accordance with their host spectrum in the endophytic phase. For instance, *Mollisia cinerea* is one of the most common colonizers of decaying wood, especially *Quercus* and *Fagus* (Breitenbach & Kränzlin, 1981; Rehm, 1896), but as an endophyte it seems to prefer coniferous trees (Tab. 6a). *Pezicula cinnamomea* is known especially from *Quercus* and *Castanea* (Sutton, 1980; Johansen, 1949; Wollenweber, 1939), but its host range in the endophytic phase is much larger and not limited

to deciduous trees. On the contrary, *P. cinnamomea* was most common on branches of *Abies alba*. Taxonomic work in progress in our laboratories may show that the taxon found on coniferous hosts is distinct from *P. cinnamomea*, even if no cultural differences are evident. *Pezicula livida* is known only from conifers (Dennis, 1978; Wollenweber, 1939), and this is confirmed by our isolations, where *P. livida* occurred frequently on conifers, especially *Pinus*, but was found only in a few branches of deciduous trees.

Host specificity of *Phomopsis* is hard to determine, as most keys delineate the species by substrate only and the genus is in urgent need of taxonomic treatment. Only *Phomopsis occulta* is supposed to be typical for conifers (Grove, 1935; Hahn, 1930; Diedicke, 1911). In a different taxonomical scheme, *Ph. occulta* and several other species from deciduous trees are seen as the anamorphs of *Diaporthe eres* Nits. (Wehmeyer, 1933).

Verticicladium trifidum, so far known only from dead pine needles (Kowalski, 1988; Gremmen, 1960), was isolated as an endophyte from seven tree species. Surprisingly, *Pinus* branches were not among those most commonly colonized by this fungus. Petrini & Fisher (1988) isolated *V. trifidum* to a much higher degree for *Pinus* and thus classified it as a host specific endophyte.

Influence of site and isolation procedure

The composition and frequency of host-specific fungal species seems to be site-dependent. Of the three most common *Quercus robur* endophytes found in this investigation, only *Coryneum umbonatum* (Teleomorph: *Pseudovalsa longipes)* was previously recorded in England (Petrini & Fisher, 1990), whereas *Colpoma quercinum* and *Amphiporthe leiphaemia* were not. At other localities, none of the three species were found in living branches (Boddy & Rayner, 1984). *Asterosporium asterospermum*, the most common species on *Fagus sylvatica* in this study, was not recorded by Petrini & Fisher (1988). Sieber (1989) isolated *Phomopsis occulta* from *Abies alba* in Switzerland as the most common endophyte, which is in agreement with our results. *Agyriellopsis caeruleo-atra* Höhn., the third most common taxon in Sieber's (1989) isolations from *Abies alba*, was not found at all here. *Corniculariella abietis* and *Pocillopycnis umensis* (Bubak & Vleugel) Dyko & Sutton, relatively common on the *Picea abies* samples examined by Sieber (1989), were absent in the branches of our samples; on the contrary, *Mollisia cinerea* and *Phialocephala* species were absent in Switzerland.

Differences in composition of the endophytic flora in branches of forest trees can have several causes. The degree of colonization of a tree tissue by endophytes may be dependent on the diversity of the

plant community. In addition, the amount of fungal inoculum might influence which fungi appear dominant. Sieber (1989) registered differences among three localities in Switzerland for *Picea* and *Abies*. Discrepancies between Sieber's study and those of other authors may also be influenced by the position from where samples were taken. Previous investigations were not concerned with the branch base, but rather with branch and twig segments of different age (Fisher & Petrini, 1990; Sieber, 1989; Petrini & Fisher, 1988; 1989). In this study endophytic colonization was more or less strongly dependent on branch diameter, which only to a certain degree reflects the age of the branch.

Our isolation procedure relies on the one used by Sieber (1989), but differs from that described by other authors (Griffith & Boddy, 1988). Too short superficial sterilization times may result in a higher percentage of ubiquitous fungi such as *Epicoccum nigrum, Cladosporium* spp. and several fast growing fungi such as *Sordaria fimicola* and *Verticicladium trifidum*. These may prevent detection of other fungi.

Role of tissue state

Only dead bark tissues host a large diversity of fungi, and this leads to high overall colonization rates on living branches. Living bark tissue and to a greater degree xylem seem to be colonized selectively by fungi that may be tissue specific (Fisher & Petrini, 1990; Petrini & Fisher, 1988). Previous studies on endophytes in branches mainly considered bark and xylem separately. In the present investigation, the subdivision of bark into peridermal and subperidermal tissues showed that the physiological condition of the tissue is of great importance. Some fungi do not possess the capability to colonize living bark tissue and active xylem, but can only exist saprophytically in dead or dying outer bark cells of living branches. For these fungi, the physiological condition of the tissue rather than tissue specificity are apparently the limiting factor. The presence of these fungi as branch pruners in xylem after death of the branch supports this hypothesis (Kowalski & Butin, 1989; Butin & Kowalski, 1983a; 1983b; 1986; 1990).

For several reasons, it seems necessary to define bark and xylem endophytes more accurately. The state of the tissue could be a means to do so. For instance, only fungi isolated from living tissue should be called endophytes, and we propose the term "phellophytes" for those isolated from dead bark layers. This differentiation should be of interest for pathologists who would like to know whether a fungus can be a latent pathogen (Sinclair, 1991), or merely lives in dead tissues from where it might attack living tissue at times of reduced

host vigour. Within a given spectrum of fungal endophytes, differences in colonization frequency of various tissues indicate that some fungi are able to pass from dead into living tissue only to a limited extent.

Comparison with branch-pruning fungi

To elucidate whether the most common branch pruning fungi are already present in living branches, the results presented here have been compared with those obtained for dead branches of the same eleven tree species (Kowalski & Butin, 1989; Butin & Kowalski, 1983a; 1983b; 1986; 1990). Many fungi observed on living branches are also the most frequent colonizers of dead branches. Presumably, the "endophytic phase" gives these fungi an advantage in colonizing branches which slowly die due to light deficiency. Worth mentioning in this respect are *Amphiporthe leiphaemia, Colpoma quercinum, Phialocephala* spp., *Diaporthe carpini, Pezicula* spp., *Trimmatostroma betulinum, Splanchnonema pupula, Aposphaeria* spp., *Grovesiella abieticola, Phomopsis* spp., *Cryptospora suffusa, C. betulae, Sirodothis* spp., *Therrya* spp. and to a certain degree *Mollisia cinerea* and *Lecytophora (Phialophora) hoffmannii*. Several species show the same colonization frequency on living as on dead branch bases. In a few cases this correspondence is even present at the diameter level (compare Tab. 4 with Kowalski & Butin, 1989; Butin & Kowalski, 1983a; 1983b; 1986; 1990). This is true for *Colpoma quercinum, Asterosporium asterospermum, Fusicoccum macrosporum, Diaporthe carpini,* and also for *Pezicula livida* and *Sirodothis* spp. on *Pinus*.

Not all branch pruning fungi, however, seem to have adapted to endophytic life. This is evident for the bark colonizers *Hercospora taleola, Aleurodiscus amorphus* and *Lachnellula calycina,* and for colonizers of wood such as *Durella commutata* and *D. atrocyanea.* These were frequent in wood of dead, mostly debarked branches but were hardly isolated from living branches. The same applies to *Rhinocladiella atrovirens* on *Pinus sylvestris* (Butin & Kowalski, 1990). Basidiomycetes also colonized branches after death, with very few exceptions.

Some fungi, in contrast, seem to be totally adapted to an endophytic life but do not colonize extensively the branch after death. The Xylariaceae were represented by many genera or species, even if, with the exception of the *Geniculosporium*-anamorph of *Hypoxylon serpens* (Chesters & Greenhalgh, 1964), they were not frequent. On dead branches, however, xylariaceous fungi were very rare (Kowalski & Butin, 1989; Butin & Kowalski, 1983a; 1983b; 1986; 1990). This is surprising as they are generally known as xylotrophic endophytes (Chapela, 1989; Chapela & Boddy, 1988) and as saprobic colonizers

of stumps, logs and branches (Rogers & Callan, 1980). It seems possible that these fungi colonize living bark of all tree parts endophytically but require larger branch diameters or more constant moisture conditions in ground vicinity in order to become established in the succession of decay fungi.

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