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Review

Endophytic fungi in forest trees: are they mutualists?

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ABSTRACT

Forest trees form symbiotic associations with endophytic fungi which live inside healthy tissues as quiescent microthalli. All forest trees in temperate zones host endophytic fungi. The species diversity of endophyte communities can be high. Some tree species host more than 100 species in one tissue type, but communities are usually dominated by a few host-specific species. The endophyte communities in angiosperms are frequently dominated by species of Diaporthales and those in gymnosperms by species of Helotiales. Divergence of angiosperms and gymnosperms coincides with the divergence of the Diaporthales and the Helotiales in the late Carboniferous about 300 million years (Ma) ago, indicating that the Diaporthalean and Helotialean ancestors of tree endophytes had been associated, respectively, with angiosperms and gymnosperms since ≥300 Ma. Consequently, dominant tree endophytes have been evolving with their hosts for millions of years. High virulence of such endophytes can be excluded. Some are, however, opportunists and can cause disease after the host has been weakened by some other factor. Mutualism of tree endophytes is often assumed, but evidence is mostly circumstantial. The sheer impossibility of producing endophyte-free control trees impedes proof of mutualism. Some tree endophytes exhibit either a pathogenic or a putatively mutualistic behaviour depending on the situation. The lifestyle (mutualism, commensalism, parasitism) of most tree endophytes is, however, not known. They are just there in the tissue and resume growth at the onset of natural senescence of the host tissue on which they eventually sporulate. Density of colonization of conifer needles by endophytes increases with needle age. It is postulated that the needles die as soon as colonization density reaches a threshold value. Normally, the threshold is not reached before the onset of natural senescence. The threshold value may, however, be reached earlier under some adverse conditions, e.g. lack of light in dense stands. As a consequence, endophytes kill the needles prematurely. Needle endophytes could, thus, be useful to eliminate "parasitic" needle mass, i.e. needles which consume more than they produce.

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1. Introduction

Fungi are omnipresent on organic compounds. The majority are saprobes and decompose dead organic matter. Many, however, are specialized to attack and infect living organisms. Some of these are pathogens, disease symptoms becoming manifest after a comparatively short period of incubation. Others infect living organisms but symptoms do not develop,

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because, once inside the tissue, they assume a quiescent (latent) state either for the whole lifetime of the infected plant tissue or for an extended period of time, i.e. until environmental conditions are favourable for the fungus or the phase disposition of the host changes to the advantage of the fungus. These fungi are considered 'endophytes'. If the symbiosis occurs in leaves or needles the plant-fungus entity is sometimes termed 'mycophylla' corresponding with 'mycorrhiza' - the symbiosis of primary roots and fungi.

Two groups of endophytic fungi are recognized, clavicipitalean and non-clavicipitalean endophytes (Carroll 1988; Petrini 1991; Schulz & Boyle 2005; Stone & Petrini 1997; Stone *et al.* 2004). Species of the Clavicipitaceae form symbioses almost exclusively with grass hosts. Grass endophytes colonize their hosts systemically (except the roots) and several species are transmitted vertically by seeds to the next host generation. Grass endophytes enhance host fitness by the production of both alkaloids, that inhibit insect herbivory, and metabolites that stimulate plant growth (Clay 1991). In contrast, host colonization by non-clavicipitalean endophytes is non-systemic and is restricted to disjunctive, endophytic microthalli which may consist of only a few cells (Stone 1987). Non-clavicipitalean endophytes represent a broad range of species from several families of ascomycetes and probably occur in all plant species of the temperate zones including grasses (Sieber *et al.* 1988).

Many excellent reviews about endophytic fungi in woody plants have already been published (Carroll 1988; Petrini 1991; Schulz & Boyle 2005; Stone & Petrini 1997; Stone *et al.* 2004). In this review, I will explore the different lifestyles (mutualism, commensalism, parasitism) of endophytic fungi. A list of hosts, number of species isolated, names of dominant endophytes and their taxonomic affiliation are presented as a basis to discuss evolution of endophytes. Confirmation of co-evolution of host and endophytes will lead me to explore mutualism and parasitism of endophytes. Emphasis is on endophytes in aerial plant tissues of forest trees in the temperate zones. Those interested in root endophytes are referred to other reviews (Addy *et al.* 2005; Jumpponen & Trappe 1998; Sieber 2002; Sieber and Grünig 2006). Referencing is not comprehensive, but I have tried to include key references which may provide access to additional literature on fungal endophytes.

2. Diversity, taxonomy and evolution

Surveys of many tree species during the past 30 y have shown that colonization by endophytic fungi is ubiquitous (Tables 1 and 2). The number of species detected depends on biotic, abiotic and experimental factors, e.g. the host species, type and phase disposition of the plant organ, edaphic and climatic conditions, the isolation procedure and the number and size of samples. Species diversity of internal mycobiota is high in many tree species, e.g. more than 120 species were detected in twigs of *Carpinus caroliniana* and needles of *Abies alba* (Bills & Polishook 1991; Sieber-Canavesi & Sieber 1987, 1993) (Tables 1 and 2). Species diversity is usually high even within very small volumes of tissue. Up to six species of endophytes were detected within 1.5 cm^2 of bark of 2-yr-old coppice shoots of chestnut (Bissegger & Sieber 1994). Carroll (1995) microdissected Douglas fir needles and was able to isolate up to four different species per needle. Not only species diversity but also within-species diversity, i.e. genotype diversity, within small volumes of plant tissue can be high. Isolates from Norway spruce needles revealed single needles colonized by several different genotypes of *Lophodermium piceae* (Müller *et al.* 2001).

Endophyte isolates often remain sterile making morphology-based identification impossible. If sporulation occurs identification is frequently possible to the genus only because the species is either not described or the morphology of the fructification in culture deviates significantly from the one produced on the host. Most species descriptions are based on fungal morphology formed on the host, making identification based on cultures difficult. Comparison of the culture morphology of endophyte isolates with that of pure cultures originating from fructifications formed on host tissues sometimes helps. Alternatively, DNA sequences of both endophyte isolates and cultures originating from fructifications on the host can be compared. However, many fungi occur as endophytes on a broad range of hosts but sporulate only on one or a few of them (Baayen *et al.* 2002; Petrini & Petrini 1985), making such 'comparison' approaches a Herculean effort. In addition, the high diversity of fungal endophyte-DNA sequences, which do not match any of the sequences currently available from DNA databases (Higgins *et al.* 2007), and my own experience with sporulating but unidentifiable cultures of endophytic fungi indicate that many endophytes are undescribed species. Probabilistic considerations lead to the same conclusion. If there were two host-specific fungal endophytes per plant species, a minimum of between 500'000 and 600'000 endophyte species would exist, assuming that there are between 250'000 and 300'000 plant species worldwide (Schmit & Mueller 2007; Wilson 1988). About 79'000 species of fungi have been described with only 35'000 of them being plant-associated microfungi (Schmit & Mueller 2007). If we assume that all of them were occurring as endophytes, at least 465'000 endophyte species would be undescribed.

Since host tissues can be sampled methodically, studies on endophytic fungi are useful to discover and estimate fungal diversity and to monitor changes of this diversity. The concomitant use of several selective isolation methods is, however, important to get a complete picture of the hidden diversity (Bills 1996). Extraction and amplification of fungal DNA directly from plant tissues for detection, quantification and identification of endophytes is an alternative. Identification is performed by comparisons of DNA sequences with those available from databases (Ganley & Newcombe 2006). This approach speeds-up diversity surveys and will allow identification of fungi that do not grow or do not sporulate in culture. Elimination of epiphytic DNA might, however, constitute a problem since classical surface-sterilization, which is based on a sequence of immersions in ethanol and either sodium hypochlorite or hydrogen peroxide, kills the organisms on the surface but does not remove the DNA. Thus, some procedure that includes the use of nucleases must be developed. Another problem occurs if erroneous sequences and/or sequences of misidentified fungi are deposited in these databases. In addition, ITS sequences may be highly diverse

Table 1 – Dominant fungal endophytes in leaves or needles of various tree hosts

Host	Country	Number of species	Dominant species	Order[a]	References
Aceraceae					
Acer macrophyllum	British Columbia	16	*Phomopsis sp.*	Diaporthales	Sieber and Dorworth (1994)
			Diaporthe eres	Diaporthales	
Acer pseudoplatanus	Germany	22	*Phloeospora aceris*	Mycosphaerellales	Pehl and Butin (1994)
			Cryptodiaporthe hystrix	Diaporthales	
Betulaceae					
Alnus rubra	British Columbia	23	*Gnomonia setacea*	Diaporthales	Sieber *et al.* (1991a)
			Gnomoniella tubiformis	Diaporthales	
Betula pubescens	Switzerland	15	*Venturia ditricha*	Pleosporales	Barengo *et al.* (2000),
	Finland		*Phomopsis sp.*	Diaporthales	Helander *et al.* (1993)
Cupressaceae					
Calocedrus decurrens	Oregon	>3	*Linodochium sp.*		Petrini and Carroll (1981)
			Geniculosporium sp.	Xylariales	
Chamaecyparis lawsoniana	Oregon	>9	*Scolecosporiella sp.*	Pleosporales	Petrini and Carroll (1981)
			Nodulisporium sp.	Xylariales	
Juniperus communis	Switzerland	83	*Kabatia juniperi*	Dothideales	Petrini and Müller (1979)
			Anthostomella formosa	Xylariales	
Juniperus occidentalis	Oregon	>5	*Sarea difformis*	Agyriales	Petrini and Carroll (1981)
Sequoia sempervirens	California	26	*Chloroscypha chloromela*	Helotiales	Carroll and Carroll (1978),
			Cryptocline sp.	Helotiales	Espinosa-Garcia
			Pezicula livida	Helotiales	and Langenheim (1990)
Thuja plicata	Oregon	>6	*Chloroscypha seaveri*	Helotiales	Petrini and Carroll (1981)
			Geniculosporium sp.	Xylariales	
Fagaceae					
Fagus crenata	Japan	14	*Apiognomonia sp.*	Diaporthales	Kaneko and Kaneko (2004)
			Geniculosporium sp.	Xylariales	
			Ascochyta sp.	Pleosporales	
			Tritirachium sp.		
			Periconiella sp.	Xylariales	
Fagus sylvatica	Germany	64	*Apiognomonia errabunda*	Diaporthales	Pehl and Butin (1994), Sieber
	Switzerland		*Diaporthe eres*	Diaporthales	and Hugentobler (1987)
			Dicarpella dryina	Diaporthales	
Quercus alba	Maryland	18	*Dicarpella dryina*	Diaporthales	Cohen (1999)
			Dicarpella subglobosa	Diaporthales	
Quercus cerris	Italy	7	*Dicarpella dryina*	Diaporthales	Gennaro *et al.* (2003),
			Cladosporium cladosporioides	Mycosphaerellales	Ragazzi *et al.* (2003)
Quercus emoryi	Arizona	>12	*Asteromella sp.*	Pleosporales	Faeth and Hammon (1997b)
Quercus garryana	Oregon	5	*Apiognomonia quercina*	Diaporthales	Wilson and Carroll (1994)
Quercus ilex	Switzerland	33	*Phyllosticta ilicina*	Dothideales	Collado *et al.* (1996),
	Spain		*Phomopsis glandicola*	Diaporthales	Fisher *et al.* (1994)
	UK		*Acremonium strictum*	Hypocreales	
Quercus petraea	Austria	78	*Apiognomonia quercina*	Diaporthales	Halmschlager *et al.* (1993)
			Aureobasidium apocryptum	Mycosphaerellales	
Quercus pubescens	Italy	6	*Cladosporium cladosporioides*	Mycosphaerellales	Ragazzi *et al.* (2003)
			Ulocladium sp.	Pleosporales	
Quercus robur	Italy	25	*Dicarpella dryina*	Diaporthales	Gennaro *et al.* (2003), Pehl
	Germany		*Apiognomonia quercina*	Diaporthales	and Butin (1994),
			Ulocladium sp.	Pleosporales	Ragazzi *et al.* (2003)
			Trichoderma viride	Hypocreales	
Pinaceae					
Abies alba	Switzerland	127	*Cryptocline abietina*	Helotiales	Sieber-Canavesi and Sieber
			Gloeosporidiella sp.	Helotiales	(1987, 1993)
Abies amabilis	Oregon	>4	*Phyllosticta sp.*	Dothideales	Carroll and Carroll (1978)
	Washington		*Lophodermium sp.*	Helotiales	
Abies balsamea	New Brunswick	>10	*Phyllosticta sp.*	Dothideales	Johnson and Whitney (1989)
			Lophodermium sp.	Helotiales	
Abies concolor	Oregon	>6	*Phyllosticta sp.*	Dothideales	Carroll and Carroll (1978)
	Washington		*Cryptocline sp.*	Helotiales	

(*continued on next page*)

Table 1 – (*continued*)

Host	Country	Number of species	Dominant species	Order[a]	References
Abies grandis	Oregon Washington	>7	*Phyllosticta sp.* *Cryptocline sp.*	Dothideales Helotiales	Carroll and Carroll (1978)
Abies lasiocarpa	Oregon Washington	>5	*Cryptocline sp.*	Helotiales	Carroll and Carroll (1978)
Abies magnifica	Oregon Washington	>6	*Phyllosticta sp.* *Cryptocline abietina*	Dothideales Helotiales	Carroll and Carroll (1978)
Abies procera	Oregon Washington	>4	*Phyllosticta sp.* *Lophodermium sp.*	Dothideales Helotiales	Carroll and Carroll (1978)
Larix sibirica	Finland Iceland Russia	79[b]	*Monilinia laxa*	Helotiales	Kauhanen *et al.* (2006)
Picea abies	Switzerland	100	*Lophodermium piceae* *Tiarosporella parca*	Helotiales Helotiales	Müller *et al.* (2001), Sieber (1988)
Picea glauca	Québec	14	*Lophodermium piceae* *Mycosphaerella sp.*	Helotiales Mycosphaerellales	Stefani and Bérubé (2006)
Picea mariana	New Brunswick	10	*Cryptocline abietina*	Helotiales	Johnson and Whitney (1992)
Picea sitchensis	UK Oregon Washington	>11	*Lophodermium piceae* *Rhizosphaera kalkhoffii* *Phomopsis sp.*	Helotiales Pleosporales Diaporthales	Carroll and Carroll (1978), Magan and Smith (1996)
Pinus attenuata	Oregon Washington	>4	*Cyclaneusma sp.* *Lophodermium sp.*	Helotiales Helotiales	Carroll and Carroll (1978)
Pinus banksiana	Québec	9	*Coccomyces sp.* *Phomopsis sp.*	Helotiales Diaporthales	Legault *et al.* (1989)
Pinus contorta	Oregon Washington	>5	*Lophodermium sp.*	Helotiales	Carroll and Carroll (1978)
Pinus densiflora	Japan	>8	*Lophodermium pinastri* *Phialocephala sp.*	Helotiales Helotiales	Hata and Futai (1995), Hata *et al.* (1998)
Pinus lambertiana	Oregon Washington	>4	*Lophodermium sp.* *Cyclaneusma minus*	Helotiales Helotiales	Carroll and Carroll (1978)
Pinus monticola	Idaho Oregon Washington	82[c]	*Lophodermium sp.* *Hormonema sp.*	Helotiales Dothideales	Carroll and Carroll (1978), Ganley and Newcombe (2006)
Pinus mugo	Germany Switzerland	11	*Cenangium ferruginosum* *Cyclaneusma minus* *Lophodermium pinastri*	Helotiales Helotiales Helotiales	Sieber *et al.* (1999)
Pinus nigra	Slovenia	n.a.	*Cyclaneusma niveum* *Cenangium ferruginosum*	Helotiales Helotiales	Jurc *et al.* (2000)
Pinus ponderosa	Oregon Washington	>8	*Lophodermium sp.* *Sydowia polyspora*	Helotiales Dothideales	Carroll and Carroll (1978)
Pinus resinosa	Québec	14	*Lophodermium sp.* *Pragmopycnis sp.*	Helotiales Helotiales	Legault *et al.* (1989)
Pinus strobus	Ontario	n.a.	*Lophodermium nitens* *Hormonema sp.*	Helotiales Dothideales	Deckert and Peterson (2000), Deckert *et al.* (2002)
Pinus sylvestris	Poland	86	*Anthostomella formosa* *Lophodermium seditiosum* *Cyclaneusma minus* *Cenangium ferruginosum* *Lophodermium pinastri*	Xylariales Helotiales Helotiales Helotiales Helotiales	Kowalski (1993)
Pinus thunbergii × *densiflora*	Japan	>8	*Lophodermium pinastri* *Phialocephala sp.*	Helotiales Helotiales	Hata *et al.* (1998)
Pseudotsuga menziesii	Oregon Washington	>11	*Rhabdocline parkeri* *Phyllosticta abietis*	Helotiales Dothideales	Carroll and Carroll (1978), Stone (1987)
Tsuga heterophylla	Oregon Washington	>11	*Cryptocline sp.*	Helotiales	Carroll and Carroll (1978)
Tsuga mertensiana	Oregon Washington	>9	*Lophodermium sp.* *Phyllosticta sp.*	Helotiales Dothideales	Carroll and Carroll (1978)
Salicaceae					
Populus tremula	Spain	9	*Penicillium sp.* *Cladosporium maculicola*	Eurotiales Mycosphaerellales	Santamaria and Diez (2005)
Taxaceae					
Taxus brevifolia	Oregon Washington	>6	*Phyllosticta sp.*	Dothideales	Carroll and Carroll (1978)

Table 1 – (*continued*)

Host	Country	Number of species	Dominant species	Order[a]	References
Tiliaceae					
Tilia cordata	Germany	17	*Apiognomonia tiliae* *Mycosphaerella punctiformis*	Diaporthales Mycosphaerellales	Pehl and Butin (1994)

a according to Kirk *et al.* 2001.
b morphotypes, operational taxonomic units (OTUs).
c ITS-sequence types (Ganley & Newcombe 2006).

within the same species and can, thus, lead to an overestimation of the number of species (Grünig *et al.* 2004).

Members of the Betulaceae, Fagaceae, Cupressaceae and Pinaceae have been most intensively examined for the presence of endophytic fungi (Tables 1 and 2). Although species diversity of endophytes within and among tree species is high, the communities in host species of the same plant family are dominated by closely related endophyte species. Relatedness of dominant endophytes decreases with decreasing relatedness of the host trees. Differences are most pronounced between gymnosperms and angiosperms. Most of the dominant endophytes of the broadleaved trees, i.e. Aceraceae, Betulaceae and Fagaceae, belong to the Diaporthales whereas those of the trees with scale-like or needle-like leaves, i.e. Cupressaceae and Pinaceae, belong to the Helotiales. Divergence of angiosperms and gymnosperms was estimated to have occurred about 300 Ma ago based on molecular data (Schneider *et al.* 2004), and Diaporthalean and Helotialean ascomycetes were estimated to have diverged at the same time (Fig 1) (Berbee & Taylor 2001; James *et al.* 2006). Fungi on conifers evolved into today's Helotialean fungi and those on angiospermous trees into the Diaporthalean fungi. Thus, the dominant endophytes have been co-evolving with their hosts since more than 300 Ma.

Members of both the bitunicate ascomycetes (Dothideales, Pleosporales and Mycosphaerellales) and the Xylariales can be dominant endophytes in angiosperms and gymnosperms. The 'Bitunicatae' probably have diverged more than 300 Ma ago from the common ancestor of the Helotiales and Diaporthales, and, consequently, before the divergence of gymnosperms and angiosperms (Fig 1). This might be the reason for the occurrence of bitunicate ascomycetes as dominant endophytes in both conifers and woody angiosperms (Tables 1 and 2).

A further indication for host-endophyte co-evolution is the degree of relatedness of dominant endophytes in needles of *Abies, Tsuga* and *Pinus* species. Whereas *Abies* and *Tsuga* are closely related, *Pinus* is only distantly related to *Tsuga* and *Abies*. Correspondingly, species of *Phyllosticta* (anamorphic forms of *Guignardia* spp.) are dominant only in needles of *Abies* or *Tsuga* species, and *Cyclaneusma* spp. only in pine needles (Table 1). Congeneric tree species are often colonized by the same species or by a ''sister'' species of the same fungal genus, e.g. *Apiognomonia quercina* on *Quercus* spp., *Lophodermium pinastri* on *Pinus* spp., or *L. piceae* on *Picea* spp. and *Abies* spp. (Table 1). It is often impossible to differentiate ''sister'' species on different hosts based on morphology. The species limits between morphologically identical fungi are a subject of constant debate and several methods to define such limits have been proposed (Grünig *et al.* 2007; Taylor *et al.* 2000). Reproductive isolation was demonstrated to occur among populations of the same morphological species. These reproductively isolated populations are considered separate cryptic species, e. g. cryptic species of the dark septate endophyte *Phialocephala fortinii* s. l. can occur sympatrically adjacent to each other in the same root (Sieber and Grünig 2006). Thus, it is advisable to split rather than to lump species in future taxonomic works. An exception to this rule is the genotypic identity (as determined by ITS sequencing) of *Guignardia mangiferae* isolates from a wide host range all over the world (Baayen *et al.* 2002; Rodrigues *et al.* 2004).

Host specificity of some Xylarialean and Dothidealean endophytes is low. They are rarely dominant but occur sporadically as endophytes in a wide range of plant species, e.g. *Hypoxylon serpens* and *Guignardia mangiferae* (Baayen *et al.* 2002; Petrini & Petrini 1985). A few individual thalli of *H. serpens* are always isolated during census works irrespective of the host species. Similarly, *G. mangiferae* occurs worldwide as an endophyte in many plant species. Colonization by non-host-specific endophytes increases diversity and probably enhances fitness, protecting the tree in situations of adverse biotic or abiotic stresses. Perhaps, these endophytes possess traits which are advantageous under extreme conditions. Support for this idea comes from some *Colletotrichum* species which are pathogenic on the 'main' host species but symptomless endophytes on 'non-disease' host species, providing mutualistic benefits such as disease resistance, drought tolerance, and growth enhancement (Redman *et al.* 2001). This differential behaviour may result from differences in fungal gene expression in response to the plant or differences in the ability of the plant to respond to the fungus.

Mutualistic endophytes are often considered to have evolved from parasitic or pathogenic fungi (Carroll 1988; Saikkonen *et al.* 1998). However, the reverse is equally conceivable. Symbioses of roots and fungi have existed since the move of plants to land. The same may apply to endophytic fungi in aerial plant tissues. The direction of evolution may have changed several times from pathogenic to non-pathogenic and back again in response to changing selection pressures. Endophytes on one host often are more closely related to congeneric pathogens on the same host than to congeneric endophyte species in another host (Fig 2). For example, endophytic *Lophodermium pinastri* on pines (*Pinus* spp.) are more closely related to pathogenic *L. seditiosum* on pines than to endophytic *L. piceae* on spruce (*Picea* spp.). Closely related pathogenic and endophytic fungi possess a common ancestor, although the lifestyle of this ancestor is, however, unknown.

Table 2 – Dominant fungal endophytes in twigs (< 5 cm) of various tree hosts

Host	Country	Number of species	Dominant species	Order[a]	References
Aceraceae					
Acer macrophyllum	British Columbia	16	*Cryptodiaporthe hystrix*	Diaporthales	Sieber and Dorworth (1994)
			Pezicula livida	Helotiales	
Acer pseudoplatanus	Germany	15	*Petrakia irregularis*		Kowalski and Kehr (1992)
	Poland		*Phomopsis spp.*	Diaporthales	
			Phialocephala dimorphospora	Helotiales	
Betulaceae					
Alnus glutinosa	Germany	>21	*Ophiovalsa suffusa*	Diaporthales	Fisher *et al.* (1991), Kowalski and Kehr (1992)
	Poland		*Pezicula cinnamomea*	Helotiales	
	UK		*Pleurophomopsis lignicola*		
Alnus rubra	British Columbia	27	*Phomopsis sp.*	Diaporthales	Sieber *et al.* (1991a)
			Ophiovalsa suffusa	Diaporthales	
Betula pendula	Germany	14	*Ophiovalsa betulae*	Diaporthales	Kowalski and Kehr (1992)
	Poland		*Pseudovalsa lanciformis*	Diaporthales	
Betula pubescens	Switzerland	19	*Ophiovalsa betulae*	Diaporthales	Barengo *et al.* (2000)
			Trimmatostroma betulinum		
Carpinus betulus	Germany	17	*Pezicula carpinea*	Helotiales	Kowalski and Kehr (1992)
	Poland		*Diaporthe carpini*	Diaporthales	
Carpinus caroliniana	New Jersey	155	*Pestalotiopsis guepinii*	Xylariales	Bills and Polishook (1991)
			Trichoderma harzianum	Hypocreales	
Cupressaceae					
Juniperus communis	Switzerland	82	*Kabatia juniperi*	Dothideales	Petrini and Müller (1979)
			Pezicula cinnamomea	Helotiales	
Fagaceae					
Castanea sativa	Switzerland	14	*Cryptodiaporthe castanea*	Diaporthales	Bissegger and Sieber (1994)
			Pezicula cinnamomea	Helotiales	
Fagus crenata	Japan	<13	*Phomopsis sp.*	Diaporthales	Sahashi *et al.* (1999)
Fagus sylvatica	Germany	44	*Apiognomonia errabunda*	Diaporthales	Danti *et al.* (2002), Kowalski and Kehr (1992), Petrini and Fisher (1988), Sieber and Hugentobler (1987), Toti *et al.* (1993)
	Italy		*Pezicula livida*	Helotiales	
	Poland		*Botryosphaeria quercuum*	Dothideales	
	Switzerland		*Diaporthe eres*	Diaporthales	
	UK		*Asterosporium asterospermum*		
			Neohendersonia kickxii		
Quercus cerris	Italy	14	*Phomopsis quercina*	Diaporthales	Gennaro *et al.* (2003), Ragazzi *et al.* (2003)
			Diplodia mutila	Dothideales	
			Dicarpella dryina	Diaporthales	
			Dendrodochium sp.		
Quercus ilex	Spain	64	*Biscogniauxia sp.*	Xylariales	Collado *et al.* (1996), Fisher *et al.* (1994)
	Switzerland		*Nodulisporium sp.*	Xylariales	
	UK		*Phoma sp.*	Pleosporales	
Quercus petraea	Austria	45	*Colpoma quercinum*	Helotiales	Halmschlager *et al.* (1993)
			Apiognomonia errabunda	Diaporthales	
Quercus pubescens	Italy	13	*Phomopsis quercina*	Diaporthales	Ragazzi *et al.* (2003)
			Apiognomonia quercina	Diaporthales	
Quercus robur	Germany	23	*Amphiporthe leiphaemia*	Diaporthales	Gennaro *et al.* (2003), Kowalski and Kehr (1992), Petrini and Fisher (1990), Ragazzi *et al.* (2003)
	Italy		*Phomopsis quercina*	Diaporthales	
	Poland		*Colpoma quercinum*	Helotiales	
	UK		*Trichoderma viride*	Hypocreales	
			Nodulisporium sp.	Xylariales	
			Eutypella sp.	Xylariales	
			Dicarpella dryina	Diaporthales	
Oleaceae					
Fraxinus excelsior	Germany	18	*Phomopsis sp.*	Diaporthales	Kowalski and Kehr (1992)
	Poland				
Pinaceae					
Abies alba	Germany	48	*Diaporthe eres*	Diaporthales	Kowalski and Kehr (1992), Sieber (1989)
	Poland		*Grovesiella abieticola*	Helotiales	
	Switzerland		*Pezicula sp.*	Helotiales	

Table 2 – (continued)

Host	Country	Number of species	Dominant species	Order[a]	References
Larix decidua	Germany	17	*Tympanis sp.*	Helotiales	Kowalski and Kehr (1992)
	Poland		*Phialocephala dimorphospora*	Helotiales	
Picea abies	Germany	58	*Mollisia sp.*	Helotiales	Barklund and Kowalski (1996),
	Poland		*Tryblidiopsis pinastri*	Helotiales	Kowalski and Kehr (1992),
			Mollisia cinera	Helotiales	Sieber (1989)
			Pezicula livida	Helotiales	
			Tympanis sp.	Helotiales	
			Pocillopycnis umensis		
Pinus sylvestris	Germany	18	*Pezicula livida*	Helotiales	Kowalski and Kehr (1992)
	Poland		*Tympanis sp.*	Helotiales	
Pinus tabulaeformis	China		*Rhodotorula pinicola*	Sporidiales	Zhao *et al.* (2002)
Salicaceae					
Populus tremula	Spain	9	*Valsa sordida*	Diaporthales	Santamaria and Diez (2005)
			Trichoderma viride	Hypocreales	
Salix fragilis	UK	>10	*Cryptodiaporthe salicella*	Diaporthales	Petrini and Fisher (1990)
			Daldinia sp.	Xylariales	
			Microsphaeropsis sp.	Pleosporales	

a according to Kirk *et al.* (2001).

3. Are tree endophytes pathogens?

The endophyte community in some tree species is dominated by endophytes that are considered pathogens, e.g. species of *Apiognomonia*, *Ophiovalsa*, *Pezicula* or *Phomopsis* (Tables 1 and 2). However, these 'pathogens' have co-evolved with their hosts and can, thus, not be highly virulent, and symptoms are observed only very rarely and limited to single localities where symptoms usually develop on just a few branches of a single tree. These outbreaks either are incited by another external factor that is mostly unknown or are due to virulent, rarely

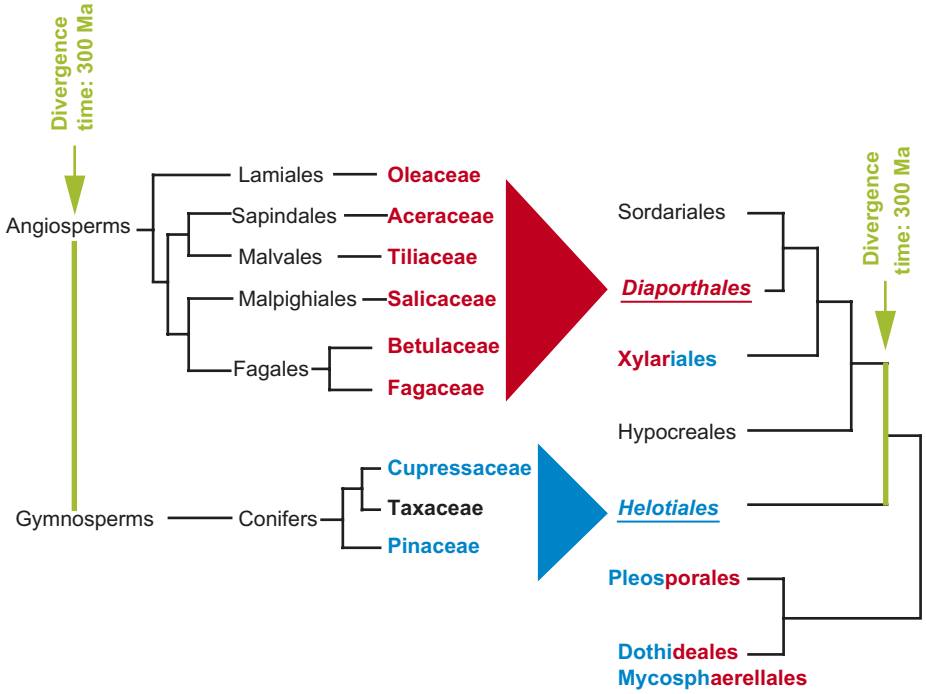

Fig. 1 – Comparison of the genealogical trees of the orders of ascomycetes which comprise tree endophytes and the plant families which comprise tree species hosting these endophytes. Red and blue colours and the two large arrowheads indicate the relationship between the family membership of host trees and the affiliation of the endophytes dominating on these hosts. Branch lengths do not correspond to the phylogenetic distance among taxa. Green coloured branches indicate the coincidence between the divergence of the gymnosperms and the angiosperms and the divergence of the Diaporthales and the Helotiales about 300 million years ago. The genealogies were reconstructed according to James *et al.* (2006) for the ascomycetes and according to Soltis and Soltis (2000) for the tree families. Divergence times are from Berbee and Taylor (2001) for the ascomycetes and from Schneider *et al.* (2004) for the host families.

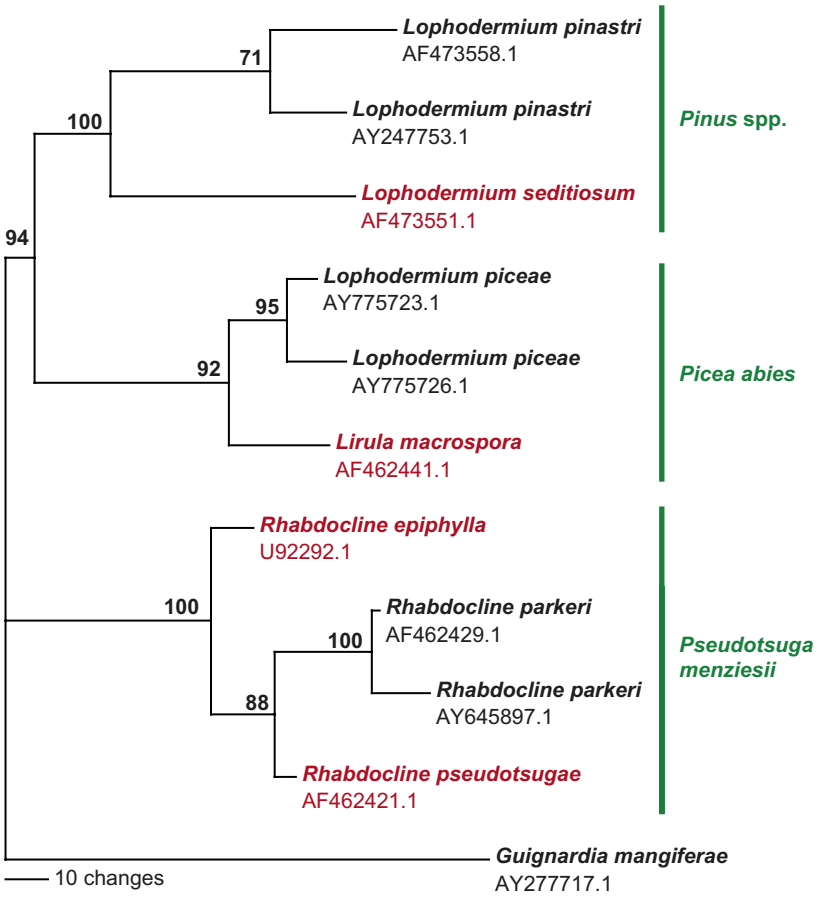

Fig. 2 – Maximum parsimony tree derived from DNA sequences of the ITS regions of rhytismatacean endophytes (printed in black) and pathogenic relatives (printed in red) of some conifers. Numbers above branches indicate percentage of bootstrap support (1000 replicates). *Guignardia mangiferae* served as an out-group.

occurring genotypes of the endophyte. In contrast, when fungi are introduced from other continents they encounter plant species with which they did not co-evolve. Resistances to these fungi could not develop and, consequently, hosts are highly susceptible. Some of these introduced fungi are serious pathogens and had and still have devastating effects on their host populations, e.g. the causal agents of chestnut blight, Dutch elm disease etc. Most of these fungi were introduced inadvertently because they do not cause any serious problems in their native range. Some may even be harmless endophytes on their natural hosts. Plant quarantine should, therefore, include testing the pest risk of endophytic fungi (FAO 2004). Since virulence and plant susceptibility are high, introduced pathogens are rarely if ever isolated from healthy tissues because the latency period is short and symptoms develop soon after infection. The two introduced pine-needle pathogens *Mycosphaerella pini* (*Dothistroma septosporum*) and *M. dearnessii* (*Lecanosticta acicola*) have not been detected as endophytes in Europe although disease symptoms are occassional observed (Holdenrieder & Sieber 1995). In contrast, the virulent form of *Cryphonectria parasitica*, an introduced pathogen that causes chestnut blight, was detected, though only rarely, in healthy coppice shoots of *Castanea sativa* in Southern Switzerland (Bissegger & Sieber 1994). Likewise, endemic pathogens with high virulence are rarely detected as endophytes. For example, *Nectria ditissima*, a canker-causing fungus on tree species of the Fagales was only sporadically isolated from red alder in British Columbia or from *Fagus sylvatica* in Europe (Danti *et al.* 2002; Dorworth *et al.* 1996; Sieber *et al.* 1991a). Thus, a high frequency of colonization of the internal of healthy plant tissues is a clear indication of low virulence of a fungus.

How are endophytes able to infect but assume a quiescent state once inside the plant? The initial steps of host infection are the same as those for a pathogen: recognition, germination, and penetration. The fungus has to overcome preformed and induced plant defence mechanisms. Preformed defences include water-repellent waxy layers on the cuticle, hairs (Valkama *et al.* 2005), cuticle composed of cutin, cellulose and pectin. Consistent with a chemical or physical host signal, fungal spores attach preferentially to host than non-host surfaces (Viret *et al.* 1994). Recognition of the host surface and binding to it is often mediated by lectin-like molecules, as for example in *H. fragiforme* on beech (*Fagus sylvatica*) (Chapela *et al.* 1993). After germination, most endophytes produce a cocktail of exoenzymes which soften the cuticle and the wall of epidermal cells to ease penetration of the thread-like infection hyphae or, if an appressorium is formed, to

facilitate breaching the plant cuticle by mechanical force (Petrini *et al.* 1992; Schulz *et al.* 2002; Sieber *et al.* 1991b; Thines *et al.* 2000). A quiescent state is assumed after infection. The inducible defences such as programmed cell death, papillae formation, phytoalexins, pathogenesis related proteins (Van Loon & Van Strien 1999), e.g. peroxidases, chitinases, RNases, proteases and protease inhibitors (e.g. polygalacturonase inhibitor proteins) (De Lorenzo & Ferrari 2002), are either not activated or the hypersensitive response kills only single host cells as demonstrated for *Rhabdocline parkeri*, the dominant endophyte in Douglas fir needles (Stone 1987) (Table 1). This endophyte infects only single epidermal cells. The infected cell dies but the endophyte survives as a short, multicellular thallus that stops growing until the needle senesces (Stone 1987). Probably, a hypersensitive reaction of the host, elicited by the endophyte, causes programmed death of the epidermal cell, and subsequent quiescence of the endophytic thallus may be mediated by some cytostatic host metabolites. This type of interaction is compatible with both the gene-for-gene (GFG) resistance concept (Flor 1971) and the quantitative (polygenic) resistance concept (Dickinson 2003). According to the GFG concept, an elicitor encoded by the avirulence gene (avr) of the endophyte is recognized by the product of the resistance gene (R) of the host. Recognition activates a signal transduction pathway which leads to the hypersensitive response and quiescence of the endophyte. In a pathogenic interaction either the avr or the R product or both are not produced, i.e. the host does not react, and consequently disease symptoms develop. Alternatively, once inside the host, endophytic thalli may switch to quiescence endogenously. Avoidance of recognition during quiescence may be achieved by masking the endophytic thalli. For example, a gene has been cloned from the bean anthracnose fungus *Colletotrichum lindemuthianum* which is switched on during the initial phase of colonization and switched off later during the necrotrophic phase (Perfect *et al.* 1998). This gene encodes a glycoprotein that resembles plant cell wall proteins which is believed to coat the hyphae that the plant is unable to recognize as alien.

The infection process and the subsequent dormant phase of xylem endophytes follow a slightly different pattern. Infections must occur through the periderm, lenticels, leaf scars, or scars of bud scales. Vessels or tracheids exposed after leaf/needle fall or the abscission of whole shoots constitute the only direct connection between the wood and the exterior of a tree. Usually the vessels are plugged with scar tissue, but some endophytes may be able to cross this barrier and to form small thalli in the lumina of dysfunctional vessels. Alternatively, mycelium must reach and cross the cambium, for example, in the vicinity of the invaginations formed by rays which are, however, often plugged by sclereids. Once inside the xylem the mycelium either infects single cells, establishes small intracellular thalli similar to those formed by *Rhabdocline parkeri* in Douglas fir needles (Stone 1987) or forms intercellular, flat amoeboid microthalli with very thin walls similar to those produced by *Ophiostoma novo-ulmi* under special experimental conditions (Ouellette *et al.* 1995). Similarly, Hendry *et al.* (1993) found some xylariaceous endophytes to switch to a yeast-like growth in dual cultures with beech callus. Endophytic thalli become dormant and are wrapped in the wood by the continuous growth increment of the trees. Suppression of wood endophytes in a cryptic state may result from adverse gaseous regime or low nitrogen availability (Hendry *et al.* 2002). Thalli resume their activity when suitable conditions occur, e.g. decreased water content (in sapwood), increased oxygen and/or nutrient availability, and reduced host defence. Limited oxygen and/or nutrient availability is suspected to control non-pathogenic behaviour of the two xylem endophytes *Fomes fomentarius* and *Nectria coccinea*, both of which are considered pathogens in the phytopathological literature.

The tinder fungus *F. fomentarius* seems to be a frequent quiescent colonizer of the xylem of healthy beech and birch with a preference for the stem and thick branches (Baum *et al.* 2003; Anne Danby, David Lonsdale and Lynne Boddy, personal communication). Decay caused by this fungus usually starts from small pockets, which are randomly distributed over the surface of the central part of the branch or stem and have no apparent connection to the more peripheral tissues, i.e. cambium and bark. This seems also to be true for ascomycetes latent in beech xylem (Chapela & Boddy 1988). Infections by *F. fomentarius* must have occurred many years or decades ago, especially if we consider fresh leaf scars, traces of which occur only in the very centre of stems or twigs, to offer the best paths for wood colonization. Influx of oxygen through hairline cracks formed during windstorms may reactivate dormant thalli. Cracks can reach several meters in axial direction. *F. fomentarius* fills these cracks with mats of mycelium. The cambium is killed when reached by the fungal mats. As a consequence, grooves visible from the outside of the stem form along these cracks as no growth increment occurs where the cambium has been killed (Lohwag 1931).

The discovery of the endophytic nature of *Nectria coccinea* may contribute to a better understanding of the ethiology of beech bark disease (Hendry *et al.* 2002). *N. coccinea* is considered to be one of the key players in this complex disease. The fungus apparently rapidly invades trees and kills patches of bark infested by the felted beech scale (*Cryptococcus fagisuga*) (Houston 1994; Lonsdale & Wainhouse 1987). However, the fungus has also been reported to attack and cause bark necroses in the absence of significant *C. fagisuga* infestation, e.g., following stress caused by drought (Lonsdale 1980a, b). In contrast to the 'classical' sequence of events leading to beech bark disease, we now know that *N. coccinea* is already present as an endophyte in the wood waiting for an external inciting factor which allows its pathogenic abilities.

4. Are tree endophytes mutualists?

Results from grass-endophyte systems suggest that endophytes are herbivore antagonists and enhance plant growth (Clay 1991). Correspondingly, mutualistic antagonism towards insects and pathogens has been claimed also for forest endophytes (Carroll 1995; Faeth & Wilson 1996; Stone & Petrini 1997; Wilson & Carroll 1997). Experimental demonstration of such antagonism under ''real-world'' conditions in the field, however, has been mostly inconclusive.

The four Koch's postulates can be modified to serve as guidelines for testing mutualism of tree endophytes: (1) The occurrence of the endophyte must be associated with the beneficial effect; (2) the endophyte must be isolated from the

tissue on which the beneficial effect was observed and must be grown in pure culture; (3) the cultured endophyte should cause the beneficial effect when re-introduced into an endophyte-free plant; (4) The organism must be reisolated from the experimentally infected, endophyte-free plant. Fulfilling all of Koch's postulates is a major challenge, and often impossible when working with tree endophytes. Postulate (3) is most difficult to fulfil, since endophyte-free trees are required. While production of endophyte-free tree seedlings or cuttings is feasible, it is impossible to produce adult, endophyte-free trees. Whole trees or, more realistically, single twigs could be wrapped in plastic bags shortly before bud burst (Kaneko & Kaneko 2004; Wilson 1996), since leaves in the buds are mostly endophyte-free (Toti *et al.* 1993). The endophyte could then be applied to half of the wrapped twigs. However, the bags would change the microclimate and gas exchange would be inhibited. Thus, the 'control' problem is not easy to resolve.

Studies that come close to fulfilling Koch's postulates are few. Arnold *et al.* (2003) inoculated endophyte-free leaves of 100 d-old greenhouse-grown *Theobroma cacao* seedlings with endophytes isolated from naturally infected, asymptomatic tissues and observed a significant decrease (compared to uninoculated controls) of both leaf necrosis and mortality when endophyte-inoculated seedlings were challenged with a pathogenic *Phytophthora*. Webber (1981) showed that colonization of elm bark by *Phomopsis oblonga*, an ubiquitous elm-bark endophyte that has been isolated from almost 75 % of healthy 2-yr-old twigs of *Ulmus glabra* in Switzerland (Vanden Broeck 1994), significantly reduced the number of female galleries of both elm bark beetles (*Scolytus scolytus* and *S. multistriatus*), the vectors of Dutch elm disease (*Ophiostoma novo-ulmi*), and success of larval development was close to zero. *P. oblonga* provides biological control because it reduces the population size of the two vectors.

Association of natural endophyte infections and insect mortality as well as isolation of the endophyte (postulates (1) and (2)) has been reported in several studies but the crucial infection experiments (postulate (3)) using endophyte-free trees for inoculation and control have not been performed in these studies. A cynipid wasp on *Quercus garryana* was found to suffer highest mortality when present on part of the leaf with highest endophyte density (Wilson 1995). Similarly, Pehl and Butin (1994) found correlations between mortality of gall insects and presence of endophytes on *Acer pseudoplatanus, Fagus sylvatica*, *Quercus robur* and *Tilia cordata*. Between 45 and 75 % of the larvae of the gall insect *Contarinia* sp. died when the galls were located on Douglas fir needles colonized by *Rhabdocline parkeri* as compared to at most 2-25 % on endophyte-free needles (Carroll 1995). On the other hand, leaves selected by females of the leaf mining butterfly *Cameraria* sp. for oviposition and unmined leaves were equally likely to be colonized by fungal endophytes, and long-term survival and size of surviving larvae did not differ between leafminers on control branches and leafminers on branches with elevated endophyte infections (Faeth & Hammon 1996, 1997a, b). Similarly, endophytic fungi of mountain birch (*Betula pubescens*) had negligible effects on larval performance of the leaf beetle *Phratora polaris* under natural conditions (Lappalainen & Helander 1997), and larval densities of a leaf-mining *Phyllonorycter* sp. on *Quercus gambelii* were not correlated with the frequency of infection by endophytic fungi (Preszler *et al.* 1996). Wilson and Faeth (2001) examined whether the distribution of mines of leafminers were associated with endophyte distribution. Leafminers and fungal endophytes were negatively correlated. Endophytes preferentially colonized small leaves in the sunny part of the crown, whereas miners oviposited mainly on larger leaves in the shaded part of the tree. There are two possible interpretations: (1) The leafminers select bigger leaves to provide enough food for a miner to complete development (Faeth 1991), and endophytes occur mainly on smaller leaves because the density of hairs is higher and, thus, also the probability that a fungal spore is caught is higher; (2) The leafminer actively avoided leaf areas occupied by fungal endophytes for oviposition. The two possible conclusions illustrate that correlation does not necessarily mean causality.

Endophyte metabolites have been suspected as a probable cause of herbivore antagonism, and several toxins have been isolated and characterized from tree endophytes. Bioactive constituents of extracts of *Phyllosticta* sp. and *Hormonema dematioides* endophytes from balsam fir, were reported to cause reduced growth rate and mortality of spruce budworm larvae (Calhoun *et al.* 1992). *Melanconium betulinum,* isolated from twigs of *Betula pendula* and *B. pubescens,* produced 3-hydroxypropionic acid which was selectively nematicidal against the plant-parasitic nematode *Meloidogyne incognita* (Schwarz *et al.* 2004), and endophytic *Pezicula* strains, isolated from living branches of several deciduous and coniferous trees were strongly fungicidal and herbicidal, and to a lesser extent algicidal and antibacterial (Schulz *et al.* 1995). However, all these 'antibiotic' metabolites were produced *in vitro* with fungal colonies much larger than the endophytic thalli *in planta*. It remains to be determined, therefore, whether these metabolites are produced in sufficient amounts by the endophytic thalli to have an effect.

5. The endophytic continuum

Some tree endophytes are potentially pathogenic and switch from quiescence to pathogenicity when conditions are favourable for the endophyte and/or unfavourable for the host. Some other endophytes are considered mutualists because they deter and/or kill herbivores, and again some other endophytes exhibit both lifestyles: mutualism under some circumstances and parasitism under others, e.g. species of *Apiognomonia* spp. which dominate in the leaves of many deciduous, broadleaved trees (Table 1). The endophytic thalli of these endophytes resume growth in response to an external stimulus, e.g. oviposition of gall-forming or leaf-mining insects or infection by a pathogenic fungus. Necroses develop in the leaf areas where infection or oviposition occurred, eliminating the food base of the pathogen or herbivore insect and hereby reducing the population of the antagonist. For example, oviposition of the gall-midge *Mikiola fagi* close to endophytic thalli of *A. errabunda* in beech leaves elicits such a reaction (Pehl & Butin 1994). In other cases, the endophytic thalli resume growth for, as yet, unknown reasons and develop the diseases known as anthracnose. Butin (1983) speculates that warm wet spring weather is favourable for an epidemic of *A. errabunda* on

beech. However, anthracnose of this fungus often occurs on the leaves of one branch but is absent on other branches of the same tree. Perhaps, *A. errabunda* is genetically diverse and forms a complex of morphologically identical cryptic species (Fisher *et al.* 2002; Grünig *et al.* 2007) some of which may be pathogenic and others non-pathogenic. In fact, up to four different genotypes were detected in the same beech leaf by Hämmerli *et al.* (1992).

Several mechanisms have been described which can lead to transformation of fungi from mutualist to pathogen and vice-versa: (1) a single point mutation (Freeman & Rodriguez 1993); (2) virulence genes can be transferred from one species to another as demonstrated for two pathogens on wheat creating a pathogen population with significantly enhanced virulence (Friesen *et al.* 2006); (3) viral infection of an endophytic fungus of a tropical grass confered heat tolerance to the plant host, but tolerance was lost when the fungus was virus-free (Marquez *et al.* 2007).

6. Infection time, frequency and the threshold model

Many conifer-needle pathogens only infect young needles. For example, *Chrysomyxa* spp., rust fungi on spruce needles, are able to infect current-year needles only (Gaeumann 1959). Similarly, *Meria laricis* is able to infect larch needles only during the first four weeks after emergence (McBride & Hays 1979). In contrast, needle endophytes are able to infect needles of all age classes. Susceptibility of the needles and frequency of colonization increase with needle age. Colonization of black spruce (*Picea mariana*) needles by endophytic fungi increased from 4 to 90 % between current-year and 3 y-old needles, respectively (Johnson & Whitney 1992). Similarly, the frequency of Sitka-spruce needles colonized by *Lophodermium* increased with needle age (Magan & Smith 1996), and the youngest needles of *Pinus strobus* were virtually endophyte-free whereas older needles were frequently colonized by species of *Lophodermium* and *Hormonema* (Deckert & Peterson 2000). Density of colonization *versus* needle age has been investigated only rarely. The density of infections of Douglas fir needle epidermal cells by *Rhabdocline parkeri* increases exponentially with needle age (Stone 1987). The percentage of infected cells was, however, always less than 5 % even in old needles on heavily infected trees.

Depending on the conifer species and the site conditions, the lifespan of needles is between four and twelve years. It is not known whether endophytes accelerate senescence. I postulate that needles senesce as soon as the density of colonization exceeds a certain threshold value (Fig 3). The model assumes that the endophytic thalli exist as commensals. They resume growth, kill the needle and sporulate as soon as the population density is high enough. Under normal conditions, the necessary population density is not reached before the onset of natural needle senescence. If adverse conditions occur such as lack of light in dense stands (Helander *et al.* 1994; Müller & Hallaksela 1998), infection rates can be much higher and consequently the threshold population density is reached much faster and can lead to premature needle cast.

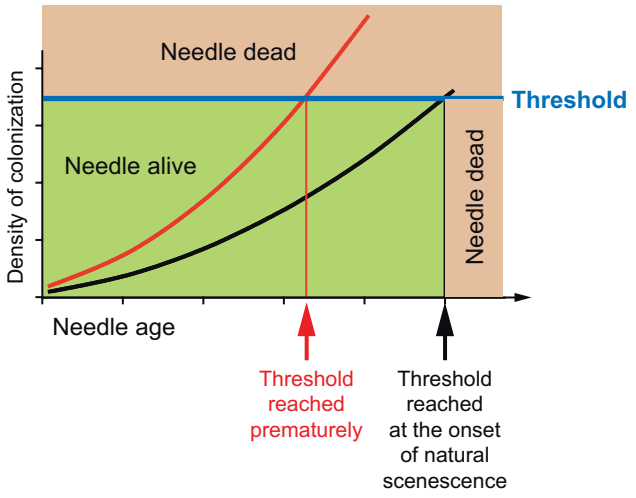

Fig. 3 – Relationship between needle age and density of colonization by endophytic thalli (number of thalli per needle volume) to illustrate the 'Threshold Model'. The black curve shows the maximum increase of density under normal conditions; the attainment of the threshold density coincides with the onset of natural senescence. The red curve shows the increase of density under adverse conditions; the threshold density is reached before the onset of natural senescence, and the needle dies prematurely.

7. Conclusions

Forest trees form symbiotic associations with endophytic fungi which live inside healthy tissues as quiescent microthalli. Usually, their presence becomes apparent only after the onset of natural senescence. All forest trees in the temperate zones host endophytic fungi and species diversity of the endophyte community in a single tree species or plant tissue can be very high. Censuses of endophytic fungi are ideally suited for the evaluation of biodiversity because the samples can be taken in a standardize manner. Species composition of the endophyte community differs among tissue types (leaves, bark, wood) and the phase disposition (age) of tissues.

Communities are dominated by a few species which are considered to be host-specific. The dominant species in angiosperm trees of the Aceraceae, Betulaceae, and Fagaceae belong to the Diaporthales, those in gymnosperm trees of the Cupressaceae and Pinaceae to the Helotiales. Divergence of angiosperms and gymnosperms coincides exactly with the divergence of the Diaporthales and the Helotiales in the late Carboniferous, early Permian about 300 million years (Ma) ago indicating that the ancestors of the Diaporthalean endophytes had been associated with angiosperms and those of the Helotialean endophytes with gymnosperms since 300 Ma. Consequently, dominant endophytes have co-evolved with their host trees. Fungi co-evolving with their hosts for such a long period of time are unlikely to be strong pathogens.

Plant resistance mechanisms against endophytes become effective only after infection. In the *Rhabdocline parkeri* – Douglas fir symbiosis (Stone 1987), the microthallus of the

endophyte is 'locked' in a single epidermal cell which is killed by a hypersensitive response.

Some fungi, which are described to be pathogens, are xylem endophytes which may remain latent for decades. The tinder fungus *Fomes fomentarius* and *Nectria coccinea* have been shown to be abundant in healthy beech wood. Growth of thalli of these endophytes is most likely inhibited by low oxygen and/or nutrient availability in the wood. *F. fomentarius* probably resumes growth in hairline cracks in the wood formed during windstorms. *N. coccinea* resumes growth after heavy attack by the beech scale *Cryptococcus fagisuga* or after a drought period.

Mutualism of tree endophytes has often been assumed based on the results from grass-endophyte systems, but evidence is mostly circumstantial. Endophyte-free controls are needed to unequivocally prove positive effects of endophytes. Production of endophyte-free trees, however, poses a major problem. Alternatives are the use of seedlings or the wrapping of branches or twigs during the time of spore production of the endophyte. Some tree endophytes exhibit differential behaviour. Depending on the situation they can be antagonistic or mutualistic. For example, *Apiognomonia errabunda*, the dominant endophyte in beech leaves, is triggered by the oviposition of gall forming insects; resumed endophyte growth results in necroses, but aborts the galls. *A. errabunda* can be considered a mutualistic symbiont of beech if we assume that the positive effects of the reduction of the insect population exceed the negative effects of necrotic tissues. In some instances, *A. errabunda* causes leaf anthracnose in the absence of insects; the behaviour of *A. errabunda* is clearly pathogenic, but the factors eliciting such reaction are not known.

To summarize, tree endophytes are mostly harmless colonizers of the internal of healthy plant tissues. Some are potentially pathogenic but disease is only caused in combination with other, mostly unknown, inciting factors. Proof of mutualism of endophyte-host symbioses has been inconclusive in most cases, but plant communities would probably not survive many environmental stresses without these symbioses. All we know for certain is that endophytes are present in any healthy plant tissue!

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