7 Biodiversity of Fungal Root-Endophyte Communities and Populations, in Particular of the Dark Septate Endophyte *Phialocephala fortinii* s. l.

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7.1 Introduction

The peripheral root tissues form a morphologically, physically and chemically complex microcosm that provides different habitats for diverse communities of microorganisms. This microcosm is not stable, and changes over space and time because the boundaries between soil, rhizosphere, and living roots are continually shifted as a result of root growth and the constant modification of nearby soil by root mechanical and metabolic activity (Foster et al. 1983). Microorganisms colonise the rhizoplane, epidermis and outer cortex in a nonrandom patchy manner and contribute to the modification of the soil-rhizosphere-root continuum. Microorganisms affect their plant hosts, and hosts reciprocally affect their symbionts, leading to a feedback that drives changes in both the microbial and plant communities (Bever et al. 1997). Many soil bacteria and fungi are able to colonise epidermal and outer cortical cells of healthy roots inter- and intra-cellularly. A comparatively small number of organisms, e.g. mycorrhizal fungi, endophytic and pathogenic fungi and bacteria, possess, however, the ability to cross the inner boundary of the rhizosphere and to penetrate deeper into the root (Bazin et al. 1990). The interaction of host and endophyte depends on the disposition of host and fungus or bacterium and the environmental conditions, but may be neutral, mutualistic or antagonistic and may change over time. Some endophytic fungi adopt mycorrhizal functions and/or place plants at a competitive advantage against herbivores, insect pests or pathogens (Carroll 1988; Hawksworth 1991). Other endophytes can switch to a pathogenic behaviour when conditions are unfavourable for the host (Schulz et al. 1999). The biodiversity of root endophyte communities varies in relation to environmental factors, type of vegetation,

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spatiotemporal patterns of the root microcosm and interactions among microorganisms. There is currently an urgent need to assess biodiversity in pristine ecosystems and to use these data as references to measure the effects of disturbances on diversity and to better enable informed decision-making on the fate of threatened natural habitats (Cannon 1997). Threats may come from a variety of sources, including exploitation by logging, machine-graded soils, urban development, pollution, climate change and input of pesticides and fertilisers. Biodiversity can be explored at several levels, i.e. in terms of communities, species and populations (Hawksworth 1991). Here, we will explore current knowledge on the biodiversity of non-mycorrhizal fungal root endophytes at all levels. The first part of this review will be dedicated to biodiversity at the community level in relation to environmental factors. In the second part, special emphasis will be placed on the diversity of dark septate endophytes (DSE), in particular of *Phialocephala fortinii* s. l.

Readers of this chapter should always bear in mind that the methods of detection are highly selective and, thus, the species list and species diversity derived for any habitat will be incomplete and will be biased in respect to physiological features selected for by the method used [Sieber 2002; Swift 1976; see Chaps. 9 (Bayman and Otero), 18 (Bloemberg and Camacho Carvajal) and 19 (Van Overbeek et al.)].

7.2 Species Diversity of Root Endophyte Communities

"Species diversity" comprises two distinct components: the total number of species, which ecologists refer to as "species richness", and "evenness" or equitability, which refers to how species abundances are distributed among the species present. An ecosystem is said to be more diverse if many species with equal population sizes are present and less diverse if some species are rare and a few are very common. Other helpful terms are "spectrum of species" or "community composition" to describe habitat or ecosystem differences with respect to the species found. The species diversity and the species spectrum of root-endophyte communities are related to various factors, which can tentatively be arranged into four groups: (1) geography and climate, (2) soil, (3) multitrophic interactions, and (4) natural and anthropogenic disturbances. This grouping is rather artificial and does not account for the intricate interplay among factors that often makes it impossible to determine the contribution of each factor. Another aspect obscuring the effects of different factors is that of site history, i.e. the dynamics of plant and endophyte communities. Nevertheless, the above grouping seems to be the most appropriate structure for this section.

7.2.1 Geography and Climate

Fungal species diversity is higher in tropical than in temperate regions owing in part to the great diversity of hosts, but also to the optimal growth conditions for many fungi as a result of the hot and moist climate (Cannon and Hawksworth 1995). Whether this relationship is also valid for fungal root endophytes remains to be tested. Compared to habitats in the temperate or the tropical zones, species diversity is distinctly reduced in arctic-alpine environments, not only because of the lower number of available host species, but also with respect to the number of endophyte species in each host. For example, only seven root endophyte species were detected in *Dryas octopetala* in arctic Spitsbergen (Fisher et al. 1995) [Table 7.1(i)]. Correspondingly, species richness in *Erica carnea* was highest at an altitude of 640 m and lowest at 2,140 m in Switzerland (Oberholzer-Tschütscher 1982). The species spectra differed greatly among sites, as expressed by very low between-site similarities [Table 7.1(ii)]. Evenness was lowest at the lowest altitude where the comparatively species-rich community was dominated by only four to five species.

There is strong evidence for a shift from arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (ECM) in temperate habitats towards symbioses of uncertain status, especially dark septate endophytes (DSE), in arctic-alpine ecosystems (Bledsoe et al. 1990; Christie and Nicolson 1983; Read and Haselwandter 1981; Väre et al. 1992). Correspondingly, the frequency of roots colonised by *Phialocephala fortinii* s. l., a ubiquitous and dominant DSE in conifer roots (see Sect. 7.3.3), was positively correlated with the altitude in forest ecosystems (Ahlich and Sieber 1996).

Weather and climatic conditions are assumed to have a weaker direct effect on species diversity of endophyte assemblages in root tissues than in aerial plant parts due to the insulating and compensating properties of soils (Fitter et al. 1985). Thus, changes in root endophyte assemblages become manifest only if the climatic conditions deviate from the "normal" over an extended period of time, i.e. if the climate changes. In fact, long-term changes in mean annual temperature, frequency and amount of precipitation, as well as enhanced CO_2 may affect root endophyte diversity through shifts in the quantity and quality of photosynthates and secondary plant metabolites translocated to the roots, the rate of root turnover, and shifts in the competivity of endophytes and other soil microorganisms (Coûteaux et al. 1999; Rillig et al. 1999; Körner 2000). However, nothing is known about the direction and magnitude of effects on root-endophyte diversity.

Table 7.1. Influence of geographical, physical, chemical and biological factors on species diversity and similarity of communities of fungal root endophytes

Hosts and factors	Observed species richness[a]	Adjusted species richness[b]	Number of very abundant species[c]	Even-ness index[d]	Total number of isolates	Sample size[e]	Pairwise similarities of communities[f] (2)	(3)	(4)	(5)	Reference
(i) *Dryas octopetala*						R					Fisher et al.
(1) Spitzbergen, site A	4	3.8 ± 0.4	2.4	0.81	42	50	0.73				1995
(2) Spitzbergen, site B	7	5.8 ± 0.4	3.8	0.83	24	50	–				
(ii) *Erica carnea*						R					Oberholzer-
(1) Fläsch (640 m)	35	26.5 ± 2.2	4.2	0.51	296	333	0.26	0.21	0.30		Tschütscher
(2) Näfels (760 m)	19	17.1 ± 1.2	4.7	0.71	219	120	–	0.20	0.19		1982
(3) Davos-Wolfgang (1,640 m)	22	21.8 ± 0.4	7.0	0.72	173	298	–	–	0.24		
(4) Davos-Schatzalp (2,140 m)	12	10.6 ± 1.0	2.7	0.68	235	300	–	–	–		
(iii) *Picea abies*						R					Kattner and
(1) Soil pH neutral	27	26.9 ± 0.3	11.9	0.70	153	480	0.57				Schönhar
(2) Soil pH acidic	29	28.8 ± 0.4	12.6	0.72	154	480	–				1990
(iv) *Alnus glutinosa*						R					Fisher et al.
(1) Submerged roots	45	44.1 ± 0.8	17.4	0.66	114	40	0.37				1991
(2) Non-submerged roots	31	29.2 ± 1.2	13.8	0.75	126	40	–				
(v) *Rhizophora mucronata*						R					Ananda and
(1) Low-tide level	6	5.3 ± 0.7	2.2	0.80	21	30	0.50	0.38			Sridhar 2002
(2) Mid-tide level	14	9.7 ± 1.2	3.6	0.87	34	30	–	0.58			
(3) High-tide level	10	9.4 ± 0.6	3.2	0.91	17	30	–	–			

Table 7.1. (continued)

Hosts and factors	Observed species richness[a]	Adjusted species richness[b]	Number of very abundant species[c]	Even-ness index[d]	Total number of isolates	Sample size[e]	Pairwise similarities of communities[f] (2)	(3)	(4)	(5)	Reference
(vi) *Phragmites australis*											Wirsel et al.
Location 1						R					2001
(1) Flooded site	10	9.5 ± 0.7	4.6	0.74	63	45	0.52	0.52	–		
(2) Dry site	13	12.9 ± 0.3	7.4	0.80	51	45	–	–	0.75		
Location 2											
(3) Flooded site	13	11.9 ± 0.9	5.6	0.71	47	45			0.58		
(4) Dry site	11	10.0 ± 0.9	4.8	0.72	52	45			–		
(vii) *Triticum aestivum*											Sieber et al.
Development stage:						R					1988
(1) One leaf – end of tillering	63	61.0 ± 1.3	15.3	0.59	547	5040	0.67				
(2) Inflorescence emerged – caryopsis hard	62	39.5 ± 2.9	6.3	0.53	1857	3360	–				
(viii) *Triticum aestivum*											Sieber et al.
Preceding crop:						R					1988
(1) Sugar beet	51	44.7 ± 2.1	9.3	0.56	623	2400	0.58	0.57	0.67		
(2) Red clover	49	48.3 ± 3.3	10.9	0.60	413	1200	–	0.60	0.59		
(3) Maize	44	35.9 ± 2.2	4.7	0.45	773	2400	–	–	0.65		
(4) Potatoes	39	33.5 ± 1.9	6.9	0.61	595	2400	–	–	–		

Table 7.1. (continued)

Hosts and factors	Observed species richness[a]	Adjusted species richness[b]	Number of very abundant species[c]	Even-ness index[d]	Total number of isolates	Sample size[e]	Pairwise similarities of communities[f] (2)	(3)	(4)	(5)	Reference
(ix) Various vegetables						R					Narisawa
(1) Eggplant	7	5.5 ± 0.9	3.2	0.71	35	45	0.67	0.55	0.67	0.86	et al. 2002
(2) Tomato	5	4.7 ± 0.4	2.7	0.78	19	45	–	0.44	0.60	0.50	
(3) Melon	4	3.9 ± 0.3	2.6	0.83	17	45	–	–	0.44	0.55	
(4) Strawberry	5	4.5 ± 0.6	2.1	0.71	21	45	–	–	–	0.67	
(5) Chinese cabbage	7	5.9 ± 0.8	4.4	0.79	29	45	–	–	–		
(x) *Betula pendula*						T					Görke
(1) Plantation in cleared windthrow	30	12.5 ± 1.7	10.6	0.59	87	160	0.34	0.29	0.38		(1998)
(2) Natural regeneration in untouched windthrow	11	9.3 ± 1.0	5.7	0.73	27	75	–	0.41	0.50		
(3) Natural regeneration in cleared windthrow	18	10.9 ± 1.5	7.4	0.60	48	100	–	–	0.57		
(4) Natural regeneration in low density forest[g]	17	9.3 ± 1.5	6.3	0.70	58	100	–	–	–		
(x) *Pinus sylvestris*						T					Görke
(1) Plantation in cleared windthrow	16	6.2 ± 1.2	6.8	0.72	56	160	0.42	0.40	0.38		(1998)
(2) Natural regeneration in untouched windthrow	8	5.5 ± 1.0	4.7	0.79	20	75	–	0.55	0.53		
(3) Natural regeneration in cleared windthrow	14	7.0 ± 1.2	6.9	0.69	26	100	–	–	0.38		
(4) Natural regeneration in low density forest	7	5.4 ± 0.9	4.8	0.80	19	100	–	–	–		

Table 7.1. (continued)

Hosts and factors	Observed species richness[a]	Adjusted species richness[b]	Number of very abundant species[c]	Even-ness index[d]	Total number of isolates	Sample size[e]	Pairwise similarities of communities[f] (2)	(3)	(4)	(5)	Reference
(xi) *Erica carnea*						R					Cevnik et al. 2000
(1) Control (Cd 1.4; Pb 171; Zn 61.8)[h]	9	8.2 ± 0.7	3.9	0.78	104	240	0.50	0.60	0.63		
(2) Low pollution (Cd 6.9; Pb 667; Zn 177)	7	6.9 ± 0.2	3.3	0.81	72	240	–	0.67	0.71		
(3) High pollution (Cd 35.8; Pb 5422; Zn 582)	11	10.5 ± 0.6	8.0	0.87	107	240	–	–	0.67		
(4) Highest pollution (Cd 87.7; Pb 31320; Zn 1330)	10	8.9 ± 0.8	3.8	0.72	142	240	–	–	–		

[a] Diversity index N0 according to Hill (1973); number of species

[b] Mean and standard error of the number of species adjusted to the lowest within-study number of isolates using rarefaction according to Hurlbert (1971)

[c] Diversity index N2 according to Hill (1973)

[d] Evenness index according to Hill (1973); this index converges towards 1 as one species tends to dominate

[e] Number of root segments (R) or trees (T) examined

[f] Soerensen index (Soerensen 1948); column numbers (in brackets) correspond to factor identifiers in the column "Hosts and factors"; $0 \leq$ Soerensen index ≤ 1, the index is 0 if two communities have no species in common, and it is 1 if all species occur in both communities

[g] Low density forest = selectively logged forest stand; aim: increased solar radiation within the stand

[h] Concentrations of heavy metals in micrograms per gram of soil

7.2.2 Soil

Soil and rhizosphere are highly variable habitats. Chemical properties such as pH or the availability of minerals and carbohydrates may vary significantly within a few centimetres of soil (Papritz and Flühler 1991). Similarly, differences in soil texture and water regime contribute to the variability of soils. In addition, roots constantly modify the nearby soil structure by depletion of minerals, ions and water and by the secretion of root exudates. Soils offer habitats for various communities of microorganisms including potential root endophytes. Plant and microbial metabolites may differentially influence the surrounding soil and change some of its properties, thus preparing the soil for the microorganisms of the next successional stage (Van Der Putten 2003).

Physical and Chemical Soil Characteristics

Soil pH had an effect on community composition but not on species diversity of endophytic fungi in Norway spruce roots (*Picea abies*) (Kattner and Schönhar 1990) [Table 7.1(iii)]. The similarity of only 57% of the endophyte communities in roots from neutral and acidic soils reflects either the selectivity of soil pH or the historical presence/absence of certain endophyte species, e.g. endophytes with low dispersion and/or survival rates. For example, *Phialocephala fortinii* preferentially occurs in roots growing in acidic soils (Ahlich et al. 1998).

Species richness was not related to soil texture in wheat roots (*Triticum aestivum*) (Riesen and Sieber 1985; Sieber et al. 1988). However, texture affected the frequency of *Microdochium bolleyi* and *Periconia macrospinosa*. *M. bolleyi* was more frequently isolated from roots originating from silty loam, whereas *P. macrospinosa* was isolated more often from roots growing in pure loam.

Root endophytes differ in their ability to metabolise minerals and carbohydrates, making some endophytes more successful than others in a given habitat. DSE are thought to be excellent metabolisers of phosphorus (P) and to mediate P uptake for their hosts (Jumpponen et al. 1998; Barrow and Osuna 2002). In fact, DSE were more abundant in habitats poor in P (Haselwandter and Read 1982; Ruotsalainen et al. 2002). Similarly, differential utilisation of carbohydrates as well as which carbohydrates were available determined fungal species diversity and endophyte-community composition in the experiments of Hadacek and Kraus (2002).

Water Regime

The water regime in soils and streams has a strong impact on species diversity and especially on the species spectrum of endophytic fungi (see Chap. 10 by Bärlocher). In roots of the same tree, 45 species were isolated from roots submerged in a river as opposed to only 31 species from non-submerged roots (Fisher et al. 1991) [Table 7.1(iv)]. The similarity of the community composition in submerged and non-submerged roots of the same individual black-alder tree was only 37%. Colonisation of submerged roots by aquatic hyphomycetes, together with the absence or scarcity of these specialists in non-submerged roots, emphasise the importance of the milieu in which roots grow in determining the composition and diversity of endophyte communities. For example, high water tables restricted the occurrence of *P. fortinii* in wetlands (Addy et al. 2000). The endophyte species diversity in roots of the mangrove *Rhizophora mucronata* strongly depended on the tidal level at which the roots were collected. Diversity was highest at the mid-tide level, i.e. the zone submerged in seawater approximately half of the time, and roots from the high-tide and the low-tide level had, on average, only 38% of species in common (Ananda and Sridhar 2002) [Table 7.1(v)]. Flooding and site conditions affected endophyte species spectra but not species richness in roots of common reed (*Phragmites australis*) (Wirsel et al. 2001) [Table 7.1(vi)]. In contrast, species spectra in bracken rhizomes (*Pteridium aquilinum*) did not differ among wetland and woodland sites (Petrini et al. 1992).

7.2.3 Multitrophic Interactions

The diversity of soil microorganisms is tremendous; 1 g soil can contain between 5,000 and 10,000 species of microorganisms (Torsvik et al. 1990). However, only 1,200 species of fungi have been isolated from soil (Watanabe 1994), perhaps because, as estimates suggest, only 17% of known fungi can be readily grown in culture (Hawksworth 1991). If this percentage were applied to the 1,200 species as suggested by Watanabe (1994), this would give an estimate of approximately 7,000 species of soil fungi (Bridge and Spooner 2001). The total length of fungal hyphae varies greatly according to soil type and soil biology and has been reported to be as high as 66,900 m in 1 g dry soil (Bååth and Söderström 1979). The high number of species and the high amount of microbial biomass in such small volumes of soil suggest that multitrophic interactions among soil bacteria, soil fungi, soil microfauna and plants are frequent. Interspecific competition may be "the" factor that overrides all others in regulating species abundance of soil fungi (Gochenaur 1984). If a community is dominated by inter- and intra-specific

competition, the resources are more likely to be fully exploited. Endophyte species diversity and spectrum will then depend on the range of available resources, including host tissues, the extent to which species are specialists, antagonism among competitors, their ability to overcome host defences and the permitted extent of habitat overlap.

Microdochium bolleyi is a frequent and successful endophyte in cereal roots, where it functions as an effective antagonist of various root pathogens. For example, its presence in wheat roots was negatively correlated with the presence of *Septoria nodorum*, the causal agent of glume blotch disease of wheat (Riesen and Sieber 1985; Sieber et al. 1988). Similarly, *M. bolleyi* inhibited various *Fusarium* species and *Gaeumannomyces graminis* var. *tritici* (Kirk and Deacon 1987; Reinecke 1978). Whether *M. bolleyi* interacts with these pathogens indirectly by inducing systemic resistance in the host plant, or directly by either parasitising pathogens or producing inhibitory metabolites, remains to be examined.

The phenological state of the roots and/or the season may influence endophyte species diversity by affecting the probability of interactions among endophytic thalli. For example, the number of dominant species was higher in young than in mature winter wheat, presumably because freshly established thalli were small. Growth was reduced due to the cold temperatures in winter, making hyphal interference less likely and/or weaker and, thus, also allowing less competitive fungi or fungi better adapted to cold temperatures to establish endophytic thalli [Table 7.1(vii)] (Riesen and Sieber 1985; Sieber et al. 1988). This situation changed in spring and summer, when the growth rate of endophytic thalli increased, making intra- and inter-species hyphal interactions more probable, leading to the dominance of the few most competitive species.

Similar to mycorrhiza, strict host specificity is the exception rather than the rule for fungal root endophytes (Bruns et al. 2002; Jumpponen et al. 2004). However, the likelihood of occurrence of some endophyte species increases in the presence of particular host species, suggesting fungal host preference or shared habitat preferences. The diversity of the plant community in which the host species grows may, therefore, influence root-endophyte diversity similarly as it has been shown to affect diversity of soil microfungi (Christensen 1981, 1989). Ahlich and Sieber (1996) presented an example of the importance of the plant community in determining the spectra of fungi associated with the host. The dominant root endophytes of European beech (*Fagus sylvatica*), *Cryptosporiopsis radicicola* and *Cylindrocarpon didymum*, were rare or absent in roots of Scots pine (*Pinus sylvestris*) growing in monoculture. Likewise, *P. fortinii*, the dominant root endophyte of Scots pine, was rare or absent in monocultures of beech. However, when the roots originated from mixed stands of Scots pine and beech, Scots pine roots showed a comparatively high rate of colonisation

by *C. radicicola* and *C. didymum*. Correspondingly, the roots of beech were frequently colonised by *P. fortinii* in mixed stands. In contrast, frequency of colonisation of *Betula papyrifera* and *Pseudotsuga menziesii* seedlings by DSE was not affected by whether or not the plants were grown in mixed culture or in monoculture (Jones et al. 1997).

In agriculture, the preceding crop may significantly affect endophyte diversity of the current crop. For example, species richness and the number of dominant species were significantly higher when wheat (*Triticum aestivum*) followed red clover than when it followed potatoes [Table 7.1(viii)] (Sieber et al. 1988). On average, only 59% of the endophyte species were indifferent to whether the preceding crop was clover or tomatoes. The range of indifferent endophyte species lay between 57% and 67% for other pairs of preceding crops [Table 7.1(viii)]. This observation may be related to differences in the spectra of endophytes that had colonised the preceding crop. Specific secondary metabolites and debris produced by the preceding crop, as well as the type and amount of agrochemicals (fertilisers, biocides, leafage killers) applied to the preceding crops may be other factors influencing both diversity and stimulation/inhibition of endophytes.

When different vegetables are grown in the same soil, some endophyte-host associations occur more frequently than others, suggesting host preference or adaptation. The similarity of the spectra of endophyte species among host species was as low as 44% in an experiment performed by Narisawa et al. (2002) [Table 7.1(ix)]. It is not known whether plants are able to actively recruit endophytes and vice-versa. Plant defence compounds probably select for certain rhizosphere microorganisms. Some evidence for such mechanisms comes from nematode and mycorrhiza research. Secondary metabolites released by roots of *Thuja occidentalis* upon attack by weevil larvae attracted entomopathogenic nematodes (Van Tol et al. 2001). Dormant propagules of mycorrhizal fungi were stimulated to germinate by chemical messengers from the host (Bruns et al. 2002). Correspondingly, mycelia of AMF were inhibited by non-host metabolites (Oba et al. 2002). Nothing is known about whether certain root endophytes release "pheromones" to attract roots of host plants.

7.2.4 Natural and Anthropogenic Disturbances

Anthropogenic and natural disturbances affect the species spectrum of plant communities and consequently also the communities of cohabiting microorganisms. Forest-management practices such as planting of trees, selective cutting or clearing of windthrows had a distinct effect on the endophytic mycobiota in the roots of forest trees (Görke 1998). Maximally

42% of the endophyte species were common to both planted and naturally regenerated trees [Table 7.1(x)]. Considering naturally regenerated trees only, species richness and the number of dominant species was highest in the cleared windthrow. Probably, endophyte diversity and community composition would also change as a consequence of gap formation by man and/or wind storm, which eliminates some hosts but creates habitats for many other hosts, i.e. ruderal plant species.

Mycorrhization and root-endophyte colonisation of naturally regenerated seedlings of *Betula platyphylla* var. *japonica* in soils of machine-graded ski slopes depended on the time elapsed since disturbance (Hashimoto and Hyakumachi 2000). Seedlings thrived well only in soil samples from soils disturbed more than 3 years previously and mycorrhization was significantly higher in these samples. In contrast, colonisation of roots by DSE was distinctly higher in seedlings sampled from soils disturbed only 1–3 years before sampling. In another study, the majority of naturally established seedlings of bishop pine (*Pinus muricata*) were colonised by DSE shortly after wildfire, indicating that a resident inoculum (chlamydospores, microsclerotia) survived the fire (Horton et al. 1998). Species richness of endophytes in roots of *Erica carnea* was highest at sites where soil pollution by heavy metals was high, but DSE occurred less frequently in the heavily polluted soils (Cevnik et al. 2000) [Table 7.1(xi)]. Endophytic fungi are either more competitive in disturbed or moderately polluted soils or better equipped to survive periods of adverse environmental conditions than mycorrhizal fungi.

The use of fungicides for crop protection can alter species diversity. Seed treatment with the systemic fungicide benomyl had no significant influence on endophyte species richness in wheat roots, but the frequency of roots colonised by seed borne *Septoria nodorum* was significantly reduced (Riesen and Sieber 1985). None of the fungicides applied to *Lolium perenne* fields at 18 sites in New Zealand had a significant effect on the root-endophyte communities (Skipp and Christensen 1989).

Fertilisation can affect fungal assemblages in roots. The frequency of *P. fortinii* in seedlings of potted *Picea glauca* was negatively correlated with the amount of nitrogen (N) applied (Kernaghan et al. 2003). Wilberforce et al.(2003) suspected N fertilisers to be one of the mechanisms by which management affects root endophyte communities in temperate grasslands. Emissions of air pollutants such as SO_2 and especially NO_x are thought to have a similar fertilising effect as fertilisers applied in agriculture. Adverse effects of these air pollutants on mycorrhizal fungi have been demonstrated in several studies (Cairney and Meharg 1999; Jansen and van Dobben 1987; Taylor and Read 1996).

7.3 Dark Septate Endophytes

Fungi with regularly septate and melanised hyphae probably constitute the most abundant and most widespread group of non-mycorrhizal root endophytes. In this section, we will briefly present the history of the term "DSE", outline the diversity of DSE and give an overview of current knowledge of the diversity and population genetics of the most prominent species complex of DSE: *Phialocephala fortinii* s. l.

7.3.1 History

Melin (1922, 1923) introduced the form taxon *Mycelium radicis atrovirens* (MRA) for sterile, melanised, septate mycelia that emerged from mycorrhizae and roots of *Picea abies* and *Pinus sylvestris*. The tree-fungus symbiosis was characterised by dematiaceous intra- and intercellular hyphae in the epidermal and cortical cells, but neither a Hartig net nor a mantle were formed. Melin (1923) coined the term "pseudomycorrhiza" for this relationship and considered it to form an antagonistic symbiosis. MRA-like fungi have been detected during numerous studies since Melin's pioneering work (Ahlich and Sieber 1996; Chan 1923; Freisleben 1934; Harley and Waid 1955; Jumpponen et al. 1998; Richard and Fortin 1973; Robertson 1954; Stoyke and Currah 1991). Since trinomials are not valid species names according to the International Code of Botanical Nomenclature, less stringent and more informal names are preferable. Read and Haselwandter (1981) introduced the term "DS hyphae" (DS = dark septate) for sterile, dark, septate hyphae and microsclerotia that occurred in roots of various alpine plants. Stoyke and Currah (1991) implemented the form taxon "dark septate endophyte" (DSE) and used it for fungi that form partly or entirely melanised, septate thalli within healthy root tissues. The taxon "DSE" serves primarily to differentiate these fungi from endophytes with septate, hyaline hyphae, and from fungi with sparsely septate, hyaline hyphae that are characteristic of AMF.

7.3.2 Biodiversity

The roots of more than 600 plant species representing about 320 genera in more than 110 families have been reported to be colonised by DSE (Ahlich and Sieber 1996; Barrow and Osuna 2002; Jumpponen and Trappe 1998b;

Kovacs and Szigetvari 2002; Ruotsalainen et al. 2002; Schadt et al. 2001). Dematiaceous mycelia are regularly received in culture during censuses of root endophytes, but it is often not known whether the endophytic thalli of these fungi are hyaline or melanised. This being the case, we must assume that DSE are much more widespread than previously assumed.

Species identity of some DSE is known because they readily sporulate in culture, e.g. *Microdochium bolleyi* and several *Phialophora* species in grasses and sedges. Many non-pathogenic *Phialophora* endophytes are related to the take-all fungi (*Gaeumannomyces graminis* var. *tritici* and var. *avenae*) of cereals and grasses in temperate areas and to *G. graminis* var. *graminis*, which causes crown sheath rot of rice in the tropics. *Phialophora radicicola* forms melanised sclerotia in cortical cells of maize roots without causing any apparent harm (Cain 1952). *P. radicicola* was also observed in the roots of three alpine grasses growing at the timberline in Bavaria (Blaschke 1986) or in roots of *Lolium perenne* in New Zealand (Skipp and Christensen 1989). The DSE abundantly observed in many alpine sedges in the Tyrolean Alps may also belong to *P. radicicola* (Haselwandter and Read 1980; Read and Haselwandter 1981). *P. radicicola* and *P. zeicola*, the maize take-all fungi from China, were recently shown to be the same species (Ward and Bateman 1999). *P. graminicola*, another non-pathogenic DSE of cereal and grass roots (Newsham 1999), provided significant control of the take-all disease by competition for senescing root tissues (Deacon 1981).

Taxonomic assignment of many DSE is problematic because sexual and asexual reproductive structures are either absent, rare, or are produced only under specific conditions. Cold treatment for up to 1 year was shown to induce sporulation in some DSE isolates, e.g. in isolates of *Chloridium paucisporum*, *Phialophora finlandica*, and *Phialocephala fortinii* (Wang and Wilcox 1985). Unfortunately, even then many DSE strains remain sterile and classification is complicated. Many mycologists have tried to bring some order into this difficult group of DSE (Harney et al. 1997; Melin 1923; Richard and Fortin 1973). Culture morphology is often used for an initial classification (Ahlich and Sieber 1996; Girlanda et al. 2002; Steinke et al. 1996; Stoyke et al. 1992). However, modern molecular biology offers a multitude of additional and potentially more reliable methods for the identification and typing of species, varieties and individuals (Carter et al. 1997; Geiser et al. 1994; White et al. 1990; Zietkiewicz et al. 1994). Some of these methods have been used to type DSE. Restriction patterns of a region on the ribosomal RNA (rRNA) genes indicated that two-thirds of the DSE from roots of subalpine plants were closely related to or conspecific with *P. fortinii* (Stoyke et al. 1992). Similarly, in a study by Harney et al. (1997), restriction site mapping of the nuclear rDNA internal transcribed spacer (ITS) regions showed that the majority of the isolates was *P. fortinii*-like and only two isolates were *Phialophora finlandica*.

According to isozyme analysis, DSE from various woody plant species belonged to two distinct groups (Ahlich-Schlegel 1997; Grünig et al. 2001; Sieber 2002). Members of the larger group were conspecific with *P. fortinii*, whereas those of the other group represented the sterile Type 1, which has been recently described as *Acephala applanata* (Ahlich and Sieber 1996; Grünig and Sieber 2005). Phylogenetic analysis of the ITS regions showed that *P. fortinii* and *A. applanata* are closely related and have *Phialocephala compacta*, *P. dimorphospora* and *P. scopiformis* as closest relatives (Grünig et al. 2002b). These five species are more closely related to members of the Leotiales such as *Gremmeniella abietina*, the causal agent of sclerroderis canker on pines, than to other *Phialocephala* species. The "*P. fortinii*-group" was also positioned within the Leotiales by phylogenetic analyses of the sequence data of the 18S and 28S subunits of the nuclear rRNA genes (Jacobs et al. 2003).

7.3.3 Diversity of *Phialocephala fortinii*

Phialocephala fortinii was shown to be the dominant DSE in coniferous and ericaceous roots in heathlands, forests and alpine ecosystems of the Northern temperate zones (Ahlich and Sieber 1996; Stoyke and Currah 1991). There is strong evidence that the roots of every Norway spruce (*Picea abies*) tree in natural forest habitats of Central Europe are colonised by this fungus (Ahlich and Sieber 1996; Grünig et al. 2004). The nature of root–*P. fortinii* symbioses and their ecological significance are largely unknown.

P. fortinii may function as a mycorrhizal fungus and mediate nutrient uptake, synthesise secondary metabolites, stimulate plant growth and/or play an important role in plant defence against root pathogens (Fernando and Currah 1996; Jumpponen and Trappe 1998a; O'Dell et al. 1993; Yu et al. 2001). Alternatively, it may behave as an opportunistic pathogen (Wilcox and Wang 1987). However, considering its widespread distribution and abundance it is very unlikely that *P. fortinii* is a primary pathogen.

We will provide a compilation of the newest findings on the genetic diversity within and among populations of *P. fortinii* and will conclude this section by forwarding some ideas and thoughts that could explain the observed diversity of this ecologically very successful species.

Genetic diversity of *P. fortinii* strains was examined on different spatial scales using isozymes, PCR-fingerprinting and analysis of the rDNA ITS regions either by polymerase chain reaction -restriction fragment length polymorphism (PCR-RFLP) analysis or sequencing. Ahlich-Schlegel (1997) studied the allelic diversity at seven isozyme loci and detected 108

different allozyme phenotypes among 194 European and North-American DSE strains. Allozyme patterns were neither host- nor site-specific. Harney et al. (1997) found many polymorphisms in the rDNA ITS regions of *P. fortinii* strains from Europe and North America by restriction mapping. Similarly, variability among rDNA ITS sequences was high (up to 12 substitutions) among 18 strains of *P. fortinii* from Central and Northern Europe (Grünig et al. 2002b). In contrast, Addy et al. (2000) detected a high degree of homogeneity among the rDNA ITS sequences of six strains of *P. fortinii* from Canada and Japan.

Strain-specific markers are necessary to study the genetic diversity at small spatial scales. In contrast to allozyme markers, ISSR-PCR markers were strain specific and allowed discrimination among isolates with identical allozyme phenotypes (Grünig et al. 2001). These markers were used to detect the population structure of DSE isolated from Norway spruce (*Picea abies*) roots collected within a 3×3 m plot of a 40-year-old plantation (Grünig et al. 2002a). Twenty-one unique ISSR-PCR genets were present among 144 strains. Identity of the isolated DSE as *P. fortinii* was confirmed by the morphology of the conidiogenous apparatus and by sequence comparisons of the rDNA ITS regions. Two genets dominated and were isolated from all sampling points within contiguous areas of at least 6.8 m^2 and 5.3 m^2 that overlapped by 3.6 m^2. Other genets were rare and were isolated only once or twice.

Jumpponen (1999) employed the random amplified polymorphic DNA (RAPD) technique to determine the population structure of *P. fortinii* at a primary succession site on a glacier forefront. In one year, 23 genets of *P. fortinii* were detected in 34 strains, in the next year 10 genets were found in 40 strains, but none of the genets was isolated in both years. Diversity of *P. fortinii* can be high even within single root pieces. For example, 8- to 10-cm-long pieces of fine root of *Picea abies* were colonised by up to six different inter-simple sequence repeat (ISSR) phenotypes (N. Nüssli and C.R. Grünig, unpublished) (Fig. 7.1). In summary, genetic diversity of *P. fortinii* seems to be high at every level. This is surprising for a supposedly asexual fungus. Therefore, studies on population genetics were initiated to find the sources of this high diversity.

ISSR-PCR and RAPD markers have many analytical drawbacks, such as dominance, and they cannot be used to infer population differentiation and recombination. In contrast, single-locus RFLP markers are codominant and supply robust data for precise population genetic analyses. In addition, data are comparable among studies and thus may be used for global analyses (Sunnucks 2000). Therefore, single-locus RFLP probes were developed for population genetic analysis of *P. fortinii* and used to find evidence for recombination, gene and genotype flow in *P. fortinii* (Grünig et al. 2003, 2004). Strains collected from three Norway-spruce plots up to 10 km apart

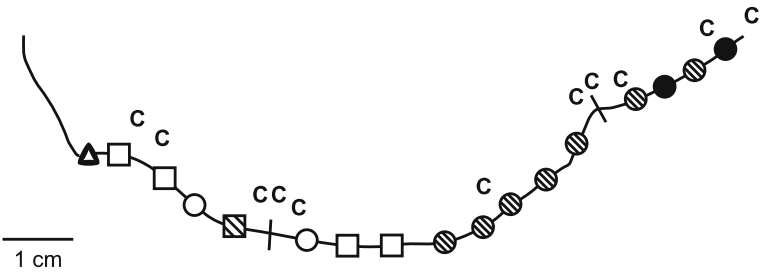

Fig. 7.1. Distribution of six inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) phenotypes belonging to three cryptic species of the root endophyte *Phialocephala fortinii* s. l. along a healthy fine root of Norway spruce (*Picea abies*). Identical symbols indicate positions on the root where the same phenotype was isolated. Symbols with identical shape represent the same cryptic species. *C* Positions on the root where *Cylindrocarpon didymum* was isolated as an endophyte

from each other were studied using 11 single-locus RFLP probes. The average gene diversity was high and up to 96 multilocus haplotypes (MLH) were observed per study plot. Significant population subdivision was detected among groups of MLH within plots, suggesting that groups were reproductively isolated and should be considered cryptic species. The RFLP data of more than 1,000 European strains indicate that *P. fortinii* s. l. is a species complex of at least eight cryptic species (C.R. Grünig, unpublished). The index of association (I_A) did not deviate significantly from zero within any cryptic species, suggesting that recombination occurs, or has occurred, within these species. Although evidence for recombination is strong for all cryptic species, it remains unclear whether sexual or parasexual processes are involved, and how often and where recombination occurs or when it last occurred (Taylor et al. 1999). Even a little sex is, however, already enough to give an organism the appearance of a recombining population (Brown 1999).

The sympatric occurrence of up to four reproductively isolated, cryptic species within a few square metres of forest floor, and sometimes even in the same root segment, is a highly interesting phenomenon and deserves a brief discussion (Grünig et al. 2004) (Figs. 7.1, 7.2). Reproductive isolation is essential for speciation. Geographically isolated populations are often reproductively isolated, and may experience allopatric speciation through genetic drift (Carter et al. 2001). On the other hand, niche or habitat specialisation may lead to sympatric speciation when local populations are confronted with heterogeneous habitats or several niches within habitats (Futuyma and Moreno 1988; Maynard Smith 1966). The patterns observed by Grünig et al.(2004) are clearly indicative of speciation. Possibly, the cryptic species were the products of allopatric speciation in the

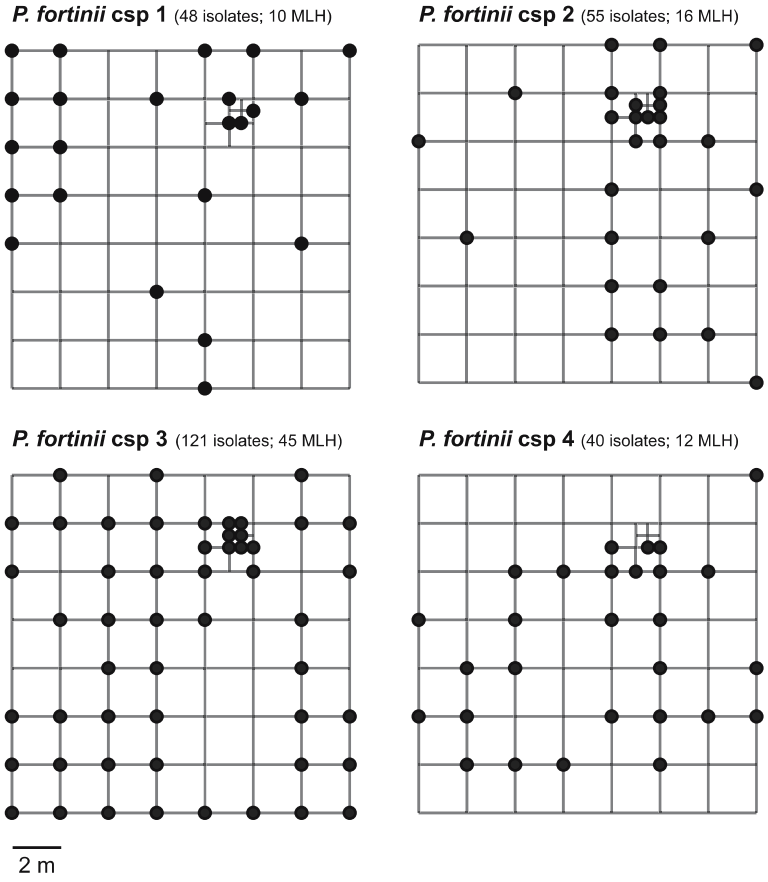

Fig. 7.2. Distribution of the four most frequently observed cryptic species (*csp*) of *Phialocephala fortinii* s. l. within healthy fine roots of Norway spruce (*Picea abies*) collected at the intersections of a 2 × 2 m grid superimposed on a forest plot (14 × 14 m) at Zürichberg, Switzerland. The four graphs represent the same study plot; the distributions of the four cryptic species are presented in separate graphs to maintain clarity. The number of isolates and the multilocus haplotypes (MLH) of each cryptic species are given in brackets

past due to geographical isolation. The ranges of these species may have subsequently overlapped (Brasier 1987). In this respect it is interesting to study the role of Quaternary climatic changes (Hewitt 2000). The succession of several glaciations and warmer inter-glacial periods had profound effects on animals, plants, and, consequently, on fungi. During the Quaternary, each species experienced many contractions/expansions of range, leading to extinctions and foundations of populations, decreases and increases in diversity and, thus, also to speciation (Taberlet et al. 1998). Refugia of relevant hosts of *P. fortinii* were often geographically isolated,

making allopatric speciation of *P. fortinii* possible. Alternatively, habitat heterogeneities are certainly present even within very small compartments of root tissues, the rhizosphere, and the surrounding soil. These heterogeneities may be pronounced enough for ecological isolation and for the development of cryptic species. Some cryptic species may be interspecific hybrids. For example, most asexual *Epichloë*-related grass endophytes appear to be such hybrids (Scott 2001). Interspecific hybrids may be better adapted to new niches such as new hosts and can provide greater or more diverse benefits to host plants (Schardl and Craven 2003). However, such hybrids were never observed for *P. fortinii* using codominantly inherited single-copy RFLP markers.

MLH with identical ISSR fingerprinting patterns were common to at least two of the sites in the study of Grünig et al. (2004). These results indicate that not only gene flow but also genotype flow most likely occurs in cryptic species of *P. fortinii*. Gene and genotype flow occur either naturally via conidia or microsclerotia transported by wind or micro- and macrofauna, or by silvicultural practices. Genotypes may be introduced by planting plants from nurseries located up to several hundreds of kilometers away (Bürgi and Schuler 2003), since nursery plants are frequently colonised by DSE including *P. fortinii* (Danielson and Visser 1990). Alternatively, machinery used during thinning and harvesting could be responsible for the import of genotypes.

Nothing is known about the significance of mutations, the ultimate source of genetic variation, for speciation within *P. fortinii* s. l. If a population is large and the mutation rate high, it is likely that mutants with higher fitness, e.g. better mutualists, will emerge (McDonald and Linde 2002). Non-lethal somatic mutations in the mitotic phase may affect the genetic diversity of a population since each nucleus has the capacity to be the founder genome of another, new mycelium (Burnett 2003). The diversity thus generated may supplement diversity generated by recombination.

7.4 Conclusions

Colonisation of roots by fungal endophytes is a common feature in the plant kingdom. In contrast to classical mycorrhizae, endophytes are regularly present in roots undergoing secondary growth. Root-endophyte species diversity is affected by climatic, physical, chemical, biological and anthropogenic factors. DSEs are among the most abundant root endophytes. They constitute a taxonomically very heterogeneous group of fungi, mostly ascomycetes, that form melanised, septate hyphae, chlamydospores or microsclerotia within the roots of the host.

Phialocephala fortinii is the most prominent DSE, especially in woody plant species. *P. fortinii* s. l. is genotypically very diverse and forms a complex of several cryptic species that can occur sympatrically. Cryptic species and selected genotypes of *P. fortinii* s. l. can now be used to test the ecological significance of these extremely abundant and successful organisms and to explain some of the contradictory results on fungus-host interactions reported in earlier studies. The elucidation of the mating mechanism(s) and the evolutionary forces that govern speciation in *P. fortinii* s. l. are other fascinating topics for future research.

We have reviewed patterns of species diversity and within-species genotypic diversity and presented several plausible explanations for these patterns, although conclusive evidence for cause and effect are still virtually lacking. Nevertheless, we would like to conclude with a motivating citation by Begon et al. (1990): "This is not so much a disappointment as a challenge to ecologists and biologists of the future. Much of the fascination of ecology and biology lies in the fact that many problems are blatant and obvious for everybody to see, while the solutions have as yet eluded us".

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