Endophytic fungi in branches of Norway spruce with particular reference to *Tryblidiopsis pinastri*

Pia Barklund and T. Kowalski

Abstract: A variety of endophytic fungi were isolated from the branches of Norway spruce (*Picea abies*). We found that as internodes age, the composition of endophytic species gradually changes, both qualitatively and quantitatively. One of the dominating species, *Tryblidiopsis pinastri*, was most common in young internodes. In contrast, three other common species, *Phialocephala scopiformis*, *Geniculosporium serpens*, and *Tapesia livido-fusca*, were most frequently isolated from old internodes. *Mollisia* spp. were also common components of the endophytic flora of the Norway spruce in Sweden.

Key words: *Picea abies*, *Tryblidiopsis pinastri*, *Phialocephala scopiformis*, *Geniculosporium* spp., *Tapesia* spp., *Mollisia* spp.

Résumé : Les auteurs ont isolé une variété de champignons endophytes à partir de branches de l'épinette de Norvège (*Picea abies*). Avec le vieillissement des entre-nœuds, la composition en espèces endophytiques change graduellement, en qualité aussi bien qu'en quantité. Une des espèces dominantes, le *Tryblidiopsis pinastri*, est la plus commune chez les jeunes entre-nœuds. Au contraire, trois autres espèces communes, *Phialocephala scopiformis*, *Geniculosporium serpens* et *Tapesia livido-fusca*, ont été plus fréquemment isolées des entre-nœuds plus âgés. Les *Mollisia* spp. sont également des composantes communes de la flore endophytique de l'épinette de Norvège, en Suède.

Mots clés : *Picea abies*, *Tryblidiopsis pinastri*, *Phialocephala scopiformis*, *Geniculosporium* spp., *Tapesia* spp., *Mollisia* spp.
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Introduction

Endophytes in living branches of conifers were not investigated until recently, even though such infections were reported from twigs of *Picea* and *Larix* over 70 years ago (Lewis 1924).

Studies have now shown that branches of trees harbour a wide range of endophytic fungi that cause no visible symptoms in their host (Petrini and Müller 1979; Sieber and Hugentobler 1987; Petrini and Fisher 1988, 1990). Specific information about endophytic fungi in branches of Norway spruce (*Picea abies* (L.) Karst.) was provided by Sieber (1989), Neumüller (1992), and Kowalski and Kehr (1992). For example, living branches of 11 coniferous and deciduous tree species were found to contain a variety of mycoflora, and a few of the fungi were confined to a single tree species (Kowalski and Kehr 1992). One species, *Tryblidiopsis pinastri* (Pers.:Fr.) P. Karsten, only occurred in Norway spruce (Kowalski and Kehr 1992). In the living basal parts of the branches investigated in Germany, many other endophytic fungi were more common than *Tryblidiopsis pinastri* (Kowalski and Kehr 1992). The three most common species were *Mollisia cinerea* (Batsch ex Merat) Karst., *Pezicula livida* (Berk. & Br.) Rehm, and *Pezicula cinnamomea* (DC.) Sacc. In a Swiss study, these three species and *Tryblidiopsis pinastri* did not occur at all in 5- to 6-year-old segments of Norway spruce branches (Sieber 1989). Instead, the most common taxa were *Sirodothis* sp. and *Gelatinosporium* sp., genera also found by Kowalski and Kehr (1992), and *Pocillopycnis umensis* (Bubák & Vleugel) Dyko & Sutton, which was not found by Kowalski and Kehr (1992). These are just a few of the examples of the differences between the two investigations by Sieber (1989) and Kowalski and Kehr (1992) in terms of the endophyte flora found.

Ascomata of *Tryblidiopsis pinastri* develop along dead branches (Livsey and Minter 1994). The fungus is primarily an endophyte, and the general consensus is that it does not harm the tree. However, Kujala (1950) suggested that *Tryblidiopsis pinastri* could attack living shoots, branches, and even trunk bark if the vitality of the tree was reduced, for example through frost damage. Recently, extensive fruitbody formation was observed on areas of trunks, where the inner bark had been dead for at least one growing season (Livsey and Minter 1994).

Although there is some evidence that endophytes may help trees defend against pests and pathogens (Carroll 1986), little is known about disease prevention that endophytic mycoflora have in the tree. Earlier work showed that colonization by the pathogen *Gremmeniella abietina* (Lagerb.) Morelet tended to occur slowly in Norway spruce twigs with a rich mycoflora of endophytic fungi, not determined to species (Barklund and Unestam 1988).

In this project whole Norway spruce branches were examined to characterize the endophytic mycoflora, with special emphasis on determining the incidence of *Tryblidiopsis pinastri*. More knowledge about endophytes is a prerequisite for assessing their influence on their host.

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Table 1. Data about the investigated Norway spruce branches and number of samples.

Part of crown	Branch No.	Branch Diam. (cm)	Branch Length (cm)	Branch No. of internodes	No. of fragments sampled Outer bark	Inner bark	Wood	Bark scales
Tree A*								
Upper	1	2.0	128	5	50	50	50	
Upper	2	1.8	104	5	50	50	50	
Middle	1	2.2	165	9	90	90	90	20
Middle	2	2.3	186	12	120	120	120	20
Lower	1	2.4	190	16	160	160	160	35
Lower	2	2.2	194	17	170	170	170	85
Tree B†								
Upper	1	1.0	78	3	30	30	30	
Upper	2	1.1	84	3	30	30	30	
Middle	1	1.2	108	5	50	50	50	
Middle	2	1.2	102	5	50	50	50	
Lower	1	1.4	147	8	80	80	80	
Lower	2	1.4	135	8	80	80	80	
Total					960	960	960	160

*Sampling time was August 7–10, 1991.
†Sampling time was August 10–12, 1991.

Materials and methods

Pieces from living branches of *Picea abies* were cultured to determine the fungal flora in (*i*) outer bark, which consisted mainly of dead bark or rhytidome as well as some living phellem lacking chlorophyll; (*ii*) inner bark, i.e., living bark consisting mainly of phloem but also with some phelloderm and cambium; and (*iii*) wood, which consisted of xylem. These tissues correspond fairly well with the peridermal bark, sub-peridermal bark, and wood, respectively, referred to by Kowalski and Kehr (1992). Branches were taken from one 25-year-old tree (tree A) and one 12-year-old tree (tree B), growing 40 km apart in pure, even-aged stands about 70 km north of Stockholm. The canopy of the older stand was closed, whereas the young stand was open. In total, six branches were taken from each tree: two branches from the upper part of the crown, two from the middle part of the crown, and two from the lower part of the crown. Branches from tree A were taken at 8, 4, and 1 m above the ground, while those from tree B were collected at 3.5, 1.5, and 0.5 m heights.

Each branch, which was removed in its entirety, had from 3 to 17 annual internodes. In total, 96 annual internodes were examined (Table 1). The total length of each branch and the diameter at its midpoint were measured. The main stem of the branch was divided at the nodes. Side branches and needles, including petioles, on the internodes were carefully removed. Only the internodes were used for the investigation. The internode sections were washed under running water to get rid of loose material, such as lichens on the surface, and then soaked in 96% ethanol for 1 min, followed by sodium hypochlorite (4% active chlorine) for 5 min, and finally 96% ethanol for 30 s. The stem pieces were then left to dry on sterile filter paper. Thirty samples (ca. 5 × 2 mm) were taken at random from each internode: 10 from the outer bark, 10 from the inner bark, and 10 from the wood. These pieces were placed on malt agar (2% malt) with 100 mg/L of streptomycin. In addition, some loose bark scales, still connected to the internode, were included in the analysis (Table 1). The methods were comparable to those used by Kowalski and Kehr (1992). The incubations were begun within 24 h after the branches had been cut in the forest. The Petri dishes were kept at +18°C for 2 months. Fungi growing from tissue pieces were transferred to new malt agar to promote sporulation. Taxonomic determinations were mainly based on the characteristics of spores produced in pure culture. In some cases, our isolates were compared with known mycelia from our personal working culture collection. Fruit bodies on dead or living twigs of *Picea abies* from the stands from which branches had been taken were used to establish pure cultures of fungi to be used for the determination of some species.

Statistical analyses were carried out. The incidence of various fungi in the different branch tissues analysed were tested by analyses of variance using the GLM procedure of SAS (SAS Institute Inc. 1992).

Results

The number of fungi isolated varied depending on the type of branch tissue used. Of the outer bark samples tested, 78–96% were colonized by fungi. Corresponding values were 7–21% for the inner bark and 0–1.7% for the wood. Up to three species were isolated per 5 × 2 mm fragment of outer bark.

Eighty-five taxa of fungi were isolated, of which 58 were determined to species or genus. The remaining 27 isolates, although distinct, did not sporulate and could not be identified even to genus (Tables 2 and 3). In both trees, the outer bark incubations yielded the most fungal species. Fifty species were isolated from tree A and 49 species from tree B (Table 2). All species that occurred in at least 3% of the incubated samples in one tree also occurred in samples from the other. Of the species isolated, only a few were common. Thus only six and five species were present in at least 5% of the outer bark samples from trees A and B, respectively. In tree B they were *Geniculosporium serpens*, *Mollisia cinerea*, *Phialocephala scopiformis*, *Tapesia livido-fusca*, and *Tryblidiopsis pinastri*, while in tree A these five species plus *Geniculsporium* sp. were common (Table 2). There were great differences in frequencies between the trees. For example, the frequency of outer bark pieces with *M. cinerea* was

Table 2. Frequency (%) of isolation for various fungal species from different tissues of *Picea abies* branches from 25-year-old tree A and 12-year-old tree B.

Taxon	Tree A			Tree B		
	Outer bark	Inner bark	Wood	Outer bark	Inner bark	Wood
Alternaria alternata (Fr.) Keissl.				2.2		
Botrytis cinerea Pers.				2.2	0.3	
Coniothyrium fuckelli Sacc.				1.6		
Epicoccum nigrum Link	0.6			2.8		
Geniculosporium serpens Chesters et Greenhalgh	14.7			5.9		
Geniculosporium sp.	5.6			1.3	0.6	
Lachnellula abietis (Karst.) Dennis	2.5	0.6		2.2	0.9	
Mollisia cinerea (Batsch ex Mérat) Karst.	5.9	0.2		36.9	0.9	
Pezicula cf. *cinnamomea* (D.C.) Sacc.	1.1					
Phialocephala cf. *dimorphospora* Kendrick	4.7			0.6		
Phialocephala scopiformis Kowalski & Kehr	26.1	3.4	0.3	11.9	4.4	
Phomopsis occulta Trav.	4.4	0.3		2.2		
Sirococcus strobilinus Preuss	1.4			3.1		
Sirodothis sp.	2.5	0.3		1.3		
Tapesia livido-fusca (Fr.) Rehm	5.9	0.2		10.6	0.9	
Tapesia sp. 1	2.3	0.3		1.6	0.6	
Tryblidiopsis pinastri (Pers.) Karst.	24.2	8.8	0.2	6.9	2.5	
Taxa isolated only from rhytidome*	10.3			10.7		
Taxa isolated from less than 1% of each type of tissue†	3.1	1.4	0.2	4.8	4.2	0.6
Total no. of isolated fungal colonies	735	96	4	355	51	2

*The following taxa constitute less than 1% of total isolations of rhytidome from each tree: *Cladosporium herbarum* (Pers.) Link ex S.F. Gray; *Cystodendron* sp. 1; *Cystodendron* sp. 2; *Cytosporella* sp.; *Daldinia* sp.; *Dasyscyphus acuum* (Alb. et Schw.) Sacc.; *Endophragmiella* sp.; *Fusarium* sp.; *Lecytophora hoffmannii* (van Beyma) W. Gams et McGinnis; *Leptostroma* sp.; *Melanconium apiocarpum* Link; *Mollisia* cf. *cembrincola* Rehm; *Mollisia* cf. *discolor* (Montagne) Phill.; *Mollisia* sp. 1; *Mollisia* sp. 2; *Pezicula livida* (Berk. et Br.) Rehm; *Phacidiopycnis pseudotsugae* (Wils.) Hahn; *Phialocephala* sp.; *Phialophora cyclaminis* Beyma; *Rhizosphaera kalkhoffii* Bub.; *Sordaria fimicola* (Rob.) Ces. et de Not.; *Topospora* sp. 1; *Trichoderma harzianum* Rifai; *Ulocladium atrum* Preuss; *Ulocladium botrytis* Preuss; *Verticicladium trifidum* Preuss; 19 nonsporulating isolates.

†The following taxa were isolated from less than 1% of the rhytidome, inner bark, and xylem from each tree: *Aureobasidium pullulans* (de Bary) Arn; *Basidiomycetes*; *Cladosporium cladosporioides* (Fresen.) de Vries; *Coniothyrium pithyophilum* (Höhn.) Pet. et Syd.; *Dasyscyphus* sp.; *Dermea piceina* Groves; *Hormonema* sp. 1; *Hormonema* sp. 2; *Lachnellula suecica* (de Bary. ex Fuck.) Nannf.; *Oidiodendron* sp.; *Phylactaena* sp.; *Rhizoctonia* sp.; *Sclerophoma pythiophila* (Corda) v. Höhn.; *Trimmatostroma* sp.; six nonsporulating isolates.

more than six times higher in tree B. On the other hand, the frequencies of *Phialocephala scopiformis* and *Tryblidiopsis pinastri* were two- and three-fold higher, respectively, in tree A.

Fifteen species were isolated from the inner bark of tree A and 20 from the inner bark of tree B (Table 2). In the inner bark of both trees, the most frequently isolated species were *Tryblidiopsis pinastri* (8.8 and 2.5%) and *Phialocephala scopiformis* (3.4 and 4.4%). All other species occurred in fewer than 1% of the pieces. The fungi occurring in the inner bark were, with a few exceptions, the same as those found in the outer bark.

Both *Tryblidiopsis pinastri* and *Phialocephala scopiformis* were only isolated from a few samples from xylem. Other fungi were only occasionally isolated (Table 2).

Geniculosporium serpens (74.4%) and *Geniculosporium* sp. (21.3%) dominated the fungal flora isolated from the bark scales. The frequencies of the other 12 species isolated from bark scales were all lower than 2% (Table 3). Both *Geniculosporium* species were more common in the bark scales than in the outer bark (cf. Table 2). Frequencies with which these two species were isolated from scales did not differ between internodes, indicating that their abundance was not affected by internode age.

Table 3. Fungi isolated from bark scales on branches from the midcrown and lower crown on the 25-year-old Norway spruce.

Taxon	No.	%
Geniculosporium serpens	120	75
Geniculosporium sp.	34	21
Total no. of isolated fungal colonies		
From 160 bark scales	173	
From sterile bark scales	16	10

Note: The following taxa were isolated to less than 2%: *Aureobasidium pullulans*; *Dasyscyphus* sp.; *Epicoccum nigrum*; *Lachnellula abietis*; *Mollisia cinerea*; *Mortierella isabellina* Oudem.; *Phialocephala* cf. *dimorphospora*; *Phialocephala scopiformis*; *Phomopsis occulta*; *Tapesia* sp. 1; two nonsporulating isolates.

Table 4. Frequency (%) of the most common fungal species isolated from branches in the upper, middle, and lower crown of the tree.

Taxon	Tree A			Tree B			*P* value
	Upper	Middle	Lower	Upper	Middle	Lower	
Outer bark							
Tryblidiopsis pinastri	42.0	31.9	13.9	10.0	9.0	4.4	<0.004
Phialocephala scopiformis	4.0	18.6	37.6	8.3	6.0	16.9	<0.004
Geniculosporium serpens	0	5.7	24.8	0	3.0	10.0	<0.0001
Geniculosporium sp.	4.0	5.7	6.1	0	0	2.5	ns
Tapesia livido-fusca	1.0	6.2	7.3	5.0	3.0	17.5	ns
Mollisia cinerea	11.0	11.4	0.9	28.3	40.0	38.1	ns
Inner bark							
Tryblidiopsis pinastri	6.0	16.7	4.5	5.0	2.0	1.9	—
Phialocephala scopiformis	0	2.4	5.2	0	4.0	6.0	—

Note: Variation in the frequency of isolation with height in the crown was evaluated, using ANOVA, for which *P* values are given for the 25-year-old Norway spruce (tree A).

Table 5. Frequency (%) of the most common fungal species isolated from branch internodes of different ages.

Taxon	Age-class of internodes			*P* value
	1975–1981	1982–1986	1987–1990	
Tryblidiopsis pinastri	6.7	15.8	46.7	<0.0001
Phialocephala scopiformis	52.0	38.4	6.3	<0.0001
Geniculosporium serpens	26.0	21.1	5.8	<0.001
Geniculosporium sp.	9.3	1.6	5.4	ns
Tapesia livido-fusca	12.7	3.5	1.7	<0.001
Mollisia cinerea	7.9	7.9	2.7	ns
No. of internodes	15	19	24	—

Note: Variation in the frequency of isolation with age-class was evaluated, using ANOVA, for which *P* values are given for the 25-year-old Norway spruce (tree A).

Overall, the incidence of fungi was lower in branches from the upper part of the crown than in the lower branches in both trees. Frequencies of the three most common species in the outer bark were influenced by the position of the branches in the crown (Table 4). The species *Phialocephala scopiformis* and *Geniculosporium serpens* were significantly more common in the lower branches of both trees. In contrast, *Tryblidiopsis pinastri* was significantly more common in the upper branches than in the lower ones. The differences were especially pronounced in the older tree (tree A), in which the height at which the upper branches had been taken was twice that at which branches had been sampled from the younger tree (tree B), which could explain the greater influence of position in the older tree. In the inner bark, the relation between position and the incidence of the two most common species there, *Tryblidiopsis pinastri* and *Phialocephala scopiformis*, was similar to those found in the outer bark (Table 4).

The total frequency of fungi in the outer bark pieces increased with internode age. The frequencies of *Phialocephala scopiformis*, *Geniculosporium serpens*, and *Tapesia livido-fusca* were significantly higher in the older internodes than in the younger ones (Table 5). In contrast, *Tryblidiopsis pinastri* was significantly more common in younger internodes. As the older tree had longer and older branches, the differences between internode age was most pronounced. The results presented are from tree A, with longer and older branches, which showed more pronounced differences (Table 5). Current-year shoots were excluded from the analysis because surface sterilization could have affected the endophytic flora of young shoots that had not yet developed a rhytidome layer.

Fungal fruit bodies were seldom seen on living branches. The only apothecia found had been produced by *Lachnellula abietis* and *Dasyscyphus* sp. and occurred in the middle of the crown in tree A.

Discussion

The great majority of fungal colonies were isolated from the outer bark. Thus, in the discussion below we are referring to results obtained from the outer bark, if not otherwise indicated.

In an attempt to explain why *Phialocephala scopiformis*, *Geniculosporium serpens*, and *Tapesia livido-fusca* were isolated significantly more frequently from the older, thick-barked parts of the branch, we suggest that the thicker rhytidome provides more protection for the fungi living near the surface, probably outside the phellogen. *Tryblidiopsis pinastri* showed the opposite pattern, apparently thriving in the

apical, thin-barked parts of the branch (Table 5). *Tryblidiopsis pinastri* was also regularly isolated from the inner bark, which indicates that the fungus colonized shoots inside the phellogen layer. Only one other species, *Phialocephala scopiformis,* was regularly isolated from the inner bark. According to Kowalski and Kehr (1992), only *Tryblidiopsis pinastri* and *Phialocephala scopiformis* should be considered true endophytes. They proposed the term phellophytes for those fungi isolated only from peridermal bark.

Initial colonization by *Tryblidiopsis pinastri* takes place in the current year's shoots during the summer, when the apothecia from dead branches mature. In August, the frequency of isolation of the fungus was slightly lower from current shoots than from 1-year-old shoots (results not shown), which indicates that the colonization of current year's shoots was probably still under way. *Tryblidiopsis pinastri* was the only fungal species to exhibit a declining frequency of isolation with increasing branch age (Table 5). As mentioned earlier, *Tryblidiopsis pinastri* was found to be highly specific to Norway spruce by Kowalski and Kehr (1992), thus *Tryblidiopsis pinastri*, compared with other endophytes in this study, has a special relationship to Norway spruce. However, since the investigation was confined to the basal parts of the branches, the authors were unaware of the occurrence of *Tryblidiopsis pinastri* in the apical parts of the branches. The frequency of the fungus was about 7% in the outer bark of the basal parts, which is about equal to the frequency found in the older internodes in the present study.

Norway spruce harbours at least two endophytes, *Tryblidiopsis pinastri* and *Lophodermium piceae* (Fuckel) Höhn., which both seem to be host specific. The life cycle of *Tryblidiopsis pinastri* (Livsey and Minter 1994) resembles that of *Lophodermium piceae* (Osorio and Stephan 1991). Both fungi mainly colonize young tissues, with the former inhabiting the current shoots and latter inhabiting the needles on these current shoots (Barklund 1987). Ascomata of both species normally develop several years after initial colonization, those of *Tryblidiopsis pinastri* on dead parts of branch internodes and ascomata of *Lophodermium piceae* on old dead needles. Both species also show clear organ specificity, the former restricted to shoot tissues and the latter to the needle lamina, and they are the dominant endophytes in the respective tissues that they colonize.

The mycoflora isolated from the thin bark scales was dominated by *Geniculosporium serpens* and *Geniculosporium* sp. Other fungi common in the bark were rare or absent in cultures from bark scales. Bark scales were a more characteristic habitat for *Geniculosporium* spp. than was the bark itself.

Many of the species identified from living basal parts of Norway spruce in Germany by Kowalski and Kehr (1992) also occurred in the present study. However, *Pezicula cinnamomea*, *Pezicula livida*, and *Lecytophora hoffmannii* were common in the German study but only occasional in the Swedish study. *Geniculosporium serpens* and *Phialocephala scopiformis* were common fungi in both countries. The latter fungus was called *Phialocephala* sp. by Kowalski and Kehr (1992). The same authors recently described it as a new species, *Phialocephala scopiformis* (Kowalski and Kehr 1995).

In an Austrian study, the most common endophytes in branches of *Picea abies* were *Mollisia* spp., *Pezicula livida*, *Phialocephala scopiformis* (as *Phialocephala* sp. later investigated by T. Kowalski), and *Geniculosporium* spp. (Neumüller 1992). Except for *Pezicula livida*, the most common species in Austria are also among the most common species in the Swedish study.

Results obtained by Sieber (1989) from Switzerland differ greatly from our findings in Sweden. For instance, *Corniculariella abietis* Karst and *Pocillopycnis umensis* were isolated relatively commonly in Norway spruce samples examined by Sieber but were not found by us in Sweden. On the other hand, *Tryblidiopsis pinastri*, *M. cinerea*, and *Phialocephala* spp., which were frequently isolated in our investigation, were not isolated by Sieber (1989).

A group of species of the closely related Ascomycete genera *Mollisia* and *Tapesia*, both genera synonymized under *Tapesia* by Aebi (1972), were isolated commonly in the present study: *M. cinerea*, *M.* cf. *cembrincola*, *M.* cf. *discolor*, *Mollisia* sp. 1, *Mollisia* sp. 2, *Tapesia livido-fusca*, and *Tapesia* sp. 1. *Cystodendron* sp. 1 and *Cystodendron* sp. 2 are included in the list, as Aebi (1972) regarded *Cystodendron* to be an anamorph of *Tapesia*. Thus nine endophytic species of the *Mollisia*–*Tapesia* complex were isolated from *Picea abies*, many more than previously reported from trees in any similar study. *Mollisia cinerea* was the only common species in this genus. This species was not host specific, being found in all 11 tree species investigated by Kowalski and Kehr (1992), including both conifers and hardwoods. *Mollisia cinerea* was also isolated from *Juniperus communis* L. (Petrini and Müller 1979).

Some of the isolates were difficult to identify to genus or species because they did not sporulate. However, for the genera *Mollisia* and *Tapesia*, the problem was primarily a lack of suitable taxonomic literature. Even when ascomata were found on dead branches, most could only be determined to genus. Similar difficulties in identifying *Mollisia* species were reported by Greenleaf and Korf (1980).

Although the endophytic branch flora of the two trees in the present study were similar, the occurrence of certain species differed greatly. For example, the two most common fungi in the outer bark in tree A were *Tryblidiopsis pinastri* and *Phialocephala scopiformis*, while in tree B, *Tapesia livido-fusca* and *M. cinerea* were the two most common (Table 4). The older tree was growing in a stand with a closed canopy and a poor field layer. In contrast, the younger tree was growing in a more open stand and had a rich field layer with grasses and herbs, thus providing a high biodiversity in the vicinity, probably also favouring a more diverse phyllosphere microbial community on the tree. Possibly the most specific fungi, the phellophytes *Tryblidiopsis pinastri* and *Phialocephala scopiformis*, might have had difficulties competing within the phyllosphere early in the infection process. Instead, *M. cinerea*, reported by Kowalski and Kehr (1992) from a number of tree species and therefore more unspecific, seemed to be favoured in the same area (cf. Table 4).

From living branches, the frequency of isolation of *Tryblidiopsis pinastri* is generally highest in apical parts of the branch (Table 5). However, on dead branches of Norway spruce, fruit bodies of *Tryblidiopsis pinastri* are often uniformly distributed, with greater concentrations occurring around the nodes (Livsey and Minter 1994). This shows that much remains to be learned regarding the expression of these

fungi as mycelia or fruit bodies. Such information would help us understand the life cycles of the different species and the roles of different fungi in connection with the host tree.

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References

Aebi, B. 1972. Untersuchungen über *Discomyceten* aus der Gruppe *Tapesia–Trichobelonium*. Nova Hedwigia, **23**: 49–112.

Barklund, P. 1987. Occurrence and pathogenicity of *Lophodermium piceae* appearing as an endophyte in needles of *Picea abies*. Trans. Br. Mycol. Soc. **89**: 307–313.

Barklund, P., and Unestam, T. 1988. Infection experiments with *Gremmeniella abietina* on seedlings of Norway spruce and Scots pine. Eur. J. For. Pathol. **18**: 409–420.

Carroll, G.C. 1986. The biology of endophytism in plants with particular reference to woody perennials. *In* Microbiology of the phyllosphere. *Edited by* N.J. Fokkema and J. van Heuvel. Cambridge University Press, Cambridge. pp. 203–222.

Greenleaf, M.A., and Korf, R.P. 1980. *Mollisia* in Macronesia: an exercise in frustration. Mycotaxon, **10**: 459–472.

Kowalski, T., and Kehr, R.D. 1992. Endophytic colonization of branch bases in several forest tree species. Sydowia, **44**: 137–168.

Kowalski, T., and Kehr, R.D. 1995. Two new species of *Phialocephala* occurring on *Picea* and *Alnus*. Can. J. Bot. **73**: 26–32.

Kujala, V. 1950. Über die Kleinpilze der Koniferen in Finnland. Commun. Inst. For. Fenn. **38**: 48–49.

Lewis, F.J. 1924. An endotrophic fungus in the *Coniferae*. Nature (London), **114**: 860.

Livsey, S., and Minter, D.W. 1994. The taxonomy and biology of *Tryblidiopsis pinastri*. Can. J. Bot. **72**: 549–557.

Neumüller, A. 1992. Untersuchungen zum Zweig- und Aststerben an Fichte (*Picea abies* (L.) Karst.) in Schöneben. Dissertation, Universität für Bodenkultur, Wien.

Osorio, M., and Stephan, B.R. 1991. Life cycle of *Lophodermium piceae* in Norway spruce needles. Eur. J. For. Pathol. **21**: 152–163.

Petrini, O., and Fisher, P.J. 1988. A comparative study of fungal endophytes in xylem and whole stem of *Pinus sylvestris* and *Fagus sylvatica*. Trans. Br. Mycol. Soc. **91**: 233–238.

Petrini, O., and Fisher, P.J. 1990. Occurrence of fungal endophytes in twigs of *Salix fragilis* and *Quercus robur*. Mycol. Res. **94**: 1077–1080.

Petrini, O., and Müller, E. 1979. Pilzliche Endophyten, am Beispiel von *Juniperus communis* L. Sydowia, **32**: 224–251.

Sieber, T.N. 1989. Endophytic fungi in twigs of healthy and diseased Norway spruce and white fir. Mycol. Res. **92**: 322–326.

Sieber, T.N., and Hugentobler, C. 1987. Endophytische Pilze in Blättern und Ästen gesunder und geschädigter Buchen (*Fagus sylvatica* L.). Eur. J. For. Pathol. **17**: 411–425.