# Endophytic mycobiota in stems and branches of *Betula pendula* to a different degree affected by air pollution

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Received 23. 2. 1998

**Key words:** Endophytic mycobiota, endophytes of *Betula pendula*, air pollution.

**Abstract:** Communities of fungal endophytes in stems and branches of *Betula pendula* from three stands growing under the effect of various concentrations of air pollutants are presented. The effect of emission on the endophyte assemblages is discussed. Moreover, the differences in the occurrence of endophytes depending on the type of tissue (bark, xylem), the organ (stem, branches), and the location of tissues in stems and branches, as well as differences between individual trees are pointed out.

**Zusammenfassung:** Gemeinschaften endophytischer Pilze in Stämmen und Ästen von *Betula pendula* von drei Standorten, die unter der Einwirkung verschiedener Konzentrationen von Luftschadstoffen stehen, werden vorgestellt. Die Auswirkung der Emissionen auf die Endophytengemeinschaften wird diskutiert. Weiters werden die Unterschiede im Vorkommen von Endophyten gezeigt, die auf der Art des Gewebes (Borke, Xylem), dem Organ (Stamm, Äste), und der Position der Gewebe in Stämmen und Ästen beruhen, sowie die Unterschiede zwischen den einzelnen Bäumen.

Air pollution may affect the occurrence of pathogenic fungi (GRZYWACZ & WAŻNY 1973, KOWALSKI & WRZALIK 1996, LAURENCE 1981), as well as the abundance of fungi in microbial communities of the phyllosphere (HELANDER & al. 1993 b, MAGAN & MCLEOD 1991). It is not clear, however, what effect has air pollution on endophytic fungi. It would be important to know this for at least two reasons. Firstly, the endophytes live within plant tissue, and are not subjected to rapid changes as are the epiphytic microorganisms. Thus, the examination of endophyte assemblages might be a better indicator of long-term effects of environmental changes (BARKLUND & ROWE 1983, HELANDER & al. 1994). Secondly, it is believed that the endophytic fungi, besides a positive effect on a host, may cause disease symptoms in case of disturbance in the equilibrium between a fungus and a host (CARROLL 1988, SIEBER 1989). Therefore, the determination of changes in populations of the endophytic fungi upon the effect of air pollution could bring nearer the problems of epidemiology of the diseases in industrial regions, where the weakness pathogens become particularly active (KOWALSKI 1981). Experiments, not numerous so far, indicate that a simulated acid rain reduces the occurrence of endophyte populations (HELANDER & al. 1993 a, 1994).

The purpose of this study was to determine a species composition of endophyte assemblages in stems and branches of *Betula pendula* ROTH., and to observe differences in the populations obtained, depending on the degree of the effect of industrial

emissions. Moreover, it will be possible to compare the results obtained with fungi which are most frequently associated with mortality of branches or whole trees of *B. pendula* in the industrial regions (KOWALSKI 1981).

**Material and methods**

Material for this study was collected from trees of *B. pendula* appearing healthy, and growing in three approximately 20-year-old stands situated in areas of different effect of industrial emissions. In all three stands *Pilus sylvestris* L. was the main forest tree species, while *B. pendula* formed 20-30% admixture. The stand 1, situated in the Myślenice forest district (compartment 2 k), about 40 km south west of Kraków, was free of the effect of air pollution, and is referred in this paper as "zone F". The stands 2 and 3 were situated in the Upper Silesia, and were under the effect of emissions, mainly from Zink and Lead Smelter "Miasteczko Śląskie". The stand 2 was situated about 12 km west of the smelter, in zone of low pollution (zone L = < 0.05 mg $SO_2$ $m^{-3}$). The stand 3 was situated 1.2 km from the emission source in zone of high emission concentration (zone H = >0.09 mg $SO_2$ $m^{-3}$). The latter two stands were growing under the effect of not only $SO_2$ but also $NO_X$ and high dust fallout. For example, in 1992 the concentration of $NO_2$, 0.5 km away from the source, was 0.61 mg/$m^2$/month (measured on plate with $K_2CO_3$), and the dust fallout was 219.0 kg/ha/year.

In September and October 1996, three trees of *B. pendula*, 7.5-10.5 m in height, and growing within the distance not greater than 10 m from one another, were felled in each stand. Only trees with deep bark cracks reaching the heigh of about 0.5 m on the butt were selected for the study. Because of these cracks, each stem was divided into two basal sections, 0.5 m long, and a number of one-metre sections, right to the top of a tree. From the bottom end of each section, a small section, 10 cm long, was cut off for a subsequent study. Moreover, from middle part of the crown 5 branches with the diameter 1.0-1.7 cm at the base were selected on each tree, and from the basal, middle and top parts of each branch a section, 15 cm long, was cut out. In total, 95 stem sections and 135 branch sections, cut out from 45 branches, were subsequently processed within 24 hours. The sterilization of sample surface was accomplished using the same technique as used by KOWALSKI & KEHR (1992). Fragments, 2 x 5 x 2 mm in size, were cut out from the bark and xylem of the stem sections (12-24 bark and 6 xylem fragments from each section), and only from the bark of the branch sections (12 fragments from each part: basal, middle, and top). Altogether, 3762 fragments were incubated at room temperature in darkness for 4-12 weeks (Table 1). Subcultures of growing mycelia were inoculated on 2% malt agar on slants or petri dishes.

Table 1. Numbers of samples and isolated fungal cultures

| Tissue type | Number of samples<br>Number of fungal cultures | | | Total |
|---|---|---|---|---|
| | Zone F | Zone L | Zone H | |
| Stems (bark) | 528<br>413 | 540<br>439 | 504<br>406 | 1572<br>1258 |
| Stems (xylem) | 180<br>10 | 198<br>1 | 192<br>11 | 570<br>22 |
| Twigs (bark) | 540<br>342 | 540<br>454 | 540<br>515 | 1620<br>1310 |
| Total | 1248<br>765 | 1278<br>893 | 1236<br>932 | 3762<br>2590 |

Overall frequency of fungal colonization was defined as the number of fragments of a given tissue type, yielding at least one species, in relation to the total number of fragments taken from this tissue type. Friedman's nonparametric analysis of variance was used in statistical computations.

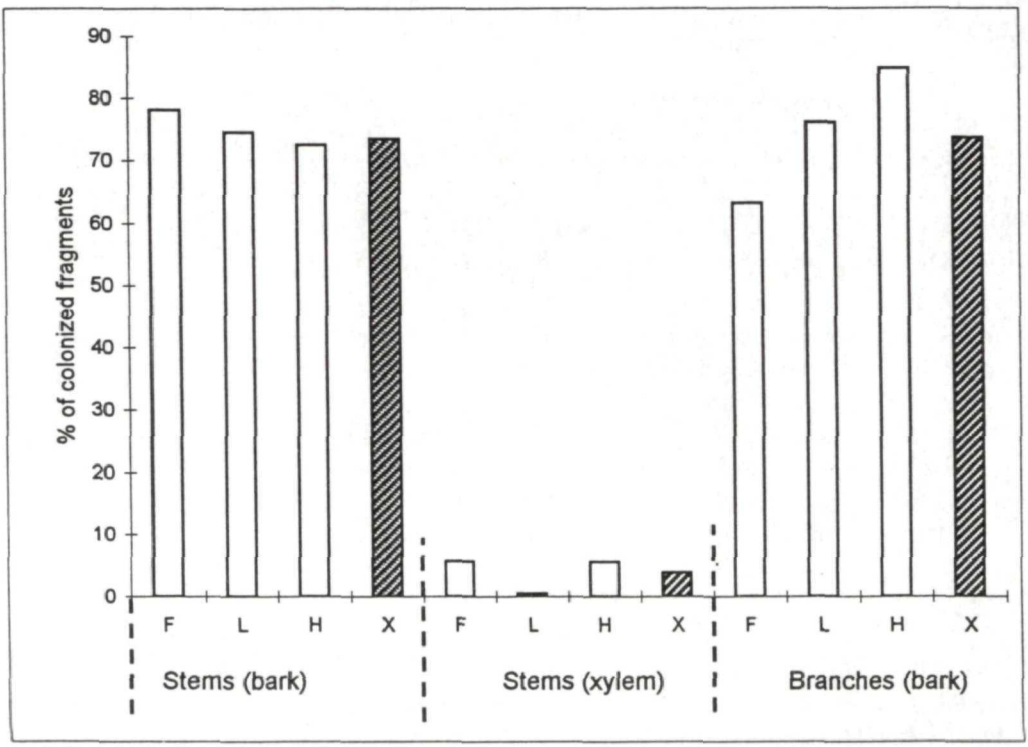


Fig. 1. Colonization of different living tissues of *Betula pendula* with endophytic fungi depending on zone of pollution with industrial emissions (*F* zone free of air pollution, *L* zone of low, *H* zone of high concentration of emissions). Black diagram indicates mean values.

## Results

Out of 3762 incubated fragments taken from stems and branches of *B. pendula*, 2372 (63.1%) were colonized with fungi. The frequency of colonization of the bark fragments of stems and branches was very similar, 73.5% and 73.7% respectively, while xylem was colonized to a very small degree (3.9%) (Fig. 1). In case of the branches, there were considerable differences in fungal colonization depending on air pollution. The branches in zone H were most frequently colonized. In case of the stems the differences were inconsiderable, although a tendency reversed to that observed for the branches may be noticed.

Table 2. Numbers of fungal species found in living *Betula pendula* depending on zone of pollution with industrial emissions

| Tissue | Numbers of fungal species | | | Total |
|---|---|---|---|---|
| | Zone F | Zone L | Zone H | |
| Stem (bark) | 20 | 24 | 37 | 52 |
| Stem (xylem) | 5 | 1 | 7 | 12 |
| Twigs (bark) | 17 | 19 | 29 | 41 |
| Total | 28 | 34 | 47 | 69 |

Altogether 2590 colonies of fungi were isolated (Table 1), and 69 species separated, although it was impossible to identify 17 species, even to the genus, because of lack of sporulation or clear characters of their cultures (Tables 2 and 3). Specific variability of fungi colonizing living trees of *B. pendula* depended on air pollution. The fungi in zone H were most differentiated (Table 2). Moreover, the number of species in the stems was by 25 % greater than in the branches (Table 2), in spite of a greater number of colonies obtained from the branches than from the stems (Table 1).

Despite a great specific variability of fungi colonizing living trees of *B. pendula* the most species were characterized by a low frequency of occurrence. Only eight species in the stems, and seven species in the branches were isolated with frequency above 1% (Table 3). They were as follows: *Aposphaeria* sp., *Coryneum brachyurum*, *Disculina betulina*, *Fusicoccum betulae*, *Godronia cassandrae*, *Melanconium betulinum*, *Pezicula cinnamomea*, *Phialocephala* cf. *dimorphospora*, *Prosthemium betulinum* and *Trimmatostroma betulinum* (Table 3). Some of them occurred exclusively, or nearly so, in the stems (*Godronia cassandrae*, *Phialocephala* cf. *dimorphospora*, *Pezicula cinnamomea*). On the other hand *Prosthemium betulinum* occurred almost 20 times more frequently in the branches than in the stems. The branches were also preferred by *Melanconium betulinum* and *Disculina betulina*. While *Coryneum brachyurum* colonized the bark of stems and branches with a very similar frequency. The occurrence of fungi on the stems depended on the distance from the ground level. For four the most frequent species this relationship is shown in Fig. 2. *Phialocephala* cf. *dimorphospora* occurred first of all in the butt, next to the root collar. *Pezicula cinnamomea* dominated at the heigh of about 0.5 m, and its percentage, similarly as of the previous species, decreased with increasing distance from the ground level. *Coryneum brachyurum* was most frequently isolated from sections taken at the heigh of 2-4 m, while *Disculina betulina* at 5-7 m. *Disculina betulina* belongs to the group of species which colonized most of the stem with greatest regularity. As compared with the stems the various parts of the branches were colonized with individual species of fungi with greater regularity (Table 4). However, there were some exceptions: *Fusicoccum betulae* occured mainly on the branch tops, and *Prosthemium betulinum* was 2 to 3 times less frequent on the branch tops than in the middle and basal parts.

Most of the most frequent species were present on birches irrespective of air pollution degree (Table 5). Some of them, however, occurred in particular pollution zones

Table 3. Frequency (%) of isolation for various fungal species from different tissues of living *Betula pendula*

| Fungal taxa | Stems | | Twigs |
|---|---|---|---|
| | Xylem | Bark | Bark |
| *Aposphaeria* sp. 1 | 0.5 | 1.1 | 0.4 |
| *Aposphaeria* sp. 2 | 0.4 | 0.5 | 2.3 |
| *Coryneum brachyurum* LINK | | 7.4 | 6.9 |
| *Disculina betulina* (SACC.) HÖHN. | 0.5 | 31.2 | 47.1 |
| *Fusicoccum betulae* COOKE | 0.2 | 0.3 | 2.8 |
| *Godronia cassandrae* PECK | | 1.6 | 0.1 |
| *Melanconium betulinum* KUNZE & SCHM. | | 2.1 | 6.0 |
| *Pezicula cinnamomea* (DC.) SACC. | 0.4 | 9.7 | 0.1 |
| *Phialocephala* cf. *dimorphospora* KENDRICK | 0.2 | 8.3 | |
| *Prosthemium betulinum* KUNZE ex SCHLECHT. | | 0.3 | 5.9 |
| *Trimmatostroma betulinum* (CORDA) HUGHES | | 4.8 | 3.1 |
| Other species* | 0.9 | 10.6 | 4.8 |
| Not identified | 0.7 | 1.5 | 1.4 |
| Number of examined fragments | 570 | 1572 | 1620 |

* Taxa which constitute less than 1% of each type of tissue:

*Acremonium* sp., *Alternaria alternata* (FR.) KEISSLER, *Apiospora montagnei* SACC., *Aureobasidium pullulans* (DE BARY) ARN., Basidiomycetes (3 spp.), *Botrytis cinerea* PERS., *Candida* sp., *Chaetomium* sp., *Cladosporium cladosporioides* (FRESEN) DE VRIES, *Coniochaeta velutina* (FUCKEL) MUNK, *Coniothyrium fuckelii* SACC., *Cylindrocarpon destructans* (ZINSSM.) SCHOLTEN, *Cystodendron* sp., *Diatrypella favacea* (FR.) CES. & DE NOT., *Didymosphaeria igniaria* BOOTH, *Epicoccum nigrum* LINK, *Geniculosporium serpens* CHESTERS & GREENHALGH, *Hormonema* sp., *Hypoxylon* cf. *deustum* (HOFFM.: FR.) GREV., *Lecytophora* sp., *Mollisia* sp., *Mortierella isabellina* OUDEM., *Mucor* sp., *Myxosporium* sp., *Nectria viridescens* BOOTH, *Penicillium* sp., *Pezicula* cf. *alba* GUTHRIE, *Pezicula* sp., *Phialemonium obovatum* W. GAMS & MCGINNIS, *Phialophora fastigiata* (LAGERB. & MELIN) CONANT, *Phialophora* sp., *Phoma* sp., *Rhizoctonia* sp. 1, *Rhizoctonia* sp. 2, *Sordaria fimicola* (ROB.) CES. & DE NOT., *Trichoderma* sp., *Taeniolella* sp., *Verticicladium trifidum* PREUSS, *Xylaria* sp.

with different frequency. For example, *Coryneum brachyurum* was almost twice as frequent in zone F as in zone H, *Prosthemium betulinum* was in the first place present in zone L, and *Melanconium betulinum* and *Trimmatostroma betulinum* most frequently occurred in zone H. The frequency of some species of fungi was different on individual trees growing in the same pollution zone (Table 6). For example *Pezicula cinnamomea* in zone F was over 5 times as frequent on the stem of tree No. 1 as on the stem of No. 2, and *Coryneum brachyurum* occurred almost exclusively on the branches of tree No. 3. In zone L *Pezicula cinnamomea* and *Phialocephala* cf. *dimorphospora* were particularly frequent on the stem of tree No. 6, and *Prosthemium betulinum* on the branches of the same tree, while *Melanconium betulinum* colonized mainly the branches of tree No. 4. In zone H, the absence of *Godronia cassandrae* and *Phialocephala* cf. *dimorphospora* in the stem of tree No. 9 is evident, as well as a very frequent isolation of *Godronia cassandrae* from the stem of tree No. 8. *Coryneum*

Table 4. Frequency (%) of the most common species of fungi isolated from basal, middle and top parts of living branches of *B. pendula*. For each fungus, values followed by identical letters are not significantly different ($P < 0.05$)

| Fungi | Part of branch | | |
|---|---|---|---|
| | Basal | Middle | Top |
| *Aposphaeria* spp. 1 + 2 | 0.7 a | 2.8 b | 4.6 c |
| *Candida* sp. | 1.9 a | 0.2 b | 0.4 b |
| *Coryneum brachyurum* | 6.5 a | 8.7 b | 5.6 c |
| *Disculina betulina* | 51.7 a | 48.0 b | 41.7 c |
| *Fusicoccum betulae* | 0.2 a | 0.6 a | 7.8 c |
| *Melanconium betulinum* | 4.3 a | 6.3 a | 7.8 c |
| *Prosthemium betulinum* | 8.7 a | 6.3 b | 2.8 c |
| *Trimmatostroma betulinum* | 1.5 a | 2.0 a | 3.7 c |
| Other species | 6.5 a | 3.9 b | 6.1 a |
| Number of fragments studied | 540 | 540 | 540 |
| Number of fragments colonized with fungi | 76.7 | 71.3 | 73.1 |

Table 5. Frequency (%) of the most common species of fungi in the bark, stems and branches of living *B. pendula* depending on zone of pollution with industrial emissions. For each fungus values followed by identical letters are not significantly different ($P < 0.05$)

| Fungi | Zone F | Zone L | Zone H |
|---|---|---|---|
| *Aposphaeria* spp. 1 + 2 | 1.8 a | 0.4 b | 4.4 c |
| *Coryneum brachyurum* | 11.0 a | 4.4 b | 6.1 c |
| *Disculina betulina* | 39.3 a | 44.4 b | 33.9 c |
| *Fusicoccum betulae* | 0 a | 1.5 b | 3.3 c |
| *Godronia cassandrae* | 0 | 0 | 2.5 c |
| *Melanconium betulinum* | 0.5 a | 2.1 b | 9.9 c |
| *Pezicula cinnamomea* | 5.3 a | 5.5 a | 3.7 c |
| *Phialocephala* cf. *dimorphospora* | 4.7 a | 5.5 b | 2.1 c |
| *Prosthemium betulinum* | 0.1 a | 7.9 b | 1.4 c |
| *Trimmatostroma betulinum* | 2.0 a | 0.4 b | 9.7 c |

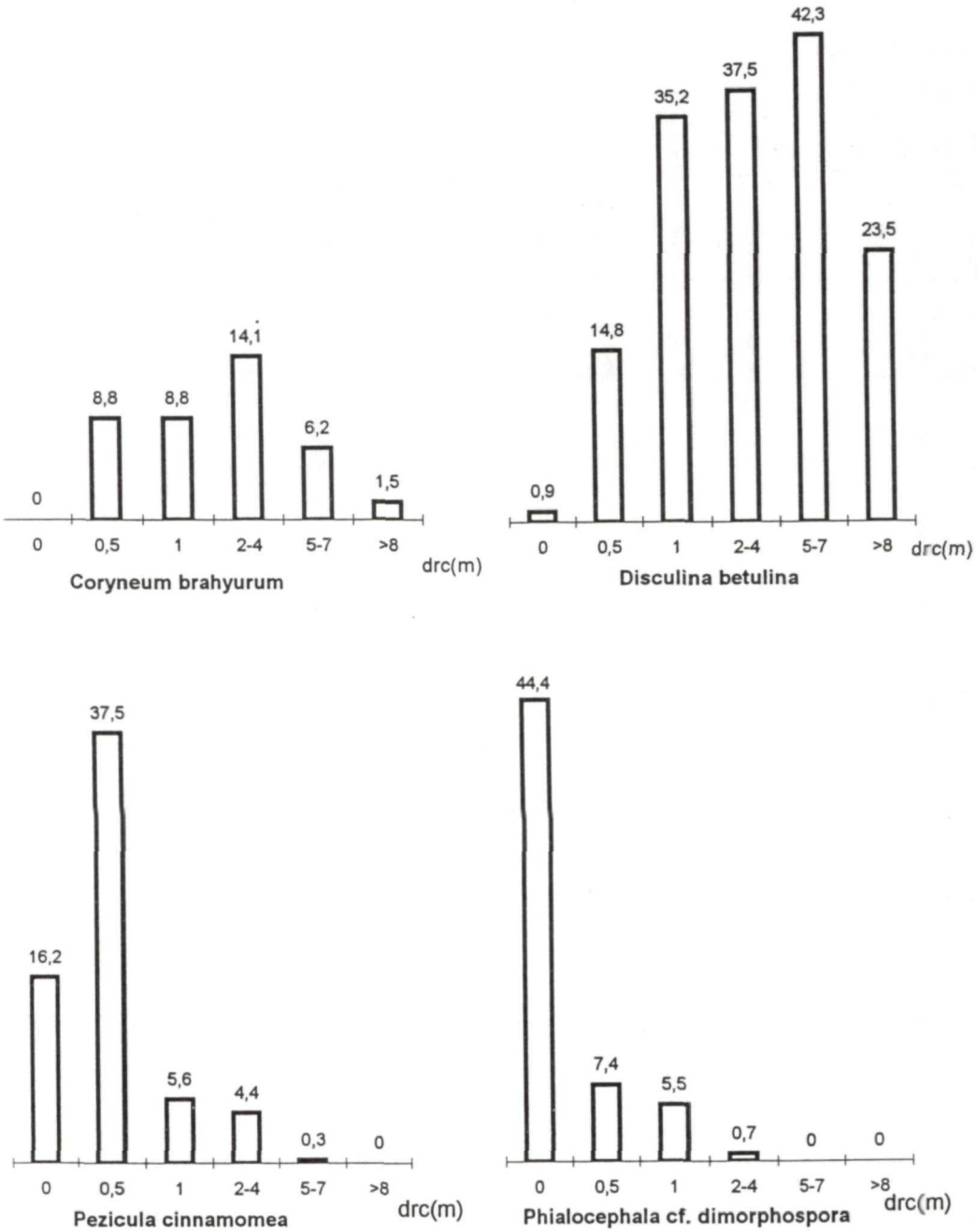


Fig. 2. Frequency (%) of four most frequent species of fungi in the bark of stems of *Betula pendula* depending on the distance from the root collar (= drc).

Table 6. Frequency of some species of fungi on individual trees of *B. pendula* (1 to 9 = tree numbers)

| Fungi | Zone F | | | | Zone L | | | | Zone H | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | *- P < 0.05 | 4 | 5 | 6 | *-P < 0.05 | 7 | 8 | 9 | *- P < 0.05 |
| Stems | | | | | | | | | | | | |
| *Coryneum brachyurum* | 19.9 | 12.8 | 17.7 | * | 1.7 | 5.2 | 7.1 | * | 1.3 | 1.2 | 0 | - |
| *Disculina betulina* | 24.4 | 38.9 | 31.8 | * | 43.3 | 39.6 | 32.1 | * | 18.6 | 22.6 | 26.1 | * |
| *Godronia cassandrae* | 0 | 0 | 0 | | 0 | 0 | 0 | | 1.3 | 13.7 | 0 | |
| *Melanconium betulinum* | 0.6 | 0 | 0.5 | - | 0 | 0 | 0 | | 10.3 | 3.6 | 5.0 | * |
| *Pezicula cinnamomea* | 17.3 | 3.3 | 11.5 | * | 5.0 | 10.9 | 17.3 | * | 9.6 | 8.3 | 5.6 | * |
| *Phialocephala* cf. *dimorphospora* | 7.7 | 8.9 | 11.5 | * | 3.3 | 10.9 | 19.0 | * | 10.9 | 3.0 | 0 | * |
| *Trimmatostroma betulinum* | 0.6 | 1.1 | 1.6 | - | 0 | 0 | 0 | | 9.6 | 13.7 | 17.8 | * |
| Branches | | | | | | | | | | | | |
| *Coryneum brachyurum* | 0.6 | 0 | 15.6 | * | 6.1 | 3.9 | 2.8 | * | 16.1 | 15.6 | 1.7 | * |
| *Disculina betulina* | 42.2 | 59.4 | 37.8 | * | 50.0 | 47.8 | 53.3 | * | 52.8 | 34.4 | 46.1 | * |
| *Fusicoccum betulae* | 0 | 0 | 0 | | 8.9 | 0 | 0 | * | 2.8 | 4.4 | 8.9 | * |
| *Melanconium betulinum* | 0 | 1.1 | 0.6 | - | 8.3 | 2.8 | 1.7 | * | 5.0 | 4.4 | 30.5 | * |
| *Prosthemium betulinum* | 0 | 0 | 0 | | 8.9 | 5.6 | 32.8 | * | 0 | 5.0 | 1.1 | * |
| *Trimmatostroma betulinum* | 1.7 | 4.4 | 2.2 | * | 1.1 | 0 | 1.1 | - | 7.2 | 8.3 | 1.7 | * |

*brachyurum* was particularly scarce in the branches of tree No. 9, while *Melanconium betulinum* was particularly frequent there. Moreover, Table 6 shows that *Disculina betulina* occurred on the stems as well as the branches of all nine *B. pendula* trees with the most regular frequency.

**Discussion**

There is a great variability in the density of colonization of living trees of *Betula pendula* with fungi depending on the type of tissue. The great majority of fungal colonies were isolated from the bark, while xylem was colonized only sporadically. Similar proportions were observed by BARKLUND & KOWALSKI (1996) during their study on endophytes of spruce branches, and KOWALSKI & KEHR (1992) on branches of various species of coniferous and broadleaved trees. There were 0-1.7% and 0.7-9% of xylem fragments colonized with fungi respectively. Only FISHER & PETRINI (1990) reported a relatively high colonization density of xylem of *Alnus* sp., amounting to 16-35%.

The frequency of colonization of the bark of stem and branches of *B. pendula*, amounting to 73.5%, was a little lower or oscillated around the mean values for other tree species. In the papers cited above, these data referring only to the bark of the branches were: 64-88% for *Alnus* (FISHER & PETRINI 1990), 78-96% for *Picea* (BARKLUND & KOWALSKI 1996), and for 11 species of broadleaved and coniferous trees 83.7% on the average (KOWALSKI & KEHR 1992).

A different number of fungal species may be present in different plant organs (PETRINI & al. 1992). A total of 69 fungal taxa isolated during this study indicates that living trees of *B. pendula* are as attractive for many fungi as some other plants. For comparison it may be quoted that from 41 (*Acer pseudoplatanus* L.) to 67 (*Fagus sylvatica* L.) fungal taxa were reported only for the basal parts of the branches (KOWALSKI & KEHR 1992). For fir branches 37 different species were reported (SIEBER 1989), while for *Alnus* spp. 85 species (FISHER & PETRINI 1990). Some literature data concerning the specific variability much differ from one another. For example, for spruce branches 85 different fungal species were reported in Sweden (BARKLUND & KOWALSKI 1996), while only 43 in Switzerland (SIEBER 1989). Most likely this difference resulted from differences in site conditions under which the trees grew. It seems that the results may also be affected by: the number of samples, age of a tree, diameter and age of branches, and location of the branches on the stem in relation to the ground level (BARKLUND & KOWALSKI 1996, HALMSCHLAGER & al. 1993, KOWALSKI & KEHR 1992, SIEBER 1989).

In spite of a great variability of fungi which colonized living trees of *B. pendula* only few species occurred with high frequency. These relations, as well as the fact that the discovered fungi belong almost exclusively to *Ascomycotina* and *Deuteromycotina*, is not a particularity for the birch stem and branches. A similar characterization was shown by most populations obtained from various organs of woody plants (BARKLUND & KOWALSKI 1996, HALMSCHLAGER & al. 1993, KOWALSKI 1993, KOWALSKI & KEHR 1992, PETRINI & al. 1992, SIEBER 1989). Among the populations isolated from the stems and branches of birch trees various ecological groups of fungi may be distinguished according to host attachement. Most of the fungi occurring with highest frequency may be included in a group of host-specific species, especially:

*Disculina betulina*, *Coryneum brachyurum*, *Melanconium betulinum*, *Prosthemium betulinum* and *Trimmatostroma betulinum*. The remaining dominant fungi are characterized by a wider or narrower host spectrum. There are, however, considerable differences between them. While *Pezicula cinnamomea* and *Phialocephala* cf. *dimorphospora* may very often occur in living branches of various coniferous and broad-leaved trees, *Godronia cassandrae* was reported, apart from birch, from not more than 5% of branches of *Abies alba* MILL. and *Picea abies* (L.) H. KARST. (KOWALSKI & KEHR 1992). In all these cases *G. cassandrae* was identified on the basis of a conidial stage formed in cultures in petri dishes. It may not be excluded that the species isolated from birch was not quite identical with the species isolated from other tree species. It exists the opinion that one of the forms of this species, *G. cassandrae* f. sp. *betulicola* GROVES, occurs on *Betula* (DESPREZ-LOUSTAU & DESSUREAULT 1988). Moreover, it should be emphasized that in the present study there were no cosmopolitan species among the dominant fungi. Such fungi as *Alternaria alternata*, *Cladosporium cladosporioides* or *Epicoccum nigrum* were isolated very rarely. Also the representatives of the family *Xylariaceae*, abundant endophytes in various organs of many plants (PETRINI & PETRINI 1985), were isolated sporadically.

A species composition of endophytes obtained by KOWALSKI & KEHR (1992) from the basal parts of the branches of *Betula pendula* in Germany was very similar to that obtained during the present study. The species which dominated in this study were also very numerous in the previous investigations. This may indicate that certain species are so strongly attached to *B. pendula* that they occur in its tissues independently of site conditions, although their rates of infection may differ.

The present results provide subsequent data on the factors affecting the dissimilarity of assemblages of endophytic fungi of birch. One of these factors is the distance of stem tissues from the ground level. It may be connected with proximity of the reservoir of infectious material. From the bark of stem, next to the root collar the soil born fungi: *Cylindrocarpon destructans*, *Mortierella isabellina*, *Trichoderma* sp. and *Penicillium* sp. were isolated more abundantly than higher up the stem. Also the form of the bark may be an important factor. The bark up to a height of about 0.5 m above the ground was deeply cracked. Different bark types have different physiological properties creating different microclimatic habitats (NICOLAI 1986). There is more moisture in the bark cracks in the butt part of the stem, thus the spores have better germination conditions. It may not be excluded that these factors caused a more frequent occurrence of *Phialocephala* cf. *dimorphospora* and *Pezicula cinnamomea* in the butts. The results also indicate that there may be different communities of endophytic fungi in various parts of the branches. KOWALSKI & KEHR (1992) showed that the species composition and frequency of occurrence depend, among other things, on the branch diameter. Since the branch diameter did not vary too much in the present study, because the samples were taken from the middle parts of the crowns of trees of similar height, the differences may be explained by the difference in age between the branch tops and their basal parts, and also the difference in the bark character. A similar relationship was observed by BARKLUND & KOWALSKI (1996) in *Picea abies* where *Tryblidiopsis pinastri* (PERS.) KARST. was most abundant in the youngest top parts of the branches, while *Phialocephala scopiformis* KOWALSKI & KEHR in the oldest ones. HELANDER & al. (1994) concluded that the endophyte colonization rates varied widely among individual trees. These conclusions could be confirmed by the present study

in case of some fungi. Since trees of *B. pendula* grew within a single zone, few metres apart, perhaps certain genetic characters, or differences in their health condition, not manifested by external symptoms so far, caused a more frequent colonization with some endophytic fungi. Three zones in which samples were taken from living birches varied in the degree of air pollution. However, a very careful interpretation of the results obtained is necessary since it was impossible to eliminate the dissimilarity of other factors deciding about local site conditions (see also HELANDER & al. 1994). The present results seem to indicate that the industrial emissions are not indifferent for the colonization of trees with endophytic fungi. This effect in case of birches seems to be expressed by a higher colonization density of branches with endophytic fungi, an increased species variability of fungi in the bark of the stems and branches, and changes in the frequency of occurrence of individual species of fungi. Some fungi may increase, and the other decrease their abundance. The mechanism of the effect of the emissions on the endophytic colonization by fungi may vary. It is known that upon the effect of emissions some fungi may disappear, and others may increase their occurrence, thus the abundance of infectious material may change (GRZYWACZ & WAŹNY 1973). The reservoir of infectious material may also increase for many fungi due to a more frequent dying of branches and whole trees under the effect of industrial emissions (KOWALSKI 1981). On such a substratum many species of fungi may sporulate, and later cause a symptomless infection (BUTIN & KOWALSKI 1986, KOWALSKI 1981). Moreover, the changes mentioned above may result from the fact that the industrial emissions lower the health condition of trees, make the infection for some fungi easier, which initially live as endophytes, and under favourable conditions act as weakness pathogens.

It is worth to emphasize that the species of fungi which most frequently were found to be endophytes in trees of *B. pendula* were by and large also the species which were most frequently associated with dying trees in industrial regions (KOWALSKI 1981). Often, these are the same species which may accelerate the process of natural pruning of *B. pendula* (BUTIN & KOWALSKI 1986).

These investigations were carried out under the project No. 5 P06M 029 12. The authors thank Mrs Mgr J. MICHALIK for help in laboratory work.

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