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Fungi isolated from living symptomless shoots of *Pinus nigra* growing in different site conditions

TADEUSZ KOWALSKI
PAWEŁ ZYCH

Department of Forest Pathology
29-Listopada 46
PL-31-425 Kraków, Poland

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Abstract: Communities of fungal endophytes in symptomless shoots of *Pinus nigra* from three sites differing, among others, in the degree of harmful effect of industrial emissions and disease activity of pathogenic fungi are presented. Over 2100 colonies of fungi were isolated from almost 2600 shoot fragments, among which 49 taxa were identified. *Alternaria alternata*, *Crumenulopsis pinicola*, *Fusicoccum* spec., *Lecytophora hoffmannii*, *Mollisia cinerea*, *Pezicula eucrita*, *Phialemonium* spec., *Phialophora* spec. 1, *Phomopsis occulta*, *Phomopsis* spec., *Sclerophoma pythiophila*, *Sirodothis* spec. 1, *Trimmatostroma* cf. *abietis* and non-sporulating fungus No. 32 colonized over 10% of shoots. The frequency of occurrence of fungi depended on the shoot age (1, 2, and 3-year-old shoots were investigated), type of tissue (between leaf scales, within leaf scales), and position in the crown (upper, lower). Attention was paid to whether there are fungi able to cause diseases of *P. nigra* shoots among the endophytic assemblages found.

Zusammenfassung: Es werden Gesellschaften von Pilz-Endophyten in symptomfreien Sprossen von *Pinus nigra* von drei Standorten vorgestellt, die sich unter anderem im Grad der Schädigung durch industrielle Emissionen und durch pathogene Pilze unterscheiden. Mehr als 2100 Pilzkolonien wurden aus fast 2600 Sproßfragmenten isoliert, von denen 49 Taxa identifiziert wurden. *Alternaria alternata*, *Crumenulopsis pinicola*, *Fusicoccum* spec., *Lecytophora hoffmannii*, *Mollisia cinerea*, *Pezicula eucrita*, *Phialemonium* spec., *Phialophora* spec. 1, *Phomopsis occulta*, *Phomopsis* spec., *Sclerophoma pythiophila*, *Sirodothis* spec. 1, *Trimmatostroma* cf. *abietis* und der nicht-sporulierende Pilz Nr. 32 kolonisierten mehr als 10% der Sprosse. Die Häufigkeit des Pilzvorkommens hing ab vom Alter der Sprosse (1, 2 und 3 Jahre alte Sprosse wurden untersucht), Gewebe-Typ (zwischen Blattschuppen, innerhalb Blattschuppen), und Position in der Krone (obere, untere). Es wurde beachtet, ob unter den Endophyten auch Pilze vorkommen, die in *P. nigra*-Sprossen Krankheiten hervorrufen können.

Pinus nigra ARN. belongs to the tree species relatively few susceptible to toxic substances present in industrial emissions (DAVIS & WILHOUR 1976). For this reason it is presently introduced in Poland in considerable numbers in regions of high concentration of industry. In the Upper Silesia Industrial District, where convertion of dying stands was initiated earlier than in other areas, there are pure stands of *P. nigra* or mixed stands with high percentage of *P. nigra* of various age, from very young plantations to stands over 80-years old (KOWALSKI 1987). Also, almost 20 years ago, many seed plantations of *P. nigra* were established.

However, the health condition of many of these stands is unsatisfactory. *Pinus nigra* is severely attacked by *Gremmeniella abietina* (LAG.) MORELET, and in some ar-

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eas this tree species has been completely eliminated from plantations (KOWALSKI 1987, KOWALSKI & DOMAŃSKI 1983). It is also very susceptible to infection by *Crumenulopsis sororia* which causes cankers on stems and branches. In some areas such symptoms are present on over 50% of the trees (KOWALSKI & al. 1994). Recently, more and more frequently, shoots of *P. nigra* die due to the infection by *Sphaeropsis sapinea* (FR.) DYKO & SUTTON. For many decades in the past diseases of *P. nigra* needles were of no importance in Poland. This situation changed in 1998, when an epidemic disease of *Dothistroma septospora* (DOROG.) MORELET occurred in some areas (KOWALSKI & JANKOWIAK 1998, KOWALSKI & al. 1998). Sometimes *P. nigra* in stands situated not far away from one another is attacked by diseases to a different degree. Different local site conditions and different stand structure may be the causes of this phenomenon. However, other factors may also be of importance.

Table 1. Characteristics of sampled stands of *Pinus nigra*

Stand - No.	Stand location Forest District Forest Range compartment	Zone of pollution with industrial emissions, location	Forest site type	Stand age (years)	Occurrence of pathogenic fungi (frequency)*1
Site A	Miechów Goszcza, 71	I - low emission concentration (25 km north east from Steelworks in Kraków)	uppland broadleaf forest	16	*Cenangium ferruginosum* (+++) *Dothistroma septospora* (+++) *Gremmeniella abietina* (+)
Site B	Świerklaniec Repecko, 58	II - medium emission concentration (12 km west from Zink and Lead Smelting Works in Miasteczko Śląskie - Upper Silesia)	fresh coniferous forest	24	*Cenangium ferruginosum* (++) *Gremmeniella abietina* (+++) *Crumenulopsis sororia* (+++)
Site C	Świerklaniec Imielów, 203	III - high emission concentration (1.2 km east from Zink and Lead Smelting Works in Miasteczko Śląskie - Upper Silesia)	fresh coniferous forest	26	*Cenangium ferruginosum* (++) *Gremmeniella abietina* (++)

*1 Frequency of occurrence: high (+++), medium (++), low (+)

It is known that some fungi may cause a latent infection and live in plant tissues as endophytes. When trees are weakened these fungi may contribute to the appearance of disease symptoms (CARROLL 1988, PETRINI 1986). Endophytic fungi of *P. nigra* are little known. Mainly needles were investigated, and over small areas only (CARROLL & al. 1977, JURC & JURC 1995, KOWALSKI & ZYCH 2002). The investigations presented in this paper were devoted to endophytes in shoots of *P. nigra*. The purpose of this study was to determine the qualitative and quantitative composition of mycobiota

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depending on the kind of tissues, age of shoots, and their position in the tree crown.

Materials and methods

Materials for investigations were collected in autumn 1998 in three pure stands of *Pinus nigra*, from 16 to 26 years of age, growing in southern Poland. The stands investigated differed in the degree of pollution with industrial emissions and the intensity of occurrence of pathogenic fungi (Table 1). In a stand on site A no threat by pathogenic fungi was observed from the time of its establishment until 1997 when the epiphytotic infection by *Dothistroma septospora* was observed (KOWALSKI & JANKOWIAK 1998). This was the first report of the occurrence of this pathogen in Poland. As a result of strong infection of needles the lower crown branches were dying, where cupules of *Cenangium ferruginosum* FR.: FR. appeared commonly. The shoot infection by *Gremmeniella abietina* was sporadic. On sites B and C trees of *P. nigra* are infected to a various degree by *Cenangium ferruginosum* and *Gremmeniella abietina* since 1980 (KOWALSKI & DOMAŃSKI 1983; KOWALSKI, unpubl.). Besides, the stems and branches of *P. nigra* on site B are frequently attacked by *Crumenulopsis sororia* (KOWALSKI & al. 1994).

From 12 trees on site A, and 12 trees on sites B and C, (6 trees each) growing in a central part of a stand, two branches without disease symptoms were cut. One branch was cut in the upper crown (third whorl from the top), and the other in the lower crown (second living whorl from the bottom). In total, 48 branches were cut. In the laboratory, from each branch a 1-year-old shoot (1998), a 2-year-old shoot (1997), and a 3-year-old shoot (1996) were cut out.

Isolations were made on 2% malt agar medium in Petri dishes during 12-24 hours after collection. The surface of samples was disinfected by submersion in 96% ethyl alcohol for 1 min, 4% natrium hypochlorite for 3 min, and again in 96% ethyl alcohol for 0.5 min, after which they were dried in sterile filter paper. 12 fragments were cut out from each shoot section: six fragments, 3 x 2 mm in size, from the area between the leaf scales including outer and inner bark, and six fragments, about 5 mm^2, from the leaf scales. In total 144 shoots were investigated, from which 2592 fragments were placed on malt agar and then incubated in the dark at room temperature (Table 2). Colonies growing from shoot fragments were split off on the same medium and then identified. The frequency occurrence of each fungus species was expressed as the percentage of colonized shoots.

Results

A total of 2100 colonies of fungi was isolated from living, symptomless shoots of *Pinus nigra*, among which 49 fungal species were identified. Colonies of 12 species were separated without their identification due to lack of spores. Almost exclusively they were anamorphs of *Ascomycota* or mitosporous fungi (*Deuteromycota*). *Basidiomycota* were represented by one species (Table 2). The following species colonized over 10% of the shoots: *Alternaria alternata*, *Crumenulopsis pinicola*, *Fusicoccum* spec., *Lecytophora hoffmannii*, *Mollisia cinerea*, *Pezicula eucrita*, *Phialemonium* spec., *Phialophora* spec. 1, *Phomopsis occulta*, *Phomopsis* spec., *Sclerophoma pythiophila*, *Sirodothis* spec. 1, *Trimmatostroma* cf. *abietis* and non-sporulating fungus No. 32. Moreover 11 species were found in 5-10% of the shoots. The species which were isolated from less than 5% of the shoots were the most numerous ones (Table 2).

Species diversity and the numbers of mycobiota in the shoots depended on the site where a tree was growing. Thus, there were 48 species found on site A, 38 species on site B, and 35 species on site C (Table 2). *Crumenulopsis pinicola* was over twice as numerous on site A than on site B, and it was not found on site C. *Sirodothis* spp. were not isolated from site B, and on site A they were twice as frequent as on site C. *Verticicladium trifidum* was over 10 times less frequent on site A than on sites B and C. Some species were found only on one site, e.g., *Geniculosporium* spec. on site A, *Leu-*

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cocytospora kunzei and *Didymosphaeria igniaria* on site B, while *Epithyrium resinae* on site C.

Table 2. Number and frequency (%) of living symptomless shoots of *Pinus nigra* colonized by fungi

Fungi	Site A		Site B		Site C		Total	
	number	(%)	number	(%)	number	(%)	number	(%)
Alternaria alternata (FR.) KEISSLER	11	(15.3)	4	(11.1)			15	(10.4)
Anthostomella formosa KIRSCHST.	1	(1.4)					1	(0.7)
Aureobasidium pullulans (DE BARY) ARN.	1	(1.4)					1	(0.7)
Basidiomycetes spec.	1	(1.4)					1	(0.7)
Botrytis cinerea PERS.	1	(1.4)			1	(2.8)	2	(1.4)
Cenangium acuum COOK ex PECK					1	(2.8)	1	(0.7)
Cenangium ferruginosum FR.: FR.	2	(2.8)	1	(2.8)			3	(2.1)
Cladosporium cladosporioides (FRES.) DE VRIES			1	(2.8)	2	(5.6)	3	(2.1)
Cladosporium herbarum PERS.: FR.	1	(1.4)					1	(0.7)
Coniothyrium fuckelii SACC.	6	(8.3)	2	(5.6)	1	(2.8)	9	(6.3)
Crumenulopsis pinicola (REBENT.) GROVES	23	(31.9)	5	(13.9)			28	(19.4)
Didymosphaeria igniaria BOOTH			2	(5.6)			2	(1.4)
Drechslera spec.			1	(2.8)			1	(0.7)
Epicoccum nigrum LINK	2	(2.8)					2	(1.4)
Epithyrium resinae (SACC. ex BERL.) SACC.					3	(8.3)	3	(2.1)
Fusicoccum spec.	17	(23.6)	3	(8.3)	9	(25.0)	29	(20.1)
Geniculosporium cf. *serpens* CHESTERS & GREENHALGH	8	(11.1)			3	(98.3)	11	(7.6)
Geniculosporium spec.	8	(11.1)					8	(5.6)
Gremmeniella abietina (LAG.) MORELET	1	(1.4)	4	(11.1)	1	(2.8)	6	(4.2)
Hypoxylon spec.	1	(1.4)					1	(0.7)
Lecytophora hoffmannii (VAN BEYMA) W. GAMS & MCGINNIS	6	(8.3)	6	(16.7)	5	(13.9)	17	(11.8)
Leucocytospora kunzei (SACC.) URBAN			2	(5.6)			2	(1.4)
Lophodermium pinastri (SCHRAD. ex HOOK) CHEV.	2	(2.8)	1	(2.8)			3	(2.1)
Melanconium spec.	3	(4.2)	3	(8.3)	3	(8.3)	9	(6.3)
Mollisia cinerea (BATSCH ex MERAT) P. KARST.	36	(50.0)	27	(75.0)	17	(47.2)	80	(55.6)
Mollisia spec.	2	(2.8)					2	(1.4)
Pezicula eucrita (P. KARST.) P. KARST.	52	(72.2)	27	(75.0)	24	(66.7)	103	(71.5)
Phialemonium spec.	19	(26.4)	15	(41.7)	5	(13.9)	39	(27.1)
Phialocephala cf. *dimorphospora* KENDRICK	3	(4.2)	4	(11.1)	1	(2.8)	8	(5.6)
Phialophora spec. 1	18	(25.0)	14	(38.9)	2	(5.6)	34	(23.6)
Phialophora spec. 2	4	(5.6)	5	(13.9)			9	(6.3)
Phialophora spec. 3			1	(2.8)			1	(0.7)
Phomopsis occulta TRAV.	14	(19.4)	1	(2.8)			15	(10.4)
Phomopsis spec.	23	(31.9)	9	(25.0)	3	(8.3)	35	(24.3)
Rhizoctonia spec.	10	(13.9)			1	(2.8)	11	(7.6)
Sclerophoma pythiophila (CORDA) HÖHN.	39	(54.2)	18	(50.0)	26	(72.2)	83	(57.6)
Scoleconectria cucurbitula (TODE: FR.) BOOTH			6	(16.7)	7	(19.4)	13	(9.0)
Sirodothis spec. 1	27	(37.5)			7	(19.4)	34	(23.6)
Sirodothis spec. 2	2	(2.8)			1	(2.8)	3	(2.1)
Sphaeropsis sapinea (FR.: FR.) DYKO & SUTTON	1	(1.4)	1	(2.8)			2	(1.4)
Taeniolella spec.	4	(5.6)	1	(2.8)	1	(2.8)	6	(4.2)
Therrya fuckelii (REHM) KUJALA	1	(1.4)					1	(0.7)
Trimmatostroma cf. *abietis* BUTIN & PEHL	25	(34.7)	5	(13.9)	15	(41.7)	45	(31.3)
Verticicladium trifidum PREUSS	1	(1.4)	6	(16.7)	7	(19.4)	14	(9.7)
Xylaria spec.	10	(13.9)					10	(6.9)
Non-sporulating fungus No. 28			6	(16.7)	6	(16.7)	12	(8.3)
Non-sporulating fungus No. 29			7	(19.4)			7	(4.9)
Non-sporulating fungus No. 32	24	(33.3)	7	(19.4)	13	(36.1)	44	(30.6)
Other non-sporulating fungi (12 species)	13	(18.1)	11	(30.6)	25	(69.4)	49	(34.0)

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Bacteria	22 (30.6)	20 (55.6)	3 (8.3)	45 (31.3)
Number of examined shoots	72	36	36	144
Number of examined fragments	1296	648	648	2592
% of fragments colonized by microorganisms	75.1	85.3	72.1	76.9
Number of isolates obtained	1026	622	502	2150

The distribution of individual species in the shoots varied. The tissues were colonized most intensively by *Pezicula eucrita* and *Mollisia cinerea*, while *Alternaria alternata*, *Coniothyrium fuckelii*, *Geniculosporium* cf. *serpens*, and *Verticicladium trifidum* were characterized by more limited occurrence (Table 3).

Table 3. Occurrence of common fungi in shoots of *P. nigra* depending on their age and position in the crown

Fungi	Index of distribution of fungi in shoots *	Colonized shoots (%)			Colonized shoots (%)	
		1-year-old	2-year-old	3-year-old	upper crown	lower crown
Alternaria alternata	0.07	22.9	6.3	2.1	12.5	8.3
Coniothyrium fuckelii	0.07	8.3	6.3	4.2	4.2	8.3
Crumenulopsis pinicola	0.10	22.9	22.9	12.5	18.1	20.8
Fusicoccum spec.	0.11	20.8	29.2	10.4	26.4	13.9
Geniculosporium cf. *serpens*	0.08	0.0	6.3	16.7	5.6	9.7
Lecytophora hoffmannii	0.09	4.2	14.6	12.5	11.1	9.7
Melanconium spec.	0.06	6.3	10.4	2.1	2.8	9.7
Mollisia cinerea	0.20	18.8	66.7	81.3	43.1	68.1
Pezicula eucrita	0.26	27.1	95.8	91.7	69.4	73.6
Phialemonium spec.	0.16	16.7	27.1	33.3	23.6	27.8
Phialophora spec. 1	0.09	12,5	27.1	31.3	19.4	27.8
Phomopsis occulta	0.12	6.3	20.8	4.2	12.5	8.3
Phomopsis spec.	0.11	25.0	39.6	8.3	23.6	25.0
Sclerophoma pythiophila	0.16	68.8	58.3	45.8	56.9	58.3
Sirodothis spec. 1	0.16	20.8	25.0	25.0	20.8	26.4
Trimmatostroma cf. *abietis*	0.16	50.0	20.8	22.9	34.7	27.8
Verticicladium trifidum	0.07	4.2	8.3	16.7	9.7	9.7
Bacteria	0.11	18.8	35.4	39.6	31.9	30.6
Number of examined shoots		48	48	48	72	72
Number of examined fragments		864	864	864	1296	1296
% of fragments colonized by micro-organisms		61.7	83.6	77.9	73.5	80.3
Number of isolates obtained		558	793	799	1018	1132

* index = $\frac{\text{number of fragments from which a given taxon was isolated}}{\text{number of shoots from which a given taxon was isolated}}$

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All more frequent species of fungi, with the exception of *Geniculosporium* cf. *serpens* colonized shoots of *P. nigra* irrespective of their age (Table 3). However, some differences in their colonization frequency may be observed. *Sirodothis* spec. 1 colonized shoots with similar frequency, irrespective of their age. *Alternaria alternata*, *Trimmatostroma* cf. *abietis*, and *Sclerophoma pythiophila* most frequently colonized 1-year-old shoots. Such species as *Mollisia cinerea*, *Pezicula eucrita*, *Phialemonium* spec., *Phialophora* spec. 1, and *Verticicladium trifidum* colonized older shoots more frequently than 1-year-old shoots. In the case of *Melanconium* spec., *Phomopsis occulta*, and *Phomopsis* spec. a considerable decrease in frequency of occurrence on 3-year-old shoots was observed in comparison with 2-year-old shoots (Table 3).

It was found that the frequency of occurrence of some species of fungi in the investigated two types of tissues differed (Fig. 1). This was most evident in the case of *Fusicoccum* spec. which from tissues within leaf scales was over 60 times more frequently isolated than from tissues between leaf scales. The same relationship, but to a lesser degree, was evident in the case of *Geniculosporium* cf. *serpens*, *Sclerophoma pythiophila*, and *Verticicladium trifidum*. *Pezicula eucrita*, *Phialemonium* spec. and *Phomopsis occulta* were 1.5 times more frequent in tissues between leaf scales. *Crumenulopsis pinicola* and *Trimmatostroma* cf. *abietis* colonized both types of tissues with a very similar frequency (Fig. 1).

All more common fungi occurred in the upper as well as lower crown (Table 3). Some species, e.g., *Crumenulopsis pinicola*, *Phomopsis* spec., *Sclerophoma pythiophila*, and *Verticicladium trifidum* occurred in both crown parts with the same or similar frequency. *Alternaria alternata*, *Fusicoccum* spec., and *Trimmatostroma* cf. *abietis* were a little more frequent on shoots of the upper crown, while *Mollisia cinerea* and *Phialophora* spec. 1 in the lower crown (Table 3).

Colonies of bacteria were isolated from 31.3% of shoots (Table 2). Their distribution was very limited. From among 2592 shoot fragments taken for isolation the bacteria were isolated from only 86 (3.3%) fragments. The frequency of colonization of shoots with bacteria increased as their age increased (Table 3).

Discussion

Over 60 taxa found during this study indicate that living shoots of *Pinus nigra* are equally attractive to fungi as shoots of *Pinus sylvestris* (KOWALSKI & STAŃCZYKIEWICZ 2000). Most of the species of fungi isolated from shoots of *P. nigra* during this study were also characteristic for the endophyte assemblages obtained from shoots of *P. sylvestris* growing under similar site conditions (KOWALSKI & STAŃCZYKIEWICZ 2000). However, these two pine species differed in respect of the occurrence frequency of some species of fungi. Two of the most frequent species on *P. sylvestris*, i.e. *Sclerophoma pythiophila* and *Pezicula eucrita* (as *P. livida*) occurred in 95.5% and 91.7% of the shoots respectively. In the case of *P. nigra* these two species were also the most frequent ones, but they occurred in 57.6% and 71.5% of the shoots respectively. *Crumenulopsis pinicola* and *Phomopsis occulta* were also more frequent on *P. nigra*, while *Taeniolella* spec., *Verticicladium trifidum*, and *Trimmatostroma* cf. *abietis* occurred more frequently in shoots of *P. sylvestris* (KOWALSKI & STAŃCZYKIEWICZ 2000). *Fusicoccum* spec. colonized shoots of both pine species with almost equal frequency (20.1% – *P. nigra*, 19.4% – *P. sylvestris*). Therefore, it is worth to note that

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one of the species, i.e. *Coelomycetes* with *Cytosporina*-like spores isolated from 75.0% of the shoots of *P. sylvestris* was not found in shoots of *P. nigra* at all, which seems to indicate its high host specificity. The phenomenon of a selective attack of a definite tree species is quite widely known among pathogenic fungi (BUTIN 1996). For example on site A a severe infection of the needles of *P. nigra* by *Dothistroma septospora* has been observed for several years, while *P. sylvestris* in this area is not affected at all (KOWALSKI & JANKOWIAK 1998).

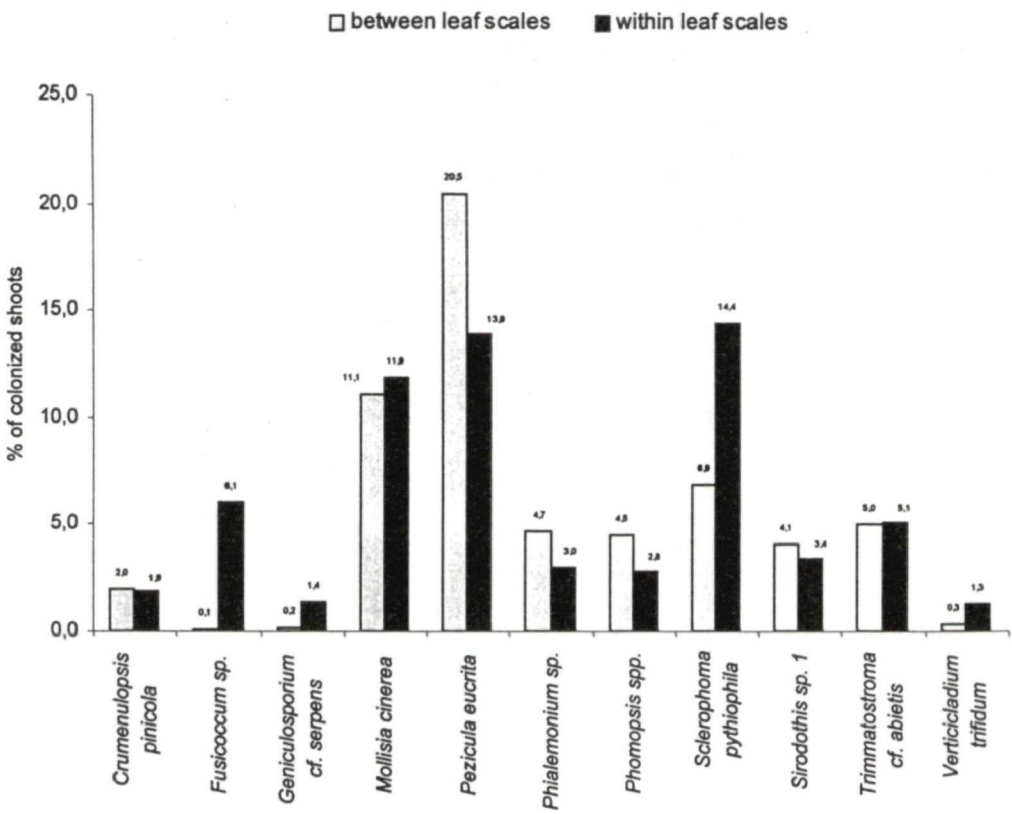

Fig. 1. Occurrence of common fungi in symptomless shoots of *Pinus nigra* depending on a type of tissue.

Some of the species isolated from living shoots of *P. nigra* may colonize endophytically many tree species, not only coniferous, but also broad-leaved ones. Especially *Lecytophora hoffmannii*, *Mollisia cinerea*, *Phialocephala* cf. *dimorphospora*, and *Verticicladium trifidum* belong to this group (KOWALSKI & KEHR 1992, KOWALSKI & GAJOSEK 1998, PETRINI & MÜLLER 1979, SIEBER 1989). The endophytic way of life of some of them, e.g., *M. cinerea* and *P.* cf. *dimorphospora* helps to colonize wood of branches dying because of lack of light (BUTIN & KOWALSKI 1990). They produce extracellular phenol oxidases and cause decay of wood typical for the so-called soft rot, and thus contribute to natural pruning (BUTIN & KOWALSKI 1992).

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From the mycological point of view the identification of one of the species as *Pezicula eucrita* requires a comment. On coniferous trees it was mainly known as *P. livida*. It was identified under this name in many papers concerning the endophytes by the author and his co-workers (e.g., BUTIN & KOWALSKI 1990, KOWALSKI & DOMAŃSKI 1993, KOWALSKI & KEHR 1992, KOWALSKI & STAŃCZYKIEWICZ 2000). The change of name in the present paper has been made on the basis of the monograph of VERKLEY (1999). He proved that *P. livida* is a synonym of *P. cinnamomea*, while in the past it was uncorrectly assumed that *P. eucrita* is a synonym of *P. livida*. Thus the name *P. eucrita* used at present concerns the fungus which in earlier papers was presented under the name *P. livida*. Such a conclusion is possible because the first author of this paper made the identification on the basis of cultures of this species, which are distinctly different from cultures of *P. cinnamomea*. KOWALSKI & KEHR (1992) were able on the basis of cultures to conclude that on coniferous trees there occur not only one, but many species of the genus *Pezicula*.

About 20% of the fungal species occurred in shoots of *P. nigra* on all three sites investigated, the remaining ones occurred on only one or two sites. Many species on these sites were characterized by various frequency of occurrence. It is well documented that endophytic communities on trees are greatly affected by the growth conditions of trees, connected with moisture, elevation, length of snow cover, availability of nutrients, and also with presence of symptoms of forest decline (CARROLL & CARROLL 1978, SIEBER 1989, PETRINI & MÜLLER 1979, SIEBER & HUGENTOBLER 1987).

A various degree of pollution with industrial emissions could have affected the composition of endophytes of *P. nigra*. This has been indicated by the results obtained in southern Poland in respect of other tree species (KOWALSKI & GAJOSEK 1998, KOWALSKI & STAŃCZYKIEWICZ 2000), as well as by the results obtained in other countries (HELANDER & al. 1994, ASAI & al. 1998). A harmful effect of SO_2 on trees becomes pronounced at a long-term concentration of 0.015 to 0.035 mg m^{-3} (HUTTUNEN & al. 1983). On site C this value was over 0.09, and on site B 0.06 to 0.09 mg m^{-3}. Not all fungi react to high concentration of emissions by a reduction in numbers. In shoots of *P. nigra Trimmatostroma* cf. *abietis*, *Sclerophoma pythiophila*, and *Scoleconectria cucurbitula* were even more frequent at high concentration of emissions than on a site where this concentration was lower. In the case of *Sclerophoma pythiophila* it was proved in vitro that its growth is not stopped in a short period of time upon the effect of SO_2 (MAGAN & al. 1996). The industrial emissions cause stresses in plants (HUTTUNEN & al. 1983). In turn, the frequency of a latent infection of endophytes depends on availability of inoculum (SIEBER-CANAVESI & SIEBER 1987).

The three sites investigated differed in the presence of inoculum of some species also for some other reasons. *Pinus nigra* yielded to the disease processes to a various degree, and this allowed the fructification of many fungi on dead branches and stems (KOWALSKI, unpubl.; KOWALSKI & JANKOWIAK 1998; KOWALSKI & al. 1994). According to the results of this study the presence of inoculum of pathogenic fungi was not reflected in the frequency of endophytic colonization of living shoots. *Crumenulopsis sororia* was not isolated at all, while *Cenangium ferruginosum*, *Gremmeniella abietina*, and *Sphaeropsis sapinea* were scarce. Relatively frequent in living shoots of *P. nigra* were fungi from the group of so called pathogens of weakness, among which representatives of the genera *Pezicula* and *Phomopsis* may be included. In industrial

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regions they are found in necrotic areas connected with contractions on stems of *P. nigra* (KOWALSKI 1990).

Sclerophoma pythiophila, *Cenangium ferruginosum*, and *Scoleconectria cucurbitula* in the case of *Pinus sylvestris* occur within necrotic areas on shoots with needles colonized by *Thecodiplosis brachyntera* (KOWALSKI 1998). In the case of *Pinus nigra* *T. brachyntera* rarely colonized the needles. The necrotic areas were occurring on shoots at their bases, usually colonized by the same species as on *P. sylvestris* (KOWALSKI, unpubl.). The results of this study showed that especially *Sclerophoma pythiophila* may stop its endophytic mode of life and become active.

On each site the occurrence of endophytic fungi on *P. nigra* depended on shoot age, their position in the crown, and the type of tissues. Such relationships were also observed on *P. sylvestris* (KOWALSKI & STAŃCZYKIEWICZ 2000). The same tendencies observed in both pine species are especially worth to note. For example, the frequency of *Mollisia cinerea* and *Verticicladium trifidum* increased as the age of the shoots increased, *Sclerophoma pythiophila* was more frequent in tissues within the leaf scales, while *Fusicoccum* spec. was more frequent in the upper crown parts. This provides certain information in reference to ecological requirements of these species. Most probably small differences in the tissue structure, their biochemical composition, moisture and temperature conditions in niches on a surface of organs are enough to be reflected in colonization by endophytes. This is why different parts of a single organ, e.g., a leaf or a needle, are infected by endophytes with different frequency (CARROLL & CARROLL 1978, STONE 1987).

The isolation of *Lophodermium pinastri* from shoots of *P. nigra* is remarkable. This species is known as a saprotroph or a weak pathogen on needles. It turned out, that it may occur endophytically not only in needles but also in shoots. This was even more evident in *P. sylvestris*, where living shoots were colonized almost five times more frequently than living needles (KOWALSKI & STAŃCZYKIEWICZ 2000). In contrast, *Lophodermium seditiosum* did not occur as an endophyte in shoots of any pine species, although it occurred in symptomless needles (KOWALSKI & STAŃCZYKIEWICZ 2000, KOWALSKI & ZYCH 2002).

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