

Fungi inhabiting stumps of *Pinus nigra* depending on the period of their exposure

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The purpose of this study was to determine the species composition of communities of fungi inhabiting stumps of *Pinus nigra* depending on the period of their exposure. After 6 and 18 months of stump exposure two species of *Basidiomycota* distinctly dominated: *Stereum sanguinolentum* and *Phanerochaete gigantea*. After 30 months of exposure the communities of fungi were the most diversified. A longer period of stump exposure resulted in the decrease of numbers of isolates of *Ph. gigantea* and *S. sanguinolentum*. However, other species belonging to *Basidiomycota* increased their numbers. As the wood decomposition progressed, however, the fungi belonging to *Deuteromycota* were isolated more often.

Key words: *Pinus nigra*, stumps, fungi, colonization.

INTRODUCTION

In Poland *Pinus nigra* due to its relatively high resistance to air pollution is often planted in stands growing in industrial regions. This tree species is recommended for conversion of dying stands growing under the influence of industrial emissions (Siuta 1978). The observations showed, however, that *P. nigra* used in conversion of degraded stands is susceptible to number of diseases caused by pathogenic fungi attacking overground portions of trees (Kowalski 1987; Kowalski et al. 1994; Kowalski and Jankowiak 1998). Dying of trees in forests growing under a harmful influence of industrial emissions may also be caused by infection with parasitic fungi attacking tree roots. *Armillaria* spp. and *Heterobasidion annosum* (Fr.) Bref. are among the most important ones. These pathogens often inhabit stumps as their natural food base, and from there they may infect roots of living trees. Apart from parasitic fungi, stumps and roots are also colonized

by other microorganisms, which may play an important role in limiting the infection by root pathogens (Kwaśna 1991). Antagonistic effect of microorganisms inhabiting stumps most often takes place through food competition or secretion of inhibitors and antibiotics, which check the development of pathogenic mycelium. For these reasons the species composition of fungi inhabiting stumps left after tree felling has a significant effect on the extent of infection danger to the next tree generations (Sierota 1995). The purpose of this study was to determine the species composition of communities of fungi inhabiting stumps of *P. nigra* with special attention paid to the possibility of spontaneous colonization by *H. annosum* and its main antagonist *Phanerochaete gigantea* in the course of stump exposure. It was also important to determine the difference between the species composition of fungi inhabiting stumps of *Pinus sylvestris* and those inhabiting stumps of *P. nigra* which hitherto have not been studied.

MATERIALS AND METHODS

The investigations were conducted in a seed orchard of *P. nigra* situated in the Miechów Forest District (Goszcza Forest Div., compartm. 71h). This plantation was established during 1985–1986 on the upland forest site after removal of 35-year-old stand of *P. sylvestris* and extraction of its stumps. In total, 8086 3 or 4-year-old seedlings of *P. nigra* were planted in eight plots using 3x3 m spacing. First thinning was carried out in 1997 when 2294 trees were removed, and the stumps left after the felling, 15 to 20 cm in diameter, were analyzed during this study. The removed pines were 14–16 years old. A more detailed description of this seed orchard was given by Kowalski et al. (1998). The evaluation of the colonization of wood of *P. nigra* stumps was carried out by two methods.

In the first method fungi were identified on the basis of mycelium isolated from stumps on 2% malt agar medium. During the period from 1997 to 1999 wood samples were collected each autumn, i.e. after 6, 18 and 30 months after tree felling. Each time blocks, 6 cm thick, were taken from 20–25 stumps selected at random. In total 65 blocks were obtained. In the laboratory the blocks were wiped with 96% ethyl alcohol. After the removal of the surface layer of wood, 44–63 fragments were taken for isolation from each block from three depths: 0.1–0.2 cm, 0.5–1.0 cm, and 2.0–4.0 cm, and also from circumferential part of the block. The wood fragments were taken from each block along two radii selected at random. In total, 3476 wood fragments were collected for isolation. The incubation of fungi took place in the dark at room temperature. The colonies of fungi were compared on the basis of the morphological and microscopic characteristics, and the pure cultures were obtained from the representative cultures for the identification of fungi.

In the second method the occurrence of fungi was determined on the basis of the presence of fructifications on stumps. For this purpose an analysis of 65 and 100 stumps selected at random, exposed for 18 and 30 months respectively, was accomplished.

RESULTS

Fungi isolated from stumps of *P. nigra*

Out of 3476 fragments of stumps the cultures of fungi were obtained from 3192 fragments (91.9%). This percentage was the lowest in the case of stumps exposed for 6 months, and the highest in the case of stumps exposed for 30 months (Table 1). Over 50 species and genera of fungi were found on investigated stumps. In respect of taxonomy the isolated fungi represented the following types: *Zygomycota*, *Ascomycota*, *Basidiomycota* and *Deuteromycota*. Out of the separated fungi only *Trichoderma harzianum* and *Phanerochaete gigantea* occurred in all groups of investigated stumps after 6, 18 and 30 months of stump exposure.

After 6 months of exposure of stumps over 20 species of fungi were found on their upper surface. A similar number of species was found in circumferential parts of stumps. This number was reduced by almost a half in the case of deeper layers of stumps (Table 1). After 6 months of stump exposure the following three species of *Basidiomycota* dominated: *Stereum sanguinolentum*, *Phanerochaete gigantea* and *Peniophora incarnata*. These fungi were mainly isolated from deeper layers of stumps. Over 10% frequency also characterized such species as *Trichoderma harzianum* and *Mariannaea elegans*. The latter species was abundant only in the upper layers of stumps. Also the fungi from the genera *Fusarium*, *Acremonium* and *Penicillium* as well as *Leptographium lundbergii* and *Rhinoctadiella atrovirens* were among a relatively more numerous ones (Table 1).

After 18 months of exposure the fungi belonging to *Basidiomycota* made the most numerous group. *Phanerochaete gigantea* (28.1% of isolates) and *Stereum sanguinolentum* (28.0%) were the species most often isolated. The latter one was mainly isolated from the deeper layers of stump wood. From among the *Basidiomycota* only *Hypochnicium erikssonii* was not too numerous (1.2% of isolates). In comparison with 6-month exposure the proportion of fungi from the genus *Trichoderma* was slightly higher amounting to 22.1% of isolates on the average. The remaining species isolated from stumps, mostly belonging to *Ascomycota* and *Deuteromycota*, occurred in smaller numbers (below 8% of isolates). In this group *Mucor hiemalis*, *Sclerophoma pythiophila*, *Leptographium lundbergii* and *Fusarium* spp. were relatively more frequent. These species were isolated from the upper surface of stumps as well as from their deeper layers (Table 1).

Tabela 1
Fungi isolated from stumps of *Pinus nigra*

Fungi	Number (%) of fragments from a different level of depth after stumps exposure (in months)											
	0.5 cm			0.5-1.0 cm			2.0-4.0 cm			Circumferential parts of stump		
	6	18	30	6	18	30	6	18	30	6	18	30
<i>Acremonia atra</i> Sacc.	1(0.2)	-	-	-	-	-	-	-	-	-	-	5(2.3)
<i>Acremonium murorum</i> (Corda) W. Gams	2(0.4)	6(2.5)	6(2.6)	-	-	-	-	-	-	-	-	-
<i>Acremonium</i> spp.	4(0.8)	-	10(4.4)	4(1.1)	5(2.1)	2(0.9)	29(6)	8(3.3)	5(2.2)	1(0.3)	3(1.7)	5(2.3)
<i>Alternaria alternata</i> (Fr.) Keissler	-	-	18(7.9)	-	-	-	-	-	-	1(0.3)	-	1(0.5)
<i>Alternaria tenuicoma</i> (Kunze ex Pers.) Wils.	1(0.2)	-	-	-	-	-	-	-	-	-	-	3(1.4)
<i>Arthrinium sphaerospermum</i> Fockel	-	-	10(4.4)	-	-	-	-	-	-	-	-	-
<i>Aureobasidium pullulans</i> (de Bary) Arnaud	-	-	27(11.8)	-	-	13(5.7)	-	-	-	-	-	-
<i>Candida</i> sp.	-	-	-	-	-	4(1.7)	-	-	-	-	-	6(2.8)
<i>Ceratocystis coenulescens</i> (Münch) Bakshi	1(0.2)	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium indicum</i> Corda	-	-	-	-	-	1(0.4)	-	-	-	-	-	-
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	-	-	51(21.9)	-	-	2(0.9)	-	-	2(0.9)	-	-	10(4.6)
<i>Curvularia lanata</i> (Wakker) Boedijn	-	-	1(0.4)	-	-	-	-	-	-	-	-	-
<i>Cylindrocarpon cylindroides</i> Wollenw.	1(0.2)	-	13(5.7)	-	-	-	-	-	-	-	-	-
<i>Cylindrocarpon destructans</i> (Zins.) Scholtz	12(5)	-	-	-	-	-	-	-	-	-	-	-
<i>Cylindrocarpon magnusianum</i> (Sacc.) Wollenw.	-	-	-	-	-	-	-	-	-	-	-	-
<i>Hyalodendron</i> sp.	-	-	-	-	-	-	-	-	-	-	-	16(7.4)
<i>Hypocnemium erikssonii</i> Halbnb. et Hjosl.	-	3(1.2)	15(6.6)	-	3(1.2)	11(4.8)	-	-	-	-	-	-
<i>Epicoccum nigrum</i> Link	9(1.9)	4(1.7)	21(9.2)	2(0.6)	3(1.2)	-	1(0.2)	-	4(1.7)	6(1.7)	6(3.4)	25(11.6)
<i>Eurotium</i> sp.	-	-	-	7(1.9)	-	-	12(2.5)	-	1(0.4)	3(0.9)	-	2(0.9)
<i>Fusarium</i> spp.	33(6.9)	16(6.7)	101(44.3)	22(6.1)	10(4.2)	-	10(2.1)	2(0.8)	-	22(6.1)	16(9.1)	18(8.3)
<i>Geotrichum serpens</i> Chesters et Greenhalgh	-	-	-	-	-	-	-	-	-	1(0.3)	-	-
<i>Geotrichum candidum</i> Link ex Leman	-	-	-	-	-	-	-	-	-	5(1.4)	-	-
<i>Gliocladium catenulatum</i> Glim. et Abbott	-	-	-	-	-	-	-	-	-	-	-	-
<i>Gliocladium virens</i> Miller, Giddens et Foster	5(1.1)	-	-	-	-	-	2(0.4)	-	-	-	-	-
<i>Gliocladium viride</i> Matr.	-	-	16(7)	-	-	14(6.1)	-	-	-	-	-	-
<i>Graphium</i> sp.	-	-	-	-	-	47(20.6)	-	-	-	-	-	5(2.3)
	-	-	-	-	-	2(0.9)	-	-	-	-	-	-

After 30 months of stump exposure the communities of fungi isolated from wood were the most diversified ones. Over 30 species and genera of fungi were isolated from the upper surface of stumps and from their circumferential parts. Not too many species less were found in the deeper layers of stumps. A longer period of exposure resulted in a smaller number of isolated fungi from *Basidiomycota*. With progressive wood decay the fungi belonging to *Deuteromycota* were isolated more frequently. Such genera and species as *Fusarium* spp., *Phoma pinastrella*, *Cladosporium cladosporioides* and *Paecilomyces lilacinus* were isolated from surface layers of stumps. Their frequency was over 21% of fragments. From the deeper layers of stumps the following species were most frequently isolated: *Gliocladium* spp., *Rhinocladiella atrovirens*, *P. lilacinus* and *Penicillium* spp. The frequency of these fungi was lower, and did not exceed 7% (Table 1). In comparison with 6 and 18 months of stump exposure an increase in numbers of fungi from the genus *Trichoderma* was observed (34.7% of isolates). The percentage of fungi causing wood discolouration such as *Ceratocystis coerulea*, *Sclerophoma pythiophila*, and *Graphium* sp. was relatively small. Only *Leptographium hundbergii* was isolated from 2.1% of fragments on the average. No *Heterobasidion annosum* was found in any of the isolates (Table 1).

Fungi found in stumps on the basis of the presence of fructifications

After 18 months of exposure the presence of 12 species and genera of fungi was observed on investigated stumps. Three belonged to *Basidiomycota* and nine to *Ascomycota* and *Deuteromycota*. The fructifications of *Coniochaeta malacotricha*, *Stereum sanguinolentum*, *Phanerochaete gigantea*, *Nectria fucikeliana* and *Pycnidium resinae* were quite abundant. The fructifications of *Pezizella chionea*, *Ascocoryne sarcoides* and *Helotium laetum* were little less numerous. No fructifications of *Peniophora incarnata* were found although this species was isolated from stumps as early as after 6 months of their exposure (Table 2).

After 30 months of exposure the number of species belonging to *Basidiomycota* had increased. But only *Phanerochaete gigantea*, *Stereum sanguinolentum* and *Hypochnicium erikssonii* occurred abundantly while the remaining ones such as *Peniophora incarnata*, *Schizophyllum commune*, *Skeletocutis amorpha* and *Lycoperdon perlatum* were sporadic (on 1–5% of stumps). The percentage of *Ascomycetes* decreased with progress of wood decay. However, the occurrence of new species such as *Creopus* sp. and *Sordaria fimicola* was observed, although they were sporadic (Table 2).

Tabela 2
Percentage of stumps *Pinus nigra* with fruitbodies of fungi

Fungi	Exposition (in months) of stumps	
	18	30
<i>Ascocoryne sarcoides</i> (Jacquin ex Gray) Groves et Wilson	9.2	0
<i>Coniochaeta malacotricha</i> (Awd. In Niessl) Traverso	100	0
<i>Creopus</i> sp.	0	5.1
<i>Dacrymyces stillatus</i> Nees: Fr.	0	2
<i>Helotium laetum</i> (Boud.) Sacc.	5.5	10
<i>Hypochnicium erikssonii</i> Halbnb. et Hjosl.	10.8	29
<i>Lycoperdon perlatum</i> Pers.: Pers.	0	5
<i>Nectria fackeliana</i> Booth	27.8	5
<i>Peniophora incarnata</i> (Pers.: Fr.) P. Karst.	0	2
<i>Pezizela chionea</i> (Fries) Dennis	16.7	5
<i>Phanerochaete gigantea</i> (Fr.: Fr.) Raftan et al.	35.4	65
<i>Phoma pinastrella</i> Sacc.	5.5	0
<i>Postia stipica</i> (Pers.: Fr.) Jülich	0	1
<i>Pycnidiaella resinae</i> (Ehrenb.ex Fr.) Höhn.	22.2	0
<i>Schizophyllum commune</i> Fr.: Fr.	0	3
<i>Scutellinia scutellata</i> (L. ex St. Amans) Lambotte	3.1	0
<i>Skeletocutis amorpha</i> (Fr.) Kotl. et Pouz.	0	3
<i>Sordaria fimicola</i> (Rob.) Ces. et de Not.	0	1
<i>Sphaerobolus stellatus</i> Tode: Pers.	0	1
<i>Stereum sanguinolentum</i> (Alb. et Schw.: Fr.) Fr.	36.9	23
Not identified <i>Discomycetes</i>	0	2
Not identified <i>Ophiostomatales</i>	3.1	3
Number of analyzed stumps	65	100

DISCUSSION

It was observed during this study that stumps of *Pinus nigra* after 6 months of exposure were mainly inhabited by fungi belonging to *Basidiomycota*. These fungi use the hydrolytic and oxydizing enzymes, which act on the macromolecules of lignin, cellulose and hemicelluloses resulting in change of structure and consistence of stump wood (Sierota 1995). Such fast and spontaneous colonization of pine stumps by *Phanerochaete gigantea* and *Stereum sanguinolentum* found in this study confirm the results of Meredith (1960), Przebórski (1969) and Kwaśna (1992). A fact of their relatively slow colonization of surface layer of stump and fast inhabiting of its deeper parts irrespective of the exposure period is worthy of notice. It is probably connected with high moisture requirements of these fungi, strong nutritional competition of microorganisms inhabiting stumps, and an antagonistic action of fungi colonizing first of all the surface layers of a stump (Kwaśna 1992). These fungi, belonging mostly to *Ascomycota* and *Deuteromycota*, were most abundantly isolated in this study

from surface layers of stumps. Similar phenomenon was observed by Mańka and Wróblewski (1969) and Przezbórski (1969). Their investigations showed that *Heterobasidion annosum* i *Ph. gigantea* colonized the upper parts of stumps much slower than the parts located deeper.

In this study, after 18 months of stump exposure, the *Basidiomycota* were the most abundant fungi in the surface as well as deeper parts of stumps. This confirms the results of the studies of Kwásna (1992), Sierota (1995), Przezbórski (1969) and Meredith (1960), where *Phanerochaete gigantea* and *Stereum sanguinolentum* were most abundant during that period. These species are among the fungi, which colonize the stumps in their early phase of ecological succession (Sierota 1995). It appears that during that period there are the most favourable conditions for colonization of stumps by these fungi. Investigations of Meredith (1960) and Sierota (1995) showed that further exposure of stumps result in the decrease in proportion of *Ph. gigantea* and *S. sanguinolentum* in favour of "new" *Basidiomycota*, a component of subsequent phase of succession. Meredith (1960) found on 3-year-old pine stumps such species as *Hypholoma fasciculare* (Huds.) Kumm., *Tricholoma rutilans* (Schaeff.: Fr.) Kummer and *Polyporus amorphus* Fr. Kwásna (1992) after 30 months of exposure of pine stumps observed the appearance of *Coniophora puteana* (Schum. ex Fries) Karst. only. While Sierota (1995) found on the cutover stumps the appearance of *Hirschioporus abietinus* (Dicks.: Fr.) Donk and *Hypholoma fasciculare* after their earlier inoculation with *Phanerochaete gigantea*. During this study, similar as in the investigations cited above, the decrease of isolates of *Ph. gigantea* and *S. sanguinolentum* was observed after 30 months of stump exposure. However, during the second study method when search was made for fructifications on stumps, the fructifications of *Ph. gigantea* were the most numerous ones. This may had been caused by the possibility that some of the fructifications found were the well preserved last year fructifications found were the well preserved last year fructifications found were the well preserved last year fructifications. The present study after 30 months of stump exposure showed, however, a much richer species spectrum. Such fungi as *Skeletocutis amorphia*, *Schizophyllum commune*, *Sphaerobolus stellatus* and *Lycoperdon perlatum* appeared on stumps. Such large differences in species composition of fungi inhabiting stumps presented in papers mentioned above may had been caused by many various factors. The fact that in this study the stumps of *Pinus nigra* were investigated while the studies cited above concerned *Pinus sylvestris* may also be of importance. Moreover, the felled trees of *P. sylvestris* whose stumps were investigated were 80–100 years old. In the present study the felled trees were much younger, which most certainly may had affected the species composition of fungi inhabiting stumps of *P. nigra*. Also the ecological factors such as climate, land configuration or wind direction may be of importance. The local conditions may have either positive or negative effect on the sporulation of individual species of fungi. A great effect of climatic conditions on the

sporulation and occurrence of fungi was reported by K ä ä r i k and R e n m e r f e l t (1957). These authors found that the height of stumps (i.e. their different moisture content) and their geographic location may considerably affect the species composition of fungi inhabiting stumps of *P. sylvestris* in Sweden.

Such high percentage of colonization of stumps by *Phanerochaete gigantea* after 6 months of their exposure may indicate that this fungus colonizes stumps of *P. nigra* fast and efficiently. However, a natural infection of stumps by this fungus is infrequent and depends on local climatic conditions (S i e r o t a 1995). Such successful colonization of *P. nigra* stumps may be connected with the most favourable for *Ph. gigantea* time of tree felling. The results of K ä ä r i k and R e n m e r f e l t (1957) showed that stumps of *P. sylvestris* are most successfully colonized by *Ph. gigantea* in the spring. As a natural antagonist of *Heterobasidion annosum* this fungus in association with other fungi inhabiting stumps may considerably limit their infection by this pathogen. This has been confirmed by present study since no *H. annosum* was found in spite of the abundance of its fructifications on stumps of *P. sylvestris* in a stand surrounding the seed orchard. An artificial inoculation of stumps with *Ph. gigantea* may be an efficient treatment limiting the development of *H. annosum* on *P. nigra*. This fact has a significant importance since stumps play an important role in infection of living trees by this pathogen (M a á k a 1992).

Also a significant role in colonization of stumps of *P. nigra* was played by fungi from the genus *Trichoderma*. K w a á n a (1992) found that these fungi are among the species colonizing stumps most early. Same was found by S i e r o t a (1995) who observed that fresh stumps were colonized most early by fungi belonging to *Mucorales*, *Trichoderma* spp. i *Penicillium* spp. This author associates them with possibility of wood decaying (S i e r o t a 1977). According to G a r r e t t (1970) these fungi utilize the simplest forms of carbohydrates and are the colonization pioneers on the one hand and produce volatile and non-volatile organic compounds limiting the growth of root pathogens on the other hand. This study showed that the fungi from the genus *Trichoderma* efficiently competed with other fungi inhabiting stumps of *P. nigra*, and were abundant during the entire period of stump exposure. These fungi colonized deeper layers of stumps to greater extent than surface layers. A same relationship was found by M a á k a and W r ó b l e w s k i (1969).

Other fungi belonging to *Ascomycota* and *Deuteromycota* were abundant on stumps of *P. nigra*, especially in their surface layers. These fungi, usually unable to degrade wood cell walls, inhabit stumps thank to the ability to utilize the products of cellulose and lignin decomposition and metabolites of other associated organisms. The amount of the metabolites, which may be used by these fungi, increases with the progress of stump decomposition (S i e r o t a 1995). Probably this is why in this study these fungi occurred most abundantly after 30 months of stump exposure.

During this study the blue stain fungi belonged to the species, which slowly colonized the wood. This appears to confirm the opinion of Garrett (1970) that the blue stain fungi are characterized by a passive colonization of stumps associated with lack of active cellulose enzymes, and this is probably why they do not play a significant role in the process of decomposition of stumps of *Pinus nigra*.

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Grzyby zasiedlające pniaki *Pinus nigra* w zależności od czasu ich ekspozycji

S t r e s z c z e n i e

Badania prowadzono w latach 1997–1999 na plantacyjnej uprawie nasiennej sosny czarnej w Nadleśnictwie Miechów. Celem badań było określenie składu gatunkowego zbiorowisk grzybów zasiedlających pniaki *P. nigra* w miarę upływu czasu ekspozycji pniaków.

Po 6 i 18 miesiącach ekspozycji pniaków wyraźną dominację wykazały dwa gatunki grzybów podstawkowych: *Stereum sanguinolentum* i *Phanerochaete gigantea*. Po 30 miesiącach ekspozycji izolowano najbardziej zróżnicowane zespoły grzybów. Wydłużający się okres ekspozycji pniaków wpłynął na zmniejszenie się liczby izolatów *Ph. gigantea* i *S. sanguinolentum*. Na pniakach zanotowano jednak wzrost liczby innych gatunków należących do *Basidiomycota*. Wraz z postępującym rozkładem drewna częściej izolowano grzyby należące do *Deuteromycota*.