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Effect of pine sawdust on the structure of fungi communities in the soils of post agricultural land

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This paper presents the results of mycological studies of soils left barren for 15, 6 and 3 years before and after addition of pine sawdust. Considerable differences in the species composition of fungi communities were found related both to the type of soil (period of lying barren) and treatment. Before the treatment the soil was dominated by such species as *Paecilomyces lilacinus*, *P. marquandi*, *Apiospora montagnei*, *Pseudogumnoscus roseus*, *Penicillium janczewskii*, *P. jensenii*, while after a year following addition of sawdust by *Trichoderma harzianum*, *T. pubescens*, *T. virens* and numerous species of *Penicillium*. The presence of *Trichoderma* species, comprising over 60% of the communities after the treatment indicated the possibility of creating conditions for efficient protective action against root infection of trees by *Heterobasidion annosum* (a cause of dangerous destruction in forests growing on post agricultural land in Poland).

Key words: pine sawdust, post agricultural land, soil fungi, *Trichoderma* spp.

INTRODUCTION

The physical, chemical and biological properties of farmland soil are different from those of typical forest soil. In the latter there is no layer of high density (the so called "plough sole") preventing capillary ascent of water to surface layers and their aggregate structure is not destroyed (T u s z y ń s k i 1990). The small proportion of lignified tissues, the absence of duff, roots and stumps are the basic differences between forest and post agricultural soils. The lack of these major sources of energy for fungi decomposing cellulose

and lignin and participating in the process of mineralization of organic substances and formation of specific structure of forest soil significantly affects the biological activity of post agricultural soils (Wentzel 1969; Trojanowski and Heider 1975; Richards 1979; Redfern 1989; Tracz 1993). These soils are also considerably rich in nitrogen (carbon: nitrogen ratio in plough layer is lower than in forest soils), deficient in phosphorous and potassium and poor in organic substance content at the depth of 20–25 cm (Federov 1960; Baule and Fricker 1971; Szujecki 1990; Królikowski 1975; Tuszyński 1990). Arable soils usually have higher reaction (pH 6–8) than the forest ones (pH 4–6) particularly in surface layers. Thus other organisms dominate such as bacteria, actinomuceate, cyanophyceate, chlorocypheate and diatoma (Pacewiczowa and Trzcińska 1961; Sierota 1976; Smyk 1984; Tuszyński 1990).

In field ecosystems, the differentiation of quantitative and qualitative species composition of soil mycocenoses is also considerably related to soil culture (crop rotation) and species composition of crops (Smyk 1984). Dorenda (1974) and Smyk (1984) indicated that in arable soils fungi communities included mostly the species of the genera *Absidia*, *Aspergillus*, *Cylindrocarpon*, *Fusarium*, *Humicola*, *Mucor*, *Penicillium*, *Stemphylium*, *Spicaria*, *Scopulariopsis*, *Trichoderma* and *Verticillium*. Particularly numerous were cellulolytic fungi species easily decomposing cellulose in neutral environment and nitrophylic fungi, e.g. *Penicillium* spp. The fungi with antagonistic potential against many pathogens, as biological control agents, occurred in a limited number (Garrett 1970, Krupa and Dommergues 1979).

In coniferous stands growing on post agricultural soil the main threat is a root disease caused by *Heterobasidion annosum* (Fr.) Bref. Among factors favouring the disease development (Mańka 1972; Twarowska and Sternak 1974; Sierota 1995) is the absence or restricted occurrence of soil and rhizosphere fungi with antagonistic activity against the pathogen (Garrett 1970; Veselinovic 1978; Przezbórski 1989; Kwaśna 1992). At the same time, the possibility of stimulating biological processes in soil by adding organic substrates such as bark, sawdust or humus mulches was indicated by Duda and Sierota (1987) and Szujecki (1990). This treatment not only led to the increase in activity of fungi competitive or antagonistic with respect to the pathogen, but also improved breeding traits of seedlings (Bellon and Buraczyk 1994).

The aim of this work was to determine the species composition of fungi communities after addition of sawdust into post agricultural soils of various period of cultivation and lying barren.

MATERIAL AND METHODS

The study was conducted in 1995 in the Głęboki Bród Forest District (Gulbin, department 320s) on post agricultural land scheduled for afforestation in 1996. Due to different barren period of each segment of the area, 3 plot blocks were selected: a) soil barren for 15 years covered with wild grass close to coniferous forest, b) post agricultural soil barren for 6 years with grass for 3 years, c) previous pasture sown yearly, barren for 3 years and one year before treatment under oats. In autumn soil samples were taken from six sites on each of the four plots representative of each evaluation variant (areas of 25 m^2 each). After preparing the average sample, part of the latter was used for determination of mechanical composition of the soil and some of its chemical properties (Tab. 1). From the remaining part of the sample fungi were isolated with Warcup's soil-plate method modified by M a ń k a (1964). The number of mycelium forming units (MFU) was determined in the mean sample of 0.008 g of soil.

In autumn 1995 pine sawdust was distributed on the plots scheduled for treatment and ploughed into the depth of about 35 cm. In spring 1996 the soil was also ploughed and harrowed. In appropriate experiment variant common pine with birch seedlings were planted out. In autumn 1996 soil samples were collected from these sites and subjected to the same chemical and mycological analyses as in the previous year (Tabs 1, 3).

RESULTS

Chemical analysis of soil

The results of analysis of the mechanical composition of soil (Tab. 1) indicated that the soil studied was composed of light clayey sand (barren for 15 years) and loose sand (barren for 6 and 3 years). Soil acidity varied: 5.1, 7.0 and 4.4 pH, respectively, which was probably related to the type of fertilizer applied and crop character prior to the study. The highest pH of soil for the area barren for the last 6 years was associated with the highest content of Ca^{+2} (143.5 mg/100 g), K (5.0 mg/100 g) and Mg^{+2} (9.2 mg/100 g).

Following sawdust addition and ploughing considerable changes occurred in soil reaction and content of each element. The soil pH was in the range of pH_{KCl} 4.7 – 5.2; the contents of carbon and nitrogen increased and the values of the C/N ratio rose over twofold. Phosphorous content decreased while the contents of potassium and magnesium increased (except for the area barren for 6 years). Initially large differences between the plots concerning the value of determined parameters stabilized at similar level after a year following sawdust addition.

Table 1

Mechanical and chemical composition of soil in three plots of different barren period (15, 6 and 3 years) in 1995 and 1996

Parameter	Barren 15		Barren 6		Barren 3	
Soil fraction:						
sand Σ%	77		89		91	
dust Σ%	10		7		4	
loam Σ%	13		4		4	
Mechanical group:	Light clayey sand		Loose sand		Loose sand	
	1995	1996	1995	1996	1995	1996
pH H_2O	5.1	5.7	7.0	6.1	4.4	5.6
pH KCl	4.1	4.7	6.0	5.2	3.8	4.7
C%	1.20	2.88	0.98	3.26	0.87	2.89
N%	0.091	0.099	0.071	0.085	0.059	0.103
C/N	13.2	29.1	13.8	38.3	14.7	28.1
P_2O_5 (mg/100g)	1.1	0.7	2.4	2.1	10.7	3.0
K	4.0	7.0	5.0	7.0	1.2	5.0
Ca	32.5	57.0	143.5	85.0	18.5	55.0
Mg	2.5	7.5	9.2	7.2	2.5	6.7

Mycological analysis of soil

The results of mycological analysis of soil carried out before and after the treatment are given in Tables 2 and 3. Before the treatment the soil barren for 15 years yielded 232 isolates representing 40 fungi species, the soil barren for 6 years — 177 isolates of 63 species and the soil barren for 3 years — 350 isolates of 58 species. The addition of sawdust to the soils resulted in significant quantitative and qualitative changes in the species composition of fungi communities.

A considerable decrease in the number of species and marked differences in the species composition of fungi communities were noted. Both before and after sawdust addition no *Heterobasidion annosum* pathogen was found while the proportion of fungi causing dangerous root diseases (*Fusarium* spp., *Phoma* spp.) was low. Before the treatment *Paecilomyces lilacinus* (13.8%), *Apiospora montagnei* (8.5%) and *Paecilomyces marquandii* (8.0%) predominated in each of the communities.

The decomposition of sawdust in the soil over a year long period resulted in a considerable increase in the number of *Trichoderma* species which are known for their antagonistic effect on root pathogens, particularly of *T. harzianum*.

Table 2

Number of mycelium forming units (MFU) of fungi before the treatment (added sawdust of pine) in soil barren for 15, 6 and 3 years

Species	Number of MFU in barren soil		
	15	6	3
1	2	3	4
Absidia coerulea Bainier	1		
A. spinosa Lendner		4	
Acremonium roseogriseum (S. B. Saksena) W. Gams		3	
A. strictum W. Gams	1	11	
Alternaria alternata (Fr.) Keissler		5	3
Alysidium resinae (Fr.) M. B. Ellis	21	2	
Apiospora montaqnei Sacc.		15	2
Arthrinum phaeospermum (Corga) M. B. Ellis		3	4
Aspergillus versicolor (Vuill.) Tiraboschi		3	
Ascomycetes con. st. *Scopulariopsis* sp.		1	
Basidiomycotina			1
Candida albicans (Robin) Berkhout		1	
Chaetomium funicola Cooke		1	2
Chaetomium sp. 1		2	
Chaetomium sp. 2			5
Chaetomium sp. 3			3
Chloridium virescens var. *chlamydosporum* (Beyma) Gams et Hol.-Jech.			3
Chrysosporium merdarium (Link) J. Carm.	2	1	1
C. pannicola (Corda) v. Oorschot et Stalpers			1
C. pannorum (Link) Hughes		2	
Cladosporium cladosporioides (Fres.) de Vries		3	4
Cochliobolus sativus (Ito et Kurib.) Drechsl. et Dastur		1	
Coniothyrium fuckelii Sacc.	7	4	26
Curvularia eragrostidis (Tsuda et Ueyama) Sivanesan			1
Fusarium aquaeductuum (Radl. et Rabenh.) Lagerh.		1	1
F. equiseti (Corda) Sacc.		5	
F. flocciferum Corda			1
F. oxysporum Schlecht.	4	1	5
F. redolens Wollenw.		2	
F. sambucinum Fuckel			1
Gliocladium catenulatum Gilm. et Abbott	1	1	
G. roseum Bainier	3		
Heteroconium chaetospira (Grove) M. B. Ellis		1	
Humicola fuscoatra Traaen		3	6
H. grisea Traaen	3	1	3
Hormonema sp.		1	2
Melanospora fimicola Hansen			2
Metarrhizium anisopliae (Metschnikoff) Sori	3	3	
Monocillium mucidum W. Gams		1	
Monodictis putredinis (Wallr.) Hughes		2	
Mortierella alpina Peyronel	1	8	2
M. cf. *hygrophila* Linnemann		2	5
M. minutissima van Tieghem		2	
M. thaxteri Bjorling			10

cont. Table 2

1	2	3	4
M. vinacea Dixon-Stewart	3		9
Mortierella sp.			1
Mucor hiemalis Wehmer		1	
Mycelium radicis atrovirens Melin			1
Ochroconis constricta (Abbott) de Hoog et Arx	1		
Oidiodendron griseum Robak			2
O. tenuissimum (Peck) Hughes			3
Paecilomyces lilacinus (Thom) Samson	32	4	
P. marquandii (Massee) Hughes	13	1	5
Penicillium adametzii Zaleski			63
P. aurantiogriseum Dierckx		5	2
P. brevicompactum Dierckx	9		2
P. citrinum Thom	1	1	
P. commune Thom		3	
P. expansum Link		1	5
P. fellutanum Biourge	1		
P. herquei Bain. et Sartory		2	
P. islandicum Sopp	2		2
P. janczewskii Zaleski		1	53
P. janthinellum Biourge	1		
P. jensenii Zaleski	29	2	16
P. lanosum Westling	3		6
P. martensii Biourge		2	
P. puberulum Bainier	1		
P. purpurogenum Stoll	1		1
P. raistrickii G. Sm.			2
P. steckii Zaleski		3	
P. waksmani Zaleski	4	5	3
Phialophora cyclaminis v. Beyma			6
P. fastigata (Lagerb. et Molin) Conant		4	
P. lasiosphaeria W. Gams			1
Phoma eupyrena Sacc.		1	
P. glomerata (Corda) Wollenw. et Hochapfel		4	1
P. medicaginis var. *pinodella* (L. K. Jones) Boerema		2	
Pseudogymnoascus roseus Raiłło	18	12	35
Pyrenochaete terrestris (Hansen) Gorenz, Walker et Larson			2
Ramichloridum schulzeri (Sacc.) de Hoog		1	
Scytalidium japonicum Udagawa, Tominanga et Hamaoka			5
Sesquicillium candelabrum (Bonorden) W. Gams	1		9
Torula sp.			1
Trichocladium asperum Harz	2	1	5
Trichoderma harzianum Rifai	5	2	
T. koningii Oudemans	19		
T. longipilis Bissett	2		2
T. polysporum (Link:Pers.) Rifai	1		
T. pubescens Bissett	7	2	2
T. viride Pers. ex Gray		2	
Trichoderma sp. con. st. *Hypocrea lactea* (Fr.:Fr.) Fr.	1		
Truncatella truncata (Lev.) Steyaert		5	
Trichosporiella cerebriformis (de Vries et Klein-Natrop) Gams		1	
Verticillium bulbillosum W. Gams et Malla	3	3	1
V. lamelliocola (F. E. V. Smith) W. Gams	2	2	

cont. Table 2

1	2	3	4
V. lecani (Zimm.) Viegas		2	
V. nigrescens Pethybr.	1		
V. nubilum Pethybr.			1
Zygorhynchus moelleri Vuill.	17	1	5
non-sporulating, black hyphae IBL 8	1		
non-sporulating, black hyphae IBL 9	4		
non-sporulating, black hyphae IBL 32		4	
non-sporulating, black hyphae IBL 60		1	
non-sporulating, black hyphae IBL 68			1
non-sporulating, white hyphae IBL 3	1		
non-sporulating, white hyphae IBL 62			2
Number of species	40	63	58
Number of isolates	232	177	350

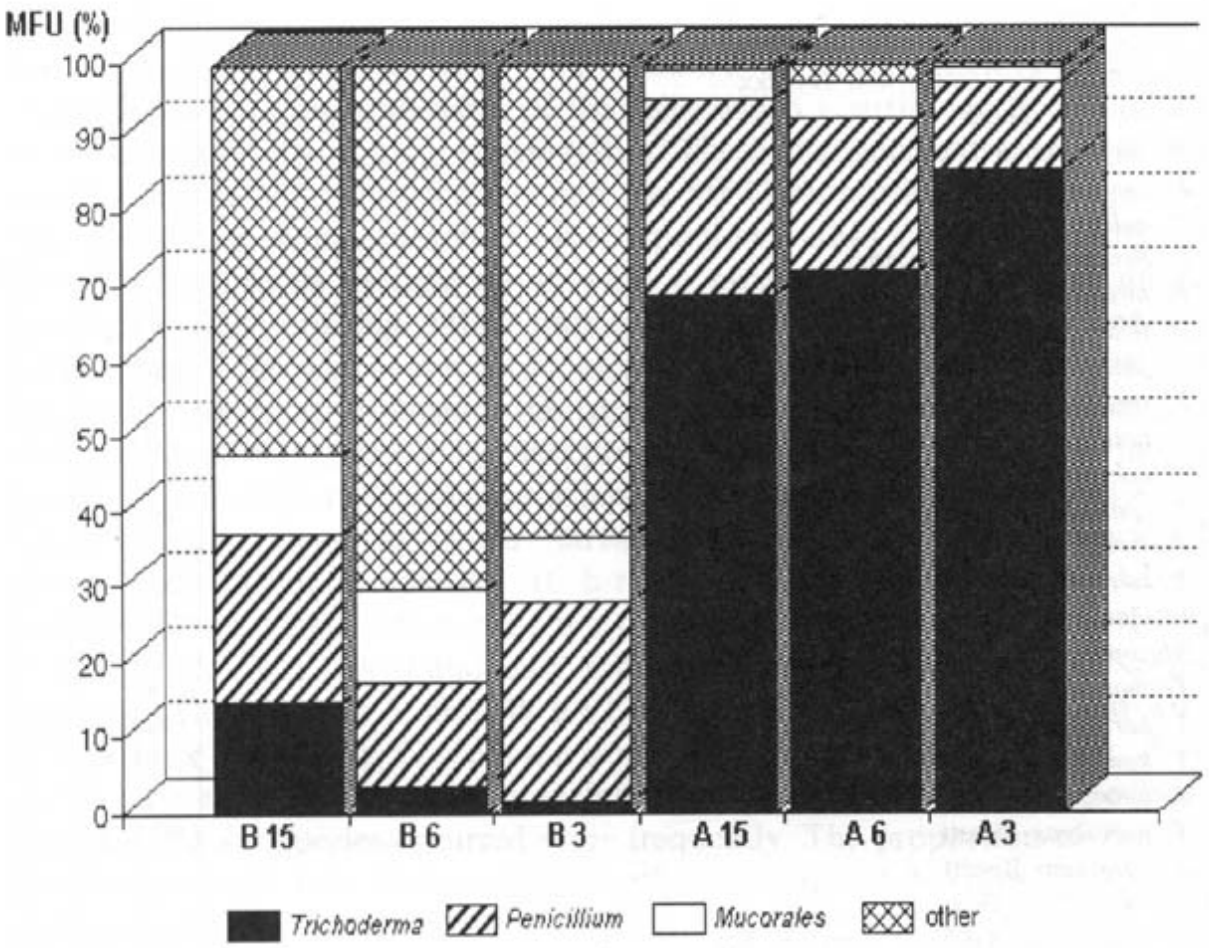

Fig. 1. Frequency (MFU%) of the most common taxa in relation to the type of soil (15, 6, 3-years-old) and treatment (B, A)
15, 6, 3 — period of soil lying barren; B — before addition of sawdust; A — 1 year after addition of sawdust

Table 3

Number of mycelium forming units (MFU) of fungi one year after the treatment (added sawdust of pine) in soil barren for 15, 6 and 3 years

Species	Number of MFU		
	15	6	3
Absidia coerulea Bainier	3	7	3
Aspergillus versicolor (Vuill.) Tiraboschi	1		
Cladosporium herbarum (Pers.) Link	1		
Coniothyrium fuckelii Sacc.			1
Fusarium oxysporum Schlecht.		5	
Humicola grisea Traaen			1
Lecythophora sp.	1	1	
Mortierella nana Linnemann	1	1	
Mortierella vinacea Dixon-Stewart	6	3	
Mucor hiemalis Wehmer	1	1	
M. plumbeus Bonorden	2	1	1
M. racemosus Fres.		1	1
M. sp.	1		
Penicillium brevicompactum Dierckx	1		
P. citrinum Thom	27		
P. corylophilum Dierckx	1		
P. coclopium Westling	2	3	2
P. daleae Zaleski	1		3
P. herquei Bain. et Sartory	2	29	4
P. hirusutum Dierckx	1		
P. fellutanum Biourge			1
P. janczewski Zaleski	6	7	
P. jensenii Zaleski		3	4
P. notatum Westling	4		8
P. ochrochlorron Biourge	7	8	
P. spinuulosum Thom	27	8	6
P. steckii Zaleski			3
P. terrestre Jensen	1		
P. vinaceum Gilman et Abbott	10	12	
Rhizopus arrhizus Fischer		1	
Trichoderma aureoviride Rifai	5		
T. harzianum Rifai	139	184	165
T. koningii Oudemans	3	8	4
T. longipilis Bissett		1	
T. pubescens Bissett	63	30	18
T. strigosum Bissett		3	2
T. viride Pers.:Gray	1		1
T. virens (Mil. Gidd. et Fost.) v. Arx	22	24	35
Zygorhynchus moelleri Vuill.		1	1
Number of species	28	23	20
Number of isolates	340	342	263

The proportion of some taxa in the fungi communities is given in Fig. 1. Before the treatment the species belonging to the genera *Trichoderma* and *Penicillium* and order *Mucorales* comprised less than 50% of the fungi communities. The addition of pine sawdust resulted in the increase of frequency of *Trichoderma* species from 14.6% to 68.5% for the soil barren for 15 years, from 3.4% to 72.2% for the soil barren for 6 years, and from 1.4% to 85.6% for the soil barren for 3 years. It is interesting to note that higher intensity of substrate colonization by *Trichoderma* species was observed in the soil barren for 3 years, irrespectively of the fact that this species was the least numerous before the treatment. The proportion of nitrophyllic species of the genus of *Penicillium* decreased only on the field barren for 3 years, which was probably due to competition with *Trichoderma*.

DISCUSSION

The addition of sawdust into the soil in autumn, and then ploughing and leaving it to decompose for 1 year resulted in considerable changes in biological activity and chemistry of the soils, which contributed to variations in the structure of soil fungi communities. These changes involved in species composition of fungi communities and the MFU proportion of each species. The abundance of pine sawdust caused predomination of fungi playing an important role in its decomposition while competition among fungi eliminated the species utilizing other nutrients from soil. *Trichoderma* species, particularly *T. harzianum* and *T. pubescens*, were hardly isolated from the soil before the treatment. Following the treatment it dominated the communities with regard to MFU (spores, hyphae) proportion. The species which previously predominated such as *Paecilomyces lilacinus*, *P. marquandii*, *Apiospora montagnei*, *Pseudogymnoascus roseus* retreated. *Paecilomyces* species associated mainly with deciduous forests (Christensen, Whittingham and Novak 1962; Gochenaur and Whittingham 1967; Christensen 1969) and only sporadically present in coniferous forest soils (Christensen and Whittingham 1965; Widden and Parkinson 1973; Gochenaur and Woodwell 1974; Söderström 1975) also retreated following the addition of pine sawdust. However, *Mucor* species occurred more frequently. The proportion of *Penicillium* species did not change significantly, although differences in species composition were noted. After the addition of sawdust the activity of *P. citrinum*, *P. herquei*, *P. spinulosum* and *P. steckii*, typical of forest soils with lower pH, increased.

Considering the increasing frequency of *Mucorales*, *Trichoderma* and *Penicillium* species, the species composition of fungi communities in post

agricultural soil following addition of pine sawdust resembles that of deciduous forests in Poland (Mańka, Łakomy and Maćkowiak 1993; Kwaśna 1995) and other countries (Bååth and Söderström 1980; Christensen et al. 1962; Domsch and Gams 1970; Wicklow, Bollen and Denison 1974; Widden and Parkinson 1973), although it is different with respect to *Trichoderma* species. In coniferous forest soils *T. viride* and *T. polysporum* predominated (Söderström 1975; Söderström and Bååth 1978; Goldfarb, Nelson and Hansen 1989; Mańka et al. 1993; Kwaśna 1995). However, a year after addition of pine sawdust, mainly *T. harzianum* was found in the post agricultural soil. It was characterized by relatively large, smooth, tear-like spores whose thick walls resembled those of *T. atroviride* Karsten, but the typical layout of phialids and spore sizes were within the limits reserved for *T. harzianum*. The other numerously represented species was *T. pubescens*. Goldfarb et al. (1989) found *T. hamatum* closely resembling *T. pubescens* on Douglas spruce stumps and roots.

In forest soils the antagonistic ability of *Trichoderma* species with respect to other fungi are commonly recognized. After reaching high frequency they eliminate some wood decomposing fungi from the subsoil and those pathogens which are relative parasites. *Trichoderma* species restrict the growth of *Heterobasidion annosum* (Sierota 1976; Kwaśna 1997) in vitro and it appears that they have the same effect under natural conditions. The antagonistic effect of *Trichoderma* is related to their abundant production of spores, the ability to produce antibiotic metabolites and colonize various substrates (nutritional and spatial competition). *Trichoderma* species is capable of paraziting other fungi, surrounding spirally their hyphae and entering them (Domsch, Gams and Anderson 1980).

The optimal pH for the growth of *Trichoderma harzianum* is 3.7–4.7. In the subsoil with lower reaction spore germination is stimulated which may account for higher frequence of fungi in more acid soils. *Trichoderma* species colonize mainly decomposing wood. Therefore, it is unlikely that they eliminate mycorrhizic fungi colonizing roots of living trees.

The neighbourhood of coniferous forest on the soil barren for 15 years and lower pH may result in the increase in the number of populations of *Trichoderma* species even before addition of sawdust. Intensive fertilization and higher lime content on the plot barren for 6 years due to the increase in pH, resulted in the decline of the populations of this species. It is probable that on the plot barren for 3 years similar effect was due to oats cultivated a year earlier. Mineral fertilization and higher lime content caused a rise in the number of fungi species on the plot barren for 6 years, while the high fresh organic substrate content after ploughing oats into the plot barren for 3 years contributed to the rise in the number of fungi species.

Penicillium and *Trichoderma* species decomposed sawdust cellulose. Subsequently the substrate was colonized by *Mucorales* which could only decompose proteins therefore, they were characteristic of the last stage of substrates colonizers. The low proportion of these fungi in the soil with pine sawdust suggested that this was not the last stage of sawdust decomposition.

The succession of fungi in soil is controlled by the amount and form of carbon in the substrate, which depends largely on the conditions of decomposition of organic matter. Under some conditions the fungi with high competitiveness can behave completely inactive in the others. In the case of *Trichoderma* species the presence of nitrogen, in addition to carbon, plays a significant role (C o w l i n g and M e r i l l 1966) as well. The increase in nitrogen content and the value of C/N ratio following addition of sawdust resulted, first of all, in the rise of *Trichoderma* spp. populations and antagonistic effect.

The results obtained indicated that the proportion of fungi antagonistic against many pathogens of roots is small in the cultivated soils left barren. The addition of substrate containing cellulose and lignin – pine sawdust – stimulated to activity and modified the mycostatic systems in soil. The enrichment of post agricultural soil with pine sawdust stimulated mainly the growth of *Trichoderma* species, which are known precursors in colonizing subsoils (B l i s s 1951; G a r r e t t 1970; D u d a and S i e r o t a 1987). The catabolic enzymes and antibiotic compounds produced by *Trichoderma* species fungi allow fill the free ecological niches and eliminated previously occurring fungal components of soil environment (D a h m 1966). The activity of these fungi to colonize the organic substrates was confirmed by the present study. Their presence can create conditions for efficient protective activity against infection of pine and birch seedlings by *H. annosum*. The addition of pine sawdust into post agricultural soil may be regarded as one of the protective measures in designing afforestations of barren land in Poland.

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Wpływ trocin sosnowych na strukturę zbiorowisk grzybów w glebach porolnych

Streszczenie

W pracy przedstawiono wyniki analiz mikologicznych gleb odłogujących 15, 6 i 3 lata – przed – i po 1 roku od zabiegu polegającego na starannym przemieszaniu z glebą trocin sosnowych. Stwierdzono znaczne różnice w składzie zbiorowisk grzybów, związane zarówno z rodzajem gleby (okresem odłogowania), jak i wpływem zabiegu. Przed zabiegiem w glebie

dominowały takie gatunki, jak: *Paecilomyces lilacinus*, *P. marquandii*, *Apiospora montagnei*, *Pseudogymnoascus roseus*, *Penicillium janczewskii*, *P. jensenii*, zaś po roku od wprowadzenia trocin: *Trichoderma harzianum*, *T. pubescens*, *T. virens* i liczne *Penicillium*. Grzyby rodzaju *Trichoderma*, znane z inhibicyjnego oddziaływania na *Heterobasidion annosum* (sprawcę groźnych szkód w lasach na gruntach porolnych w Polsce), stanowiły po zabiegu ponad 60% liczebności zbiorowisk. Wskazuje to na możliwość stymulowania warunków skutecznego oddziaływania ochronnego przed infekcją korzeni ze *H. annosum* przez dodawanie trocin sosnowych do odłogującej gleby porolnej.