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Microfungi in the soil beneath common oak and their effect on *Armillaria* occurrence

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Microfungal assemblages in a soil beneath 30- and 50-year-old oaks and their 2-year-old stumps were studied using the soil dilution plate method. A total of 98 culturable microfungi were isolated. Compared to the living oaks before felling and the control living oaks, the density of *Mortierella macrocystis, Penicillium janczewskii, Pseudogymnoascus roseus, Sporothrix schenckii, Tolypocladium inflatum* and *Umbelopsis vinacea* significantly increased in the soil beneath stumps in the 32- and 52-year-old stands. Density of *Aspergillus kanagawaensis, Monodictys lepraria, P. daleae* and sterile dematiaceous hyphomycetes increased significantly in the 32-year-old stand and *Chrysosporium merdarium* in the 52-year-old stand. These fungi are known 'stimulants' of *Armillaria* rhizomorph formation. It is suggested that the increase in density of *Armillaria* rhizomorph 'stimulants' in a soil beneath oak stumps may increase the possibility of colonization of stumps by *Armillaria*.

Key words: *Armillaria, Quercus*, soil fungi, stimulating effect

INTRODUCTION

Armillaria is the causal organism of butt and root rot and an aggressive pathogen of conifers and hardwoods. Oak stumps remaining after felling, after infection usually by rhizomorphs and rarely by basidiospores, are colonized by the pathogen and act as a source of nutrients and a prolonged 'reservoir' of inoculum. *Armillaria* basidiomes are produced abundantly in autumn on the stump surface and at the stump base. A profuse networks of rhizomorphs - the main infecting organs of *Armillaria*, are produced from stumps and roots until stump decomposition (Rishbeth 1988; Kwaśna unpubl.). Rhizomorphs can infect nearby trees. Once established in a stump, the fungus is able to utilize the substantial food base available, to attack and kill nearby trees for many years (Rykowski 1975, 1981 a, b, c, 1984; Łakomy 1998).

Oak stumps in Poland are mainly colonized by *A. ostoyae* (Romagnesi) Herink and *A. gallica* Marxmüller et Romagnesi (Rykowski 1990; Łakomy 1998; Żółciak 1999 a, b).

The effect of soil texture, compaction, temperature, moisture, water logging, droughts, pH, aeration, organic matter, nutrients and inhibitory substances content, on rhizomorph growth has been widely examined and discussed (Redfern and Filip 1991). There is, however, a paucity of information regarding the effect of soil microorganisms on the rhizomorph growth. The first observations of stimulatory effects exerted by fungi were those of Mańka (1953), Kessler and Anderson (1960), Pentland (1965; 1967), and Goheen and Hansen (1978). The increase in *Armillaria* rhizomorph formation and disease incidence were observed in the presence of *Aureobasidium pullulans* (de Bary) Arnaud, *Ceratocystis virescens* (Davids) C. Moreau, *Heterobasidion annosum* (Fr.) Bref., *Leptographium wageneri* (Kendr.) Wingf., *Mycelium radicis atrovirens* Melin, *Phaeolus schweinitzii* (Fr.) Pat. and *Phellinus weirii* (Murr.). In 1986, Watanabe carried out intensive studies on the effect of 121 individual fungi on *Armillaria* rhizomorph production. Thirty seven species, including *Macrophoma*, *Gliocephalis*, *Diplodia* and *Sordaria* effectively stimulated rhizomorph production *in vitro*.

Early observed fungal 'stimulants' of *Armillaria* growth rarely represented species found in soil, particularly in forest one, which is the main habitat of *Armillaria* in temperate zones. Therefore Kwaśna (1995; 1996 a, b; 1997 a, b, c; 2001; 2002), Kwaśna and Łakomy (1998) and Kwaśna et al. (2001) investigated the interactions between *Armillaria* spp. and saprotrophic forest tree root and soil microfungi. On and in stump roots of coniferous species, particularly of Scots pine (*Pinus sylvestris*), the increase in density of fungi antagonistic to *A. ostoyae*, mainly *Trichoderma viride* Pers. ex Fr., resulted in an increase in the suppressive effect towards the pathogen *in vitro* (Kwaśna 1997 a, b, c). In contrast, on and in hardwood stump roots, particularly in birch (*Betula pendula*), an increase in density of *Zygorhynchus moelleri* Vuill. stimulated *Armillaria* rhizomorph formation *in vitro* (Kwaśna 1996 a, b; Kwaśna and Łakomy 1998). Kwaśna (2001; 2002) found that the density of rhizosphere and root fungi on stumps of oak (*Quercus robur*) was, respectively, 2-5 and 1.5-2 times greater than in living trees, and that many of the most frequently occurring species stimulated the *Armillaria* rhizomorph formation. Microfungi from *Aspergillus*, *Chrysosporium*, *Monodictys*, *Mortierella*, *Penicillium*, *Pseudogymnoascus*, *Sporothrix*, *Tolypocladium* genera stimulated rhizomorph production *in vitro*. Each fungus, when applied in an oak disc attached to an oak segment colonized either by *A. ostoyae* or by *A. gallica* or *A. mellea* and immersed in soil, caused significant increases in all or single rhizomorph character assessed, e.g. number of rhizomorphs, number of rhizomorph apices, rhizomorph length and weight. It is presumed that the increase in density of fungi stimulating rhizomorph production in roots and rhizosphere may favour the colonization of oak stumps by *Armillaria* which is manifested by the formation of numerous basidioms on stump (Kwaśna 2001; 2002; 2003; Kwaśna et al. 2001).

After analyses of root and rhizosphere it was necessary to study the soil fungal assemblages and their possible effect on *Armillaria* growth. The purpose of the present study was to determine the changes in microfungal assemblages in a soil beneath stumps of oak (*Q. robur*) left after felling. It was necessary to investigate whether the soil fungal assemblages, beneath stumps, behave similarly to those in roots and rhizosphere of stumps, i. e. whether a total density of assemblages and contribution of *Armillaria* rhizomorph 'stimulants' increase after felling. An evaluation of the effect of changes in density of the *Armillaria* 'stimulants' in the soil beneath stumps on colonization of oak stumps by *Armillaria* is attempted.

MATERIALS AND METHODS

Soil samples were collected in September 1995, from two forest stands, i.e. from the 30-year-old and 50-year-old common oak (*Q. robur*) mixed with Scots pine (*P. sylvestris*) in areas described by Kwaśna (2001). In each stand, soil samples were collected from two plots (I and II, 30 x 50 m each) situated 200 m apart, from beneath 5 randomly selected, apparently healthy oaks in each plot. After removal of the litter, humus layer and upper soil, three 5 cm diameter cores were obtained from the B-horizon (30-50 cm deep) from among roots of each tree or stump. Between samples the corer was sterilized by rinsing with 70% ethanol and sterile water. Entire cores were placed in sterile vials. Immediately after the samples had been collected the trees were deliberately felled. Exactly two years later (September 1997), the procedure was repeated and soil samples were collected from beneath stumps of the trees sampled previously. Additionally, control soil samples were collected from living oaks located near stumps, from beneath 5 trees in each plot.

In a laboratory, soil samples were separately processed by the soil dilution plate method of Warcup (1950) modified by Johnson and Mańka (1961) and Mańka (1964). The effectiveness of the method for the isolation of soil fungi was shown by Kwaśna and Nirenberg (1994). One g of a fine fraction of the soil, sieved through a 0.6 mm sieve, was mixed with 149 g of fine, sterile quartz sand, initially for 10-20 s in a mortar with a small amount of sand, followed by slow rotation with the remaining sand for a further 10 min. in a sterile flask. A subsample (0.26 mg) of fresh soil-sand mixture was placed in an empty Petri dish and covered with 10 ml Czapek-Dox agar. Rose bengal was added (10 ml 0.3%) to eliminate the growth of bacteria and Mucorales. Ten replicates were prepared for each tree or stump. Fungi were incubated at 20-22°C for 42 days. The plates were examined microscopically after 10, 20 and 42 days, and sporulating fungi were identified. Non-sporulating colonies were transferred onto potato dextrose agar (PDA) slants and incubated at room temperature under diffuse daylight until sporulation occurred. Fungi were identified according to their morphology on PDA, synthetic low nutrition agar (SNA) (Nirenberg 1976), Czapek yeast autolysate agar (CYA), 2% malt extract agar (MEA) and 1% carrot decoct agar (CDA) (Kwaśna 2001; 2002). Sterile dematiaceous hyphomycetes were induced to sporulate under UV light (310-420 nm for 12 h a day) at 20°C or on 2% MEA at 5°C in high humidity for 12-15 months.

Diversity of the fungal community was expressed as the number of species in a sample of soil. Density was expressed as the number of isolates in a sample of soil. Frequency was expressed as the percentage of isolates in a sample of soil.

The method of analysis of the effect of 'test' fungi on *Armillaria* rhizomorph formation was performed according to Kwaśna (2001; 2002).

Chemical properties of soil were assessed with Walkley and Black, Tiurin, Kjeldahl and Olsen methods (Ostrowska et al. 2001).

The statistical significance of differences in (i) values of chemical properties of two soils, beneath the living oaks and stumps, (ii) numbers of isolates of fungi in two different samples, (iii) numbers of rhizomorphs, rhizomorph apices, rhizomorph length and weight in the fungus/*Armillaria* treatment and a control, and (iv) frequencies of species in two different samples, were determined by the χ^2-test.

RESULTS

Soil parameters. All soils were acidic with pH 4.15-4.25. Soils beneath stumps had lower pH, higher organic matter content, more carbon, nitrogen, potassium, and nearly twice the amount of available phosphorus. Parameters of soils beneath the living trees in 1997 were similar to those of 1995 (Tab. 1). Differences between all values were statistically insignificant at $P \leq 0.05$.

Table 1
Chemical properties of the soils

	30-year-old stand			50-year-old stand		
	Living trees 1995	Living trees 1997	Stumps 1997	Living trees 1995	Living trees 1997	Stumps 1997
pH (KCl, 1:2.5)	4.25	4.25	4.15	4.15	4.20	4.15
Soil organic matter (mg kg^{-1})[a]	3.24	3.20	4.10	3.13	3.10	4.0
C (mg kg^{-1})[b]	3.2	3.1	3.7	3.1	3.1	3.6
N (mg kg^{-1})[c]	3.8	3.7	4.3	3.6	3.5	4.2
K as K_2O (mg kg^{-1})	1.6	1.8	2.2	1.7	1.8	2.0
P as P_2O_5 (mg kg^{-1})[d]	5.5	5.6	10.3	5.35	5.4	9.8

Explanations: [a] - Walkley and Black method of detection of soil organic matter in soil; [b] - Tiurin method of detection of carbon in soil; [c] - Kjeldahl method of detection of nitrogen in soil; [d] - Olsen method of detection of phosphorus in soil.

Twelve fungal assemblages were isolated from the soil collected in autumn beneath living trees and stumps of *Q. robur* in 30-32- and 50-52-year-old stands, in two plots (I and II) in each stand. A total of 98 taxa of culturable microfungi were isolated. Only 25 taxa belonged to the dominating fungi (Tab. 2). There were 22-35, 21-33 and 33-40 species in soils beneath the living oaks before felling in 1995, the control living oaks in 1997, and 2-year-old stumps in 1997, respectively, including also the rarely occurring fungi. There were 111-343, 102-302 and 293-495 fungal isolates obtained from soil samples from beneath the living oaks before felling in 1995, the control living oaks in 1997, and 2-year-old stumps in 1997, respectively. The density of fungi, expressed by the total number of isolates, increased usually significantly in soils beneath stumps, compared to the living oaks before felling in 1995 and the control living oaks in 1997. The density of fungi in the soil beneath the living oaks before felling in 1995 and the control living oaks in 1997 differed only in the 30-year-old stand, in plot II.

T a b l e 2

Effects of felling of oaks on fungal diversity (total number of species), density (total number of isolates) and on number of isolates of the selected fungal taxa in soil

	Living oaks before felling in 1995						Control living oaks in 1997						2-year-old stumps in 1997					
	30-year-old			50-year-old			32-year-old			52-year-old			in 32-year-old			in 52-year-old		
	I	II	Σ I+II	I	II	Σ I+II	I	II	Σ I+II	I	II	Σ I+II	I	II	Σ I+II	I	II	Σ I+II
Total number of species	22	35		34	34		21^{f}	33		30	28		37^{f}	40		35	33	
Total number of isolates	111^{a}	205^{a}_{g}	316^{c}	343	246^{a}	589^{c}	102^{e}	110^{e}_{g}	212^{c}	302	222^{e}	522^{c}	293^{a}_{e}	495^{a}_{e}	788^{c}	294	357^{a}_{e}	651^{c}
Absidia cylindrospora Hagem	0^{b}	1^{b}	1^{d}	4	5^{b}	9^{d}	1^{f}	1^{f}	2	5	2^{e}	7	9^{bf}	8^{bf}	17	11	18^{be}	29
Aspergillus kanagawaensis **Nehira**	36^{a}	15^{a}	51	28	7	35	30^{e}	10^{e}	40	20	10	30	94^{ae}	58^{ae}	152^{c}	21	15	36^{c}
Aspergillus versicolor Tiraboschi	1	0^{a}	1	0^{b}	0	0	1	0^{e}	1	0^{f}	0	0	6	14^{ae}	21^{d}	6^{bf}	1	7^{d}
Chloridium virescens var. *chlamydosporum* (v. Beyma) W. Gams et Hol.-Jech.	0	0	0	1	0	1	0	0	0	0^{f}	1	1	2	0	2	4^{f}	3	7
Chrysosporium cretaceus Traaen	0	0^{b}	0	0	0	0	0	0^{f}	0	0	0	0	0	8^{bf}	8	2	0	2
Chrysosporium merdarium **(L. ex G.) Carm.**	1	4	5	3^{b}	6^{a}	9	1	3	4	2^{f}	8^{e}	10	6	9	15^{c}	15^{bf}	37^{ae}	52^{c}
Clonostachys candelabrum (Bon.) Schroers	0^{b}	0^{a}	0	0	0	0	0^{f}	0^{e}	0	0	0	0	6^{bf}	12^{ae}	18^{c}	1	0	1^{c}
Geomyces pannorum (Link) Sigler et Carm.	6	9	15^{d}	2	4	6^{d}	4	5	9	4	7	11	1	7	8	2	5	7
Gymnoascus reessii Baranetzky	5	6	11^{c}	39^{a}	19	58^{c}	2	4	6^{c}	30^{f}	20	50^{c}	1	8	9^{c}	13^{af}	26	39^{c}
Humicola grisea Traaen	0	0^{b}	0^{d}	2	4^{b}	6^{d}	0	1	1	2	1	3	2	4^{b}	6	1	0^{b}	1
Monodictys lepraria **(Berk.) M. B. Ellis**	0^{b}	0	0	0	0	0	0^{e}	0	0	0	0	0	7^{be}	0	7^{d}	0	0	0^{d}
Mortierella macrocystis **W. Gams**	0	0^{a}	0	0^{b}	0^{b}	0	0	0^{e}	0	0^{f}	0^{f}	0	1	14^{ae}	15	7^{bf}	5^{bf}	12
Penicillium adametzii **Zaleski**	8	68^{g}	76^{c}	20	17	37^{c}	7	23^{eg}	30	15	18	33	8	83^{e}	91^{c}	23	19	42^{c}
Penicillium citrinum Thom	3^{d}	5^{c}	8	11^{dh}	23^{cg}	34	2	3	5	3^{h}	4^{fg}	7	1^{d}	7	8	8^{d}	12^{f}	20
Penicillium daleae Zaleski	7	15^{a}	22^{c}	83^{ah}	53	136^{c}	8	20^{f}	28^{c}	54^{fh}	53	107^{c}	6	45^{af}	51^{d}	33^{af}	40	73^{d}
Penicillium janczewskii **Zaleski**	8^{a}	26^{ah}	34^{c}	44	34^{b}	78^{c}	10^{e}	12^{eh}	22^{c}	55	26^{e}	81^{c}	39^{ae}	66^{ae}	105	56	59^{be}	115
Pseudogymnoascus roseus **Raiłło**	17	7^{a}	24^{c}	0^{bh}	4	4^{c}	12	3^{e}	15	8^{h}	9	17	16	42^{ae}	58^{c}	6^{b}	9	15^{c}
Sporothrix schenckii **Hektoen et Perkins**	1	0^{b}	1	1^{a}	3^{b}	4	1	1^{f}	2	2^{f}	0^{e}	2	4	10^{bf}	14^{d}	15^{af}	13^{be}	28^{d}

Tab. 2 cont.

Tolypocladium inflatum **W. Gams**	0	0^{b}	0	0^{b}	2	2	0	0^{f}	0	0^{f}	0^{f}	0	2	7^{bf}	9	4^{bf}	4^{f}	8
Trichocladium opacum (Corda) S. Hughes	0	0	0	0	0	0	0	0	0	0	0	0	3	3	6^{d}	0	0	0^{d}
Trichoderma viride Pers. ex Fr.	0	2	2	0	2	2	0	1	1	0	2	2	0	3	3	2	3	5
Trichoderma spp.	0^{b}	1	1	2	1	3	1	1	2	3	0	3	6^{b}	1	7	1	1	2
Umbelopsis nana (Linn.) von Arx	3	2	5^{c}	22^{bg}	4	26^{c}	3	2	5	2^{fg}	2^{f}	4	4	8	12	9^{bf}	10^{f}	19
Umbelopsis vinacea **(Dixon-Stewart) von Arx**	2^{a}	19^{b}	21^{c}	31	28^{b}	59^{c}	4^{e}	11^{e}	15^{c}	20	33	53^{c}	25^{ae}	35^{be}	60	25	50^{b}	75
Sterile dematiaceous hyphomycetes	3^{b}	0^{b}	3	1	2	3	1^{f}	2	3	3	0	3	12^{bf}	8^{b}	20^{d}	0	0	0^{d}

Explanations: Fungal name in **bold** – stimulant of *Armillaria* rhizomorph formation; [a] - the ratio for living oaks before felling in 1995 : 2-year-old stumps is significantly different from 1:1 at $P \leq 0.001$; [b] - the ratio for living oaks before felling in 1995 : 2-year-old stumps is significantly different from 1:1 at $P \leq 0.05$; [c] - the ratio for 30-(32) year-old stand : 50-(52)-year-old stand is significantly different from 1:1 at $P \leq 0.001$; [d] - the ratio for 30-(32)-year-old stand : 50-(52)-year-old stand is significantly different from 1:1 at $P \leq 0.05$; [e] - the ratio for control living oaks in 1997 : 2-year-old stumps is significantly different from 1:1 at $P \leq 0.001$; [f] - the ratio for control living oaks in 1997 : 2-year-old stumps is significantly different from 1:1 at $P \leq 0.05$; [g] - the ratio for living oaks before felling in 1995 : control living oaks in 1997 is significantly different from 1:1 at $P \leq 0.001$; [h] - the ratio for living oaks before felling in 1995 : control living oaks in 1997 is significantly different from 1:1 at $P \leq 0.05$.

Rarely occurring fungi in soil beneath the living trees: *Acremonium charticola* (Lindau) W. Gams, *A. strictum* W. Gams, *Aspergillus fumigatus* Fres, *Aureobasidium pullulans* (De Bary) Arnaud, *Candida* sp., *Ciboria batschiana* (Zopf) Buchwald, *Cladosporium cladosporioides* (Fres.) de Vries, *C. herbarum* Link ex Fr., *Geotrichum candidum* Link, *Humicola fuscoatra* Traaen, *M. ramanniana* (Moller) Linn., *Mucor racemosus* Fres. f. *racemosus*, *M. racemosus* f. *sphaerosporus* (Hagem) Schipper, *Mycelium radicis atrovirens* Melin, *Oidiodendron echinulatum* Barron, *Oidiodendron* sp., *Penicillium brevicompactum* Dierckx., *P. meleagrinum* Biourge, *P. multicolor* Grigorieva-Manoilova et Poradielova, *P. pinophilum* Hedgecock, *P. purpurogenum* Stoll, *P. steckii* Zaleski, *P. variabile* Sopp, *Penicillium* spp., *Phialophora cyclaminis* v. Beyma, *Rhizopus nigricans* Ehrenberg, cf. *Spiromastix warcupii* Kuehn et Orr, *Torulomyces lagena* Delitsch, *T. longipilis* Bissett, *T. strictipilis* Bissett,

Rarely occurring fungi in soil beneath stumps: *Acremonium persicinum* (Nicot) W. Gams, *A. pteridii* W. Gams et Frankland, *Apiospora montagnei* Sacc., *Cordana pauciseptata* Preuss, *Doratomyces microsporus* (Sacc.) Morton et G. Sm., *Embellisia chlamydospora* (Hoes, Bruehl et C. G. Shaw) E.Simmons, *Microascus trigonosporus* Emmons et Dodge, *Mortierella gracilis* Linn., *M. spinosa* Linn., *Mortierella* sp., *Mucor ambiguus* Vuillemin, *Paecilomyces farinosus* (Holmskiod) A. H. S. Brown et G. Sm., *Paecilomyces* sp., cf. *Papulaspora* sp., *Penicillium chrysogenum* Thom, *P. corylophilum* Dierckx., *P. decumbens* Thom, *P. frequentans* Westl., *P. herquei* Bainier et Sartory, *P. islandicum* Sopp, *P. lividum* Westl., *P. montanense* Christensen et Backus, *P. waksmanii* Zaleski, *Phaeostalagmus cyclosporus* (Grove) W. Gams, *Trichoderma hamatum* (Bonorden) Bainier, *T. koningii* Oud., *Verticillium bulbillosum* W. Gams et Malla, *V. lamellicola* (F. E. V. Sm.) W. Gams, *V. lecanii* (A. W. Zimmerm.) Viegas, *Zygorhynchus moelleri* Vuill.

Rarely occurring fungi in soil beneath the living trees and stumps: *Aspergillus niveus* Blochwitz, *Beauveria bassiana* (Balsamo) Vuill., *Chaetomium homopilatum* Omvik, *Coniothyrium fuckelii* Sacc., *Epicoccum purpurascens* Link, *Mortierella isabellina* Oud. et Koning, *Mucor hiemalis* Wehmer, *O. tenuissimum* (Peck) Hughes, *P. raistrickii* G. Sm., *P. spinulosum* Thom, *P. verruculosum* Peyronel, *T. polysporum* (Link et Pers.) Rifai.

Compared to the living oaks before felling in 1995, and the control living oaks in 1997, the density of *Mortierella macrocystis, Penicillium janczewskii, Pseudogymnoascus roseus, Sporothrix schenckii, Tolypocladium inflatum* and *Umbelopsis vinacea* significantly increased in the soil beneath stumps in the 32- and 52-year-old stands, while *Aspergillus kanagawaensis, Monodictys lepraria, P. daleae* and sterile dematiaceous hyphomycetes significantly increased in the 32-year-old stand, as did *Chrysosporium merdarium* in the 52-year-old stand.

There were 66 and 107 isolates of dematiaceous hyphomycetes in the soil beneath living oaks and stumps, respectively. Those included *Acremonium persicinum, Aureobasidium pullulans, Chloridium virescens* var. *chlamydosporum, Ciboria batschiana, Cladosporium* spp., *Coniothyrium fuckelii, Cordana pauciseptata, Doratomyces microsporum, Embellisia chlamydospora, Humicola* spp., *Microascus trigonosporus, M. lepraria, Mycelium radicis atrovirens, Oidiodendron* spp., *Phialophora cyclaminis, S. schenckii, Trichocladium opacum* and a few sterile species.

Armillaria sp. was isolated only from the wood of stump roots in the 30-year-old stand (Kwaśna 2002). *Armillaria* rhizomorphs were absent on roots of living trees. They occurred, however 2 years after felling of trees, at stump bases and on the surface of thicker stump roots in both stands, though more often in the 50-year-old stand, and were observed during collection of samples.

The age of the stand (30- or 50-year-old) and the plot (I or II) did not affect the diversity of fungal assemblages but did significantly affect the density of the certain species beneath both, the living trees and stumps.

Among the rarely occurring fungi there were 31 and 30 species occurring only beneath the living trees and stumps, respectively.

DISCUSSION

The effect of the presence of oak stumps on the extent of *Armillaria* infection has been noticed since the observations of Childs and Zeller (1929) and Rishbeth (1988). *Armillaria* colonizes newly created stumps mainly via rhizomorphs, either after their fast growth from pre-existing lesions on roots held earlier in check by host resistance, or invasion from an epiphytic position on roots. A few weeks after colonization, the pathogen starts to produce rhizomorphs which can infect nearby trees for many years. In studies of Rishbeth (1972) rhizomorph yield from colonized oak stump roots increased as a function of time and reached the highest level after 14 years. This is due to the presence of organic matter in the form of roots being degraded and mineralized. The occurrence of fresh and newly available organic substrates causes also the increase in density of soil microfungal assemblages. This phenomenon had already been observed by Christensen (1969) and Wicklow and Whittingham (1974) but nobody, so far, connected it with incidence of the root and butt rot fungi. The higher density of fungi in soil beneath stumps correlated with the higher organic matter content as well as carbon (C), potassium (K), nitrogen (N) and phosphorus (P) concentrations in soil.

Kwaśna (2001; 2002) and Kwaśna et al. (2001) observed that *A. kanagawaensis, Ch. merdarium, M. lepraria, M. macrocystis, P. adametzii, P. janczewskii, P. roseus, S. schenckii, T. inflatum* and *U. vinacea* may stimulate the rhizomorph for-

Table 3

Stimulatory effect of 'test' fungi from *Q. robur* roots on number of rhizomorphs of *Armillaria ostoyae* and *A. gallica*, and on branching, length and weight of rhizomorphs in oak sections *in vitro* (after Kwaśna 2001, 2002; Kwaśna et al., 2001)

Test fungus	Number of rhizomorphs		Number of rhizomorph apices		Length of rhizomorphs (mm)		Weight of rhizomorphs (mg)	
	Recalculated per 1 rhizomorph in control							
	A. ostoyae	*A. gallica*	*A. ostoyae*	*A. gallica*	*A. ostoyae*	*A. gallica*	*A. ostoyae*	*A. gallica*
Control	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Aspergillus kanagawaensis 1	0	9.0	0	3.2	0	68.3[b]	0	9.11
A. kanagawaensis 2	0	4.0	0	2.0	0	97.5[a]	0	7.5
Chrysosporium merdarium	0	0.8	0	4.93[a]	0	7.56[a]	0	10.3[b]
Monodictys lepraria	3.0	8.0	3.0	4.0	70.0[a]	156.3[a]	14.3[a]	21.88[a]
Mortierella macrocystis	1.75	0.93	18.0[a]	1.52	37.0[a]	2.5[a]	45.75[a]	1.0
Penicillium adametzii 1	0	0.9	0	5.86[a]	0	6.3[a]	0	4.5[a]
Penicillium adametzii 2	0	1.1	0	2.4[b]	0	3.1[a]	0	1.7[b]
Penicillium adametzii 3	0	1.3	0	7.2[a]	0	6.3[a]	0	6.4[a]
Penicillium adametzii 4	1.25	1.0	2.0	4.9[a]	3.5	4.4[a]	7.5[b]	3.7[a]
Penicillium adametzii 5	0.25	0.9	0.25	3.4[a]	0.25	4.4[a]	0.5	3.1[a]
Penicillium adametzii 6	2.5	1.4	5.5	3.9[a]	14.25[a]	4.5[a]	17.5[b]	2.2[a]
Penicillium janczewskii 1	0	12.0	0	2.3	0	62.5[a]	0	62.5[a]
Penicillium janczewskii 2	0.5	16.0	1.0	4.2	25.0[a]	175[a]	2.0	15.6[b]
Pseudogymnoascus roseus	0.75	0.8	3.75	1.7	6.25	2.68[a]	7.0[b]	1.44
Sporothrix schenckii	2.0	-	1.0	-	10.0[b]	-	0.5	-
Tolypocladium inflatum	1.75	-	1.75	-	37.5[b]	-	5.0	-
Umbelopsis vinacea	1.0	-	1.0	-	11.25[b]	-	0.7	-

Explanations: [a] - the ratio of treatment : control is significantly different from 1:1 at $P \leq 0.001$; [b] - the ratio of treatment : control is significantly different from 1:1 at $P \leq 0.05$.

mation in *A. ostoyae*, *A. gallica* and *A. mellea in vitro*. The 'stimulants' increase mostly the rhizomorphs' length and weight and only rarely the number of rhizomorph apices (Tab. 3).

Representatives of 'stimulants' were often the dominating fungi in the soil beneath oaks. Their density usually significantly increased in the soil beneath oak stumps compared to the soil beneath the living oaks before felling and the control living oaks.

The increase in the density of *Armillaria* rhizomorphs 'stimulants' was usually correlated with their increased frequency in fungal assemblages, which suggests that the observed phenomenon is not only a result of a general increase in density of these fungi but also a consequence of shifts among species due to the change of the nutritional conditions after felling of trees (Tab. 4).

The differences in densities of fungi in soil in stands at different age show that the phenomenon of the increase in density of fungi generally, and of *Armillaria* rhizomorph formation 'stimulants' particularly, depends on the local environmental conditions. There was a higher density of fungi beneath living trees in the 50-52-year-old stand compared to 30-32-year-old one, and beneath stumps in the 32-year-old stand compared to the 52-year-old one. Environmental factors in the 50- and 30-year-old stands differ mainly due to the various tree densities. The lower tree density in the 50-year-old stand, followed by more insolation and higher soil temperature, possibly stimulated acivity of the soil fungi and increased their populations. The higher moisture of a soil in the 30-year-old stand followed by slower degradation of the stump wood and supply of substrates at various stages of decomposition, probably increased not only the density but also the diversity of fungal assemblages.

There was higher density of the dematiaceous hyphomycetes in soil beneath stumps. This was probably related to their stronger xylan and cellulose degrading abilities and their power to withstand more extreme environmental conditions after felling (Bååth and Söderström 1980; Butler et al. 2001). The dematiaceous hyphomycetes belong to the strongest stimulants of *Armillaria* rhizomorph formation and their increased population beneath stumps plays probably a major role in enhancing *Armillaria* to colonize the stump (Mańka 1953; Kwaśna unpubl.).

In mycobiota, apart from the 'stimulants', there are also 'inhibitors' of *Armillaria* rhizomorph formation. The well-known antagonist, used often in a biological control of *Armillaria*, is *T. viride* (Hagle and Shaw 1991). Kwaśna (2001) observed that also *Clonostachys candelabrum* (Bon.) Schroers inhibited the formation of *Armillaria* rhizomorphs *in vitro*. The stumps of broadleaved trees are not usually inhabited by 'inhibitors' (Kwaśna 1996 a, b; 2001, 2002). *Clonostachys candelabrum*, however, appeared in the soil beneath stumps in the 32-year-old stand. It was the only 'inhibitor' detected. Considering that its population was low, it is presumed that the fungus might not disturb the activity of the much more frequent 'stimulants'. The density of *T. viride* remained similar beneath living trees and stumps. The studies confirmed that *T. viride* does not inhabit broadleaved wood which does not contain nitrogen required by the fungus (Cowling and Merrill 1966).

Although the differences in the stimulating effect among isolates of 'stimulants' were observed by Kwaśna (2001; 2002) it is presumed that the increase in density of fungi stimulating the *Armillaria* rhizomorphs formation in the soil beneath oak stumps may contribute to the process of colonization of stumps by *Armillaria*. These

T a b l e 4

Frequency of *Armillaria* rhizomorph 'stimulants' in the fungal assemblages

Fungus	Living oaks before felling				Control living oaks				2-year-old stumps			
	30-year-old		50-year-old		30-year-old		50-year-old		30-year-old		50-year-old	
	I	II	I	II	I	II	I	II	I	II	I	II
Aspergillus kanagawaensis	32.4	7.3	8.2	2.8	29.4	9.0	6.6	4.5	32.1	11.7	7.1	4.2
Chrysosporium merdarium	0.9	1.9	0.9	2.4[a]	0.9	2.7	0.6	3.6	2.0	1.8	5.1	10.3[a]
Monodictys lepraria	0	0	0	0	0	0	0	0	2.3	0	0	0
Mortierella macrocystis	0	0	0	0	0	0	0	0	0.3	2.8	2.3	1.4
Penicillium adametzii	7.2	33.2	5.8	6.9	6.8	20.9	4.9	8.1	2.7	16.8	7.8	5.3
Penicillium janczewskii	7.2	12.7	12.8	13.8	9.8	10.9	18.2	11.7	13.3	13.3	19.0	16.5
Pseudogymnoascus roseus	15.3	3.4	0	1.6	11.8	2.7	2.6	4.1	5.5	8.5	2.0	2.5
Sporothrix schenckii	0.9	0	0.3	1.2	0.9	0.9	0.6	0	1.4	2.0	5.1	3.6
Tolypocladium inflatum	0	0	0	0.8	0	0	0	0	0.7	1.4	1.4	1.1
Umbelopsis vinacea	1.8[a]	9.2	9.0	11.4	3.9	10.0	6.6	14.9	8.5[a]	7.0	8.5	14.0

Explanations: [a] - the ratio of living oaks before felling : 2-year-old stumps is significantly different from 1:1 at $P \leq 0.05$.

associations are the consequence of successional, consistent, natural and irreversible relationships occurring in forest after felling of trees.

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Mikrogrzyby glebowe dębu i ich wpływ na występowanie *Armillaria*

Streszczenie

Studiowano skład zbiorowisk mikrogrzybów glebowych spod 30- and 50-letnego dębu szypułkowego i jego 2-letnich pniaków powstałych po ścięciu drzew, stosując metodę rozcieńczania próbki gleby. Wyizolowano 98 gatunków grzybów. Frekwencja *Mortierella macrocystis, Penicillium janczewskii, Pseudogymnoascus roseus, Sporothrix schenckii, Tolypocladium inflatum* i *Umbelopsis vinacea* wzrosła istotnie w glebie pod pniakami zarówno w drzewostanie 32- jak i 52-letnim, w porównaniu z glebą pod drzewami przed ich ścięciem oraz drzewami kontrolnymi. Frekwencja *Aspergillus kanagawaensis, Monodictys lepraria, P. daleae* i kilku sterylnych grzybów ciemnozabarwionych wzrosła pod pniakami w drzewostanie 32-letnim, a *Chrysosporium merdarium* pod pniakami w drzewostanie 52-letnim. Wymienione grzyby mają zdolność stymulowania wzrostu ryzomorf *Armillaria*. Przypuszcza się że wzrost frekwencji 'stymulantów' *Armillaria* w glebie pod pniakami może sprzyjać kolonizacji pniaków przez *Armillaria*.