

## Contribution to the knowledge of the mycoflora in roots of oaks with and without tracheomycotic symptoms

DAVID NOVOTNÝ

Hřbitovní 47, 466 01 Jablonec nad Nisou, Czech Republic / Czech  
collection of micro-organisms, Faculty of Sciences,  
Masaryk University Brno, Tvrdeho 14, 602 00 Brno, Czech Republic

Novotný D. (2001): Contribution to the knowledge of the mycoflora in roots of oaks with and without tracheomycotic symptoms. – *Czech Mycol.* 53: 211–222

The mycoflora of roots of three species of oak (*Quercus robur*, *Q. petraea*, *Q. rubra*) from two localities near Moravské Budějovice (southwest Moravia, Czech Republic) with and without tracheomycotic symptoms was studied. Fifty-seven species of fungi were identified from the samples. The most frequently isolated were *Fusarium solani*, *Penicillium glandicola*, *P. glabrum*, *P. simplicissimum* and *Acremonium curvulum*. In the roots of trees with tracheomycotic symptoms two species of ophiostomatoid fungi were recorded. Abiotic conditions (insufficiency of precipitation and higher average temperature) are considered to be primary reasons of oak decline.

**Key words:** *Quercus robur*, *Q. petraea*, *Q. rubra*, oak decline, ophiostomatoid fungi, *Penicillium*, Czech Republic, Moravia

Novotný D. (2001): Příspěvek k poznání mykoflóry kořenů dubů s tracheomykózními příznaky a bez nich. – *Czech Mycol.* 53: 211–222

V roce 1994 byla na dvou lokalitách v blízkosti Moravských Budějovic zkoumána mykoflóra kořenů tří druhů dubů (*Quercus robur*, *Q. petraea*, *Q. rubra*). Bylo studováno 16 stromů se symptomy onemocnění s tracheomykózními příznaky a 3 stromy bez nich. Celkově bylo v kořenech zjištěno 57 druhů hub. Nejčastěji se vyskytujícími byly *Fusarium solani*, *Penicillium glandicola*, *P. glabrum*, *P. simplicissimum* a *Acremonium curvulum*. Ophiostomatální houby byly zaznamenány pouze na kořenech prosychajících stromů. Za primární příčinu hynutí dubů jsou považovány abiotické podmínky (nedostatek srážek a zvýšená průměrná roční teplota).

### INTRODUCTION

Oak decline is a serious problem in oak stands of many parts of Europe observed during the twentieth century (Anonymus 1990, Oleksyn and Przybyl 1987, Ragazzi et al. 1989, Siwecki and Liese 1991). In the Czech Republic this disease is called "tracheomycosis" or "with tracheomycotic symptoms" (Jančařík 1991, 1995). The reasons or its ethiology are not fully explained, but it is caused by a complex of abiotic and biotic factors (Oleksyn and Przybyl 1987). Fungi cause some of the symptoms, but they are not the primary reason of oak decline (Kowalski 1991, 1996, Przybyl 1995).

Typical symptoms of this disease are: yellowing, atrophy and premature fall of leaves, dying of branches, thinning of crown, creation of numerous water sprouts,

brown mucous exudation, necrotic patches in bark and phloem, discoloration of sapwood, loosening of bark (Kowalski 1991, Przybyl 1995).

Oak decline was for the first time recorded in the Czech Republic in the 1950s. It was recorded very frequently in the 1970s, 1980s and 1990s (Jančařík 1995) and characterised by yellowing and premature fall of leaves, dying of branches in the crown and creating sprouts on stems (Jančařík 1991). Similar symptoms were observed on many other trees (*Pinus*, *Larix*, *Fagus*, *Alnus*) (Jančařík 1992a, b, 1993a, b, Jančařík et al. 1991). Necrotic patches in bark or phloem of the oaks were not noticed.

The mycoflora associated with oak decline was investigated in many countries of Europe (e.g. Germany, Polen). Most researchers studied fungi associated with aboveground parts (stems, branches, leaves) of *Quercus robur* (Kowalski 1991, 1996, Kehr and Wulf 1993, Przybyl K. 1995, 1996). Several mycologists did research on the mycoflora of oaks from different other views, too (endophytes – Halmschlager et al 1993, Petrini and Fisher 1990, mycoflora of oaks damaged by air pollution – Kowalski 1983, mycoflora of natural pruning – Butin and Kowalski 1983).

The mycoflora of oak roots was studied by Kowalski (1983) (roots of dead oaks), Kowalski and Bartnik (1990) (occurrence of ophiostomatoid fungi), Amos and True (1967) (oaks with symptoms of oak-wilt in North America) and Jung and al. (1996) (occurrence of *Phytophthora*).

In the Czech Republic microscopic fungi of oaks with tracheomycotic symptoms were studied by Kubátová and Prášil (1995) and Fassatiová et al. (1995) only. They investigated the mycoflora of stems, branches and roots of three species of *Quercus* (*Q. petraea*, *Q. pubescens* and *Q. robur*).

Two methods are used for studying the mycoflora of trees. The wet chambers method was used by Kubátová and Prášil (1995) and Fassatiová et al. (1995). Methods based on strong surface sterilisation and incubation on agar medium were used by Amos and True (1967), Kowalski (1983, 1991, 1996), Kehr and Wulf (1993), Przybyl K. (1995) and others.

#### MATERIALS AND METHODS

The study was conducted in southwest Moravia (Czech Republic) near Moravské Budějovice, in two middle aged oak stands. The first stand (Bučina in Syrovický les, stand number 113C5, 57 years old) was composed of *Quercus robur* (74%), *Q. petraea* (24%) and *Q. rubra* (2%) and classified as a "fresh, oak-beech wood moderately rich in nutrients". The second stand (Na křivánkách in Černý les, stand number 104F4, 39 years old) was composed of *Quercus robur* (46%), *Q. petraea* (52%) and *Larix decidua* (2%) and classified as a "water-deficient oak-beech wood". In April and September 1994 three trees

(two *Q. robur* and one *Q. rubra*) remained without symptoms of oak decline and 16 trees (12 *Q. robur* and four *Q. petraea*) recently died or with disease symptoms in various stages were sampled. On branches, stems and roots of these trees no necrotic black or dark spots were observed. The trees were classified according to the health state of aboveground parts based on canopy cover (methods of Jančařík 1990). A tree marked 0 is healthy (without symptoms), a tree marked 4 is dead or missing at least 70% of leaves of canopy cover.

Temperature and precipitation were measured by the meteorological station at Moravské Budějovice. Over the years 1990–1994 the average temperature was 8.54 °C and the average annual precipitation 429.7 mm. Over the years 1965–1989 the average temperature was 7.61 °C and the average annual precipitation 531.7 mm per year (Dolejský 1997).

The samples were taken from two to five skeleton roots (2–7 cm thick) of each selected tree. The roots were cut into slices 0.5–2 cm thick. The slices were brushed under running water, then washed in a 0.47% solution of sodium hypochlorite (NaClO) or in 0.18% solution of peracetic acid (CH<sub>3</sub>CO<sub>3</sub>H) for 5 minutes and finally submerged in sterile water for 7–10 minutes. The slices were put in sterile glass wet chambers with sterile cotton wool and sterile filter paper. They were incubated at room temperature (20–25 °C) for 4–7 weeks in the dark. For identification the isolated fungi were cultivated on diagnostic agar media. During this study 443 of strains of fungi and three strains of bacteria were isolated.

## RESULTS

The observed root systems of trees with symptoms were never damaged more than the aboveground parts. Root systems of trees in health categories 3 and 4 were strongly reduced. They were composed of skeleton and coarse roots. Fine roots were absent. Rhizomorphs of *Armillaria* spp. were observed on many roots. Ophiostomatoid fungi were observed more times on slices than they were successfully isolated. They were found on roots of 81.8% of trees with symptoms and were not recorded on roots of healthy trees. Frequency of these fungi was different within the same health category in roots and aboveground parts of trees (Table 1).

Fifty-seven species of fungi (including sterile mycelia) and one species of bacteria were identified from the roots of the three species of oak. Twenty-four taxa were isolated from the roots of healthy trees of *Q. robur*, 42 taxa (including one species of bacteria) from the roots of diseased trees of *Q. robur*, 22 taxa from the roots of diseased trees of *Q. petraea* and 17 taxa from the roots of healthy trees of *Q. rubra* (Table 2).

*Cylindrocarpon destructans*, *Fusarium solani* and *Penicillium glandicola* were found in roots of both healthy trees of *Q. robur* (Table 2).

Table 1. Observation of ophiostomatoid fungi in oak roots.

Health category of aboveground part	Health state of roots Health state of roots	Number of roots colonised by ophiostomatoid fungi	Trees species	Locality
0	healthy	—	<i>Q. rubra</i>	Bučina
0	healthy	—	<i>Q. robur</i>	Na křivánkách
0	healthy	—	<i>Q. robur</i>	Bučina
0–1	one root dead, other healthy	2	<i>Q. robur</i>	Bučina
1	healthy	4	<i>Q. robur</i>	Bučina
1	healthy, reduced	—	<i>Q. robur</i>	Bučina
1–2	healthy	3	<i>Q. petraea</i>	Na křivánkách
1–2	healthy	4	<i>Q. robur</i>	Na křivánkách
2	healthy	1	<i>Q. robur</i>	Bučina
2	healthy and dead	1	<i>Q. robur</i>	Bučina
2–3	healthy, reduced	—	<i>Q. robur</i>	Bučina
3	healthy	3	<i>Q. robur</i>	Bučina
3	healthy	—	<i>Q. robur</i>	Na křivánkách
3	healthy, reduced	2	<i>Q. petraea</i>	Bučina
3	healthy and dead	3	<i>Q. petraea</i>	Na křivánkách
4	dead	4	<i>Q. robur</i>	Bučina
4	healthy and dead	3	<i>Q. petraea</i>	Na křivánkách
4	healthy	2	<i>Q. robur</i>	Bučina
4	dead	3	<i>Q. robur</i>	Na křivánkách

Table 2. Fungi isolated from the roots of three different species of oak.

Species of fungi	<i>Quercus robur</i>		<i>Quercus petraea</i>	<i>Quercus rubra</i>
	Healthy (2 trees)	Diseased (12 trees)	Diseased (4 trees)	Healthy (1 tree)
<i>Absidia cylindrospora</i> Hagem		1		
<i>Acremonium butyri</i> (van Beyma) W. Gams		1		
<i>Acremonium curvulum</i> W. Gams		8	2	
<i>Alternaria tenuissima</i> (Kunze: Fries) Wiltshire	1			
<i>Aspergillus fumigatus</i> Fresenius		1		
<i>Aspergillus versicolor</i> (Vullemijn) Tiraboschi		2		
<i>Beauveria brongniartii</i> (Saccardo) Petch			1	
<i>Botrytis cinerea</i> Persoon: Fries			1	
<i>Cladosporium cladosporoides</i> (Fresenius) de Vries				1
<i>Cylindrocarpon destructans</i> (Zinssmeister) Scholten	2	5	1	1
<i>Cylindrocarpon</i> sp.				1
<i>Doratomyces stemonitis</i> (Persoon:Fries) Morton et G. Smith		1		
<i>Engyodontium</i> sp.		1		

Table 2. (Cont.)

Species of fungi	<i>Quercus robur</i>		<i>Quercus petraea</i>	<i>Quercus rubra</i>
	Healthy (2 trees)	Diseased (12 trees)	Diseased (4 trees)	Healthy (1 tree)
<i>Epicoccum nigrum</i> Link		1		
<i>Fusarium oxysporum</i> Schlechtendal: Fries	1		1	
<i>Fusarium solani</i> (Martius) Saccardo	2	10	3	1
<i>Fusarium</i> sp.				1
<i>Gliocladium catenulatum</i> Gilman et Abbott	1	6		1
<i>Graphium</i> sp.		2		
<i>Mariannaea elegans</i> (Corda) G. Arnaud ex Samson		1		
<i>Mucor hiemalis</i> Wehmer		1		
<i>Oedocephalum glomerulosum</i> (Bulliard: Fries)		1		
<i>Ophiostoma piceae</i> (Münch) H. et P. Sydow		1	1	
<i>Ophiostoma</i> sp. 1		5	2	
<i>Penicillium arenicola</i> Chalabuda		3		1
<i>Penicillium aurantiogriseum</i> Dierckx *		1		1
<i>Penicillium canescens</i> Sopp			1	
<i>Penicillium chrysogenum</i> Thom		1		1
<i>Penicillium commune</i> Thom		2		
<i>Penicillium daleae</i> Zalesky		6		1
<i>Penicillium expansum</i> Link		2		
<i>Penicillium funiculosum</i> Thom		1		
<i>Penicillium glabrum</i> (Wehmer) Westling	1	9	3	1
<i>Penicillium glandicola</i> (Oudemans) Seifert et Samson	2	10	2	1
<i>Penicillium griseofulvum</i> Dierckx		1		
<i>Penicillium hordei</i> Stolk		1		
<i>Penicillium implicatum</i> Biourge		1		
<i>Penicillium janczewski</i> Zalesky		1		1
<i>Penicillium janthinellum</i> Biourge	1	2		
<i>Penicillium manginii</i> Duché et R. Heim		1		
<i>Penicillium minioluteum</i> Dierckx	1	4		1
<i>Penicillium roquefortii</i> Thom	1	2	1	
<i>Penicillium simplicissimum</i> (Oudemans) Thom	1	9	1	1
<i>Penicillium</i> cf. <i>soltium</i> Westling		2		
<i>Penicillium spinulosum</i> Thom	1	7	1	1
<i>Penicillium thomii</i> Maire			1	
<i>Penicillium</i> ser. <i>Minioluteum</i> Pitt			1	
<i>Sesquicillium candelabrum</i> (Bonorden) W. Gams	1	4	1	
<i>Streptomyces</i> sp.		2		

Table 2. (Cont.)

Species of fungi	<i>Quercus robur</i>		<i>Quercus petraea</i>	<i>Quercus rubra</i>
	Healthy (2 trees)	Diseased (12 trees)	Diseased (4 trees)	Healthy (1 tree)
<i>Trichoderma atroviride</i> P. Karsten sensu Bissett		7		
<i>Trichoderma citrinoviride</i> Bissett		1		
<i>Trichoderma hamatum</i> (Bonorden) Bainier	1	4	1	
<i>Trichoderma harzianum</i> Rifai		4	1	
<i>Trichoderma koningi</i> Oudemans	1	1	1	
<i>Trichoderma minutisporum</i> Bissett	1			1
<i>Trichoderma viride</i> Persoon: Fries agg.	1	3	1	
<i>Zygorhynchus moelleri</i> Vuillemin		1		
Sterile mycelia			1	
Number of species	17	47	22	17

\* The isolated strains belong to the complex species *Penicillium aurantiogriseum*. Identification of these strains is possible by chemical analysis of secondary metabolites only.

*Fusarium solani*, *Penicillium glandicola*, *P. glabrum*, *P. simplicissimum*, *Acremonium curvulum*, *Trichoderma atroviride*, *Penicillium spinulosum*, *P. daleae* and *Gliocladium catenulatum* were found in at least 50 % of the studied diseased trees of *Q. robur* (Table 2).

*Fusarium solani*, *Penicillium glabrum*, *P. glandicola*, *Acremonium curvulum* and *Ophiostoma* sp. 1 were isolated from the roots of at least 50 % of diseased trees of *Q. petraea*.

The mycoflora of roots of *Q. rubra* was studied on one tree only. Therefore it is impossible to give the most frequently occurring species (Table 2).

During the study, 23 species of *Penicillium* were identified from the oak roots. Twenty species were found in *Quercus robur*, 14 species in *Quercus petraea* and ten species in *Quercus rubra*. Dominant species were *Penicillium glandicola*, *P. glabrum*, *P. simplicissimum*, *P. spinulosum*, *P. daleae* and *P. minioluteum*. The first four were isolated from all studied species of oak (Table 2).

The same dominating fungi were found in roots of all species of oak.

Two ophiostomatoid fungi (*Ophiostoma piceae* s.l. – probably *O. querci* and *Ophiostoma* sp.) were observed in the roots of diseased *Quercus robur* and *Q. petraea* (Table 1).

Forty-six species of fungi were isolated from the roots of oaks growing in the locality Bučina (No. 113C5). *Fusarium solani*, *Penicillium glandicola*, *P. glabrum*, *P. simplicissimum*, *P. spinulosum*, *P. daleae*, *Acremonium curvulum* and *Gliocladium catenulatum* were found most frequently in these roots (Table 3).

Table 3. Fungi isolated from roots of three different species of oak from two localities.

Species of fungi	Locality Bučina			Locality Na křivánkách			
	All trees (12 tr.)	<i>Quercus robur</i> (10 trees)	<i>Quercus petraea</i> (1 tree)	<i>Quercus rubra</i> (1 tree)	All trees (7 tr.)	<i>Quercus robur</i> (4 trees)	<i>Quercus petraea</i> (3 trees)
<i>Absidia cylindrospora</i> Hagem	1	1					
<i>Acremonium butyri</i> (van Beyma) W. Gams					1	1	
<i>Acremonium curvulum</i> W. Gams	7	7			4	2	2
<i>Alternaria tenuissima</i> (Kunze: Fries) Wiltshire					1	1	
<i>Aspergillus fumigatus</i> Fresenius	1	1					
<i>Aspergillus versicolor</i> (Vuillemin) Tiraboschi	2	2					
<i>Beauveria brongniartii</i> (Saccardo) Petch					1		1
<i>Botrytis cinerea</i> Persoon: Fries					1		1
<i>Cladosporium cladosporioides</i> (Fresenius) de Vries	1			1			
<i>Cylindocarpon destructans</i> (Zinssmeister) Scholten	7	6		1	1		1
<i>Cylindocarpon</i> sp.	1			1			
<i>Doratomyces stemonitis</i> (Persoon: Fries) Morton et G. Smith	1	1					
<i>Engyodontium</i> sp.	1	1					
<i>Epicoccum nigrum</i> Link	1	1					
<i>Fusarium oxysporum</i> Schlechtendahl: Fries	1		1		1	1	
<i>Fusarium solani</i> (Martius) Saccardo	12	10	1	1	4	2	2
<i>Fusarium</i> sp.	1			1			
<i>Gliocladium catenulatum</i> Gilman et Abbott	7	6		1	1	1	
<i>Graphium</i> sp.	2	2					
<i>Mariannaea elegans</i> (Corda) G. Arnaud ex Samson	1	1					
<i>Mucor hiemalis</i> Wehmer	1	1					
<i>Oedocephalum glomerulosum</i> (Bulliard: Fries)	1	1					
<i>Ophiostoma piceae</i> (Münch) H. et P. Sydow					2	1	1
<i>Ophiostoma</i> sp.	5	4	1		2	1	1
<i>Penicillium arenicola</i> Chalabuda	3	3					
<i>Penicillium aurantiogriseum</i> Dierckx *	2	1		1			
<i>Penicillium canescens</i> Sopp					1		1
<i>Penicillium chrysogenum</i> Thom	2	1		1			
<i>Penicillium commune</i> Thom	2	2					
<i>Penicillium daleae</i> Zalesky	7	6		1			
<i>Penicillium expansum</i> Link	2	2					
<i>Penicillium funiculosum</i> Thom	1	1					
<i>Penicillium glabrum</i> (Wehmer) Westling	10	9		1	4	1	3
<i>Penicillium glandicola</i> (Oudemans) Seifert et Samson	10	9		1	5	3	2

Table 3. (Cont.)

Species of fungi	Locality Bučina				Locality Na křivánkách		
	All trees (12 tr.)	<i>Quercus robur</i> (10 trees)	<i>Quercus petraea</i> (1 tree)	<i>Quercus rubra</i> (1 tree)	All trees (7 tr.)	<i>Quercus robur</i> (4 trees)	<i>Quercus petraea</i> (3 trees)
<i>Penicillium griseofulvum</i> Dierckx	1	1					
<i>Penicillium hordei</i> Stolk					1	1	
<i>Penicillium implicatum</i> Biourge					1	1	
<i>Penicillium janczewski</i> Zalesky	2	1		1			
<i>Penicillium janthinellum</i> Biourge	1	1			2	2	
<i>Penicillium manginii</i> Duché et R. Heim	1	1					
<i>Penicillium minioluteum</i> Dierckx	6	5		1			
<i>Penicillium roquefortii</i> Thom	1			1	3	2	1
<i>Penicillium simplicissimum</i> (Oudemans) Thom	8	7		1	4	3	1
<i>Penicillium cf. solitum</i> Westling	2	2					
<i>Penicillium spinulosum</i> Thom	7	6		1	3	2	1
<i>Penicillium thomii</i> Maire					1		1
<i>Penicillium ser. Minioluteum</i> Pitt					1		1
<i>Sesquicillium candelabrum</i> (Bonorden) W. Gams	6	4	1	1			
<i>Streptomyces</i> sp.	2	2					
<i>Trichoderma atroviride</i> P. Karsten sensu Bissett	6	6			1	1	
<i>Trichoderma citrinoviride</i> Bissett	1	1					
<i>Trichoderma hamatum</i> (Bonorden) Bainier	5	5			1		1
<i>Trichoderma harzianum</i> Rifai	3	3			2	1	1
<i>Trichoderma koningii</i> Oudemans	3	2	1				
<i>Trichoderma minutisporum</i> Bissett	1	1			1	1	
<i>Trichoderma viride</i> Persoon: Fries agg.	6	5	1				
<i>Zygorhynchus moelleri</i> Vuillemin					1	1	
Sterile mycelia					1		1
Number of species	46	41	6	17	28	20	18

\* Isolated strains belong to the complex species *Penicillium aurantiogriseum*. Identification of these strains is possible by chemical analysis of secondary metabolites only.

Twenty-eight taxa were isolated from the roots of trees growing in the locality Na křivánkách (No. 104F4). *Penicillium glandicola*, *P. glabrum*, *P. simplicissimum*, *Fusarium solani* and *Acremonium curvulum* were found most frequently in these roots (Table 3).

Sixteen species of fungi were found in roots from both localities. Four species (*Penicillium daleae*, *P. minioluteum*, *Sesquicillium candelabrum* and *Trichoderma viride* agg.) were observed very frequently in roots from the locality Bučina



(No. 113C5), but were not isolated from roots from the locality Na křivánkách (No. 104F4) (Table 3).

#### DISCUSSION

On the roots and stems of the studied trees not any dark necrotic patches in bark or phloem were observed. These features are described by many researchers as characteristic of oak decline in other countries (Kehr and Wulf 1993, Kowalski 1991, Przybyl 1995). The observed roots of dying trees were in better health state than the aboveground parts. This difference is probably caused by water deficiency. Branches and leaves dry sooner than roots, because water easier reaches to organs in lower positions than organs in higher position. Abiotic factors are probably the primary reason of dying in these localities.

The wet chamber method was used in this study. A different spectrum of fungi than recorded by Kowalski (1983) and Amos and True (1967) was found. This difference is probably caused by using different methods. These mycologists used methods based on strong surface sterilisation and incubation on agar media. Similar results as in the present study were obtained by Kubátová and Prášil (1995) and Fassatiová et al. (1995), who used the method of wet chambers, too.

Amos and True (1967) found most frequently *Trichoderma lignorum*, *Umbelopsis versiformis*, *Penicillium* spp., *Gliocladium roseum* and *Cephalosporium* spp. (= *Acremonium* spp.). Kowalski (1983) recorded most frequently *Trichoderma viride*, *Mycelium radicans atrovirens*, *Cylindrocarpon destructans* and *Coniothyrium fuckelii*. The dominant fungi isolated in the present work were *Fusarium solani*, *Penicillium* spp. and *Acremonium curvulum*. Six species of *Trichoderma* were isolated and members this genus occurred in roots of 73.7 % of oak trees. Fungi belonging to *Mucorales* and dematiaceous anamorphs were recorded very rarely. Kowalski (1983) did not observe *Penicillia* in roots.

*Cylindrocarpon destructans* was recorded in the oak roots using both different methods. Kowalski (1983) found it in 27.3 % of trees of *Q. robur* and in 38.5 % of trees of *Q. rubra*. During this study it was recorded in 50 % of trees of *Q. robur* and in 25 % of trees of *Q. petraea*. Amos and True (1967) did not isolated this species.

The *Penicillia* were found in stems, branches or roots of oaks by Amos and True (1967), Kowalski (1991), Halmschlager et al. (1993), Kehr and Wulf (1993) and Przybyl (1995), but they did not identify them to the species level. Species of *Penicillia* isolated from roots, stems and branches of oaks were identified by Kubátová (2000). She isolated 23 species from three species of oak (*Q. petraea*, *Q. robur* and *Q. pubescens*). Dominant species were *Penicillium glandicola*, *P. glabrum*, *P. minioluteum* and *P. simplicissimum*. The spectrum of *Penicillia* found in the present work is very similar.

In the present study *Fusarium solani* was isolated very frequently. This species was recorded by Fassatiová et al. (1995) from roots and by Kowalski (1991, 1996) and Przybyl (1995, 1996) from aboveground parts, too. They observed it not so often as in this study.

Ophiostomatoid fungi were observed on the roots of trees with any symptoms of oak decline only. They were not found on the roots of trees with healthy aboveground parts, but they were present in trees with healthy roots and drying aboveground parts. Kowalski and Bartnik (1990), who used the method based on strong surface sterilisation, isolated them from 53.3 % of dead or dying trees and from 33.3 % of diseased trees. In the present study, ophiostomatoid fungi were recorded in 81.8 % of diseased trees. Sieber et al. (1995) isolated *Ophiostoma quercii* from lesions of *Quercus robur* twigs only once, but they observed it frequently in the sapwood of stumps of recently cut oaks.

In the present study two species of these fungi were isolated. *Ophiostoma piceae* s.l. (probably *O. quercii*) was found twice (once in *Q. robur* and once in *Q. petraea*). The other species was isolated from the roots of five trees of *Q. robur* and two trees of *Q. petraea*. This species is similar to *Ophiostoma stenoceras* observed by Kowalski and Bartnik (1990) in oak roots.

*Penicillium daleae*, *P. minioluteum* and *Sesquicilium candelabrum* were found frequently in roots from the locality Bučina, but have not been isolated in the locality Na křivánkách. Fassatiová et al. (1995) isolated these species from trees in the locality Bučina, too. Occurrence of these fungi may depend on soil moisture. The stand of the locality Bučina was classified as a "fresh, oak-beech wood moderately rich in nutrients" and that of the locality Na křivánkách as a "water-deficient oak-beech wood". Also *Trichoderma viride* agg. was identified in roots from the locality Bučina only. The morphology of this species is very variable and it is similar to *Trichoderma atroviride*, which was found in both localities.

#### ACKNOWLEDGEMENTS

I wish to thank Dr. K. Prášil and Dr. A. Kubátová for many advice to this study, Dr. V. Dolejský for the permission to study on his research plots and for providing a information about oak stands and climate, Dr. L. Marvanová for reading the manuscript.

The work was supported by the Ministry of Agriculture of the Czech Republic (Project No. 29-91-9106).

## REFERENCES

- AMOS R. E. and TRUE R. P. (1967): Longevity of *Ceratocystis fagacearum* in roots of deep-girdled oak-wilt trees in West Virginia. – *Phytopathology* 57: 1012–1015.
- Anonymus (1990): Oak decline and the status of *Ophiostoma* spp. on oak in Europe. – *EPPO Bull.* 20: 405–422.
- BUTIN H. and KOWALSKI T. (1983): Die natürliche Astreinigung und ihre biologische Voraussetzungen II. Die Pilzflora der Stieleiche (*Quercus robur*). – *Eur. J. For. Path.* 13: 428–439.
- DOLEJSKÝ V. (1997): Vliv taxonomické hodnoty a stanovištních podmínek na odumírání druhů dubů. – 36 p. + 29 p. att., ms. [PhD thesis: Library of Faculty of Forestry, Czech Agricultural University, Kostelec nad Černými lesy, Czech Republic].
- FASSATIOVÁ O., KUBÁTOVÁ A., NOVOTNÝ D. and PRÁŠIL K. (1995): Mikromycety chřadnoucích lesních dřevin s ohledem na výskyt ophiostomatálních hub. – 130 p., ms. [grant report: Library of Department of Botany, Faculty of Science, Charles University, Benátská 2, Praha, Czech Republic].
- HALMSCHLAGER E., BUTIN H. and DONAUBAUER E. (1993): Endophytische Pilze in Blättern und Zweigen von *Quercus petraea*. – *Eur. J. For. Path.* 23: 51–63.
- JANČAŘÍK V. (1990): Ochrana lesů před tracheomykózním onemocněním. – *TEI (Bulletin technickovědeckých informací), Řada Ochrana*, no. 3/90.
- JANČAŘÍK V. (1991): Příznaky tracheomykózního onemocnění dubů. – *Lesn. Pr.* 70: 345.
- JANČAŘÍK V. (1992a): Žloutnutí smrku. – *Lesn. Pr.* 71: 129.
- JANČAŘÍK V. (1992b): Chřadnutí buku. – *Lesn. Pr.* 71: 161.
- JANČAŘÍK V. (1993a): Usychání olší. – *Lesn. Pr.* 72: 14–16.
- JANČAŘÍK V. (1993b): Odumírání borovic. – *Lesn. Pr.* 72: 48–50.
- JANČAŘÍK V., ŠVECOVÁ M. and STRNADOVÁ L. (1991): Tracheomykózy již ohrožují i smrk. – *Lesn. Pr.* 70: 189–190.
- JANČAŘÍK V. (1995): Některé otázky spojené s hromadným hynutím dubů. – *Zpravodaj ochrany lesa* 2: 12–15.
- JUNG T., BLASCHKE H. and NEUMANN P. (1996): Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. – *Eur. J. For. Path.* 26: 253–272.
- KEHR R. D. and WULF A. (1993): Fungi associated with above-ground portions of declining oaks (*Quercus robur*) in Germany. – *Eur. J. For. Path.* 23: 18–27.
- KOWALSKI T. (1983): Vorkommen von Pilzen in durch Luftverunreinigung geschädigten Wäldern im Oberschlesischen und Krakauer Industriegebiet. IX. Mykoflora von *Quercus robur* L. und *Q. rubra* L. an einem Standort mit mittlerer Immissionsbelastung. – *Eur. J. For. Path.*, 13: 16–59.
- KOWALSKI T. (1991): Oak decline: I. Fungi associated with various disease symptoms on overground portions of middle-aged and old oak (*Quercus robur* L.). – *Eur. J. For. Path.* 21: 136–151.
- KOWALSKI T. (1996): Oak decline II. Fungi associated with various types of lesions on stems and branches of young oaks (*Quercus robur*). – *Österr. Z. Pilzk.* 5: 51–63.
- KOWALSKI T. and BARTNIK C. (1990): *Ceratocystis* species on *Quercus robur* with oak decline symptoms in southern Poland. – *EPPO Bull.* 20: 221–228.
- KUBÁTOVÁ A. (2000): Neglected *Penicillium* spp. associated with declining trees. – In: Samson R. A. and Pitt J. I. (eds.), *Integration of modern taxonomic methods for Penicillium and Aspergillus classification*, p. 299–307, Baarn.
- KUBÁTOVÁ A. and PRÁŠIL K. (1995): Ophiostomatální a další mikroskopické houby lesních dřevin s příznaky tracheomykózního onemocnění. Předběžné výsledky. – In: Čížková D. and Švecová M. (eds.): *Aktuální problémy ochrany dřevin. Sborník referátů II*, p. 18–37, Prachatice.
- OLEKSYN J. and PRZYBYL K. (1987): Oak decline in the Soviet Union – scale and hypotheses. – *Eur. J. For. Path.* 17: 321–336.
- PETRINI O. and FISHER P. J. (1990): Occurrence of fungal endophytes in twigs of *Salix fragilis* and *Quercus robur*. – *Myc. Res.* 94: 1077–1080.

- PRZYBYL K. (1995): Zamieranie dębów v Polsce. – Idee ekologiczne, Seria Zeszyty 4: 1–85.
- PRZYBYL K. (1996): Disease symptoms and fungi occurring on overground organs of *Quercus petraea*. – *Acta Mycologica* 31: 163–170.
- RAGAZZI A., FEDI I. D. and MESTURINO L. (1989): The oak decline: a new problem in Italy. – *Eur. J. For. Path.* 19: 105–119.
- SIEBER T. S., KOWALSKI T. and HOLDENRIEDER O. (1995): Fungal assemblages in stem and twig lesions of *Quercus robur* in Switzerland. – *Mycol. Res.* 99: 534–538.
- SIWECKI R. and LIESE W. (eds.) (1991): Oak decline in Europe. – Proceedings of an international symposium Kórnik, Poland, May 15–18, 1990. p. 360, Poznań.