Disease symptoms and fungi occurring on overground organs of Quercus petraea

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Przybył K.: Disease symptoms and fungi occurring on overground organs of Quercus petraea. Acta Mycol, 31 (2); 163-17(), 1996.

The observations of Quercus petraea trees growing in mixed forests of Wielkopolska National Park and Wolin National Park were carried out in 1994 and 1995. The fungi were isolated from dead sections of twigs and branches and from trunks exhibiting necrosis in secondary bark and discolorations of sapwood. Thirty one fungal species were identified on the organs studied. They belonged to saprophytes and to the group of fungi secondarily colonizing tissues of weakened trees.

Key words: Quercus petraea, disease symptoms, Fungi.

INTRODUCTION

Increased decline in oak stands has been observed in Poland since 1983. Approximately at that time studies were initiated in order to explain certain questions related mainly to the decline of pedunculate oaks as in the case of this species rapid dying of overground organs and roots was noted.

The decline of *Quercus robur* L. trees is accompanied by a complex of symptoms, most popular of which include dying of branches and twigs, brown and wet spots on the bark and in the phloem of the trunk as well as brown or dark-grey discolorations in the sapwood of branches and trunks. The discolorations assume the form of lines parallel to the shoot axis in the long section and the form of dots in the cross section (B a r t n i k, 1989; Pr z y b y ł, 1989; K o w a l s k i, 1991; Pr z y-b y ł, 1995). In some trees, diminishing of leaves was observed and was combined with their discoloration first to yellow and later to brown (B a r t n i k, 1989; K o-w a l s k i, 1991; S i w e c k i, 1992). The most significant disease symptoms identified in root systems of dying oaks include deformation of thin roots, the presence of necrosis and discolorations in thick roots and decaying of medium-sized and thick roots (B a r t n i k, 1989; Pr z y b y ł, Pu k a c k a, 1995).

Most of the fungi isolated from dying Q. robur trees belonged to the group of fungi secondarily colonizing necrotic tissues (K o w a 1 s k i, 1991; P r z y b y ł, 1995). One of the factors causing the weakening of the observed tree-stands was insufficient water supply caused by sudden fall of groundwater level (1981/1982) and prolonged periods of drought in 1982 and 1983 (P r z y b y ł, 1989).

In 1994 the investigation on decline of oaks conducted in the Institute of Dendrology of Polish Academy of Sciences was extended to include sessile oak stands located in the area of Wielkopolska and Wolin National Parks.

The objective of the present study was to determine the disease symptoms occurring on overground organs of trees and to identify fungi associated with those symptoms.

MATERIALS AND METHODS

Observations of macroscopic symptoms on overground organs of *Q. petraea* (Matt.) Liebl. trees growing in the area of Wielkopolska National Park (Górka Forest District, dep. 138 a) and Wolin National Park (Warnowo Forest District, dep. 71 f) were made in 1994 and 1995 in experimental plots encompassing 100 trees each.

Fungi were isolated from dead sections of twigs and branches and from trunks exhibiting necrosis of secondary bark and discolorations of sapwood. The material was collected in September 1994. The sampled pieces of trunks and sections of branches and twigs were washed in running water. Subsequently, particular fragments were cut out and sterilized with 0.5 % sublimate (1-3 min.) and 70 % alcohol (1 min.). The fragments prepared in this way were then placed on medium (Malt Agar Difco). Additionally, pieces of sapwood were sampled from trunk-bases showing symptoms of decay and placed on Malt Agar (Difco) containing 100 ppm of streptomycin.

Simultaneously, six hundred and ten fungi cultures were obtained from 809 fragments (225 for Wielkopolska National Park and 385 for Wolin National Park).

RESULTS

Decline symptoms

The following decline symptoms occurred on observed trees were observed in Wielkopolska National Park:

- dying of twigs and branches,
- bark cracks and local necrotic patches in the secondary bark,
- brown and dark-grey discolorations in trunk sapwood.

Dead twigs and branches were found in the top section or in the middle and lower parts of the tree crown. In the latter case, dying branches occurred most often in trees with poorly developed crown. Bark cracks and local necrotic areas in secondary bark were located in the lower parts of trunk in trees with slightly damaged crown, while the discolorations of trunk sapwood were observed on dead trees.

The following symptoms of decline were observed in trees in Wolin National Park:

- dying of branches and twigs,
- local necrotic patches in the secondary bark,
- brown and dark-grey discolorations in the sapwood of trunk,
- white rot in tree butt.

The dying of branches was observed both on trees with well and poorly developed crown, while bark and phloem necrosis and discoloration in trunk sapwood were observed only in the case of trees whose crown manifested the disease symptoms in over 75 %. Additionally, white rot was identified in tree butts of individual trees showing an advanced stage of dying. In trees affected by decay, the presence of white mycelium and of brown-black rhizomorphs was noted. Numerous St. John's shoots were found on trees with poorly developed crown, irrespective of their stage of the process of dying.

General condition of trees

The number of *Quercus petraea* trees, growing in the experimental plots in Wielkopolska and Wolin National Parks and showing decline symptoms is presented in Table 1.

Table 1

The general condition of *Quercus petraea* trees between 1994-1995

Experimental plots	Number of damaged trees %											
	1994					1995						
	0	1	2	3	4	5	()	1	2	3	4	5
Wielkopolska National Park	68	26	2	77	1	3	67	27	2	-	1	3
Wolin National Park	60	25	2	3	-		57	30	8	3	2	77

0-trees free of disease symptoms; 1-early stage of decline: dead twigs and top sections of individual branches; 2 - intermediate stage of decline: 1/4 of the crown exhibits symptoms of decline; 3 - advanced stage of decline; 1/2-3/4 of the crown exhibits symptoms of decline; 4 - dead trees; 5 - other damage: cracks in the lower part of trunk in the case of trees exhibiting an early stage of crown dying

The proportion of trees without disease symptoms located in experimental plot in Wielkopolska National Park in 1994 and 1995 amounted to 68 and 67 %, respectively and the proportion of damaged trees to 31 and 32 %, respectively. In the group of trees showing decline symptoms, the dying of 1/2 to 3/4 of the crown was observed in two trees. In the respective period of time, the proportion of damaged trees in the experimental plot in Wolin National Park amounted to 30 % in 1994 and 41 % in 1995.

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The number of trees in the intermediate stage of decline (1/4 of the crown was dead) slightly increased in 1995 and the presence of two dead trees was noted.

In general, the condition of *Q. petraea* trees in Wielkopolska National Park did not change during the two years of observations and slightly deteriorated in the case of Wolin National Park.

Fungi occurring on Quercus petraea in two National Parks

A. W i e l k o p o l s k a N a t i o n a l P a r k. Nineteen fungi species were identified on the branches and twigs of Q. petraea trees (Table 2). Four fungi (Botrytis cinerea, Colpoma quercinum, Cytospora intermedia and Discula quercina – teleomorph: Apiognomia quercina) were isolated from twigs only. Other four species (Chaetophoma quercicola, Cladosporium britannicum, Fusarium solani, Phoma sp.) occurred exclusively on branches while the remaining ones (Acremonium spp., Alternaria alternata, Aphosphaeria sp., Aureobasidium pullulans, Cladosporium cladosporioides, Humicola fuscoatra, Nodulisporium sp., Phialophora spp., Phialophora lagerbergii, Sordaria fimicola and Trichoderma harzianum) were found both on branches and twigs. The more frequent species found in more than five specimens included: Acremonium spp., Cytospora intermedia, Discula quercina, Humicola fuscoatra, Nodulisporium sp., Phialophora lagerbergii, Sordaria fimicola and Trichoderma harzianum.

B. Wolin National Park. Eighteen fungi species were identified on the twigs and branches of *Quercus petraea* trees, eight of which occurred on both organs: *Acremonium* sp., *Alternaria alternata*, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Coniothyrium fuckelii*, *Fusarium solani*, *Phialophora* spp., and *Phomopsis quercella*. Four fungi were present only on branches (*Humicola fuscoatra*, *Nodulisporium* sp., *Sordaria fimicola*, *Trichoderma harzianum*) while six others occurred exclusively on twigs (*Botrytis cinerea*, *Colpoma quercinum*, *Cytospora intermedia*, *Discula quercina*, *Pseudovalsa longipes* and *Tubakia dryina*). Among the isolated fungi the most common (over ten specimens) included: *Cladosporium cladosporioides*, *Cytospora intermedia*, *Discula quercina*, *Phialophora* spp., *Phomopsis quercella*, *Pseudovalsa longipes* and *Sordaria fimicola* (Table 2). Fifteen species fungi were isolated from dead parts of St. John's shoots. The data presented in Table 2 show that *Colpoma quercinum* was (the most frequently isolated species 11.5 %).

C. Wielkopolska National Park. Altogether, 23 species of fungi were identified on the trunk of Q. petraea trees. The quantitative and qualitative analysis of the fungi population is presented in Table 3. In the lower part of the trunk with cracks of bark and with local necrotic areas in secondary bark, eighteen species of fungi were isolated. In this group, the fungi with high frequency of occurrence (over 7%) included Alternaria alternata and fungi belonging to the genera Fusarium, Panicillium and Scytalidium. From the sapwood of dead trees showing brown and dark-grey discolorations, eleven species of fungi were isolated. The most common

representatives of this population (over 6 %) were: Acremonium spp., Exophiala spp., Fusarium solani, Penicillium spp. and Trichoderma spp.

The fruiting bodies of *Collybia fusipes* were found in the butts of several trees. Typical for this fungus symptoms of decay of bark parenchyma were not observed.

Table 2

Fungi occurring on twigs, branches and St. Johns's shoots of *Quercus petraea* trees

	Frequency of colonization %				
Species	Wielkopolska National Park	Wolin National Park			
	1	1	2		
Acremonium spp.	5.2	4.3	1.9		
Alternaria alternata (Fr.) Keissler	1.9	1.9	1.9		
Aposphaeria sp.	2.6	-	75		
Aureobasidium pullulans (de Bary) Arn.	3.9	2.9			
Botrytis cinerea Pers.	0.6	0.4	===		
Chaetophoma quercicola Cke.	0.6	= 1	-		
Cladosporium cladosporioides (Fres.) de Vries	3.2	5.3	3.8		
Cladosporium britannicum M. B. Ellis	0.6	2	1		
Colpoma quercinum (Pers. ex StAm) Wallr.	0.6	2.4	11.5		
Coniothyrium fuckelii Sacc.		3.4	3.8		
Cytospora intermedia Sacc.	4.6	8.8	5.7		
Discula quercina (Westd.) Arx	5.8	6.8	3.8		
Fusarium solani (Mart.) Sacc.	1.9	2.9	1.9		
Humicola fuscoatra Traaen	9.2	4.3	1.9		
Nodulisporium spp.	7.7	0.9	-		
Phialophora spp.	1.9	7.3	1.9		
Phialophora laderbergii (Melin et Nannf.) Conant	8.5	-	- 1		
Phoma sp.	1.9	-	1.9		
Phomopsis quercella Trav.		5.7	1.9		
Pseudovalsa longipes (Tul.) Sacc.	1	9.3	===		
Sordaria fimicola (Rob.) Ces et de Not.	4.6	5.4	3.8		
Trichoderma harzianum Rifai	6.5	4.8	36.5		
Tubakia dryina (Sacc) Sutt.		4.8	1.9		
Other species (nonsporulating and unidentified)	28.0	18.2	15.9		

^{1 -} twigs and branches (Wielk, N. P. - in sum 155 isolates; Wolin N. P. - in sum 210 isolates)

^{2 –} St. John's shoots (in sum – 52 isolates)

Table 3

Fungi occurring on trunk of Quercus petraea trees

	Frequency of colonization %					
Species		opolska nal Park	Wolin National Park			
	1	2	1	2		
Acremonium spp.	3.9	8.3	-	6.4		
Alternaria alternata (Fr.) Keissler	7.8	2.1	4.1	4.1		
Alternaria tenuissima (Kunze) Wilt.	2.0	1.7	-	2.1		
Alysidium resinae (Fr.) M.B. Ellis	3.9	77.5	-	2.1		
Armillaria sp.	+	-				
Aureobasidium pullulans (de Bary) Arn.	2.0	2.1	6.4	2.1		
Cladosporium cladosporioides (Fres.) de Vries	2,0	_	4.1	_		
Coryneum sp.	3.9	-	-	-		
Collybia fusipes (Bull.: Fr.) Quél.	-	-	_			
Epicoccum purpurascens Ehrenb. ex Schlecht.	3.9		2.0	-		
Exophiala spp.		16.7	-	6.4		
Fusarium spp.	7.8	6.3	2.0			
Fusarium solani (Mart.) Sacc.	3.9	-	20.4	4.3		
Humicola grisea Traaen	3.9	-	12.2	=		
Humicola fuscoatra Traaen	2.0		-	-		
Melanconium bicolor Nees	-	-	4.1			
Ophiostoma querci (Münch) H. et P. Sydow		4.2	-	2.1		
Penicillium spp.	15.7	10.4	-	4.3		
Phialocephala sp.	3.9	14.6	-	10.6		
Phialophora spp.	5.9	11.000	4.1	2.1		
Phialophora lagerbergii (Melin et Nannf.) Conant	-	2.1	=	2.1		
Phialophora melinii (Nannf.) Conant	-	-		8.5		
Phoma sp.	-	-	2.0			
Phomopsis quercella Trav.		-	4.1	2.1		
Scytalidium spp.	9.8	-	-	_		
Trichoderma spp.	3.9	12.5	-	12.8		
Trichoderma harzianum Rifai	123	4.2	8.2	12.8		
Other species (nonsporulating and unidentified)	12.8	16.5	24.6	19.2		

Wielkopolska National Park: 1 – bark and local necrotic area in secondary bark of lower part of trunk (in sum 51 isolates); 2 – brown and dark-grey discoloration in sapwood of dead trees (in sum 48 isolates). Wolin National Park: 1 – local necrotic area in local necrotic area in secondary bark (in sum 49 isolates); 2 – brown and dark-grey discoloration in sapwood of dying trees (in sum 47 isolates).

D. Wolin National Park. Twenty two fungi species were identified on the trunk of dying *Q. petraea* trees (Table 3). Twelve fungi were isolated from necrotic areas occurring in secondary bark, the most common baing: *Fusarium solani*, (20.4%), *Humicola grisea* (12.2%) and *Trichoderma harzianum* (8.2%). Sixteen species of fungi were isolated from brown and dark-grey streaks located in the sapwood. The most common representatives of this group included *Phialocephala* spp. (10.6%), *Phialophora melinii* (8.5%), *Trichoderma* spp. (12.8%) and *Trichoderma harzianum* (12.8%). A pure culture of *Armillaria* sp. with well developed rhizomorphs was obtained from white mycelium located in bark and periphery of sapwood of tree butt exhibiting symptoms of white rot.

DISCUSSION

The observation of *Quercus petraea* trees growing in the mixed forests of Wielkopolska and Wolin National Parks that the symptoms of decline were observed in 1994 on 32 and 30 trees, respectively, and in 1995 on 33 and 43 trees, respectively. Similar results were obtained during the observation of *Q. robur* trees in the experimental plot (site type: lowland forest) of Jarocin Forest Division (Warta Forestry Distr., dep. 177 d.). Generally, however, the number of *Q. robur* trees showing symptoms of decline and growing in other experimental plots (site type: humid forest, fresh forest, mixed coniferous forest) was higher compared to the number of *Q. petraea* trees. Irrespective of the species of oaks, however, the majority of trees exhibited an early stage of decline indicated by the dying of twigs and individual branches (P r z y b y 1, unpubl. data).

No significant qualitative differences were found between the *Q. petraea* tree-stands studied with respect to the fungi populations. The isolated fungi belonged to saprophytes and to the group of fungi species secondarily colonizing necrotic tissues. Some fungi such as *Discula quercina* (teleomorph: *Apiognomonia quercina*), *Aureobasidium pullulans* and *Colpoma quercinum* isolated from tree twigs are representatives of endophytes (H a l m s c h l a g e r, 1993). These fungi colonize the tissues of plants without showing any symptoms of disease and their pathogenic activity may be manifested only in certain conditions (K o w a l s k i, S a d ł o w s k i, 1993). On the leaves and twigs of *Q. petraea* trees studied no disease symptoms characteristic of *Apiognomia quercina* were observed.

Fusarium solani, a fungus whose pathogenicity is controversial, was isolated from necrotic area occurring in secondary bark of the trees growing in the Wolin National Park. Random specimens of this fungus did not indicate any pathogenic activity during greenhouse experiments conducted on two-year-old seedlings (Przybył, unpubl. data).

In the population of fungi associated with brown and dark-grey discolorations in trunk sapwood species from the following genera dominated: *Acremonium*, *Exophiala*, *Phialocephala*, *Phialophora* and *Trichoderma*. The reference literature (C o l e, 1973; D o m s c h, G a m s, A n d e r s o n T r a u t e - H a i d i, 1980) indicates that *Exophiala* spp. and *Phialophora* spp. are very common on decaying

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wood in which the latter species may cause blue stain. On the other hand, the occurrence of *Ophiostoma querci* in discoloured trunk sapwood was noted sporadically, although it was the dominating species in the fungi population isolated from discoloured streaks observed in sapwood of trunk and branches of *Q. robur* between 1985 and 1988 (P r z y b y ł, 1995). Moreover, no traces of *Fusicoccum quercus*, a pathogenic fungus occurring on twigs of dying *Q. robur* trees, were found on the twigs of *Q. petraea* trees (P r z y b y ł, 1995).

The results obtained confirm the theory formulated by M a n i o n (1981) according to which fungi are a contributing factor in the process of dying of trees. More significant, are such factors as, for instance, the genetic potential and age of a given tree-stand which are considered to be predisposing factors as well as drought and frost recognized as inciting factors.

The condition of *Q. petraea* trees in Wolin National Park, evaluated on the basis of decline symptoms occurring on overground organs was slightly worse compared to the condition of the same species of trees growing in Wielkopolska National Park. However, according to U f n a l s k i and R a t a j c z a k (1995) and Ufnalski (to be published soon) the more continenetal climate of the Wielkoposka National Park was less advantageous for the growth of trees, compared to the climate observed on Wolin. Without questioning the adverse impact of meteorological factors emphasized by both of the above mentioned authors, it can be concluded that other factors such as, may have contributed to the decline of both studied populations of trees (e.g. the origin and age of trees and the level of groundwater).

These studies were performed under grant 6P2050786 obtained from Polish Committee of Scientific Research (KBN).

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