Oak decline II. Fungi associated with various types of lesions on stems and branches of young oaks (*Quercus robur*)

TADEUSZ KOWALSKI
Department of Forest Pathology
29-Listopada 46
31-425 Kraków, Poland

Received 30. 4. 1996

Key words: Fungi. - *Quercus robur*. - Oak decline, disease symptoms.

Abstract: Decline of 19-23-year-old *Quercus robur* trees has been investigated in Poland. Disease symptoms, quantitative and qualitative composition of fungi in necrotic tissues are presented. Five main types of lesions could be distinguished. Over 40 fungal species were isolated. Possible causes of lesions in young oaks are discussed. Obviously, fungi infect trees injured by other factors previously and thus are not the primary cause for the oak decline.

Zusammenfassung: Das Absterben 19-23 Jahre alter *Quercus robur* Bäume wurde in Polen untersucht. Die Krankheitssymptome, die quantitative und qualitative Zusammensetzung der Pilze in absterbenden Geweben wird gezeigt. Fünf Haupttypen der Schäden konnten unterschieden werden. Über 40 Pilzarten wurden isoliert. Mögliche Ursachen der Schäden an jungen Eichen werden diskutiert. Offensichtlich infizieren Pilze Bäume, die vorher durch andere Faktoren geschädigt wurden, und sind daher nicht die primäre Ursache für das Eichensterben.

Decline of oak stands, *Quercus robur* L. and *Q. petraea* (MALT.) LIEB., has become a serious problem in most European countries since early 1980's. Similary as in the past (GEORGEVITCH 1927, KRAHL-URBAN & al. 1944, PETRESCU 1974), mainly stands of middle and old age are stricken with the disease (BARKLUND 1992, BARTNIK 1989, CECH & TOMICZEK 1986, DELATOUR 1983, DUJESIEFKEN & BALDER 1991, HARTMANN & al. 1991, KOWALSKI 1991, PRZYBYL 1995, SCHLAG 1994). However, there are some reports that decline may occur in quite young stands, and that it is not caused by a definite pathogen. EICHHOLZ (1985), SPELSBERG (1985), SKADOW & TRAUE (1986) observed this phenomenon in Germany, RAGAZZI & al. (1989) in Italy, and SIEBER & al. (1995) in Switzerland. Disease symptoms in young oak stands are quite diversified and appear on stems as well as within the crowns. For example, RAGAZZI & al. (1989) observed that on both the younger and more mature trees the symptom development was identical, but there were differences in decline rate. Trees below 25 years of age died during one growing season, while older trees died during 2-3 seasons. In Switzerland syndrome of decline of 10-25-year-old oaks had characteristics typical for "top dying", and it was possible to distinguish four types of lesions. Dying of trees in young stands of *Q. robur*, characterized by some symptoms reported for "oak decline" in older stands, has also been observed in Poland since 1989. Characteristics and frequency of these symptoms, and fungi present in the necrotic tissues, are presented in this paper. The methods applied permitted to compare the present investigations carried out in young age to those made in middle age and old stands of southern Poland (KOWALSKI 1991).

Material and methods

The investigations were conducted in the experimental area of the Swierklaniec forest district (Upper Silesia). This area was established in order to study the effects of various types of conversion of dying old Scotch pine stands on growth and development of young trees planted there (KOWALSKI 1982, 1983; LATOCHA 1986). In 1973, 26480 seedlings of nine species of trees, including 4560 3-year-old seedlings of *Quercus robur* were planted after the following three forms of cutting: clear cutting (A), strip felling (B), and shelter-wood felling (C). These trees are under the effect of industrial dusts and gases (especially SO_2) of medium concentrations, and they are exposed to autumn and spring frosts (LATOCHA 1986). Results of the investigations on diseases of *Q. robur* trees when they were 6-8 years old were published by KOWALSKI (1983). The investigations, of which results are presented in this paper, were performed during 1989-1993 on 3527 trees of *Q. robur*, 19-23 years of age, which had survived in the experimental area up to that time (Table 1). The disease symptoms were described, and the main types of lesions distinguished. The numbers of trees with particular types of lesions, and also the position of lesions with relation to global directions were determined. All dead trees, and trees with whole overground portion dead (n=140), and some randomly chosen living trees (n=56) with lesions on stems and branches, were taken to the laboratory. In three trees from the latter group the disease symptoms present in their root systems were analysed. In the laboratory, the necrotic tissues were inspected for the presence of fructifications of fungi, and if they were present, the fungi were identified. The roots of dead trees were examined for infection by *Armillaria* spp. and *Heterobasidion annosum* (FR.) BREF. Subsequently, from each of 154 samples representing lesions on living trees, 6-8 fragments of the bark and wood (in total 1205 fragments) were placed in 2 % malt agar medium in Petri dishes. Desinfection of samples and isolation of fungi were performed according to the method described by KOWALSKI (1991).

Results

Types of lesions and their frequency. Lesions on stems and branches were quite diverse. Five main types of lesions could be distinguished. Additionally, within type 4 some subtypes were distinguished.

Type 1: Local cankers in the bark, necrotic tissues dark brown to brownish black, visible after removal of surface layer of bark. They were limited to the bark only, or were reaching the wood. Sometimes they were accompanied by slime flux on the surface of the bark. In lower parts of stems they were elliptic, 2-5.5 x 1-2 cm with long axis parallel to axis of the shoot, situated mainly in bark cracks (Fig. 1). On branches and in upper parts of stems, where the bark was not cracked, these cankers were spot-like, oval-shaped or irregular, 0.5-4 x 0.5-3 cm, sometimes merged with one another (Fig. 2). In some lesions a dark necrotic tissue was surrounded by lighter zone and separated from the living tissue by a sharply marked line (Fig. 3).

Type 2: Elongated, elliptic bark lesions reaching the wood, 4-27 x 0.5-2.5 cm in size. Initially such places were depressed with the bark adhering to the wood, especially in the middle (Fig. 5). Later the necrotic bark crumbles away and the exposed wood becomes brown, while at its edges callus is formed (Fig. 4). On stems, outside the lesions, longitudinal (Fig. 5) or tile-like (Fig. 4) cracks are present in the bark.

Type 3: Strip cankers on the stem from its base up to 1.2 m in height, covering up to ¾ of the stem's circumference, constricted in upper part and terminated wedge-like (Fig. 7). Initially the bark is adhering to the wood (Fig. 7), and later it cracks, crumbles away, and the wood is then exposed (Fig. 8). The wood under the dead bark becomes brown all the way along the pith (Figs. 9, 10). The bark outside of the lesions is smooth (Fig. 7) or rough with tile-like cracks (Fig. 8).

Type 4: Trees in this group were dead overground, or they were totally dead. Nevertheless, it was possible to distinguish on their stems the following subtypes of lesions:

4.1 Bark with axial cracks on one or more sides of the stem, wood exposed, in some places empty spaces between bark and wood, wound healing attemps absent (Figs. 11, 12).

4.2 Bark with deep tile-like cracks, adhering to the wood, on some trees adventitious shoots growing out from the living basal part (Fig. 13).

4.3 Bark peeled off, wood exposed, traces of several callus rings around the canker which is becoming larger and larger. Advancing of the canker from initial lesions down the stem is by at least 20 % greater than up the stem (Fig. 14).

Type 5: Bark with axial cracks on living stem, inner bark exposed but without necrotic symptoms, cracks never reach the wood. In some cases the beginning of formation of tile-like cracks may be observed (Fig. 6).

The lesions were present on 15.6 %, i.e. on 551 out of 3527 trees of *Q. robur* inspected (Table 1). Frequency of their occurrence depended on the form of cutting used (see material and methods), and in variant A it was twice as high as in B and C. Also the predominating lesion type depended on the cutting variant, e.g., type 2 was over two times more frequent in variant B than in C and type 3 was four times more frequent in variant C than in B (Table 1). Out of 140 trees with lesion type 4, 60 trees had an alive root system. No cankers were found on such living roots of three trees randomly analysed. Among trees with type 2 of lesion 34.5 % had only one canker; 65.5 % of trees had cankers in several places, which were situated more or less in one line on 21.2 % of these trees. Lesions appearing on stems were present on all their sides (Table 2). They were, however, more frequent on the northern side in cutting variants A and B, and on the eastern side in variant C.

Table 1. Number of *Quercus robur* trees (%) with disease symptoms. Variants: trees planted in 1973 after *A* clear cutting, *B* strip felling, *C* shelterwood felling.

Variant	Number of trees in study area in 1989	Type of lesion					Total Number (%)
		1	2	3	4	5	
A	1138	12	103	105	45	20	285 (25.0)
B	1201	0	73	8	50	2	133 (11.1)
C	1188	4	31	32	45	21	133 (11.2)
Total	3527	16 (0.4)	207 (5.9)	145 (4.1)	140 (4.0)	43 (1.2)	551 (15.6)

Table 2. Location of lesions of *Quercus robur* according to global directions expressed as percentage of trees with symptoms. For variants see Table 1.

Variant	N	S	E	W
A	46.7	14.1	19.2	20.0
B	32.6	28.8	19.7	18.9
C	18.5	26.0	41.1	14.4
Total	36.9	20.1	24.5	18.5

Fungi in necrotic tissue. Sporulation of 13 species of fungi occurred within necrotic tissues on stems and branches of the *Quercus robur* trees (Table 3). Independently of the type of canker the fructifications of *Pezicula cinnamomea* were the most frequent (40.7 %). They were formed on the surface of the bark, in bark cracks, and on exposed wood. Besides *P cinnamomea*, also fructifications of *Aposphaeria* sp., *Colpoma quercinum*, *Coryneum umbonatum* and *Ophiostoma quercus* were relatively frequent. Out of 60 dead trees *Heterobasidion annosum* occured on 13.3 % of the trees, and *Armillaria* sp. on 5 %.

Over 40 species of fungi were isolated from 1205 fragments of necrotic bark and wood (lesion types 1, 2 and 3) (Table 4). Frequency of their occurrence varied. About 20 species occurred in less than 3 % of samples (Table 4). On the other hand, the following fungi were the most frequent: *Pezicula cinnamomea* (48.0 %), *Phialocephala* cf. *dimorphospora* (38.3 %), *Aposphaeria* spp. (21.3 %), *Mortierella isabellina* (18.8 %), *Ophiostoma quercus* (9.7 %), and *Acremonium* cf. *curvulum* (9.0 %). All these frequently occurring fungi could colonize the bark as well as the wood. They were isolated from all three investigated types of lesions. In the lesions of type 1 and partially of type 2 *Pezicula* sp. was quite frequent. This species in comparison with *P cinnamomea* showed considerable differences in structure and colour of the colony. Also genetic investigations (K. SCHRADER, Braunschweig, pers. comm.) confirmed that this species is different from *P cinnamomea*. In the lesions of type 2 *Fusarium solani* appeared a little more frequently. It was isolated on malt agar (Table 4), and sometimes it also produced sporodochia on samples in nature (Table 3). In total no fungi were isolated from 21.4 % of the samples incubated. Lesions of type 1 were worse in this respect since 52.9 % of their samples yielded no fungi. No fungi were isolated from 10.6 % of 1205 fragments placed on malt agar. Also in this case the highest number of sterile fragments was among the type 1 lesions.

Figs. 1-6. *Quercus robur* lesion types. Figs. 1-3. Lesion type 1 after removal of periderm. Fig. 1. Situated in the lower part of the stem. - Figs. 2, 3. Situated in the upper part of the stem. - Figs. 4-5. Lesion type 2. - Fig. 4. Wood exposed after dropping away of the bark (arrow) and callus. - Fig. 5. Necrotic bark adhering to the wood. Fig. 6. Lesion type 5, exposed inner bark without symptoms of necrosis (long arrow), initials of tile-like cracks (short arrow). Bars: 1 cm.

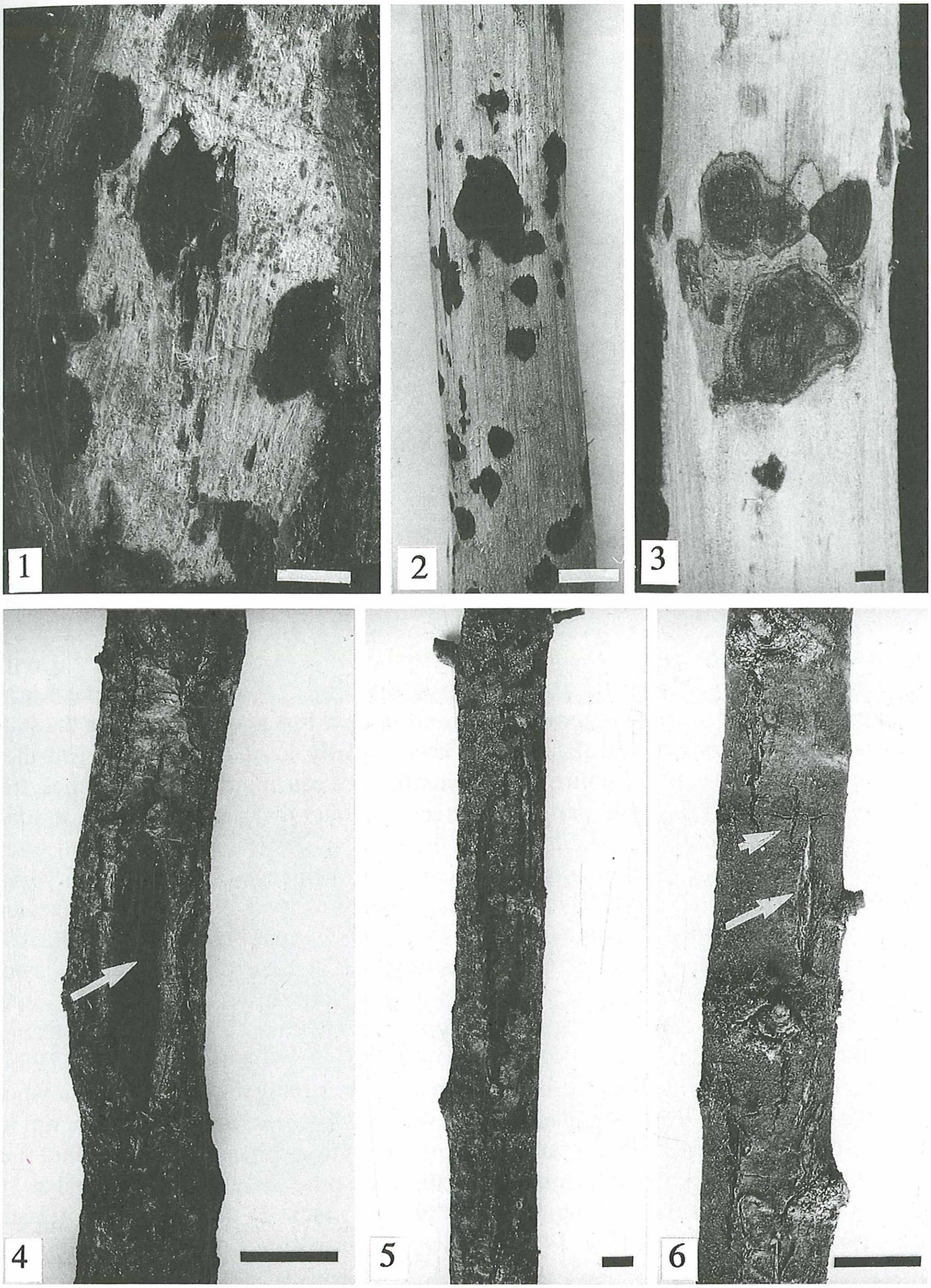
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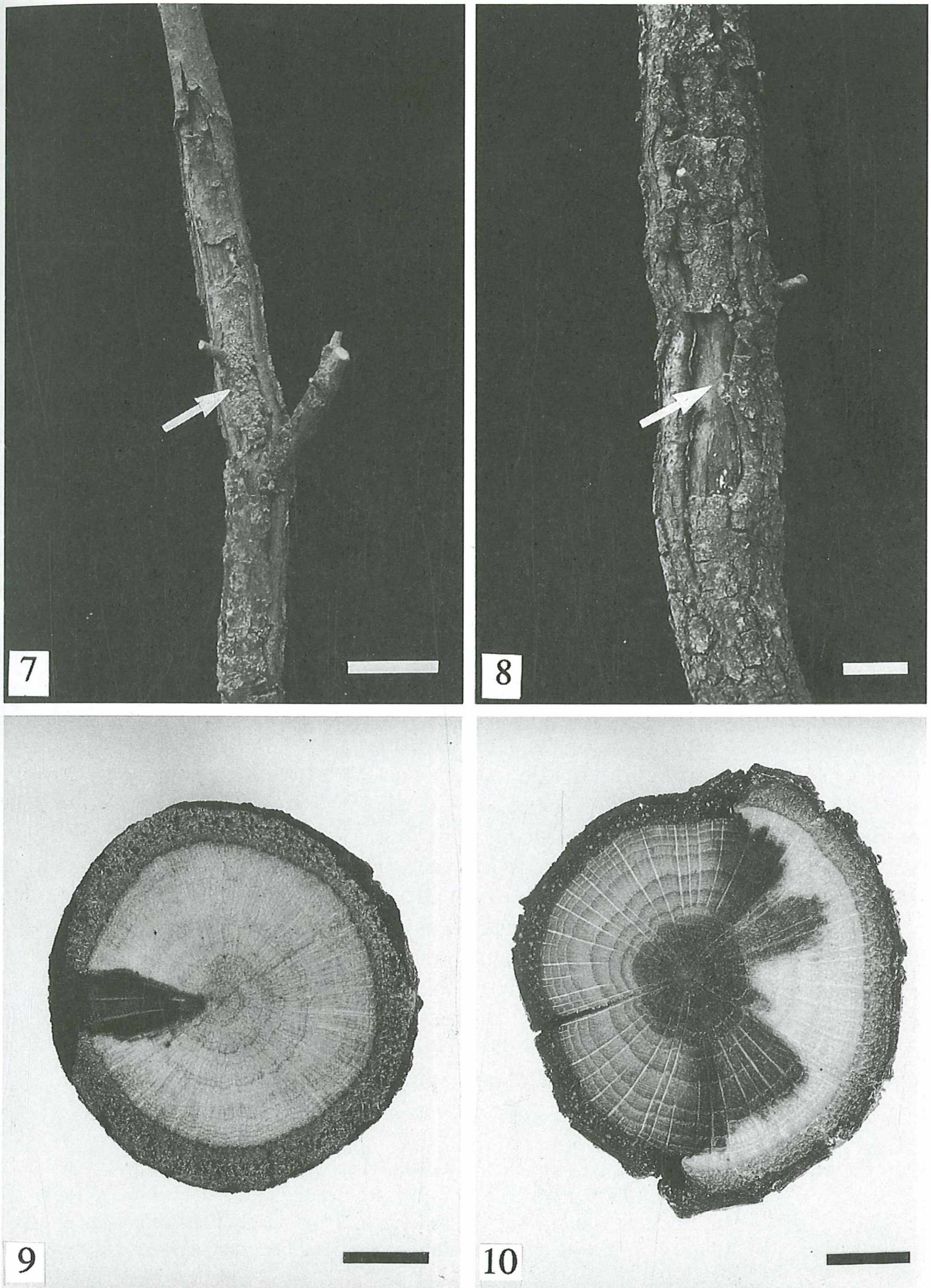
Table 3. Fungi in necrotic tissues of *Quercus robur* recognized by sporulation formed on samples in nature. *b* bark, *w* wood.

Fungi	Tissue	Number of trees with		Total
		living stem	dead stem	Number (%)
Aposphaeria sp.	b, w	7	1	8 (4.1)
Ascocoryne sarcoides (JACQUIN ex S. F. GRAY) GROVES & WILSON	w	2	1	3 (1.5)
Colpoma quercinum (PERS. ex ST. AM.) WALLR.	b	6	3	9 (4.6)
Coryneum umbonatum NEES ex STEUDEL	b	4	3	7 (3.6)
Fusarium solani (MART.) SACC.	b	2		2 (1.0)
Fusicoccum quercus OUDEM.	b	2	2	4 (2.0)
Hercospora taleola (FR.) E. MÜLLER	b	1		1 (0.5)
Mollisia cf. *cinerea* (BATSCH: FR.) KARST.	b, w	3	1	4 (2.0)
Ophiostoma quercus (GEORG.) NANNF.	b, w	5	1	6 (3.1)
Pezicula cinnamomea (DC.) SACC.	b, w	34	43	77 (39.3)
Phomopsis quercella OUDEM.	b		1	1 (0.5)
Stereum hirsutum (WILLD.) S. F. GRAY	w	2		2 (1.0)
Trimmatostroma cf. *betulae* (CORDA) HUGHES	b, w	3		3 (1.5)
Number of trees analysed		56	140	196

Discussion

Disease symptoms observed at the present time were markedly different from those occuring on stems of *Quercus robur* growing in the same area in 1975-1977, when the trees were 6-8 years old (KOWALSKI 1983). At that time fungal infections were initiated mainly from leaf scars, dormant buds and branch forks. In such places the bark had become light brown and depressed. Those initially local cankers enlarged their area and finally covered the entire stem circumference causing death of branches, tree tops or the whole overground parts of the trees. The fact that the disease was in most cases initiated in the overground parts of the trees, as many trees with dead overground part had alive roots, seems to be a common characteristic observed on young oaks in 1975-1977 and presently. In some cases death of weakened trees was accelerated by infection with root pathogens, and *Heterobasidion annosum* was more active in this respect than *Armillaria* sp. Symptoms observed at present were partly the same as those reported from young oak stands in other European countries. This is especially true for local bark cankers (lesion type 1) sometimes with mucus exudation (EICHHOLZ 1985, SIEBER & al. 1995, SKADOW & TRAUE 1986, RAGAZZI & al. 1989), and cankers (lesion type 2) of the bark which after crumbling away exposes the wood (SIEBER & al. 1995). Some symptoms, however, are different. In Poland, there are no orange-brown strip cankers and no "top dying" symptoms as described by SIEBER & al. (1995) in Switzerland. On the other hand there are other strip cankers present at the base of the stem (type 3) and longitudinal or tile-like cracks in the bark (types 4.2 and 5). Also the lesion type 4.3 (Fig. 14) is different. Its formation took several years.

Figs. 7-10. *Quercus robur* lesion type 3. - Fig. 7. Necrotic bark adhering to the wood (arrow), the bark outside the canker smooth; - Fig. 8. Necrotic bark dropped away and wood exposed (arrow), bark with tile-like cracks; - Figs. 9, 10. Cross sections of oak stems with type 3 lesions. - Fig. 9. At the top of strip canker. - Fig. 10. At the base of strip canker. Bars: 1 cm.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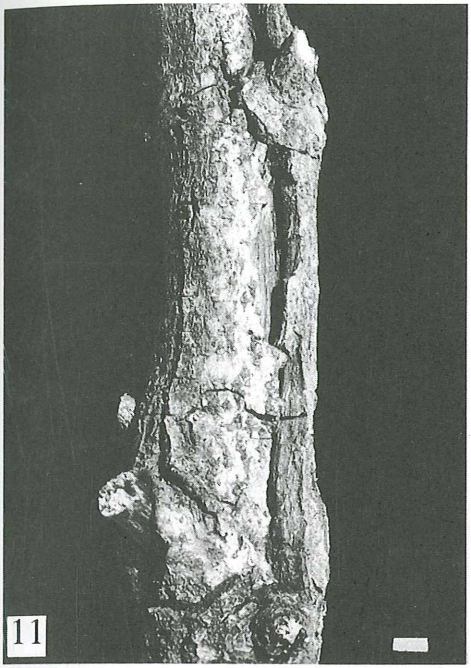
Strip cankers (Type 3) are very similar to lesions observed on 15-30-year-old *Q. rubra* L. with symptoms of *Pezicula*-canker (KEHR 1991, KOWALSKI & SADLOWSKI 1993). RAGAZZI & al. (1989) did not notice differences in disease symptoms between young and old oaks. Their observations only partially agree with the situation in southern Poland. This may be true mainly for various cankers on stems and branches of living trees. Also symptoms preceeding the death of some trees, e.g. lesion type 4.1 (Figs. 11, 12), are close to those described for older oaks by KEHR & WULF (1993) and HESKO (1987). It is doubtful whether strip cankers (type 3) observed on young oaks could be accepted as equivalent to strip cankers described for older oaks (BALDER 1989, BARKLUND 1992, HARTMANN & al. 1989), which in southern Poland have appeared rarely (KOWALSKI 1996). Species composition of fungi, discovered in necrotic tissues on the basis of sporulation on samples as well as isolation, resembles the community found on oaks during other investigations (KEHR & WULF 1993, SIEBER & al. 1995). There are, however, differences in the frequency of occurrence of some species. Sometimes, some other additional species may locally occur in high numbers, or a certain species does not occur in a site at all. This happens among young oaks or when comparison is made between young and old trees. For example, necrotic tissue of young oaks in Switzerland was often colonized by *Dichomera saubinetii* (MONT.) COOKE and *Amphiporthe leiphaemia* (FR.) BUTIN which are presently absent on the oaks studied in Poland. It is interesting that *A. leiphaemia* was observed in the same area 1975-1977 on 26.8 % of deseased *Q. robur* (KOWALSKI 1983). In comparison with the fungal community in necrotic tissues on old oaks in southern Poland (KOWALSKI 1991), *Pezicula cinnamomea*, *Phialocephala* cf. *dimorphospora* and *Mortierella isabellina* were several times more frequent on young oaks. *Ophiostomales* on young oaks were represented only by *Ophiostoma quercus*, while on old oaks they were represented by six species with their frequency depending on the kind of necrotic areas (HALMSCHLAGER & al. 1994, KOWALSKI 1991, KOWALSKI & BUTIN 1989). *Cytospora intermedia* and *Phialocephala* cf. *dimorphospora* were very often present in strip cankers of old oaks (HARTMANN & al. 1989, KOWALSKI 1996), while in similar strip cankers (type 3) on young oaks *Pezicula cinnamomea* was most frequent. *Cytospora intermedia* was not found at all presently, although in the past it was quite frequently encountered on dying tree tops (KOWALSKI 1983). *Phialocephala* cf. *dimorphospora* may be included among the species frequently occurring on injured stems irrespective of tree age. The frequency of isolation of this species may depend on the age of the cankers. It was isolated by KEHR & WULF (1993) mainly from older cankers and only sporadically from fresh ones.

Figs. 11-14. Various symptoms on dead stems of *Quercus robur* (lesion type 4). - Fig. 11. Stem with axial cracks of the bark (type 4.1). Bar: 2 cm. - Fig. 12. Cross section of the stem showing lesion type 4.1. Bar: 0.5 cm. - Fig. 13. Stem with tile-like cracks (type 4.2) and adventitious shoots at the base of the stem. Bar: 1cm. - Fig. 14. Stem with traces of callus rings around the canker (type 4.3). Bar: 1 cm.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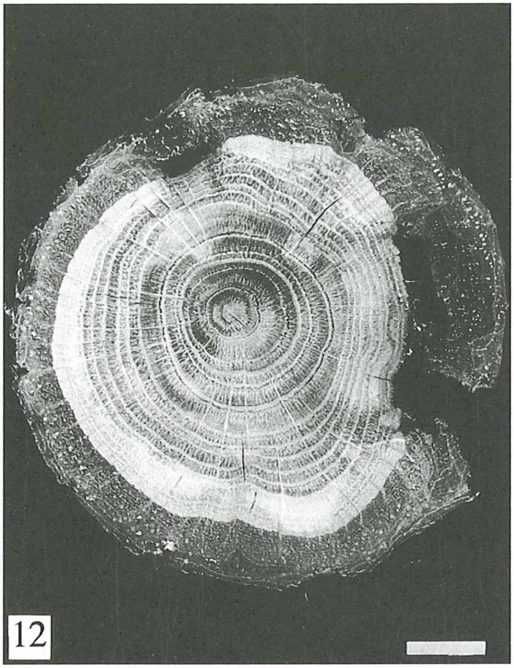
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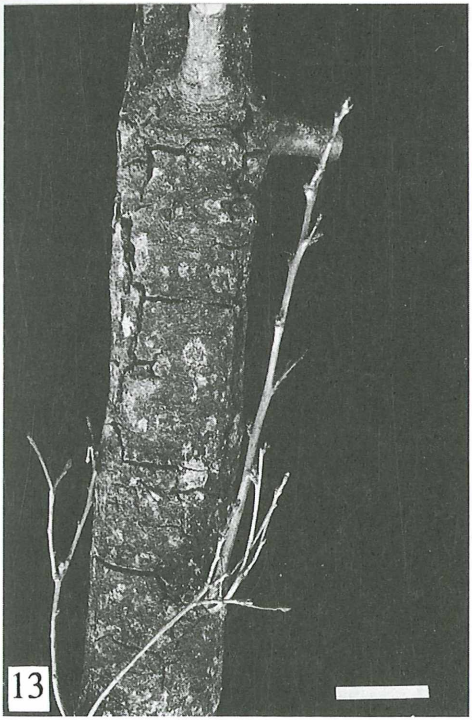
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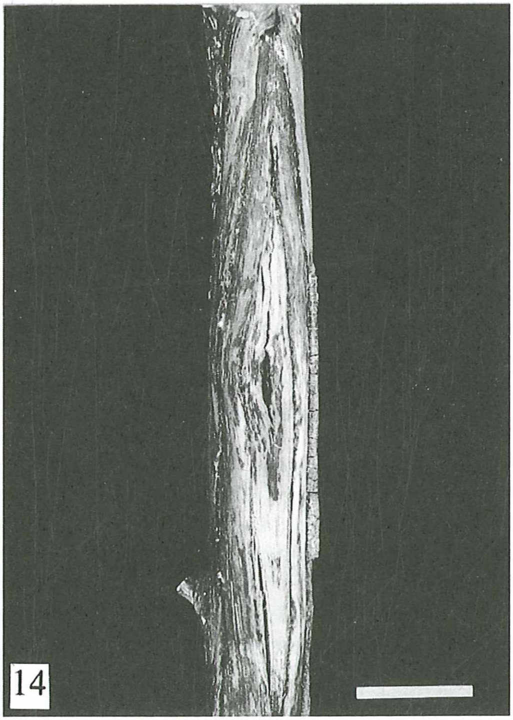
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Table 4. Fungi isolated in culture from necrotic tissues of *Quercus robur* expressed as number of samples with lesion.

Fungi	Type 1	Type 2		Type 3		Total
	bark	bark	wood	bark	wood	Number (%)
Acremonium cf. *curvulum* W. GAMS	2	1	1	3	7	14 (9.0)
Alternaria alternata (FR.) KEISSL.	1	2				3 (1.9)
Alternaria tenuissima (KUNZE ex PERS.) WILT.		1	1			2 (1.2)
Aposphaeria spp.	8	4	1	5	15	33 (21.3)
Arthrinium phaeospermum (CORDA) ELLIS			1		1	2 (1.2)
Ascocoryne sarcoides (JACQ. ex S. F GRAY) GROVES & WILSON		2	1			3 (1.9)
Basidiomycetes (2 species)			1	1	4	6 (3.8)
Chaetomium sp.				1		1 (0.6)
Cladosporium cladosporioides (FRES.) DE VRIES	1					1 (0.6)
Cladosporium herbarum (PERS.) LINK					1	1 (0.6)
Colpoma quercinum (PERS. ex ST. A.) WALLR.				4		4 (2.5)
Coniothyrium fuckelii SACC.	4		1		2	7 (4.5)
Epicoccum nigrum LINK				1		1 (0.6)
Fusarium solani (MART.) SACC.		2	4			6 (3.8)
Fusarium sp.				2	1	3 (1.9)
Hormonema sp.	1				1	2 (1.2)
Mollisia cf. *cinerea* (BATSCH: FR.) KARST.	2		1	1	2	6 (3.8)
Mortierella isabellina OUDEM.	5		1	12	11	29 (18.8)
Mucor sp.		1	1			2 (1.2)
Ophiostoma quercus (GEORG.) NANNF.	2		6	1	6	15 (9.7)
Pezicula cf. *alba* GUTHRIE	1				1	2 (1.2)
Pezicula cinnamomea (DC.) SACC.	25	6	10	9	24	74 (48.0)
Pezicula sp.	8	1	4			13 (8.4)
Phialemonium dimorphosporum W. GAMS & W. B. COOKE	1	1			2	4 (2.5)
Phialocephala cf. *dimorphospora* KENDRICK	12	1	2	11	33	59 (38.3)
Phialophora fastigiata (LAGERB. & MEL.) CON.		1	1	1	7	10 (6.4)
Phoma cava SCHULZ	1	2		2		5 (3.2)
Phomopsis quercella TRAV.				1	1	2 (1.2)
Rhizoctonia sp.				1		1 (0.6)
Verticillum lecanii (ZIMM.) VICGAS				1		1 (0.6)
Not sporulating (9 species)	7	1	1	2	8	19 (12.3)
Number of investigated samples	34	9	21	29	61	154
Number of samples which did not yield fungi	18	4	7		4	33 (21.4)
Number of investigated fragments	310	84	147	186	478	1205
Number of fragments which did not yield fungi	76	11	27	0	14	128 (10.6)

It may be supposed that, similarly as on old oaks in southern Poland (KOWALSKI 1991), the fungi were not the primary cause of these symptoms. Such a point of view may be supported by the fact that in over 20 % of the samples no fungi were found in necrotic tissues. Of the species abundant on young oaks only *Pezicula cinnamomea* is known to produce cankers, especially under stress (BUTIN 1981, KOWALSKI 1983, KEHR 1991). The most probable hypothesis is that trees were injured by other factors and then infected by fungi of which the inocula occurred in a given area or these fungi were present on oaks as endophytes before the injury. KOWALSKI & KEHR (1992) showed that such fungi as *Amphiporthe leiphaemia*, *Colpoma quercinum*, *Mollisia cinerea*, *Pezicula cinnamomea*, *Phialocephala* cf. *dimorphospora* and *Pseudovalsa longipes* (TUL.) SACC. belong to the most frequent endophytes in branches of *Q. robur*, where they are situated mainly in the peridermal bark. Injury caused by a gall midge, *Resseliella* sp., has been reported to be one of the causes of local cankers on oak stems (GIBBS 1982, DENGLER 1992). According to GIBBS (1982) the fungi are secondary inhabitants of injured tissue. There was no *Resseliella* sp. on oak trees presently investigated and also symptoms, especially cankerous swellings described by GIBBS (1982) and DENGLER (1992) were absent. From the abiotic factors, which could be the primary cause of lesions on the investigated oaks, low temperature affecting overground parts of trees seems to be most probable. The results of this study show that *Q. robur* trees planted on a clear cutting area (variant A) are more damaged than trees growing in plots with side shelter (variant B) or top shelter (variant C). This correlates well with the specific microclimatic conditions prevailing in these three variants. From 1 April to 15 July and from 1 September to 31 October, in plots situated in a clear cutting area there were about 50 % more days with the minimum temperature below 0°C than in an open area outside of the forest. Average minimum temperatures measured at ground level were lowest in variant A (clear cutting area) and highest in variant C (LATOCHA 1986). There were no frost damage marks observed on leaves during this study. Therefore, the present situation differed from that of 1977. During four days of May 1977 the temperature varied from -5°C to -11°C causing leaf injury and 2-45 cm long bark cracks (DOMANSKI 1982). Injuries caused by low temperature may mainly occur during the second half of winter, because at that time deacclimation of phloem takes place. In February these injuries, at least on old oaks, occur mainly on southern and western sides of the stems due to the climatic conditions although they may occur on all sides due to general deacclimation. From the middle of March the situation may be reversed, and the southern sides of stems may suffer less due to higher insolation (THOMAS & HARTMANN 1992). If these results are applicable to the young oaks, it may be supposed that in the area investigated the low temperature on the beginning of spring may play an important role since the lesions most often occur on northern and eastern sides (Table 2) of the trees.

These investigations were carried out under the project No. 6 P 205 070 04 financed by the Research Committee. The author thanks Mrs Mgr J. MICHALIK for help in laboratory work, and Dr I. KRISAI-GREILHUBER for correcting the English version of the text.

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