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Some aspects on *Ophiostoma roboris* (syn. *O. querci*) studies*

Abstract

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Presumed '*O. piceae*' isolates from *Quercus robur* proved to be *O. roboris* (syn. *O. querci*). Inter-mycelial recognition systems in *O. roboris* isolates obtained from oaks growing in Poland, Czecho-Slovakia and Belgium are presented and discussed.

Additional key words: Quercus, Ophiostoma roboris, vegetative incompatibility reactions.

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INTRODUCTION

Status of *O. piceae* and *O. roboris*

In a series of studies on oak dieback in Europe various *Ophiostoma* and *Ceratocystis* species were isolated e.g. *O. piceae, O. roboris, O. kubanicum, O. valachicum, O. stenoceras, C. fimbriata, C. moniliformis, C. grandicarpa* (Kryukova, Plotnikova 1979, Guseinov 1984, Skadow, Traue 1986, Zahorowska 1987, Kowalski, Butin 1989, Przybył De Hoog 1989, Kowalski, Bartnik 1990, Przybył 1990 – in print, Vajna 1990, Vannini, Luisi 1990). The first two mentioned above, *O. roboris* and *O. piceae*, were controversial from the point of view of their taxonomy, because of great morphological similarity between them (Tables 1 and 2). Several authors considered *O. piceae* and *O. roboris* synonymous (Wulf 1990), or earlier Hunt (1956) described *O. piceae* as being an inhabitant of hardwoods and conifers. Crosses between *O. piceae* isolates from conifer and hardwood hosts showed that although successful matings occurred between various hardwood isolates or between conifer isolates, conifer isolates did not mate with any of the hardwood isolates. "The conifer and hardwood isolates of '*O. piceae*' reproduc

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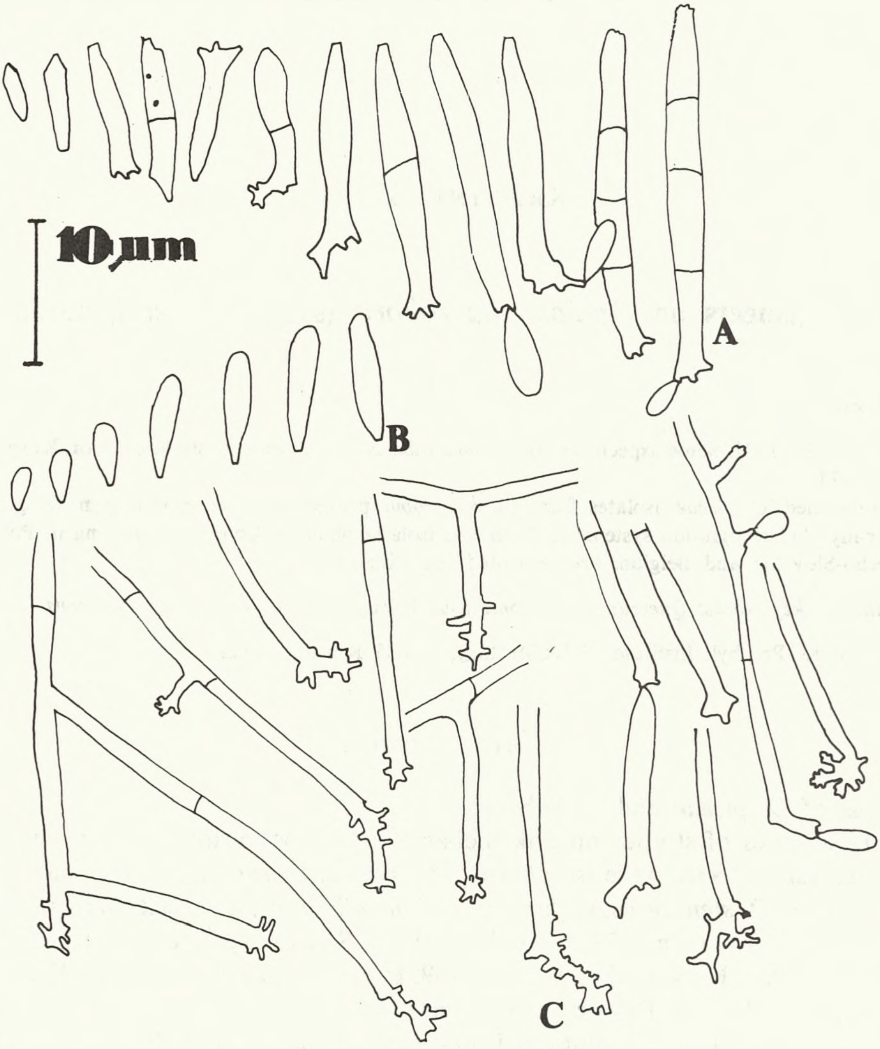

Fig. 1. *Sporothrix* state of *Ophiostoma roboris:*
A – primary conidia; B – secondary conidia; C – conidiogenous cells

tively incompatible and hence, although morphologically similar, may be sibling species of *Ophiostoma*" (Brasier and Kirk 1989). *O. piceae* is not a synonym of *O. roboris*, but the latter is a synonym of *O. querci* (Webber and Brasier 1990).

The majority of fungi isolates obtained from *Q. robur* trees in Poland were identified as *O. piceae* on the basis of comparison with the morphology of 22 *O. piceae* strains originating from different hosts e.g. *Fagus sylvatica, Picea abies, P. sitchensis, Tsuga heterophylla, Q. petraea* and *Q. robur* (Przybył and

De Hoog 1989). These authors did not find the strains similar in morphology to *O. roboris* described by *Georgescu* et al. (1948).

Table 1.

Status conidialis of *Ceratocystis* (*Ophiostoma*) *piceae* and *roboris*

Name of fungus	Status conidialis	Author (s)
C. piceae Münch (Bakshi)	Cephalosporium, Cladosporium conidia: 0-septate, 3-9 × 1-3 µm, synnemata (Graphium) stipe: 1500 µm conidia: 3-5 × 1-2,5 µm	Hunt 1956
C. piceae Münch (Bakshi)	Sporothrix-like, Hyalodendron-like conidia: 0-septate, (3-) 5-15 × 1-3,5 µm, synnemata stipe: (300-) 470-1200 (-1500) µm, conidia: 2,5-5 (-5,5) × 1-2,5 µm	Upadhyay 1981
C. piceae Münch (Bakshi)	Cephalosporium conidia: 3-9 × 1-3 µm, synnemata stipe: 1500 × 30 µm conidia: 3-5 × 1-2,5 µm	Potlaichuk, Shekunova 1985
C. piceae Münch (Bakshi)	Sporothrix conidia: 0-septate 3,5-6 × 2,3 µm, Hyalodendron conidia: 0-, 1-septate, 3,5-15 × 2-3 µm, synnemata (Pesotum) stipe: 360-800 (-1400) µm, conidia: 3,1-3,7 × 1,5-2,0 µm	Kowalski Butin 1989
O. piceae (Münch) Syd. et P. Syd.	Sporothrix primary conidia: 0-, 1-, 2-septate, (7-) 10-25 (-34) × 2-3,5 µm secondary conidia: 0-septate 3-10,5 × 1-3 µm, synnemata (Pesotum) stipes: 1. (150-) 450-600 (-700) µm 2. 600-800 (-1000) µm conidia: 2,5-5,5 × 2-2,5 µm	Przybył, De Hoog 1989
O. roboris Georg. et Teod.	Hyalodendron conidia: 0-, 1-, 2-, 3-septate, 3,5-45 × 1,2-2,5 µm, synnemata (Graphium) stipe: 336-1000 µm, conidia: (2,5-) 3,2-2,7 (-5,9) × 1,2 µm	Georgescu et al. 1948
O. roboris (C. Georg. et J. Teod.) Potl.	Hyalodendron conidia: 0-, 1-septate, 2,5-14,8 × 2,7-4,6 µm, synnemata (Graphium) stipe: 160-250 × 8-25 µm, conidia: 3-7- 5 6, × 1,9-3,7 µm	Potlaichuk, Shekunova 1985
O. roboris	Sorothrix primary conidia: 0-, 1-, 2-, 3-, septate, 7,5-45 × 1,4—2,5 µm secondary conidia: 0-septate, 3,0-10,5 × 1-3 µm, synnemata (Pesotum) stipe: 200-700 µm, conidia: 2,5-5,5 × 2,0-2,5 µm	Przybył (unpublished data)

Table 2.

Ascocarps of *Ceratocystis* (*Ophiostoma*) *piceae* and *roboris*

Name of fungus	Ascocarp	Author (s)
C. piceae Münch (Bakshi)	base: 80-180 μm, neck: 100×20 -50 (b)×5-25 (t) μm, ost. hyphae: 10-25 (-40)×2-3 μm, ascospores: 3-4,5×1,5-2 μm bean-shaped	Hunt 1956
C. piceae Münch (Bakshi)	base: 77-192 μm, neck: 500-1500 (-2900)×18-45 (-52) (b)×4,5-23 (t) μm, ost. hyphae: occasionaly lacking, ascospores: (2-) 2,5-4,5 (-5)×1,5-2 μm lunate or orange section	Upadhyay 1981
C. piceae Münch (Bakshi)	base: 192-224 μm, neck: up 1000 ×20-50 (b)×5-25 (t) μm, ost. hyphae: 10,7-21,7×3,2 μm ascospores: 2,3-4,4×1,5-2 μm	Potlaichuk Shekunova 1985
C. piceae Münch (Bakshi)	base: 80-100 μm, neck: 400-1100 ×15-45 (b)×9-20 (t) μm, ost. hyphae: 25-1 μm ascospores: 3,1-3,7×20 μm elliptical	Kowalski, Butin 1989
O. piceae (Münch) Syd. et P. Syd.	base: 70-145 μm, neck: 380-1000 ×23-25 (b)×7-12 (t) μm, ost. hyphae: up 82 μm, ascospores: 2-6×1,2 μm allantoid	Przybył, De Hoog 1989
O. roboris Georg. et Teod.	base: (95) 128-136 μm neck: 476-2000×22,2-30,4 (b) ×1,6-11 (t) μm, ost. hyphae: 12-20 (-38)×1 μm, ascospores: 3,2-3,5×0,9-1 μm allantoid	Georgescu et al. 1948
O. roboris (C. Georg. et J. Teod.) Potl.	base: 95-135×160 μm, neck: 476-200×6-30 μm, ascospores: 11-3 μm	Potlaichuk Shekunova 1985
O. roboris Georg. et Teod.	base: 96-165 μm, neck: 480-2220 ×15-33 (b)×8-22 (t) μm, ost. hyphae: 7-45×1 μm, askospores: 3-4,5 μm allantoid	Przybył (unpublished data)

Characteristics of both the anamorph and teleomorph of isolates identified as *O. piceae* are presented in Tables 1 and 2. Independently of strains of *O. 'piceae'*, I obtained, in the 1989 year, isolates which are more similar in morphology to *O. roboris* than others identified earlier (Tables 1 and 2, Fig. 1) Aim of the work.

The aim of the present studies was to revise the '*O. piceae*' strains isolated from oaks basing on cross-inoculations with *O. 'roboris'* isolates, perithecial formation and inter-mycelial recognition systems with *O. roboris.*

MATERIAL AND METHODS

Isolation of fungi

In continued studies on oak decline, carried out during the last two years, the same methods of fungi isolation from oaks and preparation single-spore cultures as described by Przybył and De Hoog (1989) were used.

Cross-inoculation

Studies were carried out with randomly selected 36 strains of *O. piceae* and *O. roboris* (Table 3). Small discs were taken from a healthy branch or stem of *Q. robur* trees and were placed on wet filter or on PDA (potato – dextrose – agar) in petri disches. Pairing of isolates mentioned in Table 3 was made in all possible combinations with the aim determinating the mating types (A and B) sexually differentiated. Pea-size pieces of single-spore cultures of two strains were placed apart on an oak disc. For the control the were inoculated by single-spore cultures of one isolate.

RESULTS

Characteristics of cultures before mating reaction

On the PDA medium some strains produced numerous protoperithecia throughout the culture. The varied appearance of all the studied cultures made it impossible to find recognizable differences between types A and B involved in the production of perithecia. The single-conidia (Sporothrix) isolations with their cultural features were assigned to three groups:

– with white, floccose or felty aerial mycelium (Fig. 2f, mating type B, no. 216 and 233);

– with scant aerial mycelium and abundant synnemata (Fig. 2 s, mating type B, no. 216, 233, 276);

– with good development of both synnemata and aerial mycelium (Fig. 2, sa, mating type B, no. 319).

The various cultural types depend on the parts of colonies from which the single-spore isolations were taken.

Mating reactions

Thirty six strains of *Ophiostoma* isolation from hardwood (32 strains) and from conifer trees (4 strains) were used in this experiment. Of the 32 hardwood

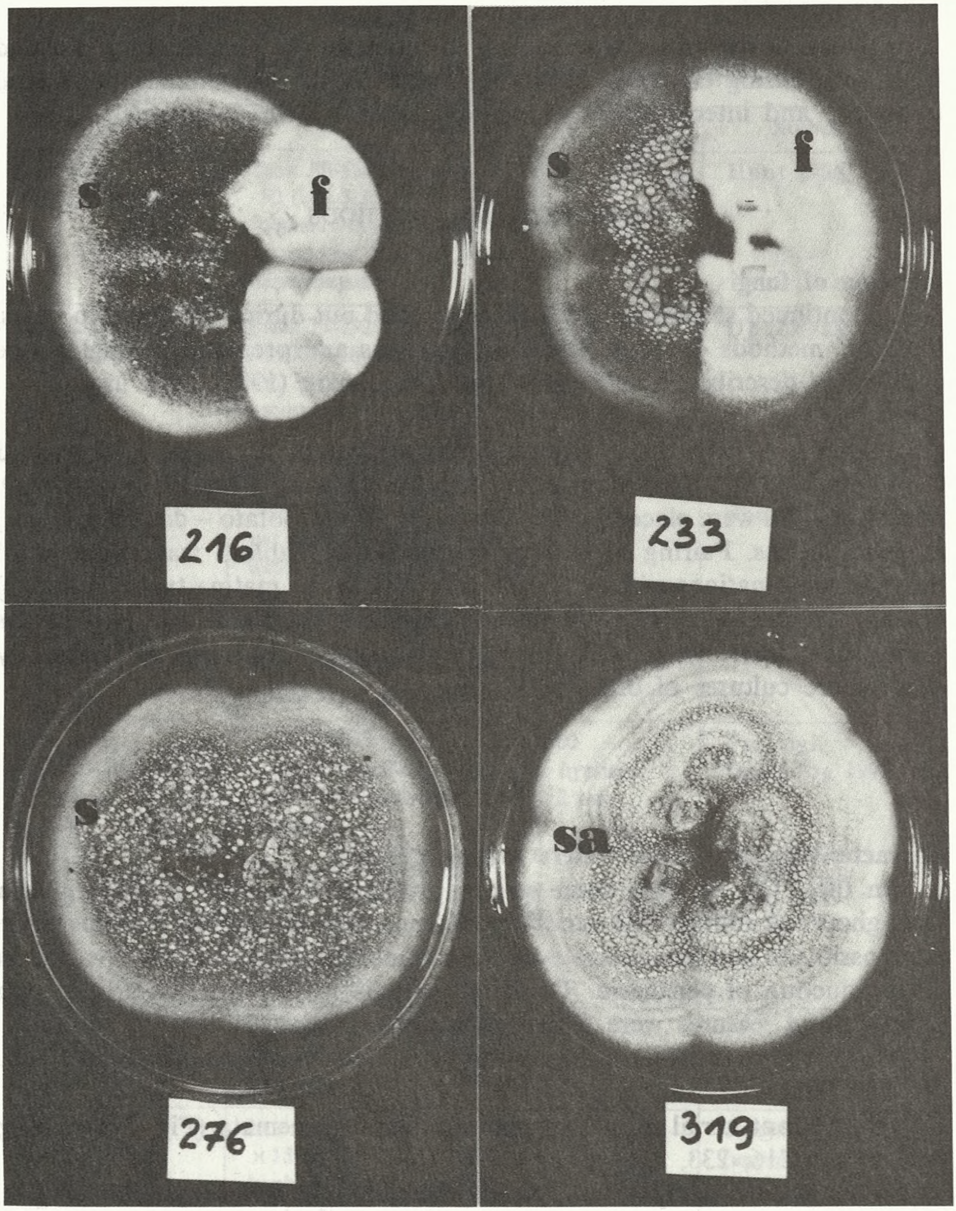

Fig. 2. Various cultural appearance of *Ophiostoma roboris* isolates:
f – floccose or felty aerial mycelium, s – scant aerial mycelium, sa – good development of synnemata and aerial mycelium (mating type B: no. 216, 233, 276; mating type A: no. 319)

isolates 31 showed monosexual characteristics. Nineteen of them appeared to be one type, herein called type B and another group of 12 isolates called type A. Strains included into one of the mentioned group consistently failed to produce perithecia when paired within the same group in all possible combinations (190 times in group B and 78 times in group A). All possible mating reactions between hardwood isolates of types A and B (228 times) gave perithecia formation. Totally 666 crosses were carried out including pairings between hardwood and conifer isolates. In the case of pairing of hardwood and conifer isolates the production of perithecia was never observed, except one strain no. 239 classed as type B of *O. 'roboris'* isolated from *P. sylvestris* in Poland. The geographic origins of the isolates shown in Table 3 did not influence the formation of perithecia. Crosses between geographically separated isolates were positive.

Table 3.

Details of *O. piceae* and *O. roboris* isolates

Culture no.	Country	Location	Name of fungus	Host
1	2	3	4	5
6I	Poland	Krotoszyn	*O. piceae*	*Q. robur*
10	Poland	Krotoszyn	*O. piceae*	*Q. robur*
10I	Poland	Krotoszyn	*O. piceae*	*Q. robur*
17	Poland	Krotoszyn	*O. piceae*	*Q. robur*
87	Poland	Krotoszyn	*O. piceae*	*Q. robur*
88A	Poland	Krotoszyn	*O. roboris*	*Q. robur*
216	Poland	Sulechów	*O. piceae*	*Q. robur*
230	Poland	Sulechów	*O. piceae*	*Q. robur*
233	Poland	Sulechów	*O. piceae*	*Q. robur*
237	Poland	Sulechów	*O. piceae*	*Q. robur*
238	Poland	Sulechów	*O. piceae*	*F. sylvatica*
239	Poland	Sulechów	*O. piceae*	*P. sylvestris*
248	Poland	Sulechów	*O. piceae*	*Q. robur*
248A	Poland	Sulechów	*O. piceae*	*Q. robur*
276A	Poland	Szczecin	*O. roboris*	*Q. robur*
294	Poland	Szczecin	*O. roboris,*	*Q. robur*
298	Poland	Sulechów	*O. roboris*	*Q. robur*
319	Poland	Tuliszków	*O. piceae*	*Q. rubra*
335	Poland	Gryfino	*O. roboris*	*Q. robur*
336	Poland	Myślibórz	*O. roboris*	*Q. robur*
451	Poland	Kórnik	*O. roboris*	*Q. robur*
236,32	Germany	–	*O. piceae*	*F. sylvatica*
H 1042	Great Britain	–	*O. querci*	–
462	Czechoslovakia	–	*O. roboris*	*Quercus* sp.
463	Czechoslowakia	–	*O. roboris*	*Quercus* sp.
464	Czechoslovakia	–	*O. roboris*	*Quercus* sp.
465	Czechoslovakia	–	*O. roboris*	*Quercus* sp.
466	Czechoslovakia	–	*O. roboris*	*Quercus* sp.
468	Czechoslovakia	–	*O. piceae*	*P. sylvestris*
469	Czechoslovakia	–	*O. piceae*	*P. abies*

1	2	3	4	5
470	Czechoslovakia	–	*O. piceae*	*P. abies*
488	Belgium	Wortel	*O. piceae*	*Q. robur*
489	Belgium	La Garenne	*O. roboris*	*Q. robur*
490	Belgium	La Gerenne	*O. roboris*	*Q. robur*
491	Belgium	La Garenne	*O. roboris*	*Q. robur*
492	Belgium	La Gerenne	*O. piceae*	*Q. robur*

451 – reisolation after inoculation with 276
236.32 – obtained from Centraalbureau voor Schimmelcultures, Baarn, identified by W. Loos
H 1042 – obtained from C. Brasier
Strains from Czechoslovakia – obtained from J. Franknechtova, identified by K. Przybył
Strains from Belgium and Poland – isolated and identified by K. Przybył.

Vegetative incompatibility

pairings of isolates, presented in Table 3, revealed four different vegetative reaction types based on the following visual features:

1) a wide (about 5mm), dense, white mycelial barrage line associated with synnematal zones occur, sometimes a dark pigment appears;

2) a narrow, dense, white mycelial barrage line (about 1 – 2 mm) occur, associated or not with synnemata concentrated close to barrage (Fig. 3A);

3) a narrow, thin, mycelial barrage line (about 1 – 2 mm) occurs, synnemata were observed (Fig. 3B);

4) a narrow, gap (about 1 mm) with scant mycelium occurs (Fig. 3C).

Features of vegetative incompatibility reaction belonging to the first ("wide") type occurred most frequently between mating types A and B hardwood isolates crossed with conifer isolates (*O. piceae*). Rarely they also occurred in crosses A × A and B × B, where one type was a Polish hardwood isolate and the other a Belgium hardwood isolate. In the studied cases either an undirectional penetration with one isolate growing faster or an equal bi-directional one was found.

"Narrow" reaction commonly occurred in A × A and B × B pairings of isolates which originated from the same locality or different localities in Poland. Occasionally this kind of reaction appeared also:

– when the two isolates involved were of different mating type (A × B) , and originated from different localities in Poland;

– in pairing mating types A × A and B × B of geographically distant isolates;

– when crossing different mating types A × B of geographically distant isolates.

In this mating group three kinds of mycelial penetration were seen: equal bi-directional, unequal bi-directional and unidirectional with one isolate growing faster.

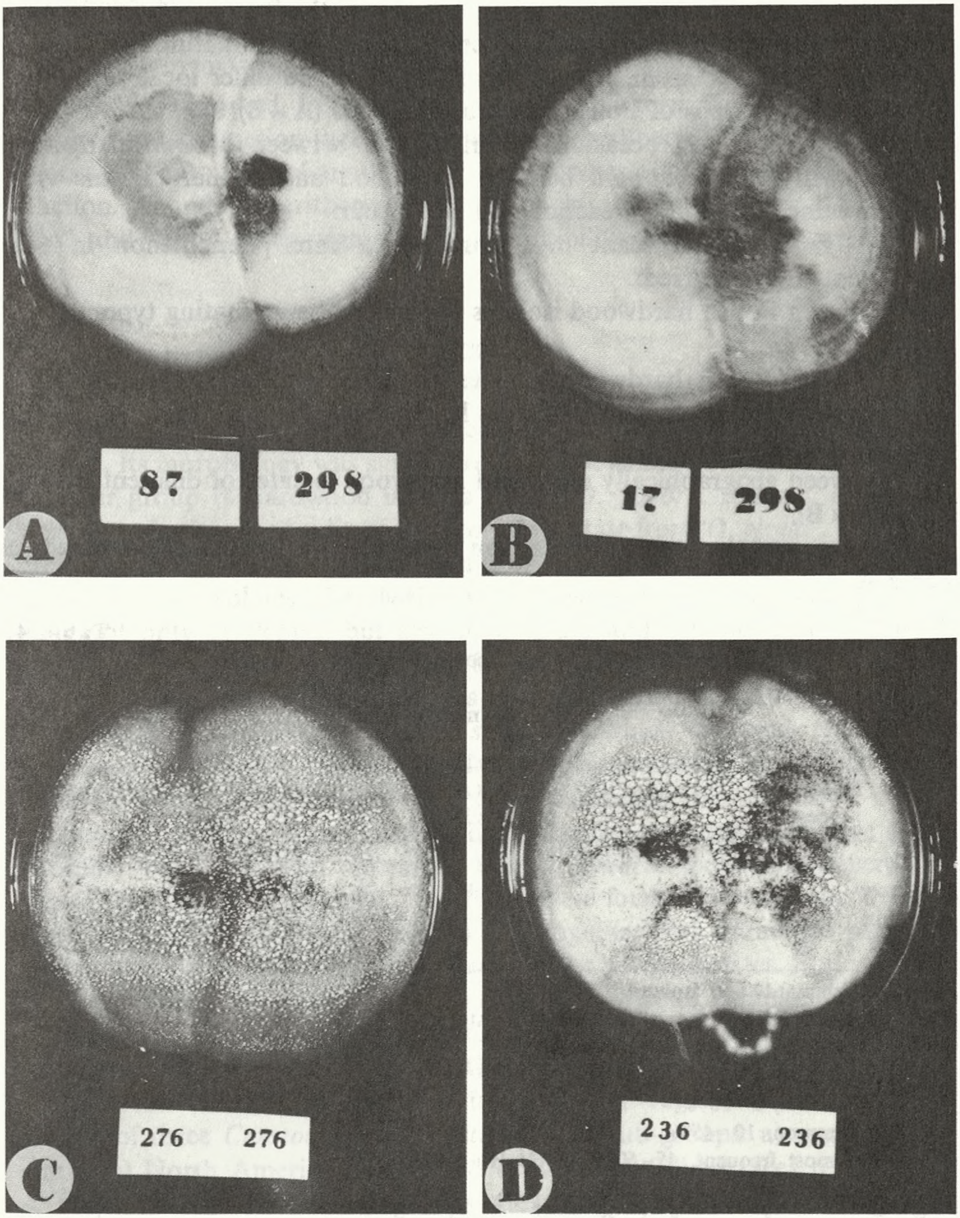

Fig. 3. Samples of vegetative incompatibility reactions in *Ophiostoma roboris*:
A – type "narrow" of vegetative incompatibility reaction;
B – type "narrow thin" of vegetative incompatibility reaction;
C – type "gap" of vegetative incompatibility reaction;
D – vegetative compatibility reaction

Features characterising the third type "narrow thin" of vegetative incompatibility reaction appeared most frequently among paired mating types A × A and B × B originated from Poland (from the same and differ localities). With opposite mating types of Polish hardwood isolates (A × B) the "narrow thin" reaction was observed occasionally. In crosses between hardwood isolates geographically separated and between hardwood and coniferous, this type vegetative incompatibility reaction did not occur.

The "gap" with a scant mycelium was a rare phenomenon in this experiment and occurred:

– between Polish hardwood isolates both of the same mating types (A × A and B × B);

– between Polish hardwood isolates of different mating types (A × B);

– between geographically separated hardwood isolates of the same mating types (A × A and B × B);

– between geographically separated hardwood isolates of different mating types (A × B).

Frequency of the vegetative reaction types described above is presented in Table 4.

Table 4.

Frequency of vegetative incompatibility reaction types

		mating types P.H.		mating types G.H.	
Relation types	C × H	A × A B × B	A × B	A × A B × B	A × B
Wide	++++	–	–	+	–
Narrow	–	+++	++	++	++
N. thin	–	++++	++	–	–
Gap	–	+	+	+	+

P.H. – Polish hardwood isolates
G.H. – geographically removed hardwood isolates
C × H – conifierous × hardwood mating
+ – scarce up to 5%
++ – occasional, 5–10%
+++ – common, 10–45%
++++ – most frequent, 45–60% (in C × H – 100%).

Vegetative compatibility (Fig. 3D)

In the vegetatively compatible reaction no evident thickening of mycelia at the junction line between two colonies was observed. The reaction occurred commonly in pairings of Polish hardwood isolates both between the same mating types A × A, B × B or in crosses where different mating types were involved (A × B).

Perithecial formation

Perithecia were not formed in the crosses between hardwood and conifer (except no. 239) isolates, in which the "wide" vegetative incompatibility reaction was observed. In the remaining, perithecia formation was more or less confined to the junction line. Small number of perithecia 10 – 20 was observed over a different width of area of both mating types of colonies in the "narrow" reaction. Abundant perithecia were formed in crosses in which the "narrow thin" and "gap" types vegetative incompatibility reactions appeared.

DISCUSSION

'Ophiostoma piceae' is easily isolated from all above-ground parts of oaks in Poland. Its morphology was similar to that of *O. piceae* obtained from conifers. Another group of hardwood isolates named *O. 'roboris'* differed, in size and structure of primary conidia of the *Sporothrix* state from '*O. piceae*'. The results presented in this paper show successful mating between hardwood '*O. piceae*' and *O. roboris* isolates. *O. roboris* (syn. *O. querci*) is a fungus widespread on oaks not only in Poland but also in Belgium and Czechoslovakia,. On the other hand in crosses between conifer isolates of *O. piceae* and hardwood isolates no perithecial formation was observed. Exception to this result was from isolate no. 239 from *Pinus sylvestris,* which behaved typically for hardwood isolates. I can explain this fact only by the fact, that I carried out the isolation from a pine-tree growing single in an oak plantation.

Between coniferous and hardwood isolates the strongest vegetative incompatibility reactions type appeared. For *O. roboris* species, however, three vegetative incompatibility reaction groups were found. The least incompatible reactions "narrow thin" and "gap" existed together with numerous perithecia, indicating a very close relationship between the paired isolates. Full vegetative compatibility, where no vegetative incompatibility genes are manifest occurred in crosses A × A, B × B and A × B mating types of Polish hardwood isolates. To summarise, different genetic populations of *O. roboris* species were found.

Brasier (1984) described the frequency of vegetative incompatibility groups of three *Ceratocystis* (*Ophiostoma*) *ulmi* sub-groups: a) non-aggressive strain, b) North America (NAN) and c) Eurasian (EAN) races of the strain. A major feature of the sub-groups are differences in their pathogenicity. Przybył (1990 – unpublished data) concluded, on the basis of a pathogenicity test, that isolates with characteristics traits of *O. roboris* are more pathogenic than those earlier described as 'O. piceae'. Discovery incompatibility reaction types of *O. roboris* isolates and differences in their pathogenicity stimulated further studies of those aspects.

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Wybrane aspekty badań nad *Ophiostoma roboris* (syn. *O. querci*)

STRESZCZENIE

W ostatnich dwóch latach wyizolowano w Polsce, z drzew *Quercus robur* wykazujących objawy zamierania, grzyb *Ophiostoma roboris* = *O. querci.* Szczepy tego grzyba różniły się morfologią stadium konidialnego od wcześniej uzyskanych z dębów izolatów oznaczonych jako '*O. piceae* ' sensu lato. Celem przeprowadzonych badań było:

– zweryfikowanie szczepów *O. piceae* wyizolowanych z drzew liściastych (*Q. robur* i *Fagus sylvatica*) i z drzew iglastych (*Pinus sylvestris* i *Picea abies*);

– wykazanie występowania zjawiska niezgodności wegetatywnej między szczepami '*O. piceae*' i *O. roboris* wyizolowanymi w różnych rejonach geograficznych (Polska, Czechosłowacja, Belgia).

Na podstawie przeprowadzonych krzyżowań z wybranymi 36 szczepami, spośród których 21 szczepów zaklasyfikowano do gatunku *O. piceae* a 15 do *O. roboris*, stwierdzono;

– tworzenie otoczni w krzyżowaniu dwóch odrębnych płciowo osobników (A × B) uzyskanych z drzew liściastych;

– występowanie 3 typów niezgodności wegetatywnej, o różnym stopniu nasilenia, między strzępkami szczepów *O. roboris* wyizolowanych z dębów rosnących w Polsce, Czechosłowacji i Belgii;

– ścisły związek między tworzeniem otoczeni a intensywnością występowania niezgodności wegetatywnej;

– brak wzrostu otoczni w krzyżówkach między izolatami '*O. piceae*' i *O. roboris* z drzew liściastych a izolatami *O. piceae* z drzew iglastych.

Z uzyskanych wyników wyprowadzono następujące wnioski:

– poddane weryfikacji szczepy '*O. piceae*' wyizolowane z drzew liściastych należą do gatunku *O. querci*;

– między strzępkami badanych szczepów *O. querci* występuje niezgodność genetyczna.

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