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Preliminary report on distribution of *Heterobasidion annosum* intersterility groups in Poland

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The study material consists of 165 *H. annosum* isolates from 25 different localities. Host species was *Pinus sylvestris, Picea abies, Betula pendula, Abies alba, Larix decidua, Pinus strobus, Prunus serotina, Quercus rubra.* Most of the *H. annosum* isolates belonged to the P group. This group was most common on pine and birch. The S group infected Norway spruce and European fir. The F group was recorded only in the south of Poland. Only three localities, where this intersterility group was present, were found in Poland.

Key words: *Heterobasidion annosum,* intersterility groups, Poland.

INTRODUCTION

Heterobasidion annosum (Fr.) Bref. is the most important pathogen causing root and butt rot in coniferous forests of the northern temperate regions (Mańka 1998; Woodward et al. 1998) Mating experiments carried out since the 70's have revealed the occurrence of intersterile groups within *H. annosum* (Korhonen 1978; Chase and Ulrich 1988; Capretti et al. 1990). Three groups, P (pine), S (spruce) and F (fir) were found in Europe (Korhonen 1978; Capretti et al. 1990) and also in Poland (Żółciak 1992; Łakomy 1996; Kowalski and Łakomy 1998).

Pines are typical hosts of the *H. annosum* P group. This group can also attack many different coniferous and deciduous species, especially when they

grow in mixed stands tpgether with pine (Greig 1979; Siepman 1988; Korhonen et al. 1992; Korhonen and Piri 1994; Sierota 1995; Swjedemark and Stenlid 1995). Very few tree species seem to be completely resistant to the P group of *H. annosum*, particularly, when trees are weakened or are under exceptionally high infection pressure (Korhonen at al. 1998). The S intersterility group is fungi of *Picea abies* forests (Żółciak 1992; Negrutski et al. 1993; Capretti et al. 1994; Munda 1994; Pagony and Shanto 1995; Tsopelas and Korhonen 1996; Korhonen et al. 1997); they can cause rather little damage to other tree species (Korhonen and Piri 1994; Piri 1996; Łakomy and Rubys 1998). This intersterility group was also recorded outside the natural distribution of *P. abies* in western Europe – in Denmark (Thomsen 1994) and in Germany (Siepman 1988, 1989). The F group infests *Abies alba* in southern and central Europe. In Greece these fungi cause damage to *A. cephalonica* Loud. and *A. borisii* regis Mattf. This group can also attack other species as *Castanea*, *Chameacyparis*, *Cryptomeria*, *Fagus*, *Juniperus*, *Larix*, *Picea* and *Pinus* (Capretti et al. 1990; Tsopelas and Korhonen 1996; Łakomy and Rubys 1998).

This work presents preliminary results of investigation on distribution of the *H. annosum* P, S and F intersterility groups in Poland.

MATERIALS AND METHODS

Study material comparises 165 *H. annosum* isolates from 25 different localities. The host species were as follows: *Pinus sylvestris* – in 64 cases, *Picea abies* – 58, *Betula pendula* – 16, *Abies alba* – 16, *Larix decidua* – 2., *Pinus strobus* – 2, *Prunus serotina* – 1, *Quercus rubra* – in 1 case. Fruit bodies as well as wood from stumps and roots were collected for the pathogen isolation. Almost half of the isolates were homokaryotic from single-spore isolation and second half were heterokaryotic from tissue or wood isolation. Single-spore cultures were isolated according to method of Korhonen and Hintikka (1980).

The material was isolated during the years 1993–1998. The isolates were identified using mating tests: each isolate was paired with homokaryotic tester strains. Three P, seven S and ten F testers were used in the tests. The P testers originated from Poland (Oborniki – 94149, Podanin – 97064, 97066), the S testers – from Estonia (Saaremaa – 91109/1, Hiiumaa – 93102/2, Vormsi – 93142/2), Finland (Kirkkonummi – 92044/5, 93254/2), Italy (Vicenza 87075/2) and Slovenia (Hotedersica – 9211/1.3), and the F testers – from France (Grande Chartreuse – 92144/1, Lyon – 92169/2), Greece (Parnon mountain – HL-2.1, Menalon Mt. – 93315), Italy (Firenze – 91280/11, 930912/1.3.1, Pistoia – 930920/3.2.2, Potenza – 92179/2), and Slovenia

(Lovrenc na Pohorju – 9210/4.2, Krekovse – 92192). The pairings were made in the centre of Petri dishes, on 1.5% malt extract agar. The distance between two inocula was 1 cm. Incubation was carried out at 25°C in the light. The mycelia were examined for the presence of clamp connections through the bottom of a Petri dish at magnification of 100–200 x, three weeks after inoculation, and again one-two weeks later. The external appearance of mycelium was also observed and any changes were noted. In some cases subcultures were also taken from the contact zone. The presence of clamps in the subcultures was checked after 7–8 days (C h a s e and U l r i c h 1983).

RESULTS

Most of the *H. annosum* isolates belonged to the P group (101). This group was most common on *P. sylvestris* and *B. pendula* in mixed stand with pine. The fungi from this group infected also *P. abies* in stands localized out of its natural range. In addition, some other species were attacked: *A. alba*, *Qu. rubra*, *P. strobus* and *P. serotina*. The S group infected *P. abies* and *A. alba*. There was no evidence of infection of *P. sylvestris*. Among 51 cultures isolated from Norway spruce growing within its natural range (Table 1, locality 3, 13, 15, 16, 17 and 20), 21 belonged to the S group, 2 to the P group and 7 to the F group (Table 1).

The F group was found only in the south of Poland. Only three localities, were thisintersterility group was present, were recorded in Poland: Węgierska Górka Forest, Sucha Forest (Mountains) and Ojcowski National Park (Fig. 1.).

The results of mating tests were very clear in the case of P group (Fig. 2). But there were some difficulties in identification of the F group, because this group is partialy compatible with the S group. In most of compatible F × S pairings clamp connections appeared later than in compatible F × F and S × S pairings. After three weeks clamps were observed in a few S × F pairings. Later there were more frequent also in subcultures. The demarcation line separated paired F and S homokaryons very clearly and their mycelia maintained homokaryotic appearance (Fig. 3).

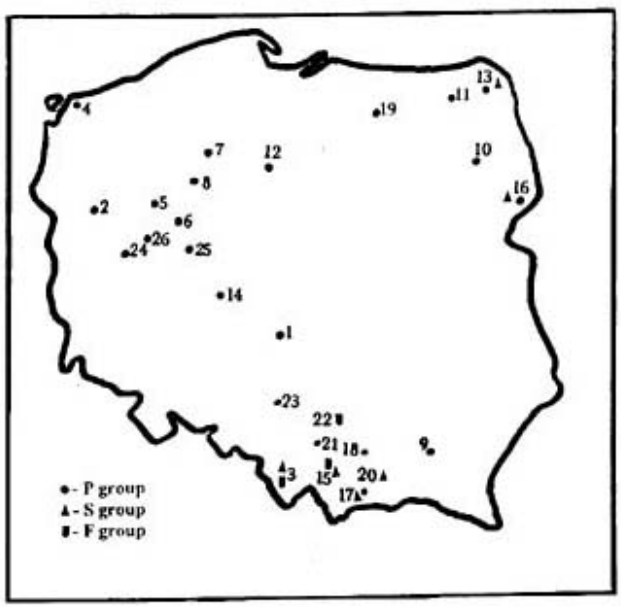

Fig. 1. Localities of *H. annosum* strains collection in Poland. The numbers of each locality refers to Table 1

Table 1

The *H. annosum* strains collection. The locality number refers to the map in Fig. 1

Locality	No. of collections	Host	Intersterility group		
			P	S	F
1. Wieluń	7	*Pinus sylvestris*	7		
2. Bogdaniec	3	*Pinus sylvestris*	3		
3. Węgierska Górka	7	*Abies alba*		1	6
	22	*Picea abies*		15	7
4. Międzyzdroje	2	*Pinus sylvestris*	2		
5. Krucz	3	*Pinus sylvestris*	3		
	2	*Betula pendula*	2		
6. Sarbia	2	*Pinus sylvestris*	2		
	1	*Betula pendula*	1		
7. Lipka	2	*Pinus sylvestris*	2		
8. Podanin	22	*Pinus sylvestris*	22		
	1	*Betula pendula*	1		
	2	*Larix decidua*	2		
9. Rzeszów	1	*Abies alba*	1		
10. Białystok	1	*Pinus sylvestris*	1		
11. Wigierski National Park	1	*Pinus sylvestris*	1		
12. Dąbrowa	3	*Pinus sylvestris*	3		
	1	*Betula pendula*	1		
13. Sejny	11	*Picea abies*	5	6	
14. Niedźwiady	1	*Pinus sylvestris*	1		
15. Sucha	6	*Abies alba*		2	4
	5	*Picea abies*		5	
16. Białowieża	4	*Picea abies*	1	3	
17. Nowy Targ	2	*Pinus sylvestris*	2		
	8	*Picea abies*		8	
18. Niepołomice	1	*Pinus sylvestris*	1		
19. Olsztyn	1	*Pinus sylvestris*	1		
20. Gorczański National Park	1	*Picea abies*		1	
21. Kraków	1	*Prunus serotina*	1		
22. Ojcowski National Park	2	*Abies alba*		2	
23. Świerklaniec	1	*Betula pendula*	1		
	1	*Quercus rubra*	1		
	2	*Pinus strobus*	2		
	1	*Pinus sylvestris*	1		
24. Pniewy	5	*Betula pendula*	5		
	7	*Picea abies*	7		
25. Zielonka	5	*Betula pendula*	5		
	10	*Pinus sylvestris*	10		
26. Oborniki	2	*Pinus sylvestris*	2		
Total	**160**		**100**	**41**	**19**

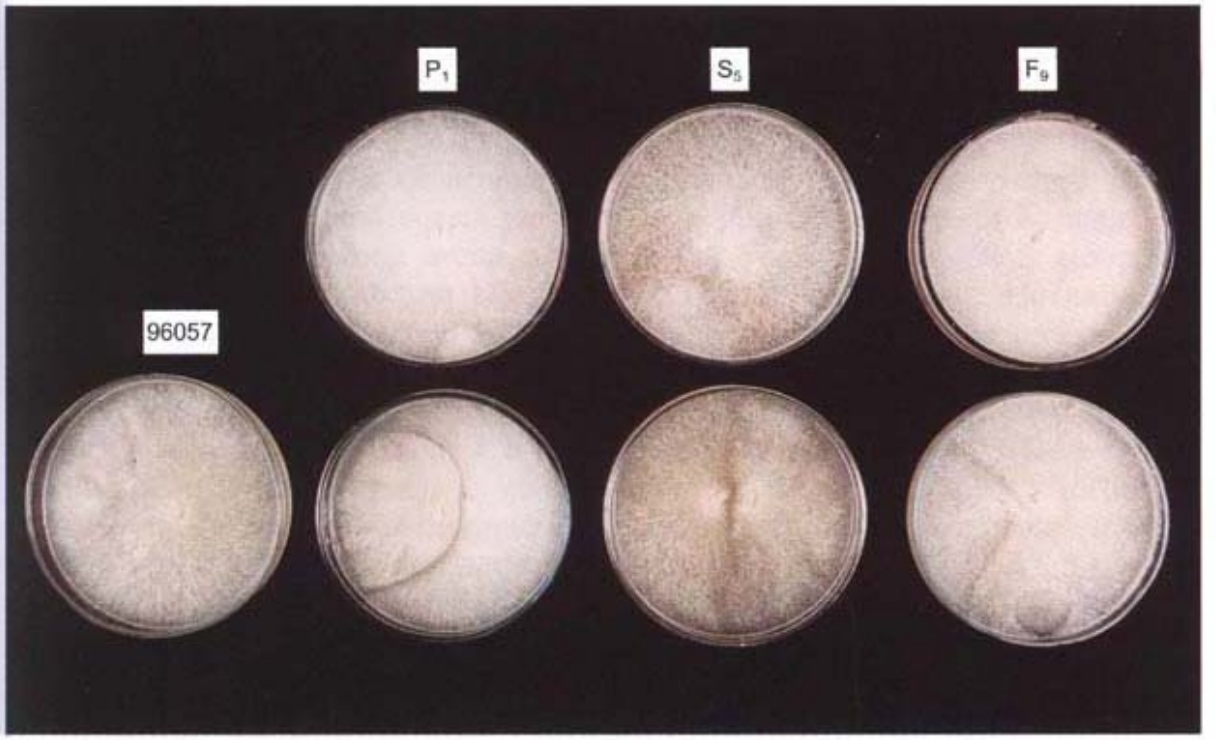

Fig. 2. Pairings between the homokaryon 96057 (Sucha locality) and homokaryotic tester strains P_1 (from Poland), S_5 (from Finland) and F_9 (from Slovenia). A line of demarcation is visible in pairings 96057 × P_1 and 96057 × F_9, but is disapearing from the pairings 96057 × S_5 owing to the formation of heterokaryon

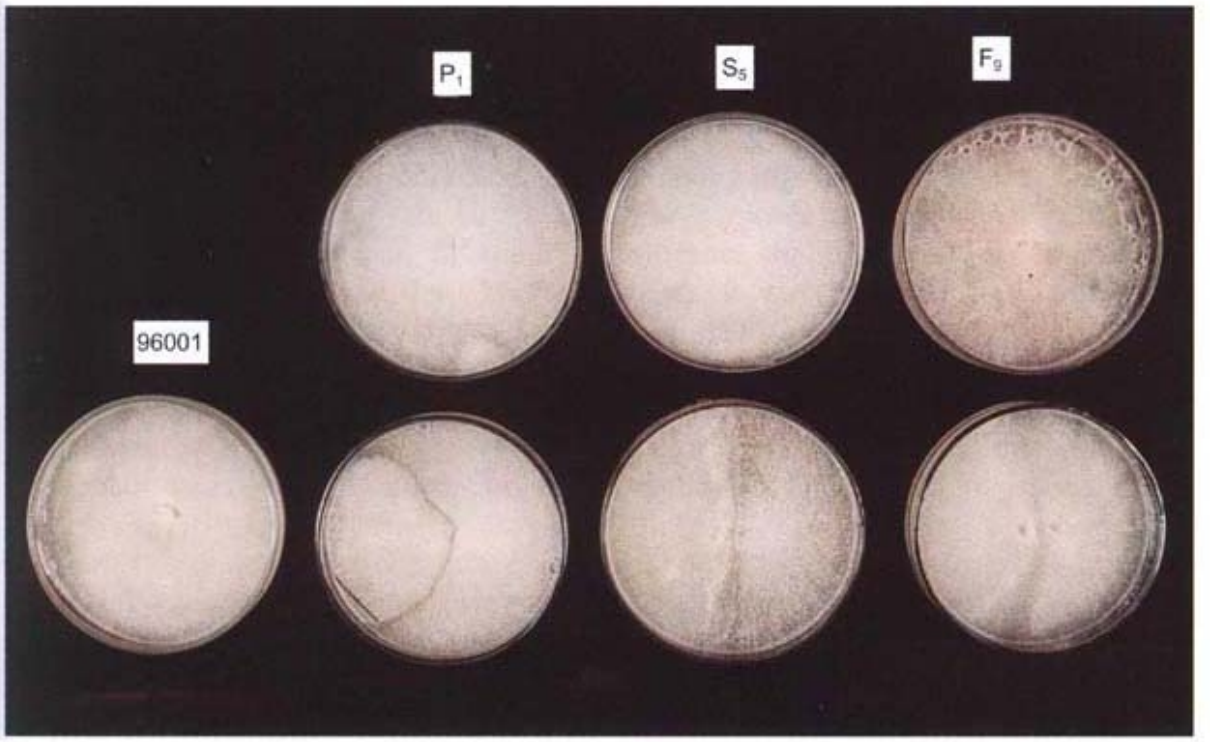

Fig. 3. Pairings between the homokaryon 96001 (Nowy Targ) and homokaryotic tester strains P_1 (from Poland), S_4 (from Finland) and F_{10} (from Slovenia). A line of demarcation is visible in pairings 96001 × P_1 and 96001 × S_4, but is disapearing from the pairings 96001 × F_{10} owing to the formation of heterokaryon

DISCUSSION

It seems that the occurrence of P, S and F intersterility groups of *H. annosum* in Poland covers the natural of occurrence range of its main tree hosts. The P group occurs almost in the whole country, the S group is present in the South and North-East and the F in the South. However, though in the case of Poland S groups the range of their occurrence is clearly differentiated, the range of F group is still not well recognized. In Poland there are known only the Mountains and highlands localization (Ł a k o m y 1996; K o w a l s k i and Ł a k o m y 1998), and the north border of European fir natural range is going far from Mountains on lowland. The P group is the most pathogenic and the most dangerous for Scots pine and birch, especially on post agricultural soils (S i e r o t a 1995; M a ń k a 1998). In the present investigation Scots pine was not infected by the S group. On the other hand, in two cases the P group attacked Norway spruce in mixed spruce-pine stand. K o r h o n e n et al. (1992) found that the P group was very destructive in spruce stands. Spruces were infected in mixed and pure stands growing on old forest soil. These authors suggested that high inoculum potential in those stands resulted from the presence of pine in previous generations. However the infection from pine to spruce via root contact was also possible. In greenhouse condition S t e n l i d and S w j e d e m a r k (1988) indicated that Scots pine is highly susceptible to the P type, but relatively resistant to the S type; Norway spruce is highly susceptible to both groups.

The Polish F group locality is the furthest of the north one in Europe. The group was isolated only from stumps, laying logs or very old dead trees. It seems to be rather rare in Poland. The presence of these fungi causes no damage in European fir stand. In Slovenia the F group appeared as a saprothroph (M u n d a 1974). However it can cause severe damage in *A. alba* stands in Italy (B i r a d h i 1962; M o r i o n d o 1970; C a p r e t t i et al. 1990). This group shows the most differential pathogenicity to *A. alba*. L a P o r t a et al. (1997) suggested that the differences in pathogenicity of *H. annosum* F group result from small genetic variation of Central Europe population in comparison to Italian F group.

The material for the future studies should be collected from *A. alba* stand especially those growing on lowlands and from Norway spruce stands within their natural range. Further investigations should show the more regions *H. annosum* P, S and F intersterility groups occurrence and explain its role for the health status of Scots pine, Norway spuce and European fir stands.

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Wstępny raport o występowaniu grup intersterylnych *Heterobasidion annosum* w Polsce

Streszczenie

Do badań użyto 165 kultur *Heterobasidion annosum* wyizolowanych z owocników lub drewna pochodzących z 25 stanowisk w Polsce. W 64 przypadkach patogena izolowano z *Pinus sylvestris*, 58 – *Picea abies*, 16 – *Betula pendula*, 16 – *Abies alba*, 2 – *Larix decidua*, 2 – *Pinus strobus*, 1 – *Prunus serotina*, 1 – *Quercus rubra*. Do identyfikacji typów intersterylnych użyto metody zgodności genetycznej grzybni (mating test). W tym celu wykładano na 1,5% pożywkę maltozową w płytkach Petriego grzybnie testowane (haploidalne i dikariotyczne) i testowe (haploidalne) w odległości 1 cm. Użyto trzech grzybni testowych typu P, 7 – S i 10 – F. Przynależność danego izolatu do odpowiedniego typu intersterylnego *H. annosum* określano po 3 tygodniach na podstawie stwierdzenia pod mikroskopem połączeń sprzążkowych.

Największa liczba izolatów patogena należała do typu P (101). Grupa ta była izolowana głównie z sosny zwyczajnej, ale także z sosny wejmutki, jodły, brzozy brodawkowatej, dębu czerwonego i czeremchy amerykańskiej. Typ świerkowy (S) i jodłowy (F) stwierdzono na świerku i jodle. Stwierdzono tylko trzy stanowiska występowania typu F w Polsce – Ojcowski Park Narodowy, Nadleśnictwo Sucha i Węgierska Górka. Występowanie trzech typów intersterylnych *H. annosum* połączone jest z naturalnym zasięgiem występowania ich głównych gospodarzy – sosny zwyczajnej dla typu P, świerka dla S i jodły dla typu F. Najgroźniejszym dla drzew wydaje się być typ P, natomiast typ F należy do najrzadszych.