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Host specialization of IS-group isolates of *Heterobasidion annosum* to Scots pine, Norway spruce and common fir in field inoculation experiments

Abstract: Werner A., Łakomy P. Host specialization of IS-group isolates of *Heterobasidion annosum* to Scots pine, Norway spruce and common fir in field inoculation experiments.
Two field inoculation experiments were conducted to study intraspecific variation in vertical spread of the P-, S- and F-IS-group isolates of *Heterobasidion annosum* in stems of *Pinus sylvestris*, *Picea abies* and *Abies alba*. Host-plants were inoculated with four isolates of each IS group after 10 mm long wounds made with a sterile knife (experiment 1) or 3 mm diameter radial holes made with a drill (experiment 2). On pine, the P-group isolates were more virulent than S and F isolates in terms of infection frequency, mortality rate and vertical spread in sapwood. The S isolates had higher incidence of infection and extensive growth on spruce than on pine. The F isolates were significantly less virulent on pine and spruce than on fir. Vertical spread of all IS groups on fir was similar. In spite of between-strain-within-IS group variation in vertical spread on each host, the study provided strong evidence for the occurrence of intraspecific differences in the host preference. In the interspecific analysis with three hosts, the isolates, IS groups and host × strain and host × IS group interactions accounted for most of the explained variation, while host-plants accounted for the smallest portion of the variance.

Additional key words: between-strain-within-hosts variation, host preference, intersterility groups, vertical spread in sapwood, wounding methods

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Introduction

Heterobasidion annosum (Fr.) Bref. consists of three intersterility (IS) groups showing different preferences to host trees (Korhonen 1978, Capretti et al. 1990). The main host of the P group is *Pinus sylvestris* L. of all ages, but it also inhabits some other plants including *Picea abies* (L.) Karst. By contrast, the S group is common on Norway spruce and only occasionally infects pines and other trees in most parts of Europe, North America and Asia (Morrison and Johnson 1999, Dai and Korhonen 1999). The F group was mainly found on *Abies alba* (Mill.) in southern and central Europe (Capretti et al. 1990, Korhonen et al. 1998, Łakomy 1996, Kowalski and Łakomy 1998, Łakomy et al. 2000).

Various inoculation methods were applied to study virulence of *H. annosum* and host resistance in the past. Since root inoculation, due to low infection frequency, is less successful (Rishbeth 1951, Boyce

1962, Werner and Łakomy – unpublished), most of the infection experiments used the direct wounding methods. Criteria for assessing the pathogenicity of the fungus and host resistance in these methods are the fungal spread in the wood and/or length of necrosis in the inner bark (Dimitri 1963; Delatour 1982; Stenlid and Swedjemark 1988). In these methods, trees in age of 2 to 100 years were used. Using this method, Dimitri (1969a, 1969b) found a correlation between the rate of infection and the depth of injury. Infection rate and extension of living mycelium in stems were usually higher in spruce than in pine.

Since intergroup hybrids are rare in nature (Garbelotto et al. 1996), and isolates of different IS groups show the ability to occur on the same host species, especially when colonize dead wood, the host specialization within *H. annosum* seems not to be strict. Considering the different host preferences of the IS groups occurring in nature and a high variability between isolates within IS groups, an attempt has been made to explain the phenomenon in term of different origin and/or saprobic and pathogenic potential of the fungal strains (Stenlid and Swedjemark 1988). However, in the study by Swedjemark et al. (1999), no significant difference in fungal growth was found between stump and tree isolates. Moreover, in long-term inoculation experiments, reisolations of the pathogen are usually unsuccessful due to strong host response to wounding and the measured virulence may be only an artifact of the inoculation experiments (Delatour 1982). These induce the studies on new methods of evaluation of the strain virulence and host reaction. Recently, variation in virulence between strains within the IS groups and the different host preferences of these groups expressed in mortality rate of *in vitro* grown pine and spruce seedlings was described by Werner and Łakomy (in press). Most of strains were more virulent on spruce than on pine, and mortality of spruce seedlings was significantly higher. The P strains displayed similar virulence on both hosts, while S strains caused higher mortality of spruce and significantly lower mortality of pine seedlings. Non-significant differences found between the effects of S and F isolates on pine, and of P and F isolates on spruce could indicate a lack of preference to non-host plants. Despite of higher mortality of spruce seedlings inoculated with F isolates when compared to pine, the host × F-group isolates interaction factor was not significant. Virulence of the P- and S-group isolates on pine and spruce in the *in vitro* inoculation experiments was similar to that observed on four-year-old pines and spruces described by Stenlid and Swedjemark (1988) and Swedjemark et al. (1999), when wounding method was used.

The objectives of the study were (i) to assess variation in aggressiveness in each of P-, S-, and F-IS group and differences in host preference between the three IS groups on four-year-old seedlings of *Pinus sylvestris*, *Picea abies* and *Abies alba* inoculated after wounding in the field; (ii) to estimate the contribution of pathogen and host-plant in spread of mycelium in stem sapwood of *P. sylvestris*, *P. abies* and *A. alba*; (iii) to evaluate the effect of wounding on the host reaction.

Materials and methods

Plants and fungi

Three-year-old trees of *P. sylvestris*, *P. abies* and *A. alba* from forest nursery (Przedborów Forest District, Poland) were planted in Polish Academy of Sciences Experimental Forest (Zwierzyniec) one year before inoculation.

Strains of *H. annosum*, four each of the P-, S- and F-IS groups selected for the study are described in Table 1. Isolates were assigned to P, S, and F intersterility groups based on their ability to heterokaryotize homokaryotic known tester mycelia (Korhonen 1978).

Inoculation procedures

Narrow wound (about 10 mm long) was made with a sterile knife (experiment 1), or round hole (3 mm in diameter), was made with a sterile drill (experiment 2) in stems of each plant (about 5 cm above the root collar) in Aprill 1999, 2000, respectively. Beech dowels colonized by *H. annosum* mycelium were inserted into the wounds which next were protected with Parafilm. The sterile beech dowels were used in control. Each *H. annosum* strain × host treatment was replicated ten times. Both experiments were of randomized block design.

Sampling and pathogen reisolation

Six months after inoculation, the plants were removed from soil and the extent of necrosis above and below the inoculum point was measured. When analysing the lesion length, all dead seedlings were excluded. Stems selected at random were surface sterilized in 2% $HgCl_2$ and devided into 5 mm thick discs with a sterile knife. Discs were incubated in Petri dishes under humid conditions and then checked for the anamorph of *H. annosum*: *Spiniger meineckellus* (Olson) Stalpers.

Statistical analysis

One- and two-way analysis of variance, based on individual data of the extent of necrosis, and Tukey's HSD test were conducted using statistical analysis software Statistica PL 1997 (StatSoft Polska Inc., USA). Data on percentage of mortality and incidence of infection were transformed before the analysis of variance according to arcsin $\sqrt{\text{percentage}/100}$ formula of C. I. Bliss (Snedecor and Cochran 1976).

Table 1. Origin of strains

Isolate No.	IS group	Year of collection	Locality (Forest stand)	Latitude	Longitude	Host
P-95107	P	1995	Klotyldzin, Poland	52°75'N	16°73'E	*P. sylvestris* (dead young tree)
P-Bor	P	1994	Borówiec, Poland	52°19'N	16°71'E	*P. sylvestris* (dead young tree)
P-Ka2	P	1994	Klęka, Poland	52°09'N	17°24'E	*P. sylvestris* (stump)
P-96092	P	1996	Klotyldzin, Poland	52°75'N	16°73'E	*P. sylvestris* (dead young tree)
S-96054	S	1996	Nowy Targ, Poland	49°25'N	20°05'E	*P. abies* (stump)
S-96057	S	1996	Sucha, Poland	49°40'N	19°27'E	*P. abies* (stump)
S-96043	S	1996	Białowieża, Poland	52°50'N	23°32'E	*P. abies* (stump)
S-95050	S	1995	Sucha, Poland	49°40'N	19°27'E	*P. abies* (stump)
S-96060*	S	1996	Węgierska Górka, Morońka, (Poland)	42°42'N	19°15'E	*P. abies* (stump)
F-94139	F	1994	Węgierska Górka, Morońka, (Poland)	42°42'N	19°15'E	*A. alba* (log)
F-97006	F	1997	Ojcowski National Park	50°13'N	19°50'E	*A. alba* (log)
F-97005	F	1997	Ojcowski National Park	50°13'N	19°50'E	*A. alba* (log)
F-96062	F	1996	Węgierska Górka, Morońka, (Poland)	42°42'N	19°15'E	*A. alba* (dead tree)

* used in experiment 2, instead of S-95050

Results

Incidence of infection and mortality rate

Mean incidence of infection was high in both experiments (Figs. 1A and 2A). The P isolates infected 96.67% and 91.97% of all seedlings of *P. sylvestris*, *P. abies* and *A. alba* in experiment 1 and experiment 2, respectively. Corresponding frequencies of S-group infections were 90.67% and 86.67%, respectively. The F isolates infected 92.67% of seedlings in the experiment 1 and 87.50% in the experiment 2. On pine, the incidence of infection was higher after inoculation with P and F isolates than after inoculation with S isolates. On spruce, the incidence of infection was the highest after inoculation with P isolates and the lowest after inoculation with F isolates. On fir, the incidence of infection was high and similar after inoculation with isolates of all the IS group in the experiment 1. Most firs were infected with F isolates in the experiment 2.

More spruces (35%) than pines (10%) died during the experiment 1. The differences in the host mortality were statistically significant at the 5% level. Mortality of spruces after inoculation with S an P isolates was similar (15 and 12.5%, respectively), whereas 10% of pines died after inoculation with P isolates (Fig. 1B). In the second experiment, 5% of pines died after inoculation with each of the P-, and F-group isolates, whereas only 2.5% after inoculation with S isolates (Fig. 2B). Mortality of spruces inoculated with P and S isolates was similar (2.5%). Mean mortality of firs after inoculation with P isolates was 4.5% and with F isolates 2.5%. The differences in mortality rates were not statistically significant.

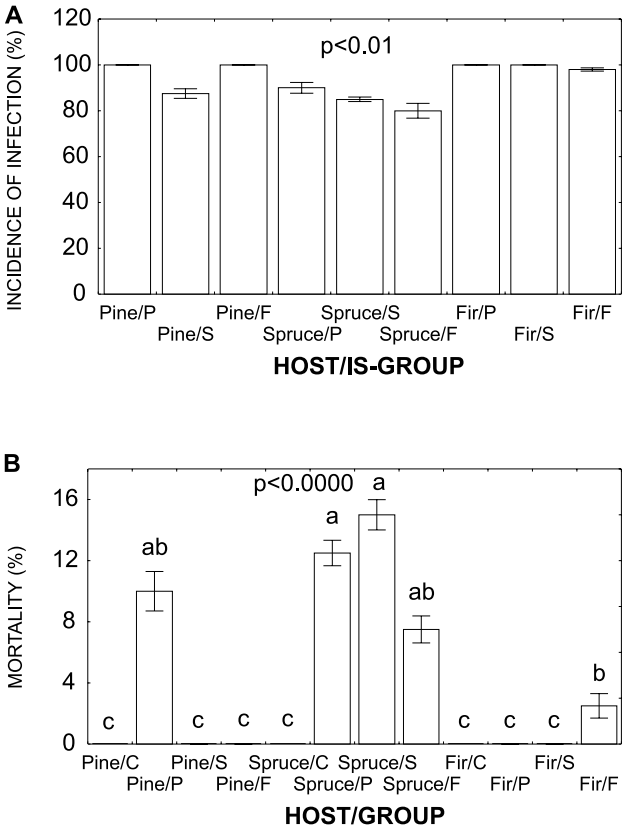

Fig. 1. Incidence of infection (A) and mortality (B) of Scots pine, Norway spruce and common fir after inoculation with isolates of the P-, S- and F-IS groups of *Heterobasidion annosum* in experiment 1. Means designated by the same letter did not differ significantly at 5% level using Tukey's HSD test. Bars indicate standard error (n=40)

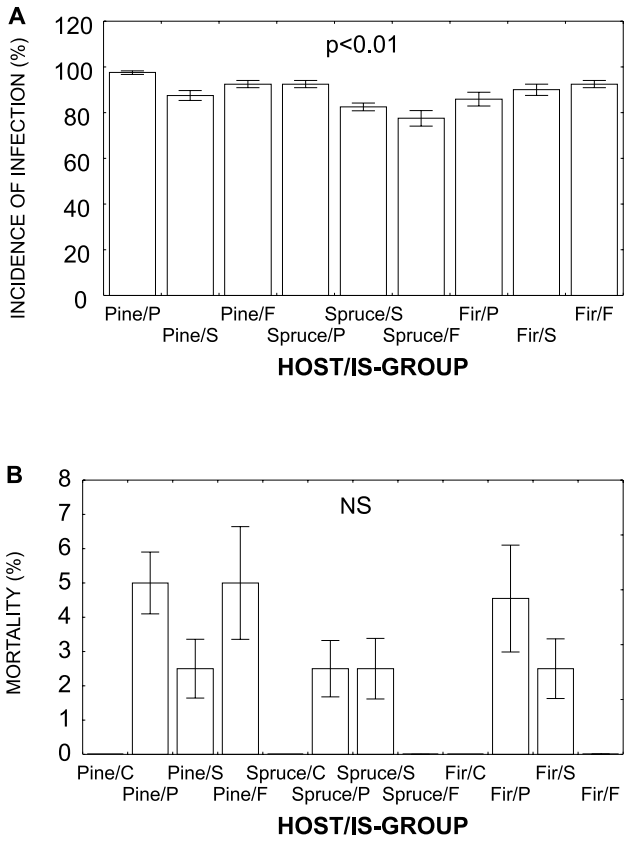

Fig. 2. Incidence of infection (A) and mortality (B) of Scots pine, Norway spruce and common fir after inoculation with isolates of the P-, S- and F-IS groups of *Heterobasidion annosum* in experiment 2. Means designated by the same letter did not differ significantly at 5% level using Tukey's HSD test. Bars indicate standard error (n=40)

Variation in vertical spread of *H. annosum* in stems

In experiment 1, mean mycelial growth on pine, spruce and fir inoculated with all isolates was 45.02, 52.22 and 50.13 mm, respectively. Corresponding values in the second experiment were 9.81, 11.71 and 10.72 mm. Spread of mycelium in pine, spruce and fir stems infected with several P, S and F isolates in experiment 2 is illustrated on Figures 3, 4 and 5.

All isolates of *H. annosum* varied significantly in vertical spread when tested on pine ($p<0.0001$) and on spruce and fir ($p<0.01$) in the experiment 1. In the experiment 2, the differences between strains in vertical spread were significant when tested on pine and spruce ($p<0.001$). On fir the differences were insignificant ($p=0.0796$). Differences in vertical spread between isolates, separately for the P-, S- and F-IS groups on pine, spruce and fir, and between the three IS groups within each host in both experiments is presented in Table 2.

Results of the analysis of variance for vertical spread of *H. annosum* strains of the P-, S-, and F-IS groups in pine, spruce and fir stems based on the data of the experiment 1 are presented in Table 3, and on the data of the experiment 2 in Table 5. In both experiments there were significant differences between

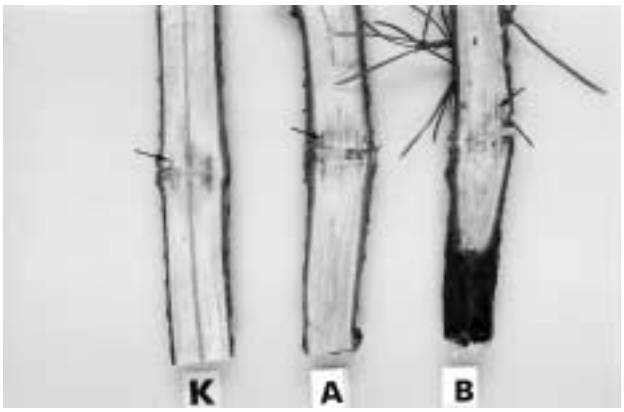

Fig. 3. Necroses in pine stems caused by wounding and subsequent vertical spread of *Heterobasidion annosum* (arrows). From left: control (K), P-96092 (A) and P-95107 (B) in experiment 2

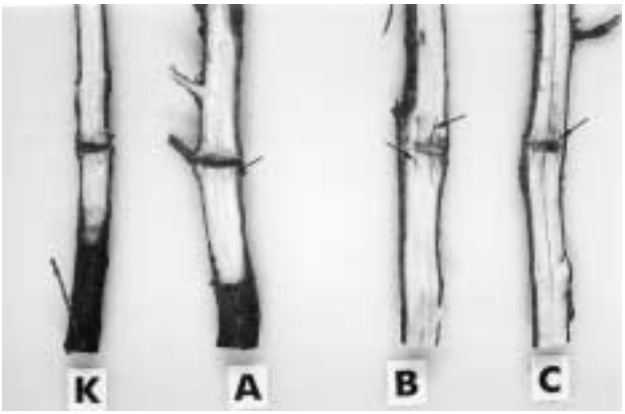

Fig. 4. Necroses in spruce stems caused by wounding and subsequent vertical spread of *Heterobasidion annosum* (arrows). From left: control (K), F-94139 (A), S-96043 (B) and P-96092 (C) in experiment 2

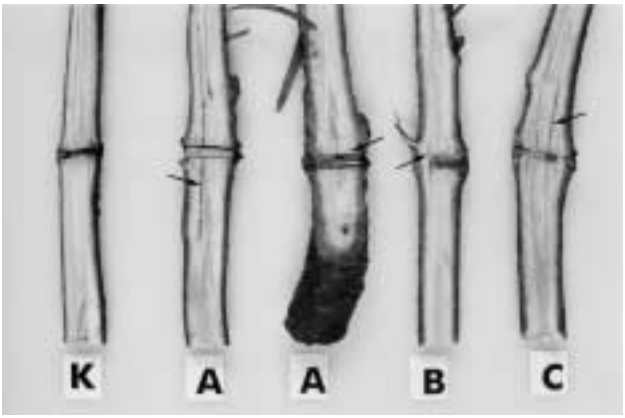

Fig. 5. Necroses in fir stems caused by wounding and subsequent vertical spread of *Heterobasidion annosum* (arrows). From left: control (K), F-96062 (A), S-95050 (B) and P-95107 (C) in experiment 2

Table. 2. Analysis of variance probabilities ($p<F$) for vertical spread of *Heterobasidion annosum* in stems of Scots pine, Norway spruce and common fir

Experiment 1			
Source of variation	Pine	Spruce	Fir
Between P isolates	NS	NS	0.0091
Between S isolates	NS	NS	NS
Between F isolates	0.0124	NS	NS
Between IS groups	0.0000	0.0001	NS
Experiment 2			
Source of variation	Pine	Spruce	Fir
Between P isolates	NS	NS	NS
Between S isolates	NS	0.0167	0.0149
Between F isolates	0.0000	0.0032	NS
Between IS groups	0.0105	0.0015	NS

hosts ($p<0.05$) and between strains ($p<0.001$). In the interspecific tests with three hosts and three IS groups, the differences between host plants were not statistically significant (Tables 4 and 6, Figs. 6A and 7A). In contrast to the experiment 1 (Table 4, Fig. 6B), there were no significant differences between IS groups in the experiment 2 (Table 6, Fig. 7B). Higly significant host × IS group factor ($p<0.001$) in the both experiments suggests the existence of host preference, most distinct among P isolates to pine (Figs. 6C and 7C) and S and P isolates to spruce (Figs. 6D and 7D). The components of variance presented in Tables 3 and 5, show that more of the variance in the vertical spread of the fungus was attributable to the strain effect than to the host-plant effect. Of the explained variance (i. e., nonerror variance), 31.86% (in the experiment 1) and 37.72 (in the experiment 2) came from strain, while the host-plant effect ac-

Table 3. Analysis of variance of vertical spread of *Heterobasidion annosum* strains representing P, S and F intersterility groups in stems of pine, spruce and fir in experiment 1

Source of variation	df	Mean square	F-value	p-value	% total variation	% explained variation
Host-plant	2	1602.01	3.1421	0.0446	1.5115	5.4760
Strain	11	1694.72	3.3239	0.0002	8.7944	31.8608
Host-plant × Strain	22	1666.57	3.2687	0.0000	17.2966	62.6632
Error	301	509.85			72.3975	
Total	336	211975.32				

Table 4. Analysis of variance of vertical spread of *Heterobasidion annosum* for host-intraspecific specialization by three IS groups on pine, spruce and fir in experiment 1

Source of variation	df	Mean square	F-value	p-value	% total variation	% explained variation
Host-plant	2	1573.70	2.8822	0.0574	1.4678	8.9069
IS group	2	5212.81	9.5473	0.0000	4.8622	29.5036
Host-plant × IS group	4	5440.93	9.9651	0.0000	10.1499	61.5895
Error	328	545.99			83.5201	
Total	336	214423.73				

Tabela 5. Analysis of variance of vertical spread of *Heterobasidion annosum* strains representing P, S and F intersterility groups in stems of pine, spruce and fir in experiment 2

Source of variation	df	Mean square	F-value	p-value	% total variation	% explained variation
Host-plant	2	104.52	3.338	0.0369	1.7869	7.1266
Strain	11	100.59	3.213	0.0004	9.4584	37.7228
Host-plant × Strain	22	73.53	2.349	0.0008	13.8282	55.1051
Error	301	31.30			74.9264	
Total	336	11698.14				

Table 6. Analysis of variance of vertical spread of *Heterobasidion annosum* for host-intraspecific specialization by three IS groups on pine, spruce and fir in experiment 2

Source of variation	df	Mean square	F-value	p-value	% total variation	% explained variation
Host-plant	2	91.98	2.618	0.0745	1.5515	17.1338
IS group	2	91.66	2.609	0.0752	1.5460	17.0732
Host-plant × IS group	4	173.61	4.942	0.0007	5.9578	67.7930
Error	328	35.13			90.9446	
Total	336	11845.14				

counted for the smallest portion. Of the remaining explained variance, the largest component came from the host × strain and the host × IS group interactions in the both experiments. As just as in the interspecific test of three hosts by twelve isolates, the IS groups accounted for higher portion of the explained variance

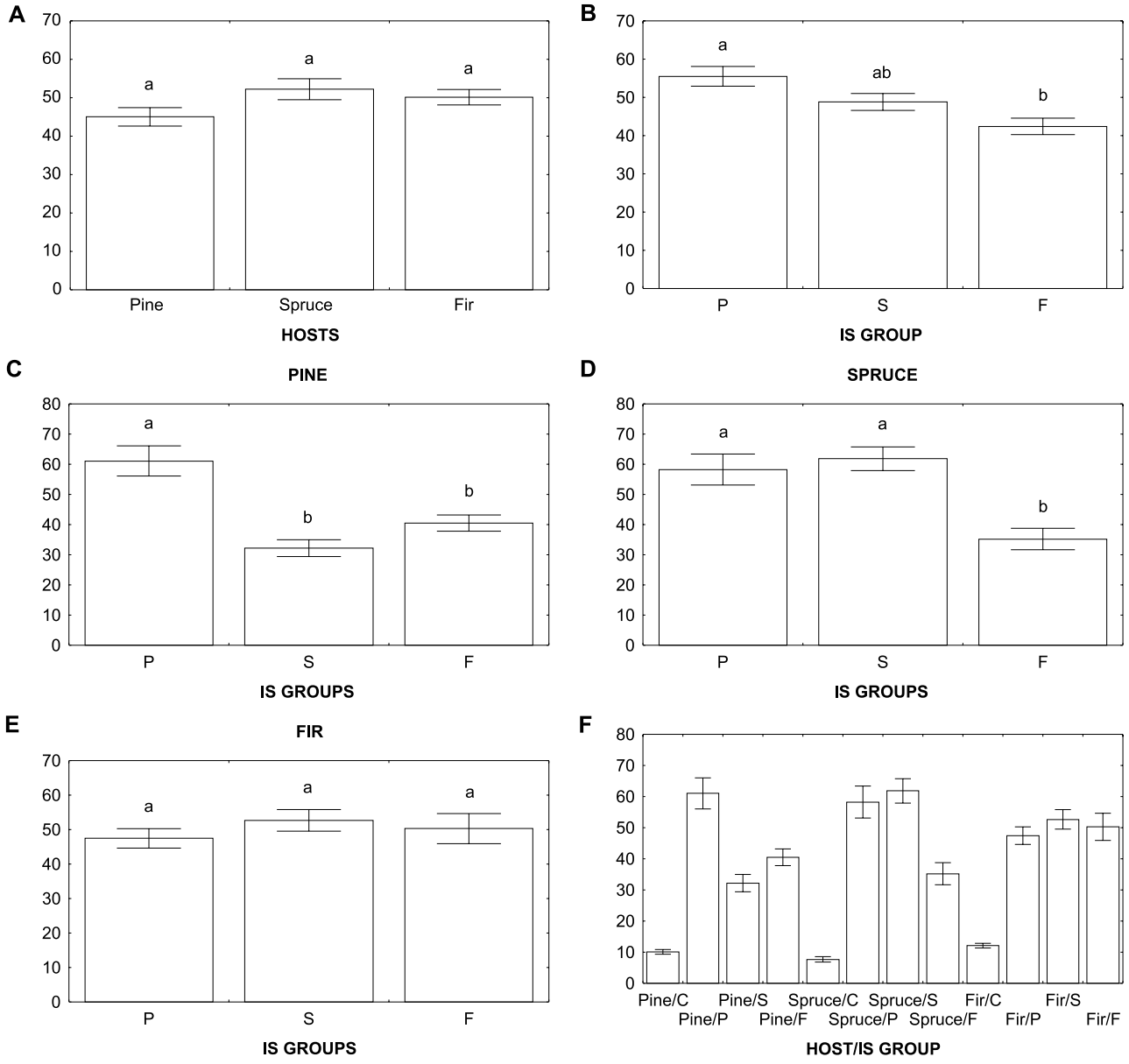

Fig. 6. Mean length of necrosis in stems of pine, spruce and fir after inoculation with twelve strains representing three IS groups of *Heterobasidion annosum* (A), in host-trees caused by the three IS groups (B), caused by each of the P-, S- and F-IS group in pine (C), spruce (D) and fir (E), and length of necrosis caused by vertical spread each of the P-, S- and F-IS group in three hosts in comparison with control (F) in experiment 1.
Means designated by the same letter did not differ significantly at 5% level using Tukey's HSD test. Bars indicate standard error (n=40–120)

in the experiment 1 (29.50%) (Table 4), whereas in the experiment 2, the host and IS group effects were similar (Table 6). In the both experiments there were no significant differences in vertical spread of the three IS groups in stems of fir (Figs. 6E and 7E). Mean length of necroses in stems of pines, spruces and firs caused by wounding and those caused by wounding and subsequent spread of mycelia of the IS groups are presented on figures 6F and 7F.

In P-group isolates, only one (P-95107) showed a higher aggressiveness on spruce in both experiments (Figs. 8 and 9). Strain P-96092 was the most aggressive on pine only in the experiment 1 (Fig. 8). Generally, the effect of the P isolates on pine and spruce was similar in the both experiments. On fir, P isolates were less aggressive. Almost all S isolates showed the similar aggressiveness on spruce and fir in the experiment 1, while with the exception of S-96057, they were more aggressive on spruce than on pine and fir in the experiment 2 (Fig. 9). Two of the F isolates (F-94139 and 97005) strongly invaded fir, while other two spread in sapwood of pines and spruces to small extent.

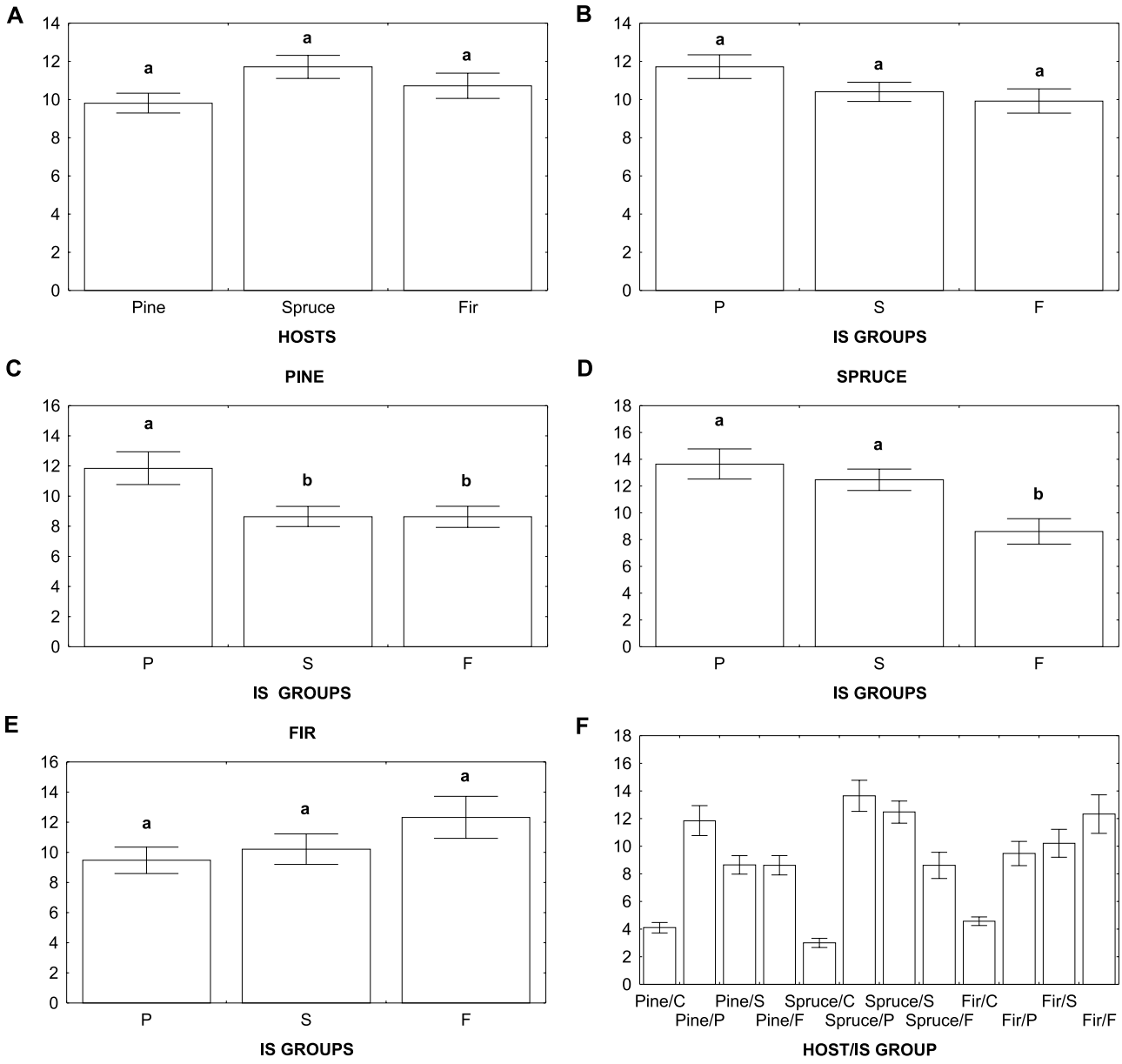

Fig. 7. Mean length of necrosis in stems of pine, spruce and fir after inoculation with twelve strains representing three IS groups of *Heterobasidion annosum* (A), in host-trees caused by the three IS groups (B), caused by each of the P-, S- and F-IS group in pine (C), spruce (D) and fir (E), and length of necrosis caused by vertical spread each of the P-, S- and F-IS group in three hosts in comparison with control (F) in experiment 2.
Means designated by the same letter did not differ significantly at 5% level using Tukey's HSD test. Bars indicate standard error (n=40–120)

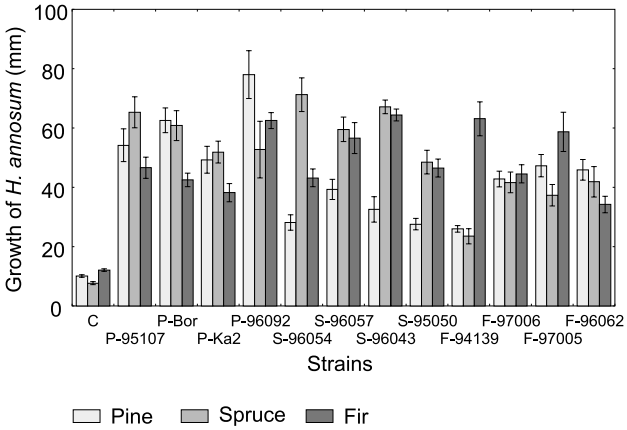

Fig. 8. Mean length of necrosis in stems of pine, spruce and fir after inoculation with twelve isolates of three IS groups of *Heterobasidion annosum* in comparison with control in experiment 1. Bars indicate standard error (n=10)

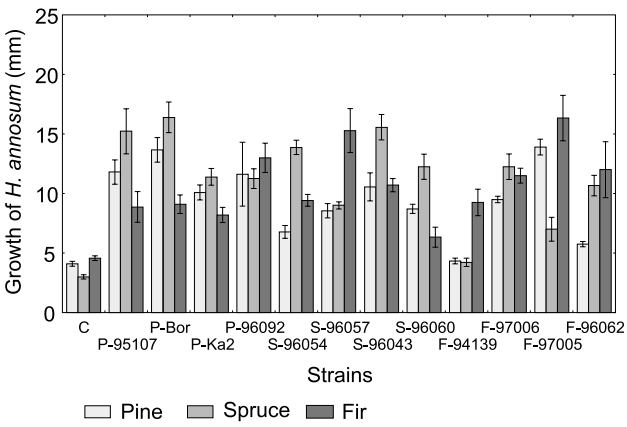

Fig. 9. Mean length of necrosis in stems of pine, spruce and fir after inoculation with twelve isolates of three IS groups of *Heterobasidion annosum* in comparison with control in experiment 2. Bars indicate standard error (n=10)

Discussion

Although reisolations of the pathogen six months after inoculation were only sporadically successful, the length of the dry zone formed in sapwood is recognized as host response to toxic compounds and hyphal growth (Coutts 1976, Johansson and Stenlid 1985) and was used as criterium for assessing the strain virulence and host susceptibility (Stenlid and Swedjemark 1988; Swedjemark et al. 1999). The similar symptoms, however, might be related with the host reaction to wounding. In pine which is more resistant to infection by *H. annosum*, the hypersensitive reaction, followed by formation of protective "barriers" and increased synthesis of resin are involved in sealing off the pathogen and rejection of the infected tissues (Werner 1993, 2001; Werner and Idzikowska 2001). The same non-specific reactions are involved in the restorative and wound healing processes (Shain 1979; Biggs et al. 1983). The difference in the length of necroses in the both experiments were mostly due to different inoculation procedures. The small wounds made with a drill in the experiment 2, caused probably a weaker reaction of the hosts than a rough wounding with a knife, used in the experiment 1. The highest mortality of spruces (more susceptible than pines) in the experiment 1, may be due to the rough wounding method and subsequent extensive infection. On the other hand, higher mortality of pines in the experiment 2, most probably was a result of a weaker host reaction to wounding and consequently a higher success of infection than in the experiment 1. Nevertheless, the results of the two experiments are not contradictory.

Results of the presented experiments confirm the occurrence of host preference in *H. annosum* complex and are in an accordance with the results of greenhouse infection experiments on four-year-old pines and spruces carried out by Swedjemark et al. (1999), and with findings of Werner and Łakomy (in press) on mortality of pines and spruces inoculated *in vitro* with strains of the three IS groups. In all the studies, the host preference was the most distinct among S-group isolates to spruce. The P isolates attacked aggressively the both hosts, whereas the S-group isolates showed limited growth on pine. The similar vertical spread of the three IS-group isolates in fir sapwood suggests the potential ability of all the isolates to spread in fir. Significantly slower mycelial growth of the F-group isolates in stems of pines and spruces and a low mortality of pine and spruce seedlings inoculated with F isolates *in vitro*, compared to results obtained with P and S isolates, (Werner and Łakomy – in press) may be due to saprobic properties of F group observed in nature (Capretti et al. 1990; Łakomy 1996; Łakomy et al. 2000).

All P isolates showed similar vertical growth on spruce and pine. Two of them, isolated from dead trees (P-950107 and P-Bor), were more aggressive on spruce. By contrast, three of four F isolates originating from logs varied in aggressiveness towards fir. Paradoxically, the less aggressive was the one isolated from a log of dead tree. Since all the S isolates were isolated from stumps, their different vertical spread in living trees seems not to be related with their origin, and consequently could not be explained in term of their different pathogenic and saprobic potential. The assumption about a higher pathogenicity of isolates originated from trees was not also confirmed in the study by Swedjemark et al. (1999) on S isolates from stumps and trees of spatially limited population.

The objective of our study was not only to assess the differences in ability to spread in sapwood of three hosts between *H. annosum* IS group-isolates to confirm their different host preference but also to

find out how much of the variance in the intraspecific specialization of the fungus was attributable to the host, strain and IS group effect. In both intraspecific analysis with three hosts and twelve isolates, hosts accounted for the smallest portion of explained variance. The strain effect was the next source of the explained variance and the interaction host × strain was the highest component of the variance. The analysis of variance with three hosts and twelve isolates split-up by three IS groups had the same pattern with the exception of similar effects of host-plant and strain in the experiment 2. While the significant host × strain and host × IS group interactions are strong evidence for the occurrence of intraspecific variation in the host preference, a higher component of variance attributable to strain effect may be explained in terms of higher variability in *H. annosum* complex and low variability in reaction to infection of the three hosts.

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References

Biggs, A.R., Davis, D.D., Merrill, W. 1983. Discussion: response bark tissues to injury and infection. Canadian Journal of Forest Research 14: 351–356.

Boyce, J.S. 1962. Greenhouse inoculations of coniferous seedlings with *Fomes annosus*. Phytopathology 52:4.

Capretti, P., Korhonen, K., Mugnai, L., Romagnoli, C. 1990. An intersterility group of *Heterobasisidion annosum*, specialized to *Abies alba*. European Journal of Forest Pathology 20: 231–240.

Coutts, N.P. 1977. The formation of dry zones in the sapwood of conifers. I. Induction of drying in standing trees and logs by *Fomes annosus* and extracts of infected wood. European Journal of Forest Pathology 6: 372–381.

Dai, Y-C., Korhonen, K. 1999. *Heterobasidion annosum* group S identified in north-eastern China. European Journal of Forest Pathology 29: 273–279.

Delatour, C. 1982. Behavior of *Fomes annosus* in the stem of Norway spruce and in the laboratory. *In* Resistance to disease and pests in forest trees. *Edited by* H.M. Heybreoek, B.R. Stephan, K. von Weissenberg. PUDOC, Wageningen. pp. 268–274.

Dimitri, L. 1963. Versuche über den Einfluss von *Fomes annosus* (Fr.) Cooke auf koniferenkeimlige. Phytopathologische Zeitschrift 49(1): 41–60.

Dimitri, L. 1969 a. Ein Beitrag zur Infektion der Fichtenwurzel durch den Wurzelschwamm *Fomes annosus* (Fr.) Cooke. Forstwissenschaftliches Centralblatt 88: 72–80.

Dimitri, L. 1969 b. Untersuchungen über die unterirdischen Eintrittspforten der wichtigsten Rotfäuleerreger bei der Fichte *Picea abies* (Karst.) Forstwissenschaftliches Centralblatt 88: 281–308.

Garbelotto, M., Ratcliff, A., Burns, T.D., Cobb, F., Otrosina, W. J. 1996. Use of taxon-specific competitive-priming PCR to study host specifity, hybridization and intergroup gene flow in intersterility groups of *Heterobasidion annosum*. Phytopathology 86: 543–551.

Johansson, M., and Stenlid, J. 1985. Infection of roots of Norway spruce (*Picea abies*) by *Heterobasidion annosum*. I. Initial reactions in sapwood by wounding and infection. European Journal of Forest Pathology 15: 32–45.

Korhonen K. 1978. Intersterility groups of *Heterobasidion annosum*. Communicationes Instituti Forestalis Fenniae 94(6): 1–25.

Korhonen, K., Capretti, P., Karjalainen, R., Stenlid, J. 1998. Distribution of *Heterobasidion annosun* intersterility groups in Europe. *In Heterobasidion annosum*: biology, ecology, impact and control. *Edited by* S. Woodward, J. Stenlid, R. Karjalainen, A. Hüttermann A. CAB INTERNATIONAL, University Press Cambridge. pp. 93–104.

Kowalski, T., Łakomy, P. 1998. A new record of *Heterobasidion annosum* (Fr.) Bref. F group occurrence in Poland in connection with interesting mycological findings. Phytopathologia Polonica 15: 49–55.

Łakomy, P. 1996. F group of *Heterobasidion annosum* found in Poland. European Journal of Forest Pathology 26: 217–222.

Łakomy, P., Kowalski, T., Werner, A. 2000. Preliminary report of *Heterobasidion annosum* (Fr.) Bref. intersterility groups distribution in Poland. Acta Mycologica 35: 303–309.

Morrison, D.J, Johnson, A.L.S. 1999. Incidence of *Heterobasidion annosum* in precommercial thinning stumps in coastal British Columbia. European Journal of Forest Pathology 29: 1–6.

Rishbeth, J. 1951. Observation on the biology of *Fomes annosus*, with particular reference to East Anglian pine plantations. (II) Spore production, stump infection and saprophytic activity in stumps. Annals of Botany 15(57): 1–27.

Shain, L. 1979. Dynamic responses of differentiated sapwood to injury and infection. Phytopathology 69: 1143–1147.

Snedecor, W., Cochran, W.G. 1976. Statistical methods. 6th edition. Ames, Iowa, USA. The Iowa State University Press. pp. 327–329.

Stenlid, J., Swedjemark, G. 1988. Differential growth of S- and P-isolates of *Heterobasidion annosum* in *Picea abies* and *Pinus sylvestris*. Transections of the British Mycological Society 90(2): 209–213.

Swedjemark, G., Johannesson, H., Stenlid, J. 1999. Intraspecific variation in *Heterobasidion annosum* for growth in sapwood of *Picea abies* and *Pinus sylvestris*. European Journal of Forest Pathology 29: 249–258.

Werner, A. 1993. Pathological anatomy of thin woody roots invaded by *Heterobasidion annosum* (Fr.) Bref. Arboretum Kórnickie 38: 114–129.

Werner, A. 2001. Growth of *Heterobasidion annosum* (Fr.) Bref. through bark of one-year-old *Pinus sylvestris* seedlings in pure culture. Dendrobiology 46: 65–73.

Werner, A., Idzikowska, K. 2001. Host/pathogen interaction between Scots pine seedlings (*Pinus sylvestris* L.) and the P strains of *Heterobasidion annosum* (Fr.) Bref. in pure culture. Acta Societatis Botanicorum Poloniae 70: 119–132.

Werner, A., Łakomy, P. 2002. Intraspecific variation in *Heterobasidion annosum* for mortality rates on *Pinus sylvestris* and *Picea abies* seedlings grown in pure culture (Mycologia – in press).