Temperature requirements for germination of conidiospores and growth of mycelia of *Heterobasidion annosum* s.s. and *Heterobasidion parviporum*

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ABSTRACT

Different temperature requirements of the two species *Heterobasidion annosum* (Fr.) Bref. (P group) and *H. parviporum* Niemelä & Korhonen (S group) should explain why the distribution areas are different in Scandinavia despite of a wide range of distribution of their host species *Picea abies* and *Pinus sylvestris* up to 68°N. Temperature ranges of P and S were observed. Germination percent, length of the germination tubes of the conidiospores and growth rate of the mycelia were measured for 6 individual strains of the S and P group, respectively. When sampled, mycelial strains of the S group showed a significantly faster growth rate at 22°C compared to the strains of the P group. Germination percent and length of the germination tubes of the conidiospores were insignificant at all temperatures. In conclusion, growth rate of the S group increased with temperature until 27°C and increase of the growth rate of the P group stagnated after 22°C. The S group had a wider temperature range than the P group.

Key words: conidiospores, germination, growth, *Heterobasidion annosum*, *H. parviporum*, temperature

ZUSAMMENFASSUNG

Die zwei Arten *Heterobasidion annosum* (Fr.) Bref. (S Gruppe) und *Heterobasidion parviporum* Niemelä & Korhonen (P Gruppe) der Gattung *Heterobasidion* wurden auf ihre unterschiedlichen Temperaturansprüche hin untersucht. Besonderes Augenmerk lag dabei auf der Keimfähigkeit der Konidiosporen, der Länge der Keimschläuche und der Wachstumsrate des Myzels. Sechs Temperaturen (2°, 7°, 12°, 17°, 22° und 27°C) und jeweils sechs Individuen pro Art wurden untersucht.

Die unterschiedlichen Temperaturansprüche der beiden Arten sollten Aufschluss auf die räumlich begrenzte Verbreitung in Skandinavien geben. Dabei wurde angenommen, dass Individuen der S Gruppe einen höheren Toleranzbereich gegenüber kalten Temperaturen aufweisen und somit schneller unter kalten Bedingungen wachsen können. Individuen der P Gruppe treten nur bis zu einer bestimmten geografischen Grenze auf, obwohl ihr Wirt (*Pinus sylvestris L.*) auch über diesen Bereich hinaus vorkommt.

Die Ergebnisse ergaben, dass *H. annosum* (Fr.) Bref. (S) einen stetigen Anstieg der Wachstumsrate unter allen untersuchten Temperaturen zeigte. Hingegen bei Individuen der P Gruppe (*H. parviporum*) nur bis 22°C eine Steigerung ihrer Wachstumsrate messbar war. Insgesamt, war eine signifikant höhere Wachstumsrate der S Gruppe bei einer Temperatur von 22°C nachweisbar. Untersuchungen über die Keimfähigkeit der Konidiosporen, sowie die Länge der Keimschläuche waren bei allen Temperaturen insignifikant.

Die unterschiedlichen Temperaturansprüche der beiden Arten zeigen keine eindeutige Erklärung für die geografische Verteilung in Skandinavien auf. Dennoch haben die beiden Arten unterschiedliche Wachstumsoptima und Toleranzbereiche.

1. INTRODUCTION

Background

The root and butt rot causing basidiomycete *Heterobasidion spp.*, which belongs to the family *Bondarzewiaceae*, is a severe problem in conifer stands in the northern temperate climate zone (Arvidson, 1954; Hartig, 1874, 1877, 1889; Laine, 1976; Neger, 1917; Rennerfelt, 1945). From an economical point of view the losses to forestry per year amount to €790 million in Europe and in Sweden about €80 million (Woodward et al., 1998). That implies that 10-20% of the mature Norway spruce (*Picea abies* (L.) Karst) is infected in southern Scandinavia (Bendz-Hellgren & Stenlid, 1998), alike in the Baltic States about 20-50% (Vasiliauskas et al., 2001) and in Germany, Austria and Switzerland is found a similar situation (Graber, 1994).

Description of the genus

Heterobasidion spp. (*Basidiomycete*) is a fungus with perennial basidiocarps with cuticulate pilei, asperulate basidiospores, slightly dextrinoid and strongly cyanophilous skeletal hyphael, and a Spininger anamorph (Gilbertson & Ryvarden, 1986; Buchanan, 1988). The genus is included in the family *Polyporaceae* of the order *Aphyllophorales*, but recently DNA analyses favour the family *Bondarzewiaceae* (Stalpers, 1980; Jülich, 1981; Gluchoff-Fiasson et al., 1983; Redhead & Norvell, 1993; Hibbert & Donoghue, 1995). The genus includes six distinct taxonomic species: *H. annosum (Fr.) Bref., H. insulare* (Murr.) Ryv.*, H. araucariae* Buchanan*, H. pahangense* Corner*, H. Perplexum* (Ryv.) Stalpers*, H. rutilantiforme* (Murrill) Stalpers (Pegler & Waterston, 1998).

Definitions

H. annosum s.l. is not a single biological species. It consists of what was earlier regarded as five intersterility groups (ISG), the S, P and F group in Europe and the S and P group in North America. Since 1998 the intersterility groups have been referred to species in Europe as *Heterobasidion parviporum* Niemelä & Korhonen*, H. annosum* (Fr.) Bref. and *H. abietinum* Niemelä & Korhonen for the S, P and F group, respectively (Korhonen, 1978; Korhonen et al., 1989; Niemelä and Korhonen, 1998). Those groups can be distinguished, because of their host preferences, pore size and distribution area. Furthermore it is possible to identify them by mating tests like the somatic compatibility test or molecular biology technique like the TSCP-

PCR (taxon-specific competitive-priming Polymerase Chain Reaction) (Dai, 2002; Garbelotto et al., 1996). Within the thesis the short names of the species will be used (S and P).

The hosts and distribution of S and P in Europe

Heterobasidion spp. infects mostly spruces, pines, firs and larch trees, though also some broadleaved trees may be attacked, when they grow in mixed stands with pine or on a site where pine was growing before (Korhonen et al., 1992). In Europe, the intersterility groups of *Heterobasidion spp.* show different host preferences. The S group (Ig-S) is often found on Norway spruce trees (*P. abies*), where it mainly causes butt rot. The main hosts of the P group (Ig-P) are pine (*Pinus sylvestris* L.) and spruce trees. The F group, which is found in the middle of Europe, prefers firs (*Abies sp.*). The distribution areas of the European S and P group reaches over whole Europe depending on their main host occurrences. P, which follows chiefly *P. sylvestris*, has been recorded in most European countries (Fig.1) with exceptions in the coldest and driest pine forests in the north and south. However S occurs throughout the natural range of its host (Fig. 2) up to 68°N in Finland (Woodward, 1998).

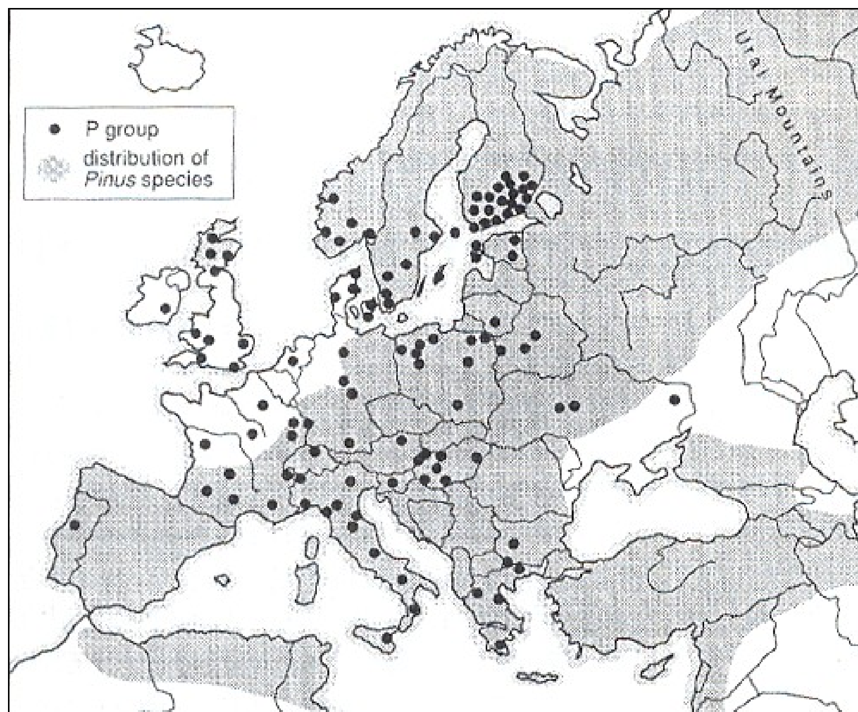

Figure 1. Distribution area of P and its host *P. sylvestris* in Europe. Figure kindly provided by Kari Korhonen.

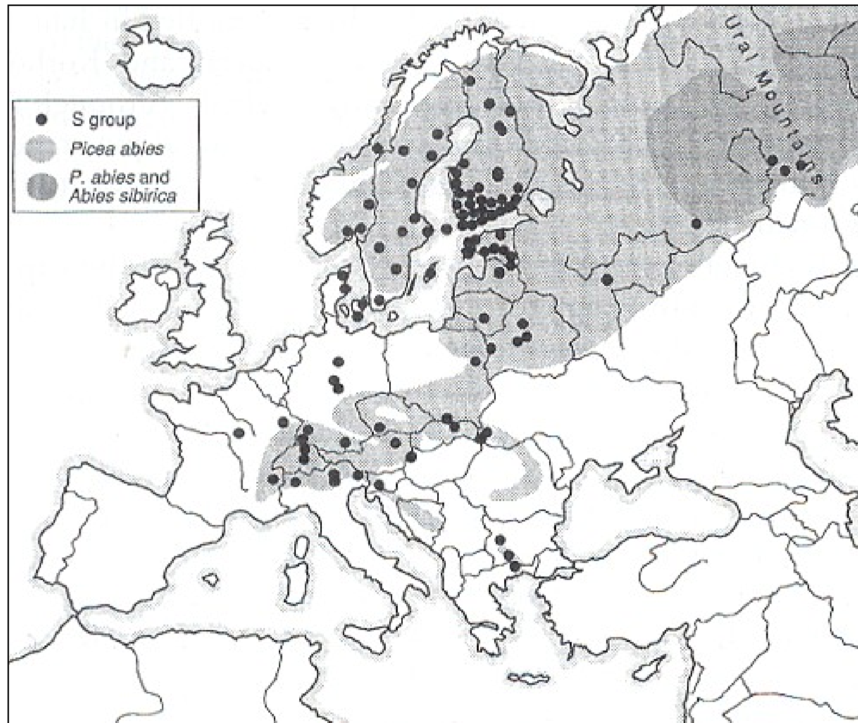

Figure 2. Distribution area of *H. parviporum* and its host *P. abies* in Europe. Figure kindly provided by Kari Korhonen.

Routes of infection

Starting points of infection are commonly after thinning or felling on freshly cut stumps and injuries at the roots by the germination and infection of airborne basidiospores (Isomäki & Kallio, 1974; Rishbeth 1951) and subsequently by vegetative growth of mycelia via root to root contacts. Conidiospores seem to be insignificant for spread, but have been recorded under the bark of stumps and dead trees, in beetle galleries, on stump tops and frequent on brash-covered stumps, which are the result of felling of decayed trees (Kallio, 1971; Rishbeth, 1957). Even though the two spore types seem to be similarly capable of infecting stumps (Piri, 1996; Swedjemark & Stenlid, 1993). Furthermore basidiocarps, which are located on the roots, the root collar on living or dead trees and on discarded logs (Rennerfelt, 1946; Schütt et al., 1979), are able to deposit 200.000 spores dm^{-2} h^{-1} (Kallio, 1970).

Forest management and climate in Scandinavia

Scandinavia (Sweden, Norway, Denmark and Finland) is situated between 55° and 70°N. The vegetation zone is temperate boreal in the north and hemi boreal and temperate in the south. Average temperatures are between -15° and 0°C from north Finland to Denmark in winter. In summer temperatures reach 10° to 17°C. Finland, Norway and the northern parts of Sweden have mostly temperatures below zero. In the northern parts the precipitation in winter is mostly snow and on average between 300 and 700 mm per year. In whole Scandinavia the

forest structure and climate is similar. Southern Sweden and Denmark differ from the northern parts, because there are mostly temperatures above zero. The area of forest is extensive in Finland, Norway and Sweden with mostly conifers and planted in large areas of monocultures. In contrast to Denmark, where is less forest than in the rest of Scandinavia. Indigenous species are *P. abies* and *P. sylvestris*. Other coniferous trees are *Larix deciduas* MILL., *Picea sitchensis* (BONG.) CARR. and *Abies* species. Broadleaves are *Betula pendula* ROTH (important in Finland, Norway and Sweden), *Fagus sylvatica* L. and *Quercus robur* L. (only in Denmark and in southern Sweden and Norway) (Bendz-Hellgren et al., 1998).

Forest management in Scandinavia is carried out with two or three thinnings in a rotation period. In all Nordic countries the main economic goal is to produce rot-free timber for the sawing and pulping industries. Manual harvesting of timber and pulpwood took place traditionally in the wintertime and also decayed wood could be sold due to the use of chlorine bleaching in the old times. Nowadays expensive harvesting machines are used all-season to remain competitive and stump treatments against root rot are recommended to use.

Sonesson (2004) described a great impact on forest ecosystem if the temperature increases in the future due to global warming. Species, which were common in more southern parts of Europe, could spread to the north. If temperature would be the limiting factor for the spread of *Heterobasidion spp.*, having a wide host range, it could attack indigenous tree species like pine or Siberian larch also in the north of Sweden (Berglund, 2005).

It has been figured out that the general rate of growth for the mycelia of *Heterobasidion spp.* is between 6,4 and 9,3 mm per day in the dark at an optimum temperature of 23°– 27°C. The conidiospores germinate in a similar temperature range after 3-4 days of germination in the darkness and after 24 hours in light (Schwantes et al., 1976).

The aim of this study was to detect the temperature requirements of two species of *Heterobasidion spp.* (P and S) with the intention of finding an explanation for the different distribution of S and P in Europe. Origin of this question was the missing spread of P further north (limit: 64°N in Scandinavia), despite of a host (*P. sylvestris*) range beyond, perhaps depending on the individual temperature requirements of the two species. Furthermore it was intended to work out if the growing behaviour of the two species is an appropriate method for the identification.

2. MATERIALS AND METHODS

2.1. Fungal isolates and experimental design

Experiment 1 – germination percent & length of the germ tubes of the conidiospores

Conidiospores of the fungal isolates of experiment 2 were used for the germination experiments.

Overgrown petri dishes from experiment 2 were used to isolate conidiospores for the germination experiments. With ionized water (5 ml) and an inoculation loop conidiospores were solved from the surfaces of the petri dishes. Those mixtures were collected in a sterile jar. Another 10 ml of ionized water were used with 5 ml each for washing the surface a second and a third time. The suspension was diluted with ionized water depending on the amount of conidiospores produced by each species (Table 2). Dilution series were used to get a sufficient spore concentration for the observations under the microscope. 750 µl of this suspension was transmitted to a new petri dish (16 ml Hagem agar). Two petri dishes for each isolate and temperature (2°, 7°, 12° and 17°C in darkness) were incubated at the same time. As soon as germ tubes started to grow obviously (visible young germ tubes) values were recorded. 30 randomly selected conidiospores of each individual of S and P were counted and the length of the germination tubes and germination percent measured.

Table 1. Fungal isolates of *Heterobasidion spp.* for experiment 1 and 2.

strain	year of inoculation	origin	country	tree host
108/1 P	1995	S. Colomba	Italy	*Pinus sylvestris*
165/1 P	1994	Sjaelland, Frederiksborg	Denmark	*Picea abies*
042/2 P	1998	Voronenezh, Zhivotinovskii	Russia	*Pinus sylvestris*
021/6 P	2005	Lazio Viterbo	Italy	*Pinus strobus*
126/3 P	1994	Inkoo	Finland	*Juniperus sp.*
076/6 P	1999	Frentino, Vigolo Vattaro	Italy	*Picea abies*
036/3 S	1998	Hämeenlinna	Finland	*Picea abies*
146/3 S	2005	Vitebskaja obl.	Belarus	*Picea abies*
057/2 S	1999	Kollari, Ylläs	Finland	*Picea abies*
251/1 S	1996	Kirkkonummi, Meiko	Finland	*Picea abies*
024/4 S	2000	Trentino, Lavarone	Italy	*Picea abies*
037/7 S	1998	Hämeenlinna	Finland	*Picea abies*

Table 2. Added ionized water for the dilution of the suspension of conidiospores depending on the production of conidiospores.

strain	added ionized water [ml]
042/2 P	10
021/6 P	10
126/3 P	85
036/3 S	10
057/2 S	85
251/1 S	85
024/4 S	10
037/7 S	85

Experiment 2 – growth rate of the mycelia

12 fungal isolates provided by Dr. Kari Korhonen of the P and S group were used. All of them were homocaryotic tester strains of a known species, which had a different behaviour of their growth rate. Six of those strains belonged to S and the other six to the P group (Table 1).
The 12 mycelia isolates of the two species (S and P) were grown under room temperature to create mother-cultures on a predefined amount of Hagem agar (24 ml, Table 2) in petri dishes (Stenlid, 1985). Inocula were prepared by small agar pieces, which have been drilled out from the mother-culture with a cork borer (diameter: 0,5 cm). The samples were incubated in two equal climatic chambers (versatile environmental test chamber (SANYO)) at 2°, 7°, 12°, 17°, 22°, 27 °C ± 0,1°C. Five petri dishes for each individual and temperature grew at the same time. Radial mycelial growth was surveyed along two axes at right angles to each other with a ruler.

2.2. Media & laboratory apparatus

For all experiments were used Hagem agar (Tab. 3) (Stenlid, 1985) and the following laboratory apparatus.

Table 3. Ingredients for Hagem agar (ph = 5,5) from MERCK.

chemicals	amount [g]
glucose	5,0
NH_4NO_3	0,5
KH_2PO_4	0,5
$MgSO_4$*7 aq	0,5
malt extract	5,0
agar	20,0
inonized water	approx.: 1 l

laboratory apparatus

- Ceramus® (Hirschmann Laborgeräte)
- Harvey SterileMax Steam Sterilizer (Barnstead International)
- CleanAir Techniek B.V. (PMV – particle measurement & validation) versatile environmental test chamber (SANYO)

2.3. Statistics

The statistical analyses were performed using the SAS computer program (SAS Institute, Cary, NC; statistical analysis system). Analysis of variance (ANOVA) was used to test the results with the GLM procedure and with the Tukey's Studentized Range Test in SAS. SAS was used to determine the relationship between the dependent variables (growth rate, germination percent and length of the germination tubes of the conidiospores) and the independent variable (temperature) based on the p-value.

3. RESULTS

3.1. Experiment 1 – germination of the conidiospores

Germination percent and length of the germ tubes of the conidiospores were measured after 19 hours at 17°C (Fig. 3, 8), 28,5 and 45 hours at 12°C (Fig. 4, 9), 72 hours at 7°C (Fig. 5, 10) and 138 and 162 hours at 2°C (Fig. 6, 11). Three P (108/1, 165/1, 076/6) and one S (146/3) species had a low spore production and for this reason they were excluded from the germination experiments. (Tab.4)

Table 4. Amount of conidiospores per petri dish for all strains of P and S species. Kindly provided by Mattias Berglund. The value for species 126/3 was not available.

strain	conidiospores/petri dish
108/1 P	53677,06
165/1 P	83497,64
042/2 P	1863000,00
021/6 P	567000,00
126/3 P	n.a.
076/6 P	83497,64
036/3 S	121500,00
146/3 S	243000,00
057/2 S	7047000,00
251/1 S	3645000,00
024/4 S	1336500,00
037/7 S	8586000,00
P mean	530134,47
S mean	3496500,00

3.1.1. Germination percent

Germination percent at 2°C were the lowest for both groups. P reached a mean of 30,11% and S of 30,32% after 162 hours (Fig. 6). At 7°C P obtained a value of 69,35% and S a value of 61,17% after 72 hours (Fig. 5), at 12°C it was 59,68% for P and 70,65% for S after 45 hours (Fig. 4) and after 19 hours at 17° germination percent amounted 39,25% for P and 65,16% for S (Fig. 3). See also table 6 for single results of each strain.

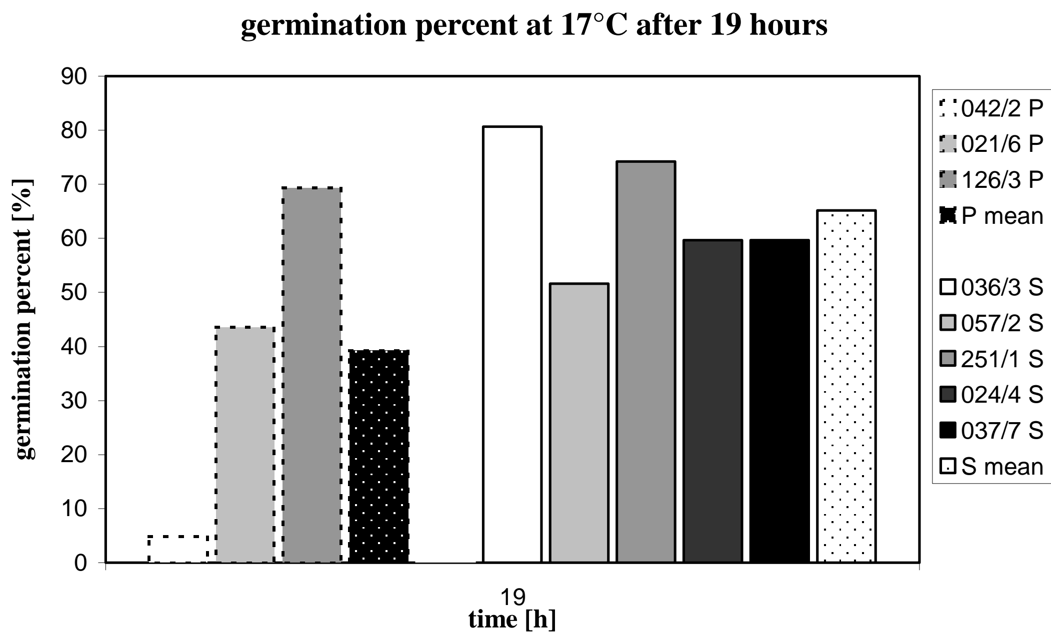

Figure 3. Germination percent of three P and five S strains after 19 hours of incubation at 17°C and the mean value of both species.

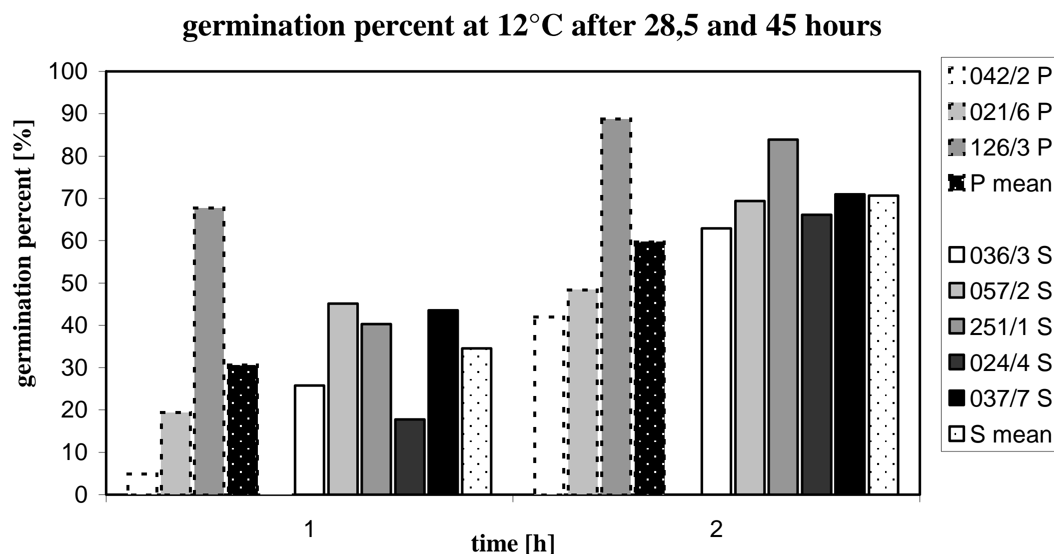

Figure 4. Germination percent of three P and five S strains after 28.5 and 45 hours of incubation at 12°C and the mean value of both species.

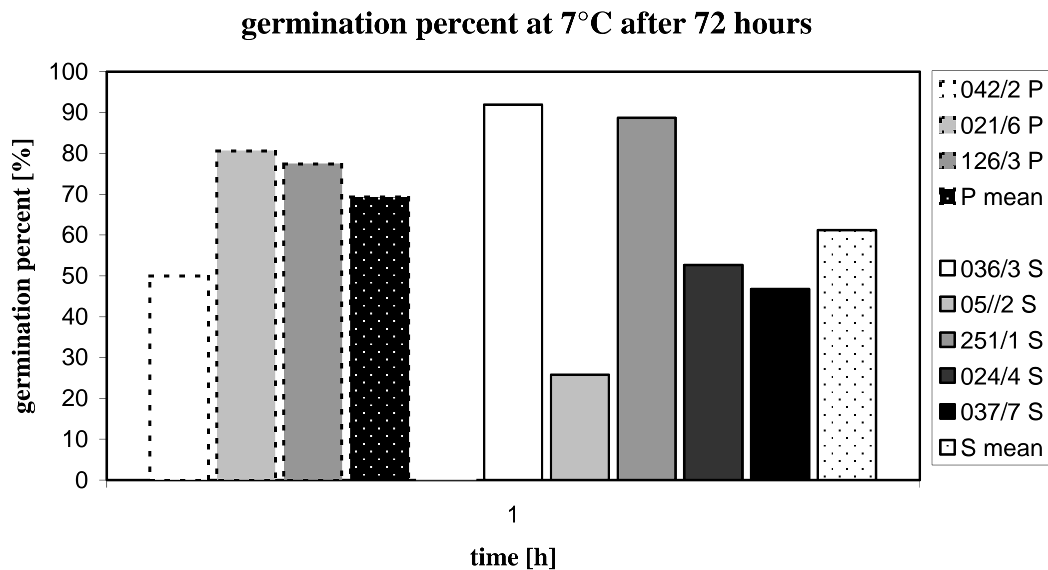

Figure 5. Germination percent of three P and five S strains after 72 hours of incubation at 7°C and the mean value of both species.

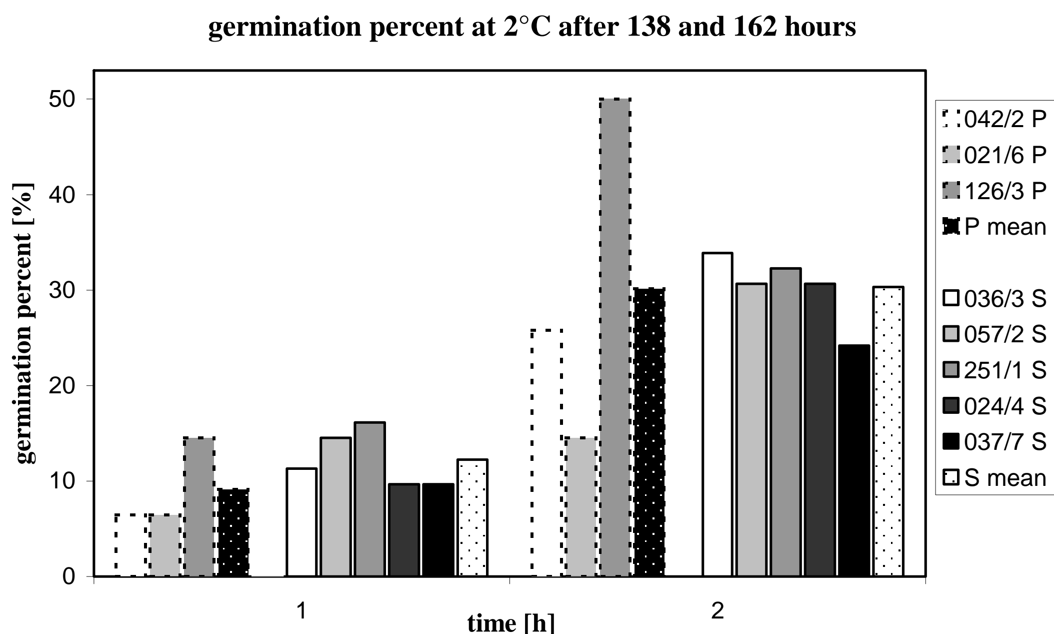

Figure 6. Germination percent of three P and five S strains after 138 and 162 hours of incubation at 2°C and the mean value of both species.

Table 5. Germination percent after 19 hours (17°C), 28,5/ 45 hours (12°C), 72 hours (7°C) and 138/162 hours (2°C) of three P and five S strains.

	germination percent at 2°, 7°, 12° and 17°C					
	2°C		7°C	12°C		17°C
species	138 h	162 h	72 h	28,5 h	45 h	19 h
042/2 P	6,45	25,81	50,00	4,84	41,94	4,84
021/6 P	6,45	14,52	80,65	19,35	48,39	43,55
126/3 P	14,52	50,00	77,42	67,74	88,71	69,35
036/3 S	11,29	33,87	91,94	25,81	62,90	80,65
057/2 S	14,52	30,65	25,81	45,16	69,35	51,61
251/1 S	16,13	32,26	88,71	40,32	83,87	74,19
024/4 S	9,68	30,65	52,63	17,74	66,13	59,68
037/7 S	9,68	24,19	46,77	43,55	70,97	59,68
S mean	12,26	30,32	61,17	34,52	70,65	65,16
P mean	9,14	30,11	69,35	30,65	59,68	39,25

Figure 7 plot the values of the germination experiment. Germination percent (Fig. 7) was not significantly affected by the different temperatures of strains of the S and P group.

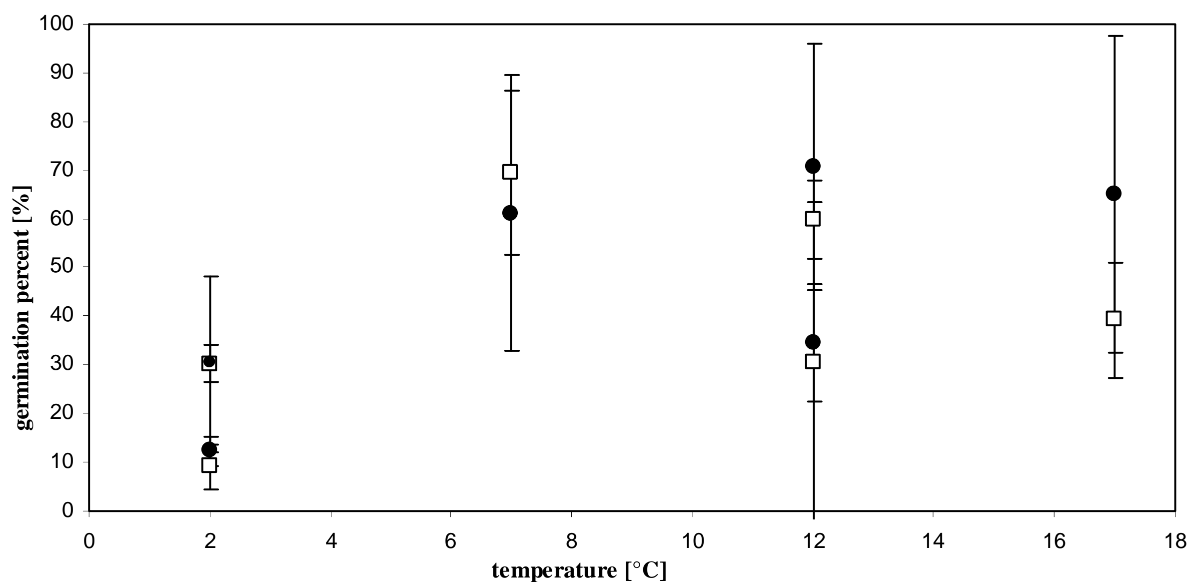

Figure 7. Mean values and standard deviations of the germination percent of the three P (white squares) and the five S (black circles) strains at 2° (138 and 162 h), 7° (72 h), 12° (28,5 and 45 h) and 17° (after 19 hours).

3.1.2. Length of the germination tubes

At 2°C P strains had a mean value of 0,0013 mm just like S strains after 138 hours. Furthermore after 162 hours at 2°C P mean was 0,0012 mm and S mean is 0,0014 mm (Fig. 11). 0,0025 and 0,0019 mm were the values for P and S at 7°C after 72 hours (Fig. 10) and 0,0013 and 0,0016 mm at 17°C after 19 hours (Fig. 8). Figure 9 shows the results for the S and P species, which were incubated at a temperature of 12°C for 28,5 and 45 hours. The mean value for S was 0,0015mm/0,0022 mm and for P 0,0016mm/0,0026mm, respectively (Table 6).

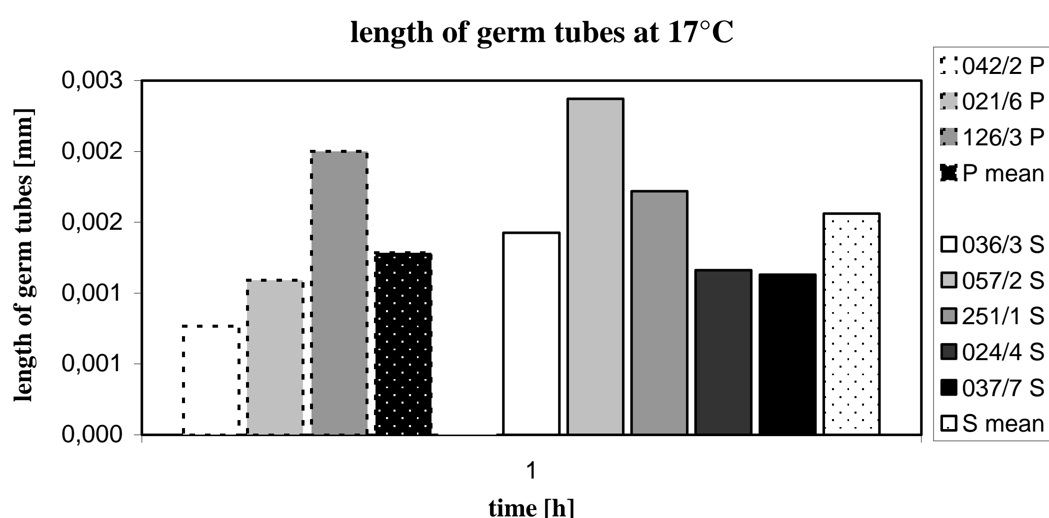

Figure 8. Length of germ tubes after an incubation of 19 hours at 17°C of three P and five S strains and the mean values of S and P species.

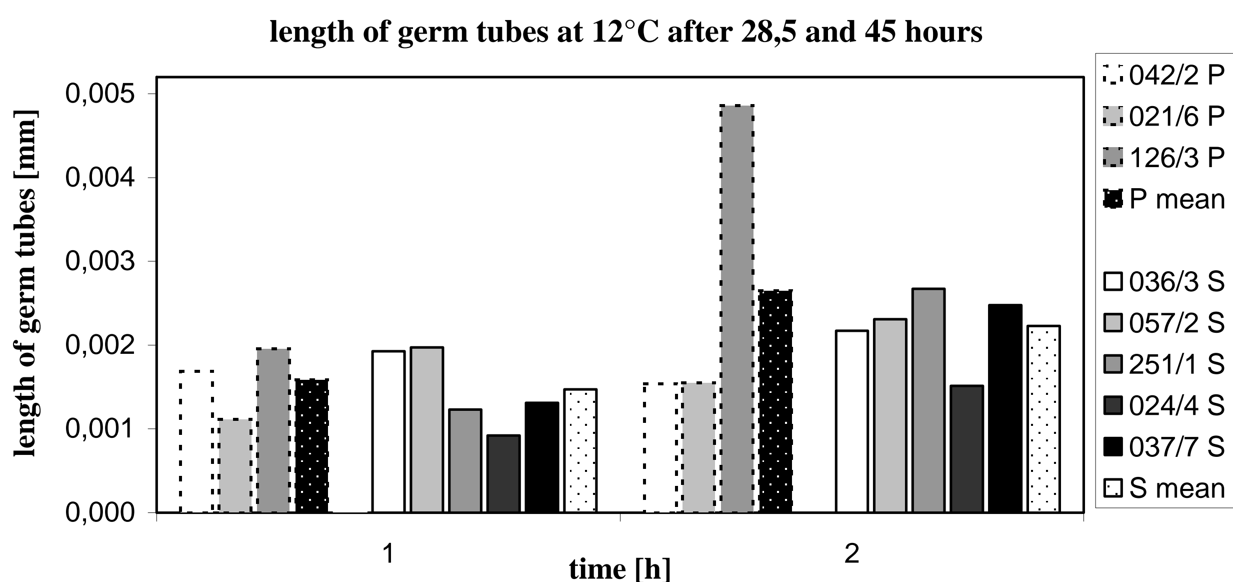

Figure 9. Length of germ tubes after an incubation of 28,5 and 45 hours at 12°C of three P and five S strains and the mean values of S and P species.

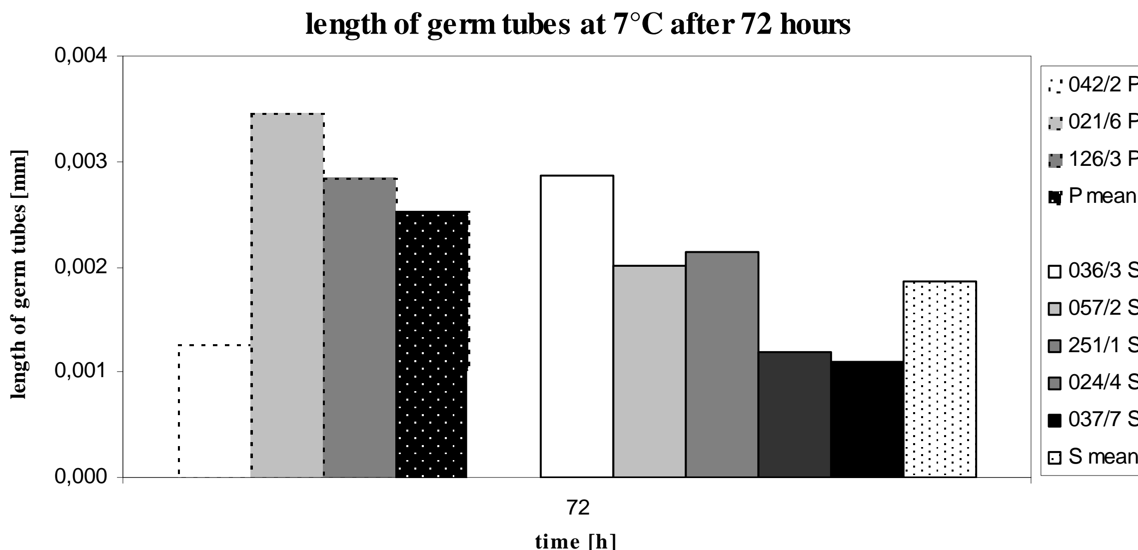

Figure 10. Length of germ tubes after an incubation of 72 hours at 7°C of three P and five S strains and the mean values of S and P species.

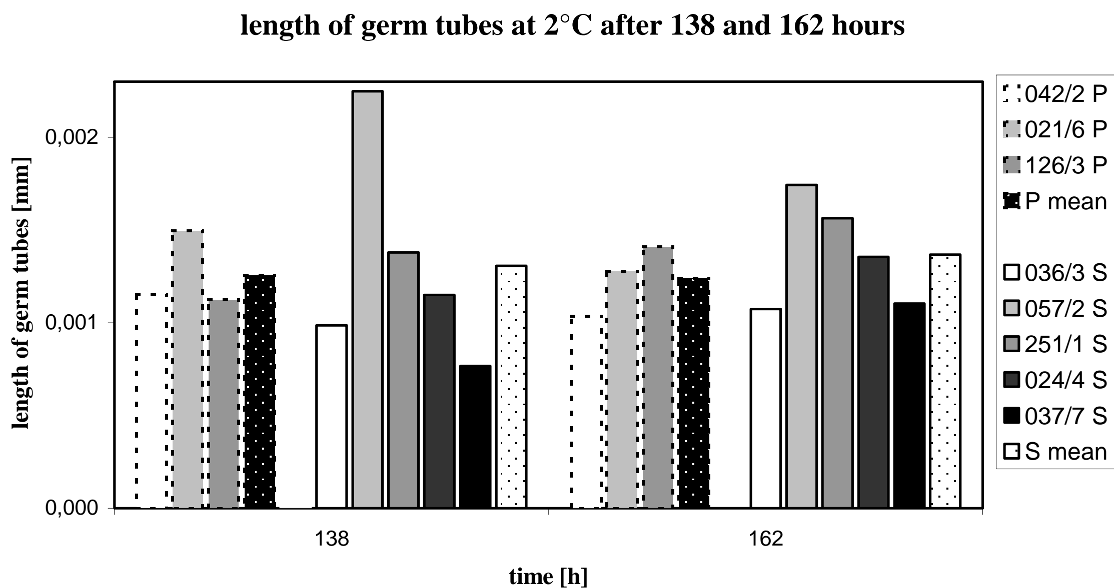

Figure 11. Length of germ tubes after an incubation of 138 and 162 hours at 2°C of three P and five S strains and the mean values of S and P species.

Table 6. Length of the germ tubes (mm) after 19 hours (17°C), 28,5/ 45 hours (12°C), 72 hours (7°C) and 138/162 hours (2°C) of three P and five S strains.

length of germ tubes at 2°, 7°, 12° and 17°C						
	2°C		7°C	12°C		17°C
species	138 h	162 h	72 h	28,5 h	45 h	19 h
042/2 P	0,0012	0,0010	0,0013	0,0017	0,0015	0,0008
021/6 P	0,0015	0,0013	0,0035	0,0011	0,0015	0,0011
126/3 P	0,0011	0,0014	0,0028	0,0020	0,0049	0,0020
036/3 S	0,0010	0,0011	0,0029	0,0019	0,0022	0,0014
057/2 S	0,0022	0,0017	0,0020	0,0020	0,0023	0,0024
251/1 S	0,0014	0,0016	0,0021	0,0012	0,0027	0,0017
024/4 S	0,0012	0,0014	0,0012	0,0009	0,0015	0,0012
037/7 S	0,0008	0,0011	0,0011	0,0013	0,0025	0,0011
P mean	0,0013	0,0012	0,0025	0,0016	0,0026	0,0013
S mean	0,0013	0,0014	0,0019	0,0015	0,0022	0,0016

Figure 12 plot the values of the germination experiments. Length of the germination tubes was not significantly affected by the different temperatures of the strains of S and P group (Fig. 12).

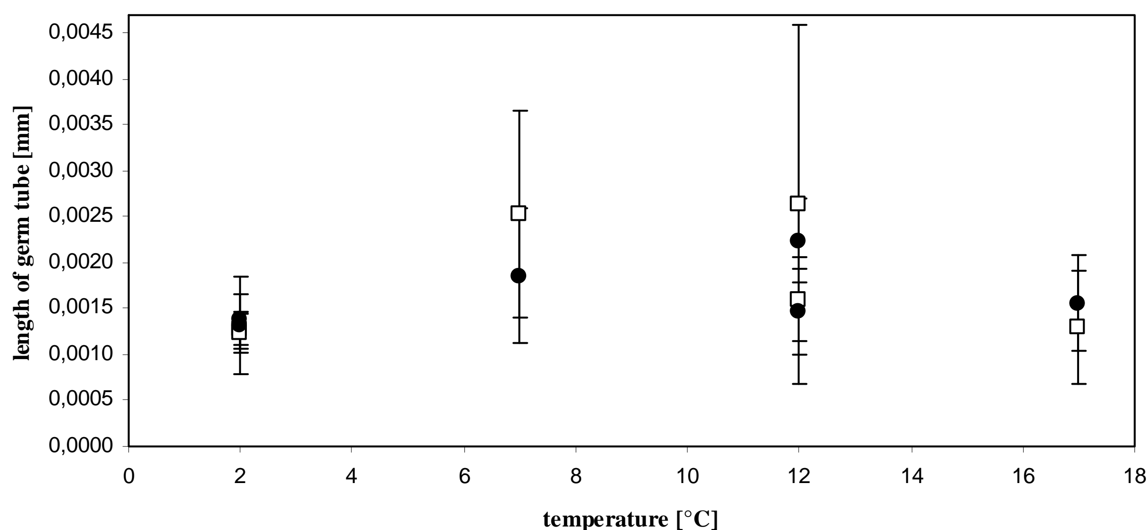

Figure 12. Mean values and standard deviations of the length of the germ tubes of the three P (white squares) and the five S (black circles) strains at 2° (138 and 162 h), 7° (72 h), 12° (28,5 and 45 h) and 17° (after 19 hours).

3.2. Experiment 2 – growth rate of mycelia

The growth of the mycelia of both species (S and P) showed a high individual variability among the different strains and species at all temperatures (Fig. 13). In general growing started after one day at 27° and 22°C, 2 days at 17°C, 3 days at 12°C, 5 days at 7°C and 13 days were needed to see hyphael growth on agar at 2°C (Fig. 14) in darkness. An increase of temperature resulted in an increase of the growth of mycelia. The mean values of the growth rate of the S group were always higher or similar compared with the mean values of the P group strains at all temperatures (Fig. 14).

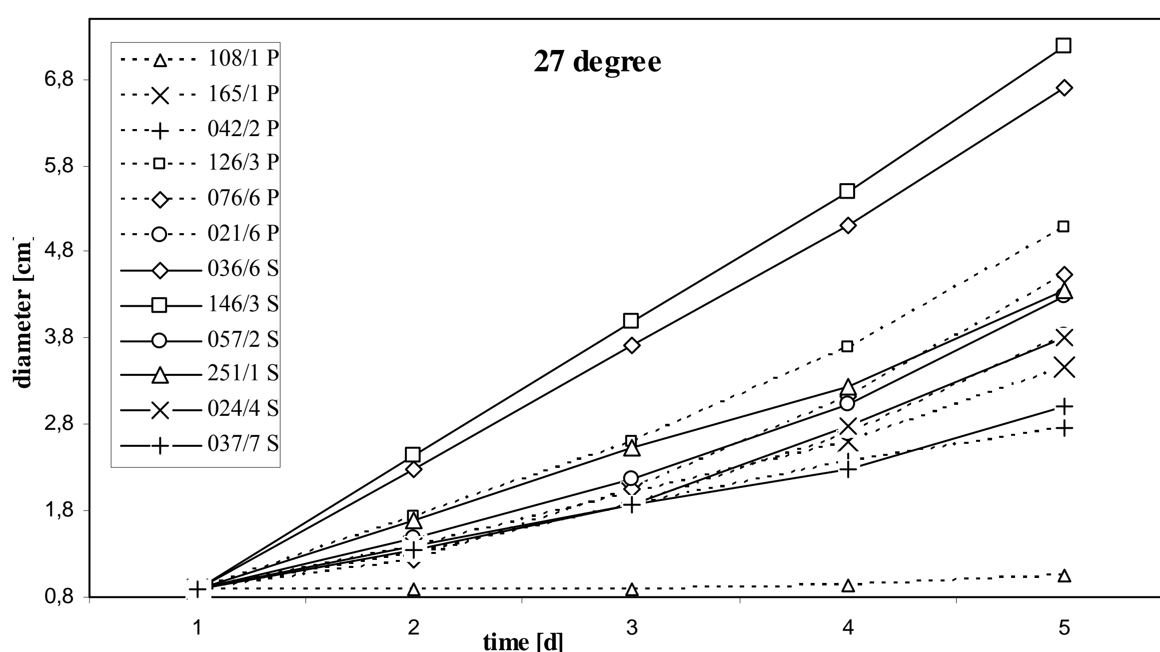

Figure 13. Mycelial growth at 27°C of all isolates of S (black line) and P (broken line) after five days of incubation in darkness.

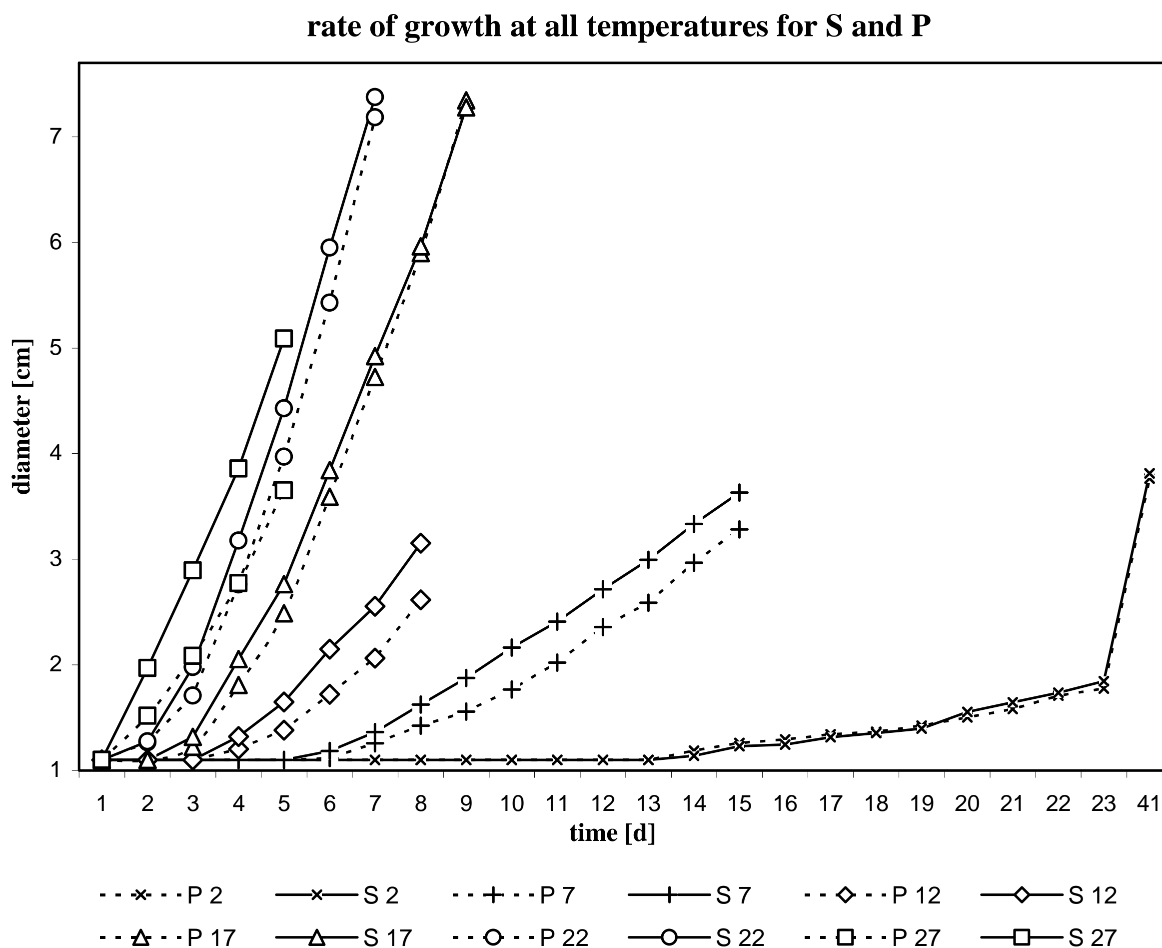

Figure 14. Mycelial growth of the S (black line) and P (broken line) species at 2°, 7°, 12°, 17°, 22° and 27°C for each day after inoculation.

An increase in temperature resulted in an increase of the growth rate until an optimum. The optimum for P was located at 17° and 22°C (3,68 mm/d) and for S at 27°C (5,42 mm/d) (Tab. 7). Slowest growth took place at 2°C for both groups (0,65 mm/d for S and 0,60 mm/d for P). Individuals of the P group reached their optimum temperature (17-22°C) earlier than individuals of the S group (27°C) (Fig. 15 and Fig. 16).

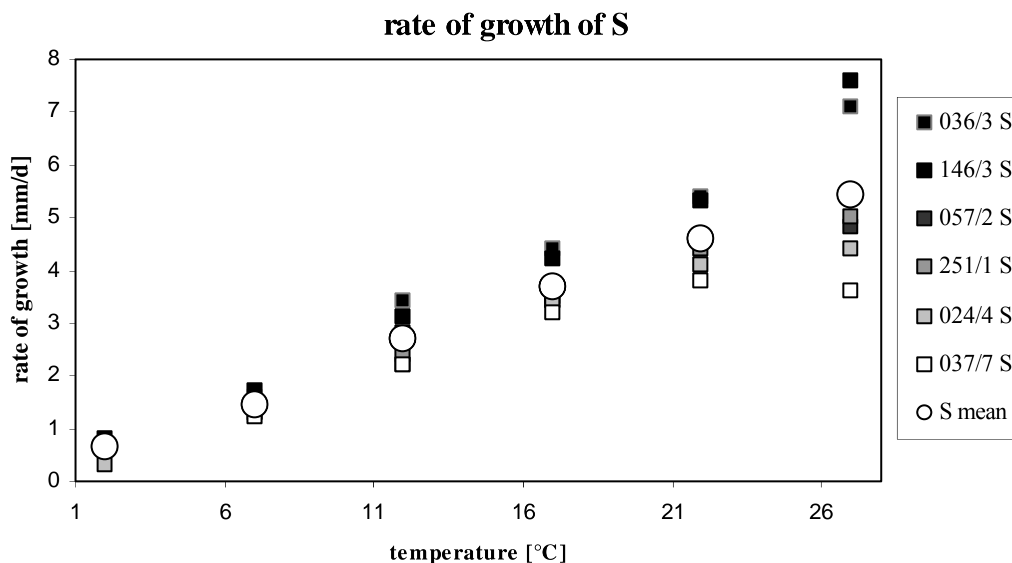

Figure 15. Rate of growth of the S species at 2°, 7°, 12°, 17°, 22° and 27°C. Each square is one strain and the circle is the mean value of species S.

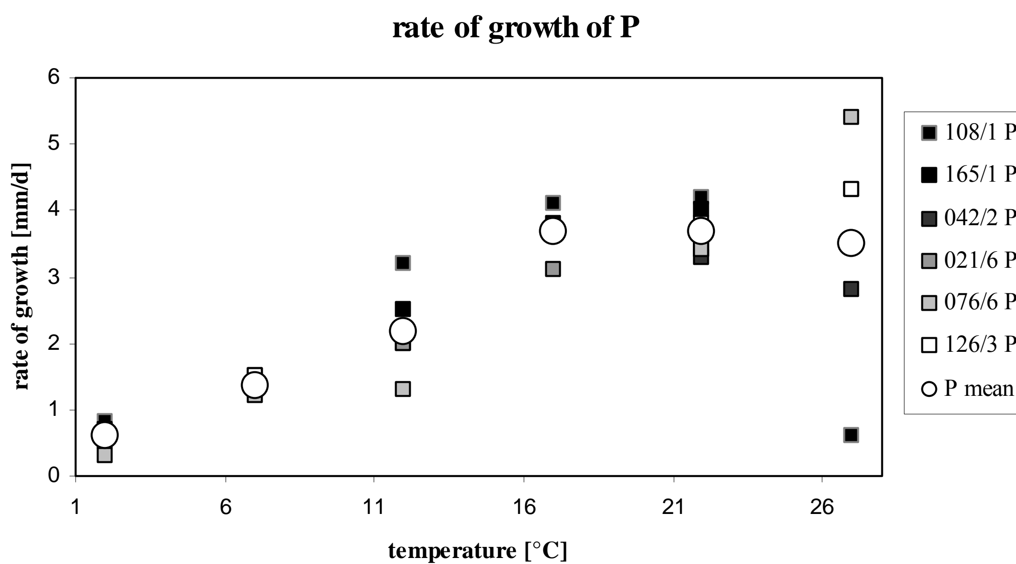

Figure 16. Rate of growth of the P species at 2°, 7°, 12°, 17°, 22° and 27°C. Each square is one strain and the circle is the mean value of species P.

Table 7. Rate of growth of the diameters of species S and P strains at 2°, 7°, 12°, 17°, 22° and 27°C and their means in mm per day.

	temperature					
strain	2	7	12	17	22	27
108/1 P	0,80	1,30	3,20	4,10	4,20	0,60
165/1 P	0,50	1,30	2,50	3,80	4,00	3,50
042/2 P	0,70	1,50	2,00	3,70	3,30	2,80
021/6 P	0,70	1,30	2,00	3,10	3,40	4,30
126/3 P	0,60	1,50	2,10	3,70	3,80	4,30
076/6 P	0,30	1,20	1,30	3,70	3,40	5,40
036/3 S	0,80	1,60	3,40	4,40	5,40	7,10
146/3 S	0,80	1,70	3,10	4,20	5,30	7,60
057/2 S	0,70	1,30	2,20	3,40	4,50	4,80
251/1 S	0,70	1,30	2,40	3,50	4,40	5,00
024/4 S	0,30	1,50	2,80	3,40	4,10	4,40
037/7 S	0,60	1,20	2,20	3,20	3,80	3,60
S mean	0,65	1,43	2,68	3,68	4,58	5,42
P mean	0,60	1,35	2,18	3,68	3,68	3,48

Statistical tests, performed by the SAS software, resulted in a significant faster growth of the S group at 22°C compared with the P group ($p < 0,0001$). Tukey's Studentized Range Test detected a significantly different Tukey Grouping of the S and P groups on the whole. Figure 17 shows the mean values and the standard deviations of all results.

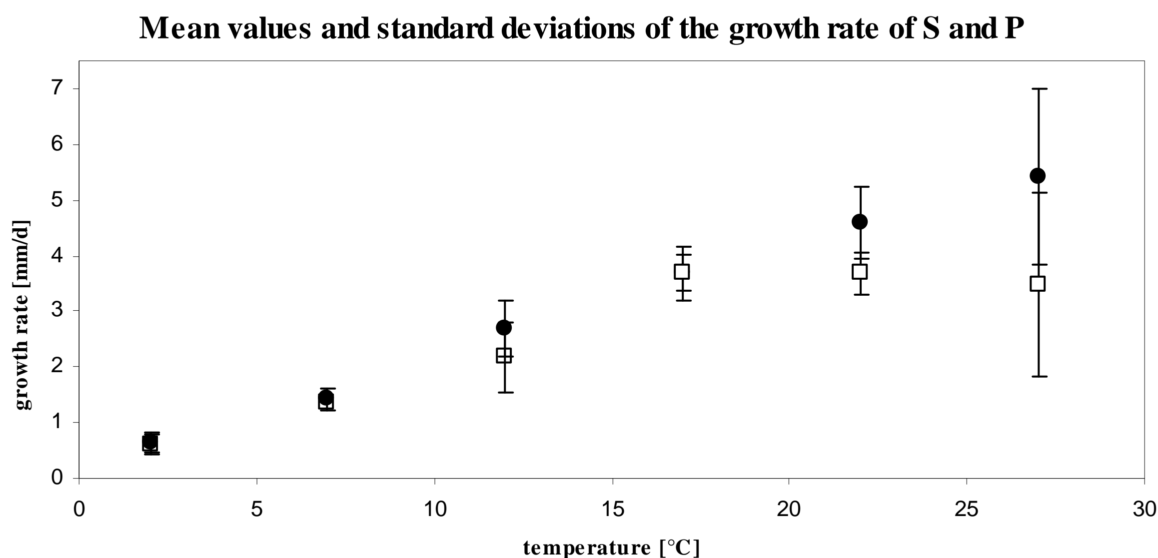

Figure 17. Mean values and standard deviations of the growth rate of all P (white squares) and S (black circles) strains at 2°, 7°, 12°, 17°, 22° and 27°C.

4. DISCUSSION

The results indicate that the temperature requirements of the two species *H. annosum* s.s. (P) and *H. parviporum* (S) were slightly different. Referred to the aim of this study, growing behaviour under different temperatures of the two species could not explain the missing spread in the north of Scandinavia of the P species. If temperature requirements are the only reason, why P is absent in north Scandinavia is not finally solved. There could be other fungi or abiotic factors, which outcompete individuals of the P species, restrict P to its range of distribution and interact with air temperature. Species are restricted by more then one environmental factor like air temperature, precipitation, food supply, predators, competitors and space and all of these factors could influence the incidence of the species. The interaction between temperature and other abiotic factors could explain the thesis over the different incidence of the two species S and P.

In the present study germination percent and length of the germination tubes were not significantly different between the two species S and P. S had however mostly a higher average germination percent and length of the germination tubes than individuals of P. For both species germination percent and length of germination tubes had high variation at all different temperatures due to too few individuals with enough conidiospores for the experiment as shown in table 4. In general germination percent and length of germination tubes have increased with temperature. In this study a minimum temperature at 2°C was obtained like in former studies already (Courtois, 1972). Maximum temperature was not observed. Temperature range of germination of conidiospores corresponds very well with temperature requirements of growth of mycelia (Courtois, 1971). Both species had a wide and similar range of temperature. This implies that germination may not be strictly independent of seasons, but has a range beyond the growing season and both species are able to infect stumps by conidiospores from already 2°C (Courtois, 1971).

Also germination tubes that grow faster have a higher chance to infect roots or stumps compared with all the other fungi or other genets. 35 different genets of *Heterobasidion spp.* were detected on one *P. abies* stump (Swedjemark & Stenlid, 2001). However interspecific or intraspecific competition between germinating conidiospores on timber wins the fastest growing individual (Swedjemark et al., 1999).

Former studies described that mycelia starts growing at a minimum temperature of 0°C and stops at a maximum of 32°-36°C (Schwantes, 1976). The optimum temperature according to Schwantes (1976) and Negrutsky (1994) may be between 24°-28°C. In the present study P reached the highest growth rate between 17°C and 22°C and S at 27°C in darkness. It is unknown if the maximum growth rate of strains of the S group would still increase by temperature above 27°C, but this was not included in the present work. S grew significantly quicker than P at 22°C in the present case and faster or similar at all other observed temperatures due to a higher rate of growth (table 7). In the present study a growth rate of 4,58 ± 0,64 mm/d for S and 3,68 ± 0,37 mm/d for P were measured at 22°C. Previous observations confirm a significant different growing response of the S and P species under a temperature spectrum between 0° and 40°C (Schwantes, 1976, Negrutsky, 1994). Korhonen (1978) and Negrutsky (1994) obtained a growth rate of 6,42 ± 0,19 mm/d for S, 7,49 ± 0,11 mm/d for the P group at 20°C and for S 6,9 ± 0,39 mm/d and for the P species 7,0 ± 0,28 mm/d at 24°C. It stick out that both former observations resulted in a higher growth rate of individuals of the P species and mostly for all measured temperatures. In the present study individuals of S grew quicker than individuals of the P species at all temperatures (Tab. 7). This contrast could be explained by high variation due to different sample sizes, origins, hosts and because of a little amount of individuals for both species. In the end values of the different studies are hard to compare. Results of Dr. Kari Korhonen were reached by testing about 780 stocks from mostly Finland and other countries. The 12 Individuals of this study are from all over Europe, Belarus and Russia. The results suggest that it is recommended to research more aimed and choose individuals from regions, which shall be investigated.

In former studies the fungus were described as one that resisted the existing pressure of natural selection and kept its temperature optima (Cowling & Kelman, 1964). Indeed the two species can exist in a wide geographical area with a wide temperature range. Schwantes (1976) described an experiment with mycelia, which was incubated at -8°C and then grew at optimum temperatures. Growth of mycelia was decreased but survived the procedure. While the same attempt with heat (36°C) killed off 50% of the individuals. According to Negrutskii (1962), mycelium could tolerate temperatures down to -30°C and spores could be frozen in liquid nitrogen to -270°C without losing vitality (Negrutskii, 1986). However cold is easier to cope with than heat. In the present study S behaved more like a generalist species, because it is able to thrive in a wide temperature range compared with strains of the P species and grew better at high temperatures (22° and 27°C). The latter (P) is more specialized for temperatures

between 0° and 17°C. The ability to grow at high temperatures and to have a large range of temperature is an advantage in competition and for stump infection, because higher temperatures on stumps effect an inactivation of the fungus or replacement through other saprophytes (Gooding, 1966). Cartwright and Findlay (1934) cherish the fact that temperature has a big influence on the growth rate of the predominant species and the competition for every piece of timber. Korhonen (1978) referred the geographical isolation of the fungus that induced to new species and later their distribution in the same area in Europe, where the predominant species and the better adapted prevails. In this study S is the better adapted species of both, because of their wider temperature range and slightly higher rate of growth.

To transfer the results to natural conditions, the experiment needs strains from Scandinavia (Finland, Sweden, Norway and Denmark) or better from the south and the north of Sweden and at least twice as many as in this study. In this study individuals were chosen randomly from all over Europe and just six individuals per species (S and P) were observed. Additionally strains had many different host trees in this case (*P. sylvestris*, *P. abies*, *Juniperus sp.* and *Pinus strobus* L.). Individuals and their hosts should be chosen unique and comparable, though Korhonen (1978) indicates that properties of the intersterility groups are independent of the host species. Additionally a change in temperature due to changes of day and night should be considered to pretend natural conditions.

The possible future increase in temperature due to global warming will have an impact to the forest ecosystems in Europe according to Sonesson (2004). Species with a more southern distribution area like P could move to the north of Scandinavia and cause severe damages on other tree species (pine, Siberian larch), which are nowadays free of root rot (Berglund, 2005). Control measures should be extended to the north and investigations about resistant tree species, which are interesting for areas with a high infection risk, have to be done. Prophylactic protection measures against the root rot pathogen with current strategies like planting trees with low susceptibility, stump removal, mixed stands (silvicultural control measures), prophylactic stump treatment like urea or borates, fungal species as competitors and antagonists (*Phlebiopsis gigantea* (Rotstop® in Fennoscandia), *Resinicium bicolour*) could be recommended on all host species in Scandinavia to prevent spread.

The intention, if growing behaviour of the two species is an appropriate method for the identification, could be disproved. Growth rates of both species were too similar to distinguish the mycelia precise.

In conclusion coniferous trees include some of the most economically important tree species of the northern hemisphere. *Heterobasidion* spp., causes the most important damage for one of the largest industries in Europe. Since Korhonen (1978) investigated the intersterility groups of *Heterobasidion* it is clear that there are different species of the fungus with different properties, niches and hosts. Likelihood of infection increases with germination percent and rate of growth of mycelia of the species S and P (Courtois, 1972). Competition between individuals of S and P and other antagonists is determined by flexible adaptation to their environment. Species of S and P possess a high range of variation by means of their wide range of temperature. S has a wider range of temperature than P, but also P could grow and germinate under cold temperatures and could survive in north Scandinavia. That means that temperature is not the only abiotic factor, which determines the range of distribution in north Scandinavia. There are more factors than temperature in nature that has to be investigated, which are determining the distribution of the two species S and P. A look at climate maps after Köppen-Geiger indicates a similarity between incidence of the P species and different climate zones. Those climate zones are determined by air temperature and annual precipitation. According to that the P species is located in the humid temperature climate with no dry season and cool summer in Scandinavia. However individuals of the S species are found in humid cold climate with short and cool summer, too. Latter climate zone includes a lot of snow and high fluctuation of temperature between 20 and 40 K (Strahler & Strahler, 2002). Potentially is the wide temperature range of S the key to survive in this climate zone. The synergistic effect between air temperature and humidity, because hot air can absorb more water than cold air, could be one approach for the next investigations.

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