Effect of Tree Susceptibility on *Peridermium pini* Lesion Development and Sporulation on Scots pine

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Abstract

Susceptibility of Scots pine progenies to pine stem rusts may be an important factor in rust epidemics. This study is an investigation of the susceptibility, lesion development, and sporulation of a resin-top disease fungus, *Peridermium pini*, on Scots pine seedlings originating from the seeds of trees showing various degrees of susceptibility to pine stem rusts. The resin-top disease symptoms and rust sporulation induced after artificial inoculation, *i.e. Peridermium pini* aecia, stem swelling and spermatial fluid, were recorded in 1995–2003.

The tree susceptibility was found to have little or no effect on the disease rate or other disease and sporulation variables. Aecial sporulation lasted longer on seedlings from resistant trees when compared to seedlings from healthy trees, but the annual lengths of stem bearing aecia and swellings were greater on the seedlings raised from healthy trees than those raised from resistant trees. The spore source was found to significantly affect the disease and sporulation variables. The northernmost spore source induced higher disease rates, greater frequency of seedlings bearing aecia and swellings, and the resultant seasonal sporulation lasted longer than when using the southern spore sources as inocula.

Key words: aecia, *Cronartium flaccidum*, lesions, *Peridermium pini*, *Pinus sylvestris*, resin-top disease, resistance, Scots pine, spermatia, sporulation, susceptibility, swelling

Introduction

Peridermium pini (Pers.) Lév. (syn. *Endocronartium pini* (Pers. emend Kleb.) Y. Hirat.) and *Cronartium flaccidu*m (Alb. & Schw.) Wint., are among the most destructive pathogens impacting on Scots pine (*Pinus sylvestris* L.) in Europe (Gäumann 1959). Locally, severe rust epidemics have been reported both in southern (Diamandis and deKam 1986) and northern Europe in recent decades (Kaitera *et al*. 1994, Kaitera 2000). A new phenomenon, however, is that rust epidemics have been observed over large areas of young pine plantations (Holmberg 2004, Krekula *et al*. 2005). The increased high susceptibility of pine provenances from non-local seed source has been proposed as one explanation for these sudden outbreaks of disease. It is known that the susceptibility of *Pinus* spp. to *Peridermium pini* is to some degree inheritable (Haack 1914, Liese 1936). Variation in the susceptibility of *Pinus* spp. provenances to *Cronartium* spp. and *Peridermium* spp. has been reported in some studies (van der Kamp 1988, 1989, Pei and Brodie 1995, Stephan 2004). Recently, however, no significant variation in the susceptibility to *Peridermium pini* has been observed among northern Finland's Scots pine provenances following artificial inoculations (Kaitera 2003).

The aim of this study was to investigate the effect of tree susceptibility to *Peridermium pini* resistance, lesion development and sporulation on Scots pine seedlings originating from seeds of trees with various rust susceptibility. A special interest was to study, whether any variation in time and duration of sporulation, slow-canker growth as evidence for rust resistance to various spore sources occurs on seedlings from various seed sources.

Materials and methods

Inoculum

Two *Peridermium pini* aeciospore sources from northern Finland (Pudasjärvi and Jokela) and one from southern Finland (Kesälahti; all mixed samples of several aecia from several Scots pines) were collected just before natural aeciospore dissemination in the early summer of 1995. The inoculum was collected from known homogenic *Peridermium pini* populations (Hantula *et al*. 1998, Kaitera *et al*. 1999), where the rust had caused severe damage and in which the aeciospores were shown to be virulent also through artificial means (Kaitera 2003). The branches bearing aecia were cut and transported to laboratory conditions, where the aeciospores were dusted onto Petri dishes through a fine mesh in a laminar flow-hood.

2. Plant material and inoculation of seedlings

Seeds were collected from a sown Scots pine stand, which had been severely damaged by *C. flaccidum* (Kaitera 2000), and from a nearby naturally-regenerated and healthy pine stand in northern Finland in the spring of 1995. A severe rust epidemic had been going on in the stand for decades, and rust sporulation was annual both on pine branches and on *Melampyrum sylvaticum* L., which is the main alternate host for the rust in the area (Kaitera 2000, Kaitera *et al.* 2005). Therefore, the pines had been receiving large numbers of inoculum annually for a long time without any evidence of lack of inoculum within the study area, and thus, reflecting true variation in long-term resistance against the rust. Seeds were collected in the severely damaged stand from open-pollinated trees bearing numerous *C. flaccidum* lesions and other disease symptoms on their branches and stems (seedlings from seed of susceptible trees are called a progeny of susceptible trees), from healthy trees without any lesions adjacent to the severely diseased trees (= progeny of resistant trees), and from healthy trees lacking any disease symptoms in the healthy stand near the damaged stand (= progeny of healthy trees).

After the seeds had been germinated, the resultant seedlings were planted in blocks of 50 seedlings. Progenies of resistant and healthy trees included 3 blocks each, but due to the low germination rate of the seed from susceptible trees, all resultant plants (20) were planted in one block. The seedlings were planted at 50 cm intervals in plastic pots containing nutrient-rich soil and peat in northern Finland in the early July of 1995. The seedlings raised from resistant and healthy trees were inoculated using three fresh *Peridermium pini* aeciospore sources (one spore source per block) in greenhouse conditions. All of the seedlings raised from susceptible trees were inoculated with one (Pudasjärvi) spore source. A few seedlings from resistant (5) and healthy (35) trees were retained uninoculated as controls. Prior to the inoculations, the seedlings were moistened using a water sprayer, and this was followed by artificially wounding them by removing two needle pairs midway along the current – year's shoot on the main stem using a sterilized scalpel (Myrholm and Hiratsuka 1993). This method has on previous occasions been used successfully in artificially establishing resin-top disease in Scots pine seedlings (Kaitera 2003). Aeciospores (50,000 - 100,000 in number) were dusted onto the needle pair scars using a fine brush, after which the inoculated seedlings were covered with a moistened plastic bag and incubated for 48 h. The germination rate of the aeciospores was 66–99% after 48 h of incubation on a substrate of 1.5% water agar. Disease symptoms and rust sporulation were recorded annually in terms of the occurrence of swellings and fruitbodies, i.e. aecia and spermatial fluid, on the inoculated stems at intervals of 1–4 weeks in June, and at intervals of two weeks to one month in July–September during the years 1995–2003. The recording interval was longer in 1997–1998 than in 1999–2002. Fruitbody production and sporulation were observed by their seasonal time of appearance (=first fruitbodies appear on the bark) and the rupturing of the aecia (=sporulation finished and fruitbodies ruptured), appearance of spermatial fluid, and the duration of sporulation (in years). In order to investigate the effect of tree susceptibility (Variable 1) and spore source (Variable 1) on different disease variables (Variable 2), I tested the hypothesis of whether the frequency distribution of Variable 2 was equal in the classes of Variable 1 by using Fisher's exact test of SAS (Anonymous 1989). The classes of the tested disease variables were disease rate (disease symptoms absent/present), occurrence of aecia, swellings or spermatial fluid (present/absent), time (years after inoculation) of the first appearance of aecia, swellings or spermatial fluid (frequency classes: 2–3/4/5–6 a combined together for testing), duration (years) of aecial sporulation (1/2/3 a), seasonal time of appearance of the aecia (early June/mid-June/late June – early July), and time of rupturing of the aecia (before July/after July). In addition, I tested the annual spread of aecia, swelling and spermatial fluid along the stem (classes: Growth greater distally/equal/proximally). During the life cycle of the rust, the spermogonia and swellings appear before aecia formation. I also tested the relationship between the occurrence of aecia and swelling and spermatial fluid (classes: Swelling or spermatial fluid present/absent before aecia formation). I compared the annual lengths of stem bearing aecia, swellings and spermatial fluid between the progenies of variously susceptible trees and spore sources using the GLM procedure (Tukey's test at $p<0.05$) of SAS (Anonymous 1989).

Results

Disease rate

The proportions of infected seedlings manifesting some of the disease symptoms (aecia, swellings or spermatial fluid) were 20%, 15% and 11% for seedlings raised from seeds of susceptible, resistant and healthy trees, recpectively. Among the spore sources, the proportion varied 9–21% (Table 1). The frequency distribution of the number of infected seedlings was

Table 1. Annual number and proportion of seedlings from seeds of susceptible, resistant and healthy trees bearing aecia, swellings and spermatial fluid (Spermat) in a 1–8 year-period following inoculation using three *Peridermium pini* aeciospore sources. Spore sources: 1=Pudasjärvi, 2=Jokela, 3=Kesälahti

Years		Tree susceptibility														Total	
		Susceptible		Resistant						Healthy							
		Aeciospore source															
		1		1		2		3		1		2		3			
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
	Aecia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	Swellings	0	0	1	2.0	0	0	1	2.0	0	0	2	4.0	0	0	4	7.7
	Spermat	0	0	1	2.0	0	0	0	0	0	0	2	4.0	0	0	3	7.9
	Aecia	0	0	0	0	2	4.0	1	2.0	1	2.0	1	2.0	0	0	5	7.9
2	Swellings	0	0	0	0	3	6.0	1	2.0	1	2.0	1	2.0	1	2.0	7	13.5
	Spermat	0	0	0	0	1	2.0	1	2.0	0	0	1	2.0	1	2.0	4	10.5
	Aecia	1	5.0	1	2.0	4	8.0	2	4.0	1	2.0	2	4.0	0	0	11	17.2
3	Swellings	0	0	1	2.0	3	6.0	2	4.0	1	2.0	1	2.0	0	0	8	15.4
	Spermat	1	5.0	1	2.0	2	4.0	0	0	1	2.0	1	2.0	0	0	6	15.8
	Aecia	3	15.0	1	2.0	7	14.0	2	4.0	5	10.0	6	12.0	2	4.0	26	40.6
4	Swellings	3	15.0	1	2.0	9	18.0	2	4.0	2	4.0	5	10.0	3	6.0	25	48.1
	Spermat	3	15.0	0	0	6	12.0	0	0	3	6.0	3	6.0	3	6.0	18	47.4
	Aecia	2	10.0	1	2.0	5	10.0	2	4.0	3	6.0	0	0	1	2.0	14	21.9
5	Swellings	0	0	0	0	1	2.0	1	2.0	2	4.0	0	0	0	0	4	7.7
	Spermat	1	5.0	0	0	1	2.0	1	2.0	2	4.0	0	0	0	0	5	13.2
	Aecia	1	5.0	0	0	2	4.0	0	0	2	4.0	0	0	0	0	5	7.9
6	Swellings	1	5.0	0	0	2	4.0	0	0	1	2.0	0	0	0	0	4	7.7
	Spermat	1	5.0	0	0	0	0	0	0	1	2.0	0	0	0	0	2	5.3
	Aecia	1	5.0	0	0	1	2.0	1	2.0	0	0	0	0	0	0	3	4.7
7	Swellings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Spermat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Aecia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Swellings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Spermat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Aecia	8	40.0	3	6.0	21	42.0	8	16.0	12	24.0	9	18.0	3	6.0	64	100.0
Total	Swellings	4	20.0	3	6.0	18	36.0	7	14.0	7	14.0	9	18.0	4	8.0	52	100.0
	Spermat	6	30.0	2	4.0	10	20.0	2	4.0	7	14.0	7	14.0	4	8.0	38	100.0

equal among the progenies of variably susceptible trees, but varied among the spore sources (Fisher's exact test, p<0.05); the frequency was higher when using the northernmost (Jokela) spore source as compared to the other two inoculum sources.

Annual occurrence and formation of aecia

Aecia developed on the inoculated seedlings 2–7 years after inoculation (Table 1). The proportion of seedlings with aecia varied between 9–20% among the progenies of variably susceptible trees and 8–19% among the spore sources. The aecia were most frequent 4 years after inoculation, and 80% of the annual aecia were formed 3–5 years after inoculation. The frequency distribution of the annual aecia was equal regardless of the progeny of the variably susceptible trees, but it differed among the spore sources; the frequency of aecia was significantly greater among the northernmost (Jokela) spore source when compared to the other spore sources (Fisher's exact test, p<0.05).

The first aecia developed on the seedlings 2–6 years after inoculation, 50% of them being observed 4 years and 82% observed 2–4 years after inoculation (Table 2). The frequency distribution of the time of appearance of the first aecia (of the combined year classes: '<4 a', '4 a' and '>4 a' after inoculation) was equal among the progenies from resistant and healthy trees and the spore sources. The average annual length of stem bearing aecia was significantly greater among the seedlings from seed of healthy trees as compared to those from resistant trees (Tukey's test, Table 5), but it was equal for the various spore sources. When combining all perennial sporulation observations, annual growth was greater distally than proximally in 58% of the cases and greater proximally than distally in 11% of the cases. The frequency distribu-

Table 2. Time (in years) of first aecial sporulation on seedlings raised from seeds of susceptible, resistant and healthy trees after inoculation using three *Peridermium pini* aeciospore sources. Spore sources: 1=Pudasjärvi, 2=Jokela, 3=Kesälahti

Years	Tree susceptibility														Total	
	Susceptible		Resistant						Healthy							
	Aeciospore source															
	1		1		2		3		1		2		3			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
2	0	0	0	0	2	15.4	1	20.0	1	20.0	1	16.7	0	0	5	13.2
3	1	25.0	1	50.0	3	23.1	1	20.0	0	0	1	16.7	0	0	7	18.4
4	2	50.0	1	50.0	5	38.5	1	20.0	4	80.0	4	66.7	2	66.7	19	50.0
5	0	0	0	0	2	15.4	2	40.0	0	0	0	0	1	33.3	5	13.2
6	1	25.0	0	0	1	7.7	0	0	0	0	0	0	0	0	2	5.3
Total	4	100.0	2	100.0	13	100.0	5	100.0	5	100.0	6	100.0	3	100.0	38	100.0

tion of the direction of annual growth either distally or proximally was equal among the progenies of the variably susceptible trees and spore sources.

Duration of annual sporulation

No aecia developed in 10% of the lesions (Table 3). Most lesions with aecia (76%) sporulated for 1–2 years with the rest of them sporulating for 3 years. The frequency distribution of the duration (in years) of aecial sporulation was equal among the spore sources, but it was different among the progenies of resistant and healthy trees (Fisher's exact test, $p<0.05$); the sporulation lasted for a longer time in the seedlings raised from seeds of resistant trees as compared to those raised from healthy trees.

Seasonal variation in aecial development

In all cases, the annual aecia developed until the mid-June and early July in 1997–1998. In 1999-2002, the aecia development in 20–71% of the cases lasted until early (8–10) June, and in 65–100% of the cases until mid- (18–24) June, and in all cases aecia development ended in early July. In regard to the progeny of variably susceptible trees, aecia developed in all cases on seedlings raised from seeds of susceptible trees in early June. The distribution of frequencies of the time of development of aecia in 1999 (classes; '<10.6.', '10.-18.6.' and '18.6.-5.7.') and in 2000 (classes; '<9.6.' and '9.–20.6.') was, however, equal for the progenies of resistant and healthy trees and spore sources (Fisher's exact test).

Sporulation ended in aecial rupturing mainly in July, although rupturing commenced already in mid-June. In 1999–2002, 40–79% of the lesions bore ruptured aecia between late June and early July. In 2000–2001, all lesions bore ruptured aecia until the end of July, while 65% of the lesions bore them until mid-July in 1999. The frequency distribution of the seasonal time of rupturing of aecia in the lesions was equal among the progenies of variably susceptible trees in 1999 (classes; '<5.7.' and '= 5.7.') and 2000 (classes; '<12.7.' and '= 12.7.'), and among the spore sources in 2000. In 1999, the frequency distribution differed among the spore sources (Fisher's exact test, $p<0.05$); the fre-

Table 3. Duration (in years) of aecial and spermatial (spermat) sporulation on seedlings raised from seeds of susceptible, resistant and healthy trees inoculated with three *Peridermium pini* spore sources. Spore sources: 1=Pudasjärvi, 2=Jokela, 3=Kesälahti

Years		Tree susceptibility														Total	
		Susceptible		Resistant						Healthy							
		Aeciospore source															
		1		1		2		3		1		2		3			
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0	Aecia	0	0	1	33	1	7	0	0	0	0	1	14	1	25	4	9.5
	Spermat	0	0	1	33	5	36	3	60	1	20	1	14	0	0	11	26.2
1	Aecia	1	25	1	33	6	43	2	40	1	20	4	57	3	75	18	42.9
	Spermat	3	75	2	67	8	57	2	40	2	40	5	71	4	0	26	61.9
2	Aecia	2	50	1	33	6	43	3	60	1	20	1	14	0	0	14	33.3
	Spermat	0	0	0	0	1	7	0	0	1	20	1	14	0	0	3	7.1
3	Aecia	1	25	0	0	1	7	0	0	3	60	1	14	0	0	6	14.3
	Spermat	1	25	0	0	0	0	0	0	3	20	0	0	0	0	2	4.8
Total	Aecia	4	100	3	100	14	100	5	100	5	100	7	100	4	100	42	100.0
	Spermat	4	100	3	100	14	100	5	100	5	100	7	100	4	100	42	100.0

quency of lesions with later aecial rupturing was then higher among the northernmost (Jokela) spore source as compared to southern spore sources.

Occurrence of swellings

Swellings were observed on the seedlings in a 1–6 year-period following inoculation in late summer (Table 1). In most cases (59%), they developed 4 years after inoculation, their proportions varying between 48–75% among the progenies of variably susceptible trees and between 45–52% among the spore sources. The frequency distribution of the occurrence of swellings (classes: 'Present' and 'Absent') was equal among the progenies of variably susceptible trees, but they were more frequently present in the northernmost (Jokela) spore source as compared to the southern spore sources (Fisher's exact test, $p<0.01$). The frequency distribution of the first development of swellings (combined classes: '1–3 a' and '4–6 a' after inoculation) was equal among progenies from seeds of resistant and healthy trees and spore sources (Fisher's exact test).

The annual length of stem bearing swellings was significantly longer among the seedlings raised from seeds of healthy trees as compared to seedlings raised from resistant trees (Tukey's test; Table 5), but equal among the spore sources. The annual growth of swellings was higher distally than proximally in 49% of the lesions and to the contrary in 37% of the cases. The frequency distribution of the annual growth of swellings along the shoot (classes: Growth higher 'distally' and 'proximally') was equal among the spore sources, but it was greater distally and lower proximally among the seedlings raised from seeds of resistant trees as compared to seedlings raised from healthy trees.

In 29% of the lesions, swellings developed one year before the aecia, while in 42% of the cases they were absent before the aecia (Table 4). In 21% of the lesions, seedlings with swellings died before aecia developed. In some cases (9%), the aecia did not precede the swellings. The frequency distribution of the occurrence of annual swellings prior to aecia (classes: 'Present' and 'Absent') was equal between the progenies of variably susceptible trees and spore sources (Fisher's exact test).

Occurrence of spermatial fluid

Spermatial fluid was detected on the seedlings in a 1–6 year-period followong inoculation (Table 1). This fluid was lacking in 26% of the infected seedlings, while most of the fluid observations (47%) occurred 4 years after inoculation. Seventy-six percent of the fluid observations occurred in a 3–5 year-period following inoculation. The frequency distribution of seedlings bearing spermatial fluid (classes 'Present' and 'Ab-

Table 4. Development of swellings and spermatial fluid (spermat) in relation (vs.) to aecia on seedlings raised from seeds of susceptible, resistant and healthy trees inoculated with three *Peridermium pini* spore sources. Spore sources: 1=Pudasjärvi, 2=Jokela, 3=Kesälahti

	Tree susceptibilty							Total	
	Susceptible	Resistant			Healthy				
	Aeciospore source								
	1	1	2	3	1	2	3		
	N	N	N	N	N	N	N	N	%
Swellings vs. aecia									
Swellings present prior to aecia	3	1	9	3	6	3	1	26	28.6
Swellings absent prior to aecia	5	2	12	5	6	6	2	38	41.8
Swellings present but aecia absent	1	1	3	1	0	1	1	8	8.8
Swellings present but seedling killed before aecia formation	1	1	6	3	1	5	2	19	20.9
Total	10	5	30	12	13	15	6	91	100.0
Spermatial fluid vs. aecia									
Spermat present prior to aecia	4	0	6	1	5	3	1	20	25.0
Spermat absent prior to aecia	4	3	15	7	7	6	2	44	55.0
Spermat present but aecia absent	1	1	0	1	0	1	1	5	6.3
Spermat present but seedling killed before aecia formation	1	1	4	0	0	2	3	11	13.8
Total	10	5	25	9	12	12	7	80	100.0

sent') was equal among the progenies from variably susceptible trees and spore sources (Fisher's exact test). The first spermatial droplets appeared in a 1–6 year-period following inoculation, and most of these first observations (55%) occurred 4 years after inoculation. The corresponding proportions varied between 46–64% among the progenies of resistant and healthy trees. The frequency distribution of seedlings bearing spermatial fluid for the first time (combined classes: '1–3 a' and '4–6 a' after inoculation) was equal among the progenies of resistant and healthy trees and among the spore sources. Spermatial fluid was observed for 1–3 years in perennial lesions, but in most cases (42%) the spermatial sporulation only lasted for one year.

Seasonally, spermatial fluid was observed mainly in August, whereas all observations of spermatial fluid were done after mid-August in 1997, between mid-July and the end of August in 1998, between late July and late August in 2000, and in August in 2001. The average annual length of stem bearing spermatial fluid was equal among the progenies of variously susceptible trees and spore sources (Tukey's test, Table 5). The annual length of stem bearing spermatial fluid was greater distally than proximally in 48% of the perennial lesions, and vice versa in 38% of the cases. The frequency distribution of the annual growth direction of the fluid along the stem (classes: 'Distally' and 'Proximally') was equal among the progenies of variably susceptible trees and spore sources (Fisher's exact test). Spermatial fluid was lacking in most cases (55%) prior to next year's aecia, but in 25% of the cases spermatial fluid was observed before the aecia. In the rest of the cases (20%) the aecia did not precede spermatial fluid on the seedlings. This was mainly due to the death of the seedling before aecia formation could occur. The frequency distribution of the occurrence of spermatial fluid prior to aecia (classes: 'Present' and 'Absent') was equal among the progenies of variably susceptible trees and spore sources (Fisher's exact test).

Discussion and conclusions

No differences in disease rates were observed in this study among the progenies of variably susceptible trees, although seedlings raised from the seeds of susceptible mother trees did become most severely infected. This is partly due to the relatively low number of seedlings from susceptible mother trees in the inoculations, but may partly be explained by the open-pollination of the mother trees by pollen from variously resistant trees, which may have altered the disease

Table 5. Annual length of stem, and distal or proximal growth (dis/pro) of aecia, swellings and spermatial fluid (spermat) from seeds of susceptible (Sus), resistant (Res) and healthy (Hea) trees on seedlings inoculated using three *Peridermium pini* spore sources. Spore sources: 1=Pudasjärvi, 2=Jokela, 3=Kesälahti. The means with the same letter are not significantly different according to Tukey's test at p<0.05

	Tree susceptibility							Total							
	Sus	Res			Hea			Sus	Res	Hea					
	Aeciospore source										Total				
	1	1	2	3	1	2	3				1	2	3	N	%
Aecia															
X	2.47	1.10	1.80	1.41	3.13	1.56	4.30	2.47*ab*	1.62*b*	2.56*a*	2.62*a*	1.73*a*	1.73*a*	–	–
STD	1.35	0.53	0.87	0.69	1.92	0.96	–	1.35	0.81	1.74	1.70	0.88	1.16	–	–
Dis>Pro	2	1	4	0	4	0	0	2	5	4	7	4	0	11	57.9
Dis=Pro	1	0	1	0	2	2	0	1	1	4	3	3	0	6	31.6
Dis<Pro	0	0	1	1	0	0	0	0	2	0	0	1	1	2	10.5
Spermat															
X	2.34	0.20	2.07	1.30	2.46	1.56	2.97	2.34*a*	1.69*a*	2.23*a*	2.20*a*	1.84*a*	2.30*a*	–	–
STD	1.52	0	1.81	1.27	1.92	0.80	3.38	1.52	1.64	1.92	1.69	1.40	2.64	–	–
Dis>Pro	2	0	5	0	3	3	1	2	5	7	5	8	1	14	48.3
Dis=Pro	2	0	0	0	1	1	0	2	0	2	3	1	0	4	13.8
Dis<Pro	1	0	3	1	3	1	2	1	4	6	4	4	3	11	37.9
Swelling															
X	1.68	1.0	1.75	0.89	1.93	1.70	4.35	1.68*ab*	1.45*b*	2.34*a*	1.66*a*	1.73*a*	2.15*a*	–	–
STD	0.38	0	1.12	0.27	0.55	0.68	1.87	0.38	0.99	1.42	0.56	0.99	2.04	–	–
Dis>Pro	1	1	10	4	1	3	0	1	15	4	3	13	4	20	48.8
Dis=Pro	2	1	2	0	0	1	0	2	3	1	3	3	0	6	14.6
Dis<Pro	1	0	3	2	5	2	2	1	5	9	6	5	4	15	36.6

resistance of the resultant seedlings, as compared to their mother trees. The infection rates were, however, generally higher among the tested seedlings than in the randomly chosen pine provenances (Kaitera 2003). High variation among *Pinus* spp. provenances in susceptibility to *Peridermium pini* (Pei and Brodie 1995) and other closely related pine stem rusts (van der Kamp 1989, Yang *et al*. 1997) has been reported. It has also been suggested that the susceptibility of Scots pine to *Peridermium pini* is a markedly inheritable trait (Liese 1936), but the results of the inoculations done in the present study do not support this view. As the type of inoculum causing the rust epidemic was basidiospores in the stand, where the susceptible and resistant trees were located, the resultant seedlings may have shown higher disease resistance against non-local inocula (aeciospores of *Peridermium pini*) as compared to local one, which may partly explain the lower variation in rust susceptibility among progenies, as compared to their mother trees than expected. A one-time inoculation may also be ineffective to reveal fully the variation among progenies in rust susceptibility compared to their mother trees that show rust resistance after several-year annual heavy natural inoculum. Scots pine seedlings have also been demonstrated to be extremely resistant to *Peridermium pini* when young, while their susceptibility seems to increase when they grow older (Mülder 1951, Murray *et al*. 1969). Significant variation was, however, observed in virulence among the spore sources: the northernmost spore source was more virulent than the southern spore sources. This was quite unexpected, as only small and insignificant variation has previously been observed among Finnish *Peridermium pini* spore sources before (Kaitera 2003). High variation in the virulence of *Peridermium* sp. has, however, been reported in North America (van der Kamp 1988).

Peridermium pini lesion development and sporulation was very similar among seedlings raised from seeds of trees showing various degrees of rust susceptibility in terms of most disease and sporulation variables. No differences were found in the annual and first formation of aecia, swelling and spermatial fluid, and seasonal formation and rupturing of aecia between the progenies of the variably susceptible trees. The virulence of the spores, however, significantly affected most of the disease variables. The first aecia developed 2 years after infection with the peak in aecial sporulation occurring in a 3–5 year-period following inoculation, which is in agreement with earlier reports of *Peridermium pini* sporulation (Haack 1914, van der Kamp 1970, Kaitera 2003). As an indication of the variation in virulence, the northernmost spore source produced aecia significantly more frequently than the other spore sources, but there were no differences in regard to the time of first sporulation among the spore sources. Earlier, Kaitera (2003) found no variation among Finnish spore sources in regard to the frequency and time of the first sporulation. The observed high variation in the frequency of aecia in the various years resembles the reported variation in *Peridermium pini* (van der Kamp 1970), *C. flaccidum* (Kaitera 2000) and some other pine stem rusts (Peterson 1971). The duration of sporulation was equal among the spore sources, but it lasted for a longer time on seedlings raised from the seeds of resistant mother trees, as compared to seedlings raised from healthy trees. This can be interpreted as being an indication of higher disease resistance.

Seasonally, the aecia sporulated between early June and early July with only insignificant variation among years regardless of the spore source. The observed sporulation is in accordance with Kaitera (2003) but in disagreement with Klingström (1967), who stated that northern spore sources sporulate earlier than southern sources. In most years, seasonal sporulation ended by aecial rupturing mainly in July regardless of the spore sources. During one year, however, the northernmost spore source finished sporulating later than the southern spore sources. Annual fruiting of the rust by expansion of the aecia along the shoot was greater distally than proximally in most perennial lesions, which agrees with earlier reports of the fruiting of *Peridermium pini* (Olembo 1971, Gibbs *et al*. 1987, Kaitera 2003) and *C. flaccidum* (Kaitera 2000). The fungal growth in different directions did not differ among the progenies of the variably susceptible trees and spore sources, but the annual length of stem with aecia was greater on seedlings derived from seeds of healthy trees compared to resistant trees, and it was equal among the various spore sources. This was an indication of the lower *Peridermium pini* resistance of the seedlings raised from seeds of healthy trees, as compared to those raised from resistant trees.

Swellings developed on the stems in the late summer starting a year after inoculation and peaking 4 years later, which is in agreement with earlier observations (Kaitera 2003). The frequencies of the annual swellings did not differ by the progeny of the variably susceptible trees, but inoculations with the northenmost spore source produced swellings more frequently than the other spore sources. Earlier, no significant variation in the number of swellings had been observed among Scots pine provenances or spore sources (Kaitera 2003). The annual length of stem bearing swelling was greater in seedlings raised from seeds of healthy trees than in seedlings raised from seed of resistant trees, which can be seen as being an indication of slow-canker growth among seedlings possessing varying rust resistance; this, has been demonstrated in other rust-host pathosystems as well (Hunt 1997). The annual growth of the swellings was greater distally than proximally with significant variation being observed in seedlings raised from seeds of resistant and healthy trees. Earlier, Olembo (1971) reported faster proximal growth of swelling than distal growth, but Kaitera (2003) did not observe any differences among the studied pine provenances. In the present study, swellings were observed commonly before the aecia, which is in agreement with earlier reports dealing with *Peridermium pini* (Klebahn 1918, van der Kamp 1970, Pei and Brodie 1995, Kaitera 2003). In a number of lesions, however, there were no swellings before aecial development regardless of the progenies of the variably susceptible trees and spore sources, which is an indication of the variation in the high general disease resistance of seedlings at the early stage of infection.

Spermatial fluid was observed at the same time and interval as annual swellings on the bark without any differences among the progenies of the variably susceptible trees and spore sources. The results agree with earlier reports on spermatial sporulation of *Peridermium pini* (Klebahn 1938, van der Kamp 1970, Pei and Brodie 1995, Kaitera 2003). In this study, spermatial droplets developed regurlarly but they were also missing prior to the aecia. This suggests that spermatization may not be necessary for aecia formation. Low genetic variation among single *Peridermium pini* and *C. flaccidum* lesions also suggests that gene flow from lesion to lesion resulting from outer spermatization may be minimal (Kasanen *et al*. 2000). As the distribution frequency of the occurrence of spermatial fluid before aecia was equal on the seedlings raised from seeds of variously susceptible trees and inoculated using various spore sources, the spermatization procedure was independent both of the susceptibility of the host and virulence of the spores. Studying other stem rusts, Hunt (1985) has shown experimentally that some aecia of *C. ribicola* J. C. Fisch. developed without any outer spermatization.

In conclusion, only small variation in disease resistance was observed between Scots pine seedlings originating from seeds of trees showing varying rust susceptibility. Greater variation was, however, observed in the disease rates among the spore sources. This suggests that the virulence of the spore source may play a more significant role in rust epidemics than the susceptibility of the progenies from seed of open-pollinated trees showing high variation in rust resistance.

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ВЛИЯНИЕ ЧУВСТВИТЕЛЬНОСТИ СЕМЯН К ПОРАЖЕНИЮ *PERIDERMIUM PINI* И СПОРООБРАЗОВАНИЮ НА СОСНЕ ОБЫКНОВЕННОЙ

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Резюме

Чувствительность семян сосны обыкновенной к ржавчиным грибам может быть важным фактором эпидемий заболеваний ржавчиными. Предметом данного исследования являются чувствительность, развитие поражения и спорообразование гриба *Peridermium pini* на саженцах сосны обыкновенной, полученных из семян деревьев с проявлением различной степени чувствительности к пузырчатой ржавчине сосны. Наблюдения за симптомами пузырчатой ржавчины и развитием заболевания ржавчиной, вызванного искусственной инокуляцией эцидиоспорами *Peridermium pini*, образованием вздутий на стволе и спермаций велись в течение 1995 –2003 гг.

Было замечено, что происхождение семян имеет несущественное или не имеет значения на интенсивность заболевания или другие показатели болезни и спорообразование. Образование эцидий проходило дольше на саженцах, полученных из устойчивых к болезни семян, по сравнению с саженцами, полученными из здоровых семян, однако годовые длины ран и вздутий на стволе были бóльшими на саженцах, полученных из здоровых семян, чем на саженцах, полученных из устойчивых к болезни семян. Было замечено, что происхождение спор имеет существенное влияние на показатели болезни и спорообразование. Споры самого северного происхождения вызвали наибольшую интенсивность заболевания, большее число стволов с эцидиями и вздутиями, и в результате сезонное спорообразование проходило дольше, чем в случае использования спор южного происхождения в качестве вакцины.

Ключевые слова: эцидиоспоры, *Cronartium flaccidum*, поражения, *Peridermium pini*, *Pinus sylvestris*, пузырчатая ржавчина сосны, сопротивляемость, сосна обыкновенная, спермации, спорообразование, чувствительность, вздутие