Botrytis cinerea and Norway Spruce Seedlings in Cold Storage

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Abstract

The risk of grey mold damage on first-year Norway spruce seedlings during cold storage was studied using conidia inoculation on spruce seedlings with different inoculation times and microclimate conditions (surface wetness, temperature, relative humidity) before storing. The temperature dependence of germination and germtube growth of *Botrytis cinerea* conidia at low temperatures was tested *in vitro.*

The germination of *Botrytis cinerea* spores *in vitro* reached 100 % in 15 hours at 6 ºC, and in about 52 hours at 0ºC. The length of the germtube in 52 hours was seven times longer at 6ºC than at 0ºC. These results indicate that the main progression of grey mold occurs in the beginning and/or thawing phase of cold storage.

In the three inoculation experiments the cold storage temperature was about -3ºC and the seedlings were stored in cardboard boxes. Uninoculated seedlings in the same boxes with inoculated seedlings had a higher disease incidence than the seedlings in boxes with no inoculated seedlings. Inoculation performed 7, 4 or 1 days before cold storage caused more disease before cold storage the earlier the inoculation was carried out. During cold storage the disease frequency doubled or increased even more.

The treatment at 6ºC, 80-90% relative humidity and surface wetness immediately before cold storage seemed to increase the number of diseased needles on the top of shoot more than the treatments at 2ºC and at 6ºC with 60-70 % relative humidity and surface wetness. The proportion of seedlings with bud burst showed a strongly negative correlation with the number of diseased needles at the top of the shoot at the end of storage. According to the results of this study, especially a high relative humidity with surface wetness on the seedlings and temperatures a few degrees above zero in the beginning of cold storage favour grey mold damage.

The experiments indicate that *B. cinerea* is a potential storage pathogen for Norway spruce seedlings even though the seedlings are, in late autumn, relatively resistant to this pathogen if not additionally stressed.

Key words *Botrytis*, grey mold, *Picea*, Norway spruce, spore germination, wetness, temperature, microclimate, cold storage

Introduction

Botrytis cinerea (Pers.:Fr.) is one of the most serious pathogens in Norway spruce (*Picea abies* (L.) Karst.) container seedling production in Scandinavia. *B. cinerea* has been also reported to cause damage on *Sequoiadendron giganteum* at storage temperatures of +1˚C - +3˚C (Smith *et al* 1973). *B. cinerea* and *Herpotrichia juniperi* (Duby) Petrak were identified on some spruce and pine seedlings in the spring 1958 (Jamalainen 1961). Cold storage damage on Norway spruce had been earlier studied by Venn (1981), who did not highlight *B. cinerea* as a serious fungus among the many other mold fungi. Cold storage damage is a result of many different factors (Venn 1980, 1981, 1982).

In Finnish forest nurseries the seedlings of Norway spruce are mainly produced in containers. Cold-storing is nowadays one way to overwinter the seedlings after the first growing season. Cold storage has many beneficial effects, *e.g.* it provides more stable winter conditions, and more time in the spring for delivering the seedlings to reforestation areas. The storage temperature is usually between -1 ˚C and -5˚C, *i.e.* at a temperature that minimizes fungal growth. Above zero temperatures before and after the stable cold-storing period may increase the risk of grey mold damage even though the optimum temperature for *B. cinerea* is about 20 ˚C (optimal relative humidity nearly 100%) (Elad *et al*. 1988, Salinas *et al*. 1989, Dugan and Blake1989).

In late autumn the susceptibility of the seedlings to grey mold is generally low (Petäistö *et al.* 2004). However, the vigour of the seedlings may decrease during cold storage (Venn 1980, Chomba *et al*. 1993, Jiang *et al*. 1994, Ögren *et al.* 1997), and thus also increase their susceptibility to *B. cinerea*. Furthermore the seedlings can be predisposed e.g. by cold, drought and shade (Zhang *et al.* 1995, Dennis *et al*. 1996, Peterson *et. al.* 1988) before storing, and unhardened seedlings are likely to be damaged.

The aim of this study is to determine whether there is a risk of grey mold damage on Norway spruce seedlings during cold storage.

Material and methods

Four experiments were performed in central Finland.

Exp 1 (2000-2001)

This experiment was performed to check if inoculation with spores produced disease during cold storing. The experiment was established on 24 October 2000, in 8 trays of Norway spruce seedlings (Plantek-81F, sown in the spring of 2000 and grown according to nursery practice but without fungicides). Grey mould monospore isolate used was BcSjk1.1 originated from a diseased spruce seedling (Petäistö *et al*. 2004). The spores were produced and inoculation performed as described in Petäistö *et al.* 2004. Half of the seedlings were sprayed with a spore suspension, 3 ml/seedling, $5x10^5$ conidia /ml, and the other half of the seedlings were sprayed with water.

The seedlings were packed 3-4 hours after the inoculation into 4 cardboard storage boxes. There were two small open cartons, each containing 37 inoculated and 37 uninoculated seedlings in each box. Another 4 boxes were packed with untreated seedlings (control control). The boxes were moved to cold storage. Temperature and relative humidity sensor (HPM231, Vaisalać, Helsinki, Finland) were placed in one experimental carton (Figure 1).

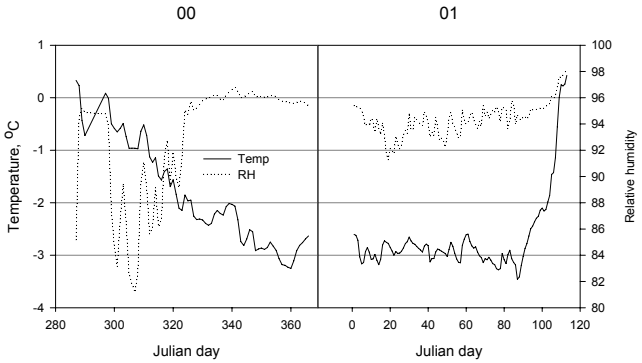

Figure 1. Exp. 1. Daily mean temperature and relative humidity in the Norway spruce storage box during cold storage in 2000-2001 (from Julian day 287)

On 8-9 May, 2001 (Julian days 128-29), when the temperature in the cold storage had remained above zero for about 2 weeks, the needles of the seedlings were examined for coloured spots (Petäistö *et al*. 2004). The number of diseased needles on each seedling was counted.

Exp 2 (2001-2002)

This experiment was performed in order to determine whether there are differences in the disease occurrence when the infection (inoculation) has occurred a few days before storing. Norway spruce seedlings (Plantek-81 F, sown in the spring of 2001 and grown according to nursery practice but without fungicides) were moved on 4 October to a growth room where the temperature was 13 ˚C during the day and 9 ˚C at night. Inoculation was carried out by spraying 3 ml spore suspension/seedling, 6 $x10^5$ spores/ml. Inoculation was performed on 9, 12 and 15 October on 300 seedlings at each time. The seedlings were packed into four cardboard boxes (two small open cartons in each box) for each inoculation time. Half of the seedlings in the small open cartons had been inoculated and half left uninoculated. Another 4 boxes were packed with untreated seedlings (control control) .The seedlings were moved to the cold storage on 16 October (Julian day 289); in other words after 7, 4 and 1 days from the inoculations. The temperature in a carton ranged between -1˚C and -4˚C and the relative humidity between 96 % and 98% (Figure 2).

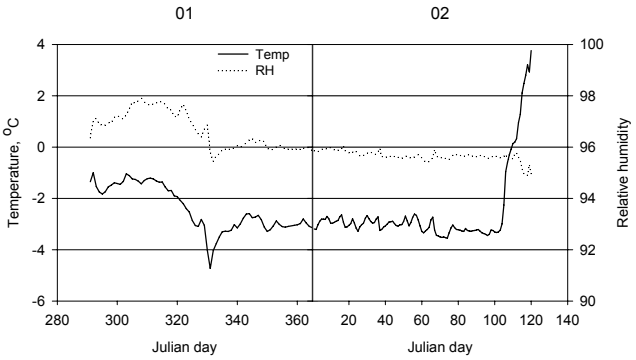

Figure 2. Exp. 2. Daily mean temperature and relative humidity in the Norway spruce storage box during cold storage in 2001-2002 (from Julian day 291)

The seedlings were examined for diseased needles on 6 May, 2002 (Julian day 126), when the boxes had been kept for about two weeks at a temperature above zero.

Exp 3 (2003-2004)

This experiment was performed to check whether there is a risk of grey mold damage when the surface of the seedlings is wet. In the experiment the storage temperature reached the target of -3˚C slowly and the following spring during thawing the seedlings remained at a temperature above zero for a couple of weeks.

The seedlings were sown on 23 June, 2003 (Plantek-81F) and grown according to nursery practice but without fungicides. In October the mean height of the seedling lot was 12.3 cm. The seedlings

for the experiment were marked and packed in small open cartons on 30 September – 1 October, 70 seedlings/carton. The cartons were moved to 3 different regulated conditions (temperature and relative humidity) on 2 October. The target regulated conditions were +2˚C and RH 50, +6˚C and RH 50, +6˚C and RH 80 and those in the nursery greenhouse (daily mean 7˚C and 90% RH for 2-21 October). In the +6˚C and RH 80 condition the target relative humidity decreased slightly because of a fault in the growth chamber system.

The surface wetness treatments were 14 days, 8 days, 2 days and no surface wetness.

The surface wetness periods were maintained by keeping the surface of seedlings moistened by spraying water on the seedlings, which were covered by plastic (Fig. 8 a). Each of the three growth chambers (+2˚C and RH 50, +6˚C and RH 50, +6˚C and RH 80) had 8 open cartons, each containing two wetness treatments (20 inoculated and 10 uninoculated seedlings for both). Each wetness treatment in each target regulated condition had four replicates.

Inoculation was performed correspondingly 14, 8 or 2 days before cold storage. 2400 µl/seedling of suspension (spore concentration 6 x 10^5 /ml) was sprayed on the seedlings (Petäistö *et al* 2004). All the seedlings (in open cartons) were moved at the same time into cardboard storage boxes and then into cold storage on 17 October (290 Julian day) 2003. One additional box containing non treated seedlings from the nursery (labelled as control control) was also placed in cold storage. On Julian day 297, the temperature in the cold storage was close to 0°C. It was assumed that on Julian day 291 and on a number of days afterwards, the temperature was above zero. The temperature and relative humidity were measured from 24 October onwards in the different wetness treatments in cartons that had been in the +6˚C and RH 50 target condition (Figure 3).

On 17 May, 2004 (Julian day 138), when the temperature in the cold storage had been above zero for about 3 weeks, the seedlings were examined for diseased needles. The location of a diseased needle was classified as being in the top (upper quarter of the shoot) and in the lower part. The needles with coloured spots and needles with a larger damaged area (Figure 8. b,c) were classified correspondingly as spotted and diseased needles. The seedlings were transferred outdoors in the open cartons, and proportion of seedlings with bud burst was determined on 21 June (Figure 8 d).

Exp. 4

The experiment was performed to test the temperature dependence of the germination and germ tube growth of spores at low temperatures.

In January 2003 the test temperatures were 0˚C, 2˚C, 4˚C and 6˚C in regulated growth chambers. A spore suspension 224000 - 256 000 spores/ml was prepared as in Petäistö *et al* 2004, but using an ice bath. One ml of suspension was spread on BDA-growth medium in Petri dishes by using a glass rode. The used PDA-Petri dishes were kept for 2 h at the test temperatures before suspension was applied.

Germination of spores was checked every 5 hours at each test temperature. Each observation was made from three different Petri dishes at each temperature. Each Petri dish was photographed (3 photos/dish, Zeiss Axioplan microscope, Carl Zeiss, Göttingen, West Germany) at 125 x magnification using a digital camera. The germination and germtube length in the digifotos were determined using the Colan program Line measurement tool (ColAn software package, ColorSoft Ltd., Lappeenranta, Finland).

In April 2003, the determination of germtube length at the temperature 4˚C and 2 ˚C was repeated.

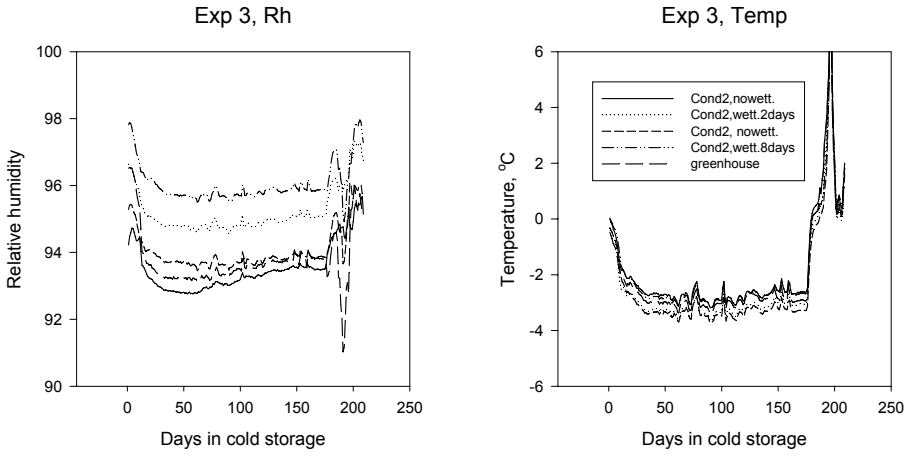

Figure 3. Exp 3. Daily mean temperature and relative humidity in a Norway spruce storage box during cold storage in 2003-2004. Relative humidity and temperature in cartons containing the seedlings subjected to condition 2 (+6˚C and RH 50) with the wetness treatment for 2 days and 8 days and with no surface wetness (nowett.) and in a box containing cartons of seedlings from the greenhouse.

Square root transformation, analysis of variance (ANOVA) and Pearson's correlation was used in data analysis in the experiments.

Results

Experiment 1

The number of diseased needles (needles with coloured spots) in the boxes where both inoculated and uninoculated seedlings were stored (boxes 1-4) was affected by inoculation (p = 0.07) (Table 1).

Table 1. The effect of inoculation (1=inoculated, 2=uninoculated) and storage box on the square root of mean number of diseased needles/seedling (sqrtsdisn). Analysis of variance (ANOVA). Exp. 1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	321.123	1	321.123	46.064	.000
	Error	52.475	7.527	6.971(a)		
inoc	Hypothesis	47.852	1	47.852	7.632	.070
	Error	18.809	3	6.270(b)		
box	Hypothesis	49.012	7	7.002	1.117	.511
	Error	18.809	3	6.270(b)		
inoc * box	Hypothesis	18.809	3	6.270	14.566	.000
	Error	377.048	876	.430(c)		

The inoculated seedlings on average had about 2 diseased needles per seedling. In the uninoculated control seedlings, which were kept in the same cartons as the inoculated seedlings, the disease on average occurred on one needle per seedling. In the boxes where no inoculations were performed (control control) there were only 0.1 diseased needles per seedling (Figure 4).

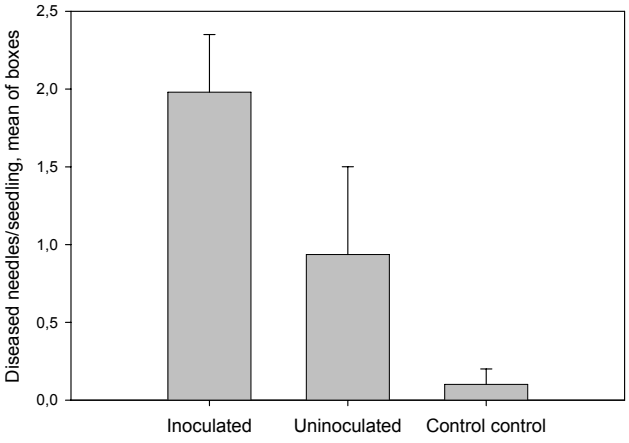

Figure 4. Exp. 1. Diseased needles/seedling, inoculated (boxes 1-4), control, uninoculated (boxes 1-4), control control, uninoculated (boxes 5-8, containing no inoculated seedlings). Mean of box means. Bars indicate standard deviation

Experiment 2

In inoculation times (7 days, 4 days and 1 day before cold storage), the number of diseased needles/seedling that developed symptoms before cold storage (inoculated minus uninoculated) was affected statistically significantly by inoculation time (p= 0.00) (Table 2a). The number of needles that developed symptoms during the cold storage was not affected statistically sicnificantly (p<0,16) by the inoculation time (Table 2b), and the result of inoculation performed 7 days before storage differed from the trend found before cold storage (Figures 5 a, b).

Table 2. The effect of inoculation time and storage box on the square root of mean number of diseased needles/seedling (inoculated minus uninoculated) a) before (sqrtbefore) and b) after storage minus before storage (sqrtstorage). Analysis of variance (ANOVA). Exp. 2

a)

Dependent Variable: sqrtbefore

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.100(a)	2	2.550	63.247	.000
Intercept	34.017	1	34.017	843.764	.000
Inoculation time	5.100	2	2.550	63.247	.000
Error	.363	9	.040		
Total	39.480	12			
Corrected Total	5.463	11			

b)

Dependent Variable: sqrtstorage

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.931(a)	2	.466	2.292	.157
Intercept	30.210	1	30.210	148.690	.000
time	.931	2	.466	2.292	.157
Error	1.829	9	.203		
Total	32.970	12			
Corrected Total	2.760	11			

In the control the number of diseased needles found before cold storage was clearly lower than that found during cold storing (Figure 5). Also in the cartons without inoculated seedlings (control control), the level of needles that developed symptoms during cold storage was almost the same. In the control of the inoculation time closest to the onset of cold storage, the number of diseased needles that appeared during winter seemed to be higher than in the other controls (Figure 5).

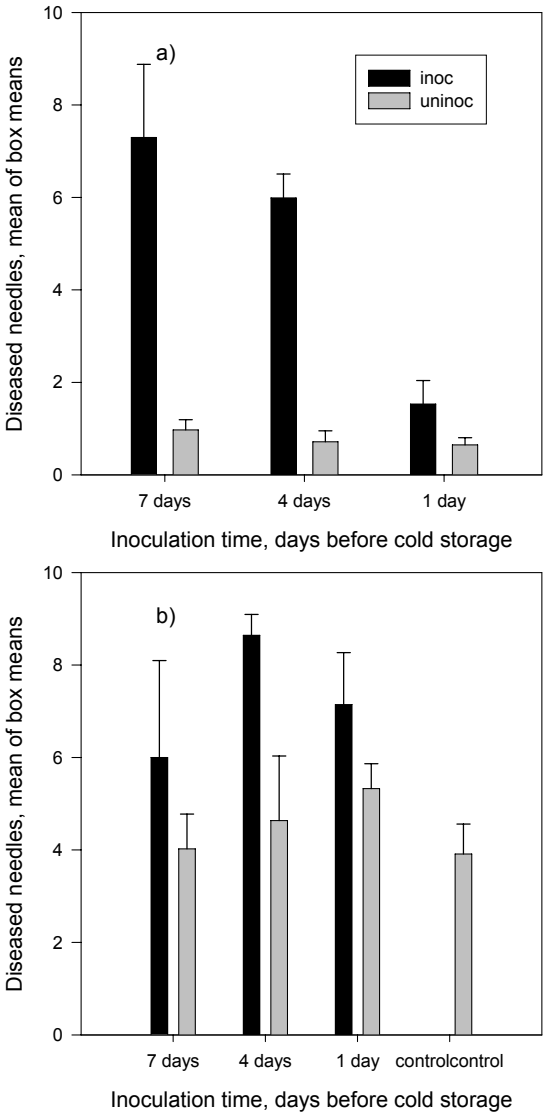

Figure 5. Exp 2. a) The number of diseased needles/seedling before cold storage, mean of the carton means. Bars indicate standard deviation. X axes: inoculation time 7 days, 4 days, 1 day before cold storage. b) The number of diseased needles/seedling diseased during cold storage

Experiment 3

Spotted needles occurred less frequently than diseased needles with larger symptomatic area (in the ration of 1 to 50). The disease incidence (spotted and diseased needles) on the top of seedlings in Exp. 3 was affected by surface wetness ($p<0.08$), by the condition (temperature/RH) before cold storage ($p<0.12$) and by inoculation ($p<0.20$). Correspondingly the disease incidence on the lower part of shoot was not affected by these factors (corresponding p-values 0.72, 0.68 and 0.38) (Table 3a,b). After cold storage the disease incidence on the top (inoculated - uninoculated) in the seedlings that had been in the +6˚C and RH 80 treatment was the highest (Figure 6). In this treatment the disease incidence decreased with decreasing duration of the wetness treatment from 8 days to 4 days and to 0 day.

Table 3. The effect of surface wetness duration (14, 8, 2, 0 days) and microclimate condition treatments 1-3 (+2˚C and RH 50, +6˚C and RH 50, +6˚C and RH 80) before storage and inoculation (1=inoc, 2=uninoc) on the square root of the mean number of spotted plus diseased needles (sqrtsdisn) a)on the top and b)on the lower part of shoot/seedling. Analysis of variance (ANOVA)

a)

Dependent Variable: sqrtsdisn on the top

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11.133(a)	23	.484	1.047	.424
Intercept	297.067	1	297.067	642.313	.000
condition	2.044	2	1.022	2.210	.117
wetness	3.326	3	1.109	2.397	.075
inoc	.777	1	.777	1.681	.199
condition * wetness	3.357	6	.559	1.210	.311
condition * inoc	.481	2	.241	.520	.597
wetness * inoc	.279	3	.093	.201	.895
condition * wetness* inoc	.868	6	.145	.313	.928
Error	33.300	72	.462		
Total	341.500	96			
Corrected Total	44.433	95			

b)

Dependent Variable: sqrtsdisn on the lower part

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.358(a)	23	.103	.332	.998
Intercept	139.706	1	139.706	452.370	.000
condition	.243	2	.122	.394	.676
wetness	.418	3	.139	.451	.717
inoc	.238	1	.238	.770	.383
condition * wetness	.363	6	.061	.196	.977
condition * inoc	.306	2	.153	.495	.611
wetness * inoc	.454	3	.151	.490	.690
condition * wetness* inoc	.336	6	.056	.181	.981
Error	22.236	72	.309		
Total	164.300	96			
Corrected Total	24.594	95			

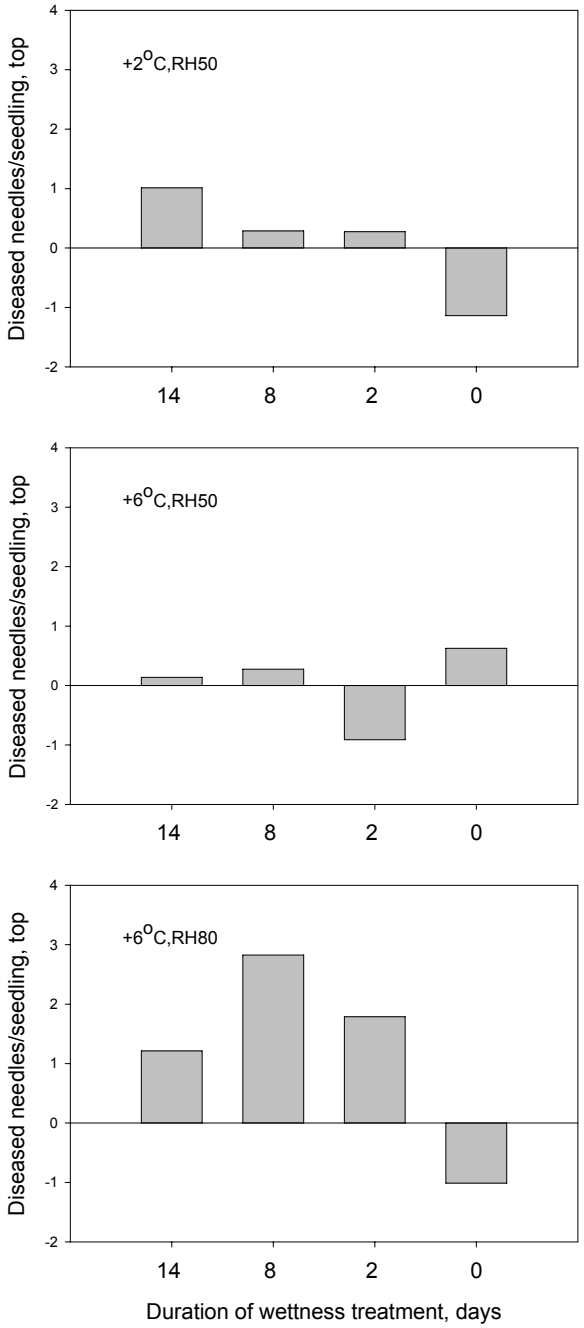

Figure 6. Exp 3. The mean number of spotted plus diseased needles/seedling at the top of the shoot in the spring of 2004, inoculated-uninoculated. The seedlings were kept in 1-3 different conditions (+2˚C and RH 50, +6˚C and RH 50, +6˚C and RH 80) and for different length of surface wetness treatment (1-4: 14, 8, 2 , 0 days) before cold storage

The number of diseased needles/seedling was over ten times higher in the control seedlings placed in the same box with the inoculated seedlings than in the box with no inoculated and untreated seedlings (data not shown).

In the following summer, the proportion of seedlings with bud burst correlated significantly (r=-0.7) with the number of diseased needles at the top of the seedling (Figure 7, Table 4). The case of null number of diseased needles/seedling was the most numerous. The colour of diseased needles on the top of the seedlings and the bud burst are shown in Figure 8.

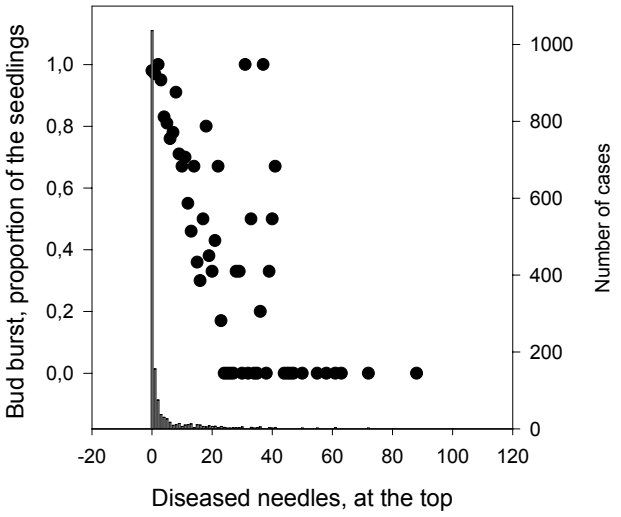

Figure 7. Exp 3. The proportion of seedlings with bud burst in the summer 2004 among the seedlings with a different number of diseased needles at the top

Table 4. Correlation between number of diseased needles at the top of the shoot and the proportion of the seedlings with bud burst

		Diseased needles. top	Bud burst
Diseased needles. at the top	Pearson Correlation	1	-.700(**)
	Sig. (2-tailed)		.000
	N	53	53
Bud burst	Pearson Correlation	-.700(**)	1
	Sig. (2-tailed)	.000	
	N	53	53

** Correlation is significant at the 0.01 level (2-tailed).

Experiment 4

The germination percent in 15 hours at 0 °C, 2 °C, 4 °C and 6 °C was 31, 82, 96 and 100, respectively (Figure 9a). Also at 0 °C the germination rate reached 100 % but took a longer time: in 52 hours it was 97.7 % and at the next measurement time, 121 days from the start, it was 100 %.

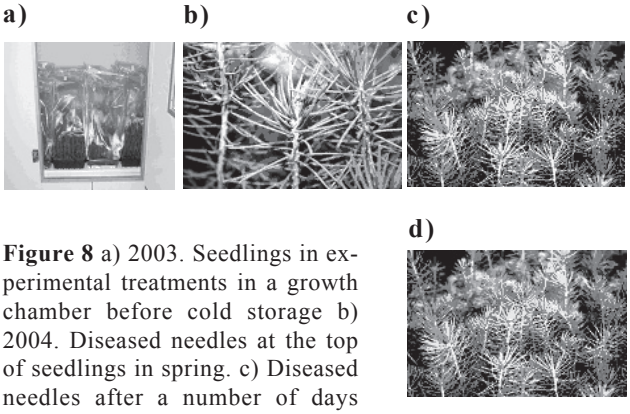

Figure 8 a) 2003. Seedlings in experimental treatments in a growth chamber before cold storage b) 2004. Diseased needles at the top of seedlings in spring. c) Diseased needles after a number of days (colour changes). d) The seedlings after bud burst in July 2004

After 52 hours the mean length of the germ tube at 2 °C, at 4 °C and at 6 °C was 2.45 (1.65, repeated test), 2.6 and 6.83 times longer, respectively, than at 0 °C (Figure 9b). The mean length of the germtubes was calculated only from germinated conidia.

Discussion and conclusions

Spruce seedlings are normally less susceptible to the *B. cinerea* inoculation in the autumn (Petäistö *et al* 2004). Thus, it can be assumed that the disease may occur in cold storage if there are changes in the constitution of the seedlings during storage, the seedlings are predisposed by some stress factors before storage or there is an especially suitable microclimate for the mold.

The *in vitro* temperature test for germ tube growth and spore germination showed that the growth of *B cinerea* is possible, although minimal, already at 0 ˚C, and spore germination reaches 100 % in about 50 hours at this temperature. For example, the growth at 6 ˚C was about seven times higher and 100% germination was reached in a 3- to 4 fold shorter time than at 0 ˚C. Little germination occurred at the test temperatures (0-6˚C) in 5 hours. Thus, due to the temperature dependence of *B. cinerea,* the risk of mold damage could increase during the freezing and thawing periods in cold storage.

Brooks (1917) also tested *B. cinerea* in Petri dishes at low temperatures and reported growth at 0˚C. Hartley *et al*. (1919) found growth at 0˚C. The temperature optimum of *B. cinerea* for germination and infection is between 9 - 21˚C, but the temperature between 2 - 25˚C is also suitable for the fungus (Elad *et al* 1989, Jarvis 1980, 1977, Marois *et al*. 1988, Salinas *et al* 1989). Dugan and Blake (1989) reported that germination occurred in 24 hours at 20-

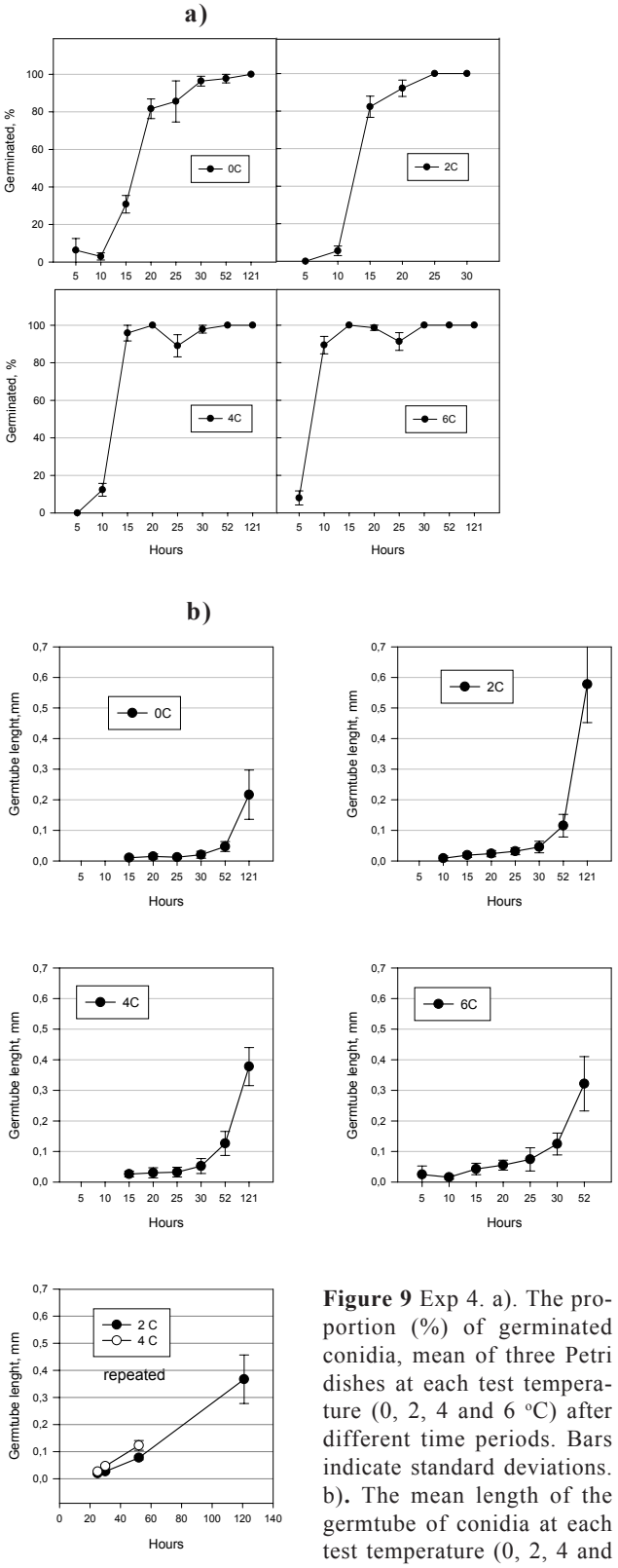

Figure 9 Exp 4. a). The proportion (%) of germinated conidia, mean of three Petri dishes at each test temperature (0, 2, 4 and 6 °C) after different time periods. Bars indicate standard deviations. b)**.** The mean length of the germtube of conidia at each test temperature (0, 2, 4 and 6 °C) after different time periods: only germinated conidia calculated. Bars indicate standard deviations

21˚C and Peterson (1995) in 3-5 hours at 15-20˚C. Symptoms in Norway spruce seedlings were found after inoculation with spores within a couple of hours, and more clearly in 14 hours in optimal microclimate conditions (Petäistö 2002). Thus the fungus is fast growing and damage can also appear rapidly.

According to the present study (Exp. 3), in addition to moisture temperature is also important for disease outbreak. When surface-wetted seedlings were put into cold storage, the disease occurrence also depended on the relative humidity and temperature. The surface wetness of the seedlings with a high relative humidity and the temperature of +6˚C seemed to favour outbreak of the disease more than surface wetness with +6˚C or +2˚C and a lower relative humidity. Surface wetness alone did not appear to favour serious damage by the fungus. These conclusions are congruent with the findings of Peterson (1995).

At +6˚C with a high relative humidity the 8-day wetness treatment caused more disease on the seedlings than the 2-day wetness treatment and the risk was lowest in the no-wetness treatment. Longer period required for spore germination and germtube growth is obviously the main reason for this. Longer lasting surface wetness also increased the relative humidity during the subsequent time in cold storage slightly more than the shorter-lasting treatments. The treatments have more effects on disease occurrence on the top than on the lower part of the shoots. The common higher humidity at the lower part of the shoots might be an explanation.

The number of diseased needles at the top of the shoot correlated with disturbances in bud burst. Grey mold can damage the bud or cause moldiness through the shoot below the bud. As the upper part of the shoot is younger and obviously not as resistant as the lower part of shoot, the most serious damage is thus likely to occur at the top of the shoot.

The study (Exp. 1) showed that grey mold damage is possible during cold-storing even without optimal temperature, surface wetness and relative humidity treatments. In these cases the disease incidence in inoculated seedlings was low but significantly differed from that of the uninoculated seedlings. The incidence of disease on the uninoculated seedlings that were in the same box as the inoculated seedlings was half that on the inoculated ones. The disease may spread in this kind of packing system e.g. via water drops and through occasional contact with the shoots of inoculated seedlings. Natural infection may also be present on the seedling material. The disease incidence in those boxes where there were no inoculated seedlings, was only one tenth that on the uninoculated seedlings that were in the same cartons as the inoculated seedlings. This observation underlines the fact that the amount of the dead needles and branches, which act as sporulation media for the fungus, must be minimized in order to avoid further infections.

If the seedlings become infected before storage, then the disease incidence was the higher, the longer the time before storing that infection occurred (Exp. 2). When already diseased seedlings were placed in cold storage, the disease incidence increased during storage from the level prevailing before storage. In Exp. 2, a two- and four – fold increase in the disease incidence during storage (inoculation 4 and 1 day before storage). After the inoculation ‘7 days before storage’ the disease increased during winter relatively less than the inoculations ‘4 and 1 day before storage’. In the ‘7 days before storage’ inoculation two of the four boxes had a clearly lower disease incidence (same level as uninoculated). If we assume that some unknown events has occurred in these boxes and the result was from the other two boxes, then the disease level on seedlings inoculated 7 days before storage was almost the same as on seedlings inoculated 4 days before storage. The disease seemed to occur more on those control seedlings in the cartons where inoculation was performed immediately before storage. This may be due to the fact that the conidia are mobile at that time due to the shorter sticking time.

The temperature at the beginning of cold storage in Exp. 1 was already close to zero on the first day, as was also the case in Exp. 2. In Exp. 3 the temperature was assumed to have been slightly above zero for a period of one week at the beginning of the storage. In this experiment it is not clear whether the fungus caused more damage in the beginning than at the end of storage. The resistance of the seedlings may have decreased during storage and perhaps the seedlings were more susceptible to the disease at the end than in the beginning of storage. The respiration of Norway spruce seedlings increases when the temperature changes from minus to plus degrees (Sandvik 1968), which may decrease the ability of seedlings to prevent fungal infection. Also during storage at -2˚C the starch content of *Picea glauca* seedlings has been reported to decrease (Jiang *et al*. 1994). It may be that the progression of damage is faster at the end of storage, although the microclimate conditions, especially moisture, before and in the beginning of storage are important.

In conclusion, spores of *B. cinerea* can germinate and the germtubes grow at zero temperatures. During storage the length of time when the temper-

ature is above zero affects the disease incidence on the seedlings. The surface wetness of the shoots at high relative humidity, combined with a temperature slightly above zero in the beginning of storage, seems to enhance the severity of gray gray mold damage. Furthermore, the number of diseased needles at the top of the shoot correlated strongly negatively with the proportion of seedlings exhibiting bud burst.

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BOTRYTIS CINEREA И СЕЯНЦЫ ЕЛИ ОБЫКНОВЕННОЙ ПРИ ХРАНЕНИИ НА ХОЛОДЕ

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Резюме

Проводилось изучение риска поражения серой гнилью однолетних сеянцев ели обыкновенной во время хранения на холоде с применением инокуляции конидией сеянцев ели в различное время и при различных микроклиматических условиях (влажность поверхности, температура, относительная влажность) перед хранением. Зависимость температуры прорастания и роста ростковой трубочки конидий *Botrytis cinerea* при низких температурах изучалась в условиях *in vitro*.

Прорастание *in vitro* спор *Botrytis cinerea* достигло 100 % через 15 часов при t 6 °C, а при t 0 °C примерно через 52 часа. Через 52 часа длина ростковой трубочки была в семь раз больше при 6°C, чем при 0°C. Эти результаты указывают на то, что основное развитие серой гнили происходит в начале и/или в стадии оттаивания при хранении на холоде.

В ходе трех экспериментов по инокуляции температура хранения была порядка -3°C, и саженцы хранились в картонных коробках. У неинокулированных саженцев, которые хранились в тех же коробках, что и инокулированные, встречаемость заболевания была более высокая, чем у саженцев в коробках, в которых не было привитых саженцев. В результате инокуляции, сделанной за 7, 4 или 1 день до хранения на холоде, заболевание было тем больше, чем раньше была произведена инокуляция. Во время холодного хранения частота заболевания возросла вдвое или увеличилась еще больше.

Обработка инокулированных сеянцев при 6°C и 80-90% относительной влажности воздуха и влажности поверхности непосредственно перед хранением на холоде свидетельствовала о большем количестве зараженных хвоинок на вершине побега, по сравнению с обработкой при 2°C и при 6°C и 60-70 % относительной влажности воздуха и влажности поверхности. Доля сеянцев с раскрывшейся почкой, показала сильную отрицательную корелляцию с количеством зараженных жвоинок на вершине побега в конце хранения. Согласно результатам данного исследования, высокая относительная влажность и влажность поверхности сеянцев, а также температура несколько градусов выше нуля в начале хранения, способствуют поражению сеянцев серой гнилью.

Экперименты показывают, что *B. cinerea* является потенциальным патогеном сеянцев ели обыкновенной при хранении, хотя сеянцы, неподвергавшиеся дополнительному стрессу поздней осенью относительно устойчивы к этому патогену,.

Ключевые слова *Botrytis*, серая гниль, *Picea*, ель обыкновенная, прорастание споры, влажность, температура, микроклимат, хранение на холоде