Effects of Light and Winter Storage Conditions on Norway Spruce Seedlings with Special Emphasis on the Occurrence of *Gibberella avenacea*

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Abstract

Fusarium-like fungi, together with *Herpotrichia*, were isolated after winter 2006 from Norway spruce seedlings in a nursery. According to ITS nucleotide sequences and morphology, the most common *Fusarium*-like species was *Gibberella avenacea*. Complete (i.e., 100%) conidiospore germination was observed *in vitro* at 0–25 °C, but its rate and germ tube growth was dependent on temperature. In early July, seedlings were moved outdoors to two conditions: shaded or exposed to sunlight. Thereafter, half of the seedlings were inoculated with *Gibberella avenacea* asexual spores once a month for four months. Following winter storage in the freezer or outdoors, localized necroses were observed on needles of inoculated and control seedlings. The number of needles and growth rate was higher in seedlings exposed to light. In control seedlings, shoot growth rate and needle dry weight percent were higher in plants stored outdoors compared to those over-wintered in the freezer. On the uppermost shoot quarter, needles were less healthy in seedlings stored in the freezer, whereas needles on the lower shoot quarters of seedlings grown in the shade were healthier than those in light. The damage caused by inoculations was not statistically significant.

Cultures of *G. avenacea* were obtained almost entirely from inoculated seedlings and identified using ITS sequences and species-specific PCR. The number of *G. avenacea* isolates was highest in seedlings grown under shade and stored in a freezer as well as in seedlings grown under light and stored outdoors. In shade grown and freezer stored seedlings, *G. avenacea* was isolated most frequently from tops, whereas it was more often detected in the lower quarters of light seedlings stored outdoors.

Key words: *Gibberella avenacea*, *Fusarium*, *Picea abies*, container seedlings, overwintering, nursery practice, light, reforestation, Fennoscandian forestry, fusariosis, Fusarium head blight.

Introduction

Every year, Finnish reforestation sites receive 170–180 million nursery seedlings, most of which are containerized Norway spruce (*Picea abies* (L.) Karst) (Finnish Statistical Yearbook of Forestry 2010). In the autumn, 40–50% of seedlings are removed from containers and packed in cardboard boxes prior to storage at -1 to -4 °C for 6–7 months. The remaining seedlings are left to overwinter under snow cover. Freezer storage of seedlings is believed to lower the incidence of problems related to outdoor storage and enables greater flexibility in springtime delivery and planting. However, fungal diseases remain a threat during freezer storage and especially during thawing (Petäistö and Laine 1999, Petäistö 2006).

In the spring of 2006, Norway spruce seedlings suffering from a needle disease were detected in a Finnish nursery. Brown mycelium typical of *Herpotrichia* was isolated and identified as *Herpotrichia juniperi* (Duby) Petrak (data not shown). Isolations from Norway spruce needles infected with *H. juniperi* have also yielded unidentified fungi producing asexual conidia typical of *Fusarium*. According to Kowalski and Andruch (2010), the aetiology of Herpotrichia needle browning on *Abies alba* Mill. (caused by *Nemastoma parasiticum* (Hartig) M.E. Barr [syn. *Herpotrichia parasitica* (Hartig) Rostr.]) is unclear and other fungi in infected tissues may contribute to needle death.

Fusarium is a large genus of ascomycete fungi widely distributed throughout the world. It contains saprotrophs and plant pathogens that are primary or secondary colonizers. Pathogenic species cause severe root and shoot diseases and damping-off in conifer nurseries (Hansen and Hamm 1988, James et al. 1991,

Lilja et al. 1992, 1995, Salerno and Lori 2007). It has also been detected in needles of *A. alba* showing symptoms of Herpotrichia needle browning (Kowalski and Andruch 2011). *Fusarium* is often found in diseased nursery seedlings, nursery soils and on conifer seeds, which can all act as inoculum (James et al. 1991, Lilja et al. 1992, 1995). Species of *Fusarium* produce spore-bearing perithecia and sporodochia on infected plants for short periods throughout the summer, from which spores are dispersed on the wind or via rain splash (Jenkinson and Parry 1994).

The aims of this study were to (i) identify the *Fusarium*-like isolates, (ii) determine the effects of environmental conditions on seedlings during the growing period and winter storage and on the pathogenicity of *Fusarium*-like isolates, and (iii) measure *in vitro* spore germination rate across a range of temperatures to determine the activity of the potential pathogen overwinter. Seedlings were grown either in an open field or in the shade and stored over winter in a freezer or outdoors under snow cover. Shade was included in the test since low light intensity is known to be a predisposing factor for foliage pathogens such as *Sirococcus conigenus* (D.C.) P.F. Cannon & Minter and *Botrytis cinerea* Pers. ex Nocca & Balb. (Wall and Magasi 1976, Zhang et al. 1994, Zhang et al. 1995).

Materials and methods

Temperature dependence of spore germination, germ-tube growth, and spore dimensions

Spores of the Pielavesi nursery isolate (*Gibberella avenacea*, see below) were produced on autoclaved barleycorn with spruce and pine needle homogenate (Petäistö and Kurkela 1993, Winder 1999). The isolate was grown for three weeks at 17 °C in light, after which an orange spore mass was collected.

Spore germination and germ-tube growth were tested in the dark at 0, 5, 10, 15, 20 and 25 °C on potato dextrose agar (PDA: 39g/l, Difco, MI, USA) media in Petri dishes. There were three plates for each temperature and monitoring time. One-ml of spore suspension containing ca. 200 000 spores was spread on each plate. Cultures were photographed with a Canon EOS 300D (Canon, Japan) fitted to a light microscope (Axioplan, Zeiss, Germany) every 5–10 hours as long as hyphal density remained low. The germination of at least 50 spores was monitored on each plate and germ-tube length was determined from photographs. It was possible to measure 5 to 47 germ-tubes for each time-temperature combination.

The Pielavesi isolate was grown on PDA media at 22 °C in the light until sporodochia were produced. At least 100 2-3 septate spores and 100 4-5 septate spores were measured.

Seedlings and inoculation

The experiment was performed in the nursery of the Suonenjoki Research Unit, Finnish Forest Research Institute (62°39”N, 27°03”E, 142 m a.s.l.). Norway spruce seedlings were sown at the end of April 2008 in 81F container trays (Lännen Oy, Iso-Vimma, Finland) filled with light sphagnum peat (Kekkilä M6W; Vapo Oy, Jyväskylä, Finland) mixed with dolomite lime and base fertilizer. Sixteen trays of seedlings were grown in the greenhouse and moved outdoors July 04 to each of the two experimental sites under shade or light (open field) conditions. In each site, light intensity (Li-Cor Li-190SB Quantum Sensor, Lincoln, Nebraska, USA) and surface wetness (Wetness SW120D, Delta_T, Burvell Cambridge, England) were measured at canopy level, and temperature and relative humidity (Vaisala HMP 143, Helsinki) immediately above seedling crowns. Seedlings received weekly fertilization at the rate of 800 ml per tray of a N-P-K 19-4-20 solution, diluted to 0.1% (July) or 0.05% (August).

Eight trays of seedlings were inoculated in both sites at the end of July, August, September and October 2008. Two-ml of a spore suspension (ca. 600 000 spores) was sprayed on each seedling. Eight trays in each site received no inoculum and served as the control.

The height of nine seedlings from each of eight shade and light control trays was measured on 25 July, 22 August and 19 September 2008. The stem diameter and growth of the new shoot of all seedlings from each of eight control trays in a shade and in light was measured at the beginning of June 2009.

Winter storage

On 21 October 2008, one day after the final inoculation, four inoculated and four control trays from each site were packed into cardboard boxes and moved to a freezer. The remaining trays at each site were moved outdoors near the climate station of the Suonenjoki Research unit. Temperature, relative humidity and snow depth were measured throughout winter storage.

Seedling health after winter storage

After winter storage, seedlings were inspected and classified according to health condition. This was carried out for seedlings stored in a freezer or outdoors between 20–23 April and 28–29 April, respectively. The main shoot of each seedling was visually divided into four quarters (1=uppermost quarter, 4=lowermost quarter) and the health of each quarter

was classified as: healthy (needles green, healthy) or diseased (needles with dead spots or dead needles). In addition, branch condition was examined from each shoot quarter.

The total number of needles was calculated from each shoot quarter in eight control seedlings grown in light and shade. The combined samples for the percent dry weight of fresh needle mass consisted of needles from four seedlings from each of four control trays in each treatment combination, i.e., light-freezer, light-outdoors, shade-freezer and shade-outdoors.

Fungal isolation and identification

On 18–20 May 2009, needles grown in 2008 were collected from each tray in the inoculation experiment. At least five diseased needles per seedling were collected from up to nine seedlings per tray. Healthy needles were collected from nine seedlings per tray. Needles were labeled as 'top' (quarter 1), 'rest' (quarters 2–4) and 'branch' and stored at -5 °C prior to analysis.

Needles were dipped for 4 seconds in 70% ethanol followed by three rinses with sterile water, cut into three 2–3 mm long pieces and incubated on PDA media at 17 °C in the dark. Isolations were made from a total of 416 seedlings.

Mycelial isolations were made from diseased needles taken from inoculated and the control trays. Additionally, isolations were made from healthy needles in each control tray, two inoculated shade grown trays (one stored in a freezer and another outdoors) and from healthy needles in two inoculated trays grown in light (one stored in a freezer and another outdoors).

Isolations from control seedlings were made from 256 healthy and 308 symptomatic needles, including 60 needles taken from branches. Isolations from inoculated seedlings were made from 40 healthy and 503 symptomatic needles, including 59 from branch needles. The number of isolations was the same for seedlings stored in a freezer or outdoors.

Fungal isolations were classified by eye according to their color: orange (similar to the isolate used in inoculation), white, pink and other colors (e.g., brown, violet, yellowish). Squash preparations were made for the detection of *Fusarium*-like spores with the light microscope.

DNA extraction

Mycelia from the Pielavesi isolate and from the according to the color classified isolates were grown on PDA media covered with a cellophane membrane to provide fresh material suitable for DNA extraction. Some isolates unfortunately died during handling and their DNA was extracted from the original PDA plates. In all cases, DNA was extracted as described by Vainio et al. (1998).

ITS-Sequencing of the isolate from Pielavesi

Sequence of the internal transcribed spacer (ITS) region of the isolate from Pielavesi was determined and compared to sequences in GenBank. The ITS region (including 5.8S rDNA) was amplified using the universal primer ITS4 (White et al. 1990) and the universal fungal primer ITS1-F (Gardes and Bruns 1993). PCR was carried out as suggested by the manufacturer of the Dynazyme thermostable polymerase (Finnzymes Ltd, Finland) and the thermal cycling profile of Gardes and Bruns (1993). The HighPure PCR purification kit (Roche, Germany) was used to purify amplification products prior to sequencing. Sequence analyses were conducted using Therm EXCEL™ II DNA sequencing kit-LC (for 66 cm gels) (Epicentre®) with the labeled-primer pair ITS1F and ITS4. Sequences were determined with an automated sequencer (LI-COR global edition IR² system, LI-COR Inc. USA) following the manufacturers instructions. DNA fragments were sequenced in both directions across the entire ITS region. Sequences were compared to those available on the NCBI public database (GenBank) via the BLAST search algorithm.

Species-specific PCR identification

In order to identify fungal isolates, two primer pairs (J1Af/r: Turner et al. 1998, and Fa17f/r: Paavanen-Huuhtanen 2000) specific to *G. avenacea* were used in successive PCRs. All isolates were first tested with J1Af/r primer pair and those that were PCR positive were subsequently confirmed using the Fa17f/r primer pair. To check false negatives, DNA quality was tested with TCG-RAMS (random amplified microsatellite) markers (Paavolainen et al. 2000). Additional primer details are provided in Table 1. The amplifications were carried out in 30 ml reaction mixtures using Dynazyme II Hot Start DNA polymerase (Finnzymes Ltd., Finland) according to the manufacturers instructions, except that the TCG-RAMS primer was supplied at 2 mM. Amplification products obtained with J1A- and TCG-RAMS primers were separated in 12% polyacrylamide gels in TBE-buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3). The Fa17f/r amplicon was electrophoresed in a 1.5% agarose gel in TAE buffer (40 mM Tris-Acetate pH 8.0, 1 mM EDTA), stained with ethidium bromide and visualized with UV trans-illumination.

Data analysis

The percent of healthy seedlings was arscin transformed and exposed to univariate analysis in SPSS 16.0 (SPSS Inc., Chicago, Illinois), and different shoot quarters were treated separately.

Table 1. Name, specificity, sequence and thermocycle profile of PCR primers used to identify *Gibberella avenacea* and *Fusarium arthrosporioides* and to confirm the quality of extracted DNA

Primer pair	Specificity	Sequence	PCR conditions	References
J1Af/r	*Gibberella avenacea, Fusarium arthrosporioides*	J1Af: GCTAATTCTTAACTTACTAGGGGCC J1Ar: CTGTAATAGGTTATTTACATGGGCG	94 °C 2 min, 35*(94 °C 30s, 58 °C 30s, 72 °C 30s), 72 °C 5min	Turner et al. 1998, Yli-Mattila et al. 2004
Fa17f/r	*Gibberella avenacea* main group II, *Fusarium arthrosporioides*[2]	Fa17f: ACCGCTTGTACCGTACCGTCAAT Fa17r: GACCGCTTGTTGCATCTAGGTAG	94 °C 3 min, 35* (94 °C 50s, 58 °C 60s, 72 °C 80s), 72 °C 3min	Paavanen-Huhtala 2000, Yli-Mattila et al. 2004
TCG-RAMS		VDH TCG TCG TCG TCG TCG V= A/C/G, D= A/G/T, H= A/C/T	95 °C 10 min 36*(95 °C 30s, 61 °C 45s, 72 °C 2min), 72 °C 7min	Paavolainen et al. 2000

Results

Identification of the Pielavesi isolate

ITS sequences of the Pielavesi isolate were identical (100% similar) to sequences of *Gibberella avenacea* Cook (GenBank isolates FJ614645, FJ980457.1 and AB272121.1). However, the sequence was also identical to isolate GU480949.1, labeled as *Fusarium lateritium* Nees. Primer pairs J1Af/r and Fa17f/r were used and, since only the first yielded an amplicon, the isolate was identified as a member of the *G. avenacea/F. arthrosporioides* clade.

A mainly orange or white mycelium combined with spore morphology confirmed the identification. The length of 2-3 septate macroconidia was 12–55 µm and 16–80 µm for 4-5 septate macroconidia. The width of the spores was 3–4 µm. Some narrow (2 µm) and 30–35 µm long spores were also present. All measured spores were borne by sporodochia.

Temperature dependence of spore germination and germ-tube growth

All spores germinated in ca. 100, 20 and 10 hours at 0, 15 and 25 °C, respectively. Germ-tube length was measured at 25 °C and at 20 °C in 5 to 10 hours, at 15 °C in 10 to 25 hours, at 10 °C in 10 to 30 hours, at 5 °C in 30 to 100 hours, and at 0 °C in 60 to 140 hours. Subsequent length measurements were limited by hyphal density. The mean germtube length was over 100 µm at 0 °C, 5 °C, 10 °C and 15 °C in 140, 60, 40, 20 hours respectively. At higher temperatures the length measurements had to be stopped earlier due to the high germination percent that led to an excessively high hyphal density.

Treatment effects on seedlings and the occurrence of damaged needles

Typically, relative humidity was slightly higher and temperature was slightly lower for shade grown seedlings than for those grown in light. In July and in of August the mean daily light intensity values (µmol/m^2/s) in light were 2.8 and 1.3 times higher than in the shade. From July 05 to October 21, the daily mean surface wetness was 0.56V in the shade and 0.61V in light.

Height growth of seedlings grown in light was slightly faster than that of seedlings grown in the shade during the summer of 2008 and spring of 2009 (Table 2). Also, stem diameter was slightly bigger for light seedlings in the spring of 2009. New growth was slightly faster for seedlings winter stored outside compared to those stored in a freezer (Table 2). The number of needles was slightly higher in seedlings grown in light, and there were more needles in the shoot quarter 1(top); means of 128 in light and 111 in shade grown plants. In the shoot quarter 2, the corresponding numbers were 67 and 49. The total number of needles/the height (cm) of seedling was 17.1±2.43 in light and 14.9±1.55 in shade seedlings.

During the winter of 2008–2009, the snow cover time lasted from the middle of November until the end of April, the maximum snow depth was 39 cm (March–April). Relative humidity was slightly higher outdoors than in the freezer during October–December but higher in the freezer than outdoors in January–April. In general, the temperature was lower in freezer storage

Table 2. The height of growth monitored control seedlings in autumn 2008 and diameters of control seedlings at tray level and length of the new shoots on 8 June 2009. Seedlings were grown in light or shade, and stored over the winter outdoors or in a freezer

	mean ±stdev height (cm)	mean±stdev diameter (mm)		mean±stdev new growth (cm)	
	autumn 2008	outdoors	freezer	outdoors	freezer
light	18.22±6.918	3.10±0.03	2.87±0.08	2.08±0.09	1.53±0.18
shade	16.43±3.571	2.69±0.12	2.63±0.06	1.59±0.16	1.32±0.12

except during January. Both temperature and relative humidity were more stable in freezer.

The percent dry weight of fresh needles from the control seedlings in spring 2009 was significantly affected by storage (P≤0.001), light treatment (P=0.017) and condition x treatment (P=0.039). Percent dry weight was lower in freezer seedlings than in those stored outdoors (p<0.01). Furthermore, shade seedlings stored in a freezer had a lower percent dry weight than light seedlings stored in a freezer (p=0.017) (Fig. 1).

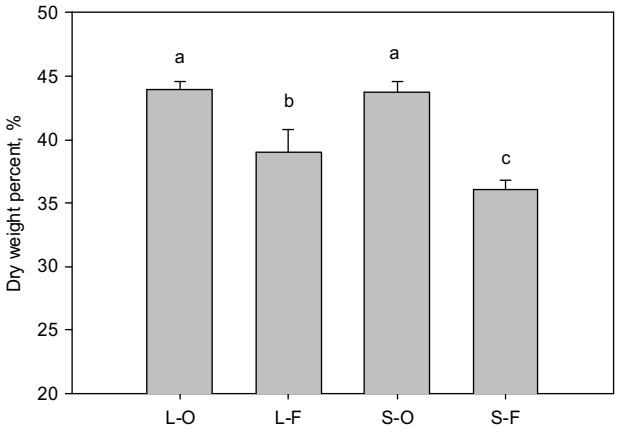

Figure 1. Mean± standard deviation percent dry weight (from fresh weight) of needle samples from four control trays in each treatment combinations: S-F, S-O, L-F and L-O. S=shade, L=light, F=freezer, O=outdoors

After winter storage, seedlings exhibited symptoms ranging from dead spots on needles to dead needles that had completely browned. The percent of healthy shoot quarter 1 (top) was higher in outdoors stored seedlings (ANOVA, on arcin transformed percent, storage effect significant, P<0,001 (Table 3a, Fig.2)). The frequencies of healthy shoot quarters 2, 3 and 4 (base) were lower in light seedlings (light treatment effect: P=0.02, P<0.001, P<0.001) (Table 3b, c, d, Fig.2). The effect of inoculation was not statistically significant.

Fungal isolation from needles and the occurrence of Gibberella avenacea

Approximately 600 and 700 isolation trials were made with shade and light seedlings, respectively. Isolations from healthy needles of inoculated seedlings resulted in 14 cases of mycelial growth, and from healthy control needles in 7 cases. Isolations resulted in 216 cased of mycelial growth from damaged needles taken from inoculated seedlings and 203 cases of mycelial growth from damaged needles from the control seedlings. In total, 419 mycelial growth from symptomatic needles and 21 from healthy needles were subjected to further analysis.

Tables 3 a-d. ANOVA of inoculation treatment, storage and light effect on arcsin transformed percent of healthy seedlings. a = shoot quarter 1(top), b = shoot quarter 2, c = shoot quarter 3, d = shoot quarter 4 (base)

a

Source	df	F	Sig.
Corrected Model	7	3.825	0.006
Intercept	1	2405.670	<0.001
treatment	1	0.734	0.400
storage	1	23.941	<0.001
light	1	0.016	0.900
treatm*storage	1	1.313	0.263
treatm*light	1	0.152	0.700
storage*light	1	0.016	0.899
treatm*storage*light	1	0.599	0.446
Error	24		

R Squared = 0.527, Adjusted R Squared = 0.389

b

Source	df	F	Sig.
Corrected Model	7	2.040	0.091
Intercept	1	1618.625	<0.001
treatment	1	0.002	0.961
storage	1	2.775	0.109
light	1	6.214	0.020
treatm*storage	1	3.045	0.094
treatm*light	1	0.779	0.386
storage*light	1	1.172	0.290
treatm*storage*light	1	0.291	0.595
Error	24		

R Squared = 0.373, Adjusted R Squared = 0.190

c

Source	df	F	Sig.
Corrected Model	7	13.060	0.000
Intercept	1	1796.874	0.000
treatment	1	1.097	0.305
storage	1	2.271	0.145
light	1	79.571	0.000
treatm*storage	1	7.156	0.013
treatm*light	1	0.143	0.709
storage*light	1	1.074	0.310
treatm*storage*light	1	0.108	0.746
Error	24		

R Squared = 0.792, Adjusted R Squared = 0.731

d

Source	df	F	Sig.
Corrected Model	7	7.762	<0.001
Intercept	1	2736.938	<0.001
treatment	1	0.003	0.954
storage	1	0.777	0.387
lightcond	1	45.175	<0.001
treatm*storage	1	3.738	0.065
treatm*lightcond	1	2.210	0.150
storage*lightcond	1	2.344	0.139
treatm*storage*lightcond	1	0.086	0.772
Error	24		

R Squared = 0.694, Adjusted R Squared = 0.604

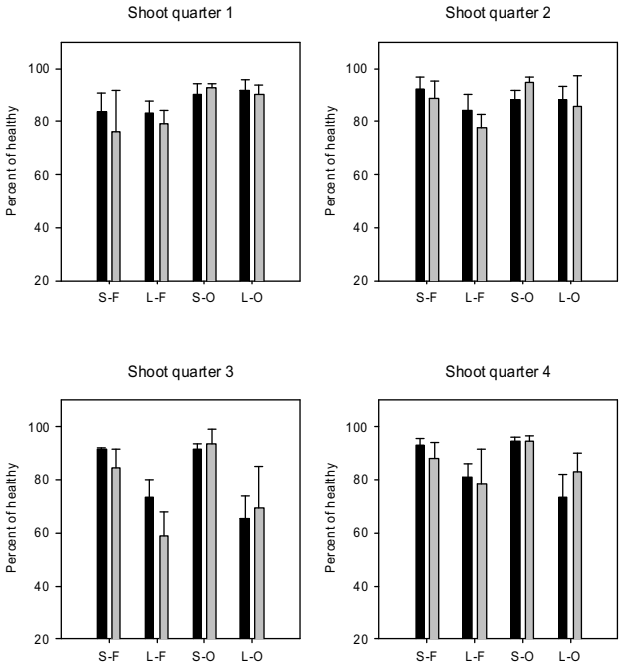

Figure 2. Mean±standard deviation tray mean percent of undamaged seedlings according to shoot quarter 1 (top), shoot quarter 2, shoot quarter 3, shoot quarter 4 (base) and treatment combination S-F, L-F, S-O and L-O. S=shade, L=light, F=freezer, O=outdoors. Control (black bar) and inoculated (gray bar) seedlings

Curved, long, narrow spores typical also to *G. avenacea* were detected in 44 isolates: 22 from shade grown and 22 from light seedlings. From these 44 sporulating isolates only three were from the control trays. From the isolates, 29 were classified orange, 12 white.

PCR products obtained with primers specific of *G. avenacea* showed that the inoculation isolate belonged to *G. avenacea /F. arthrosporioides* rather than the main group II of *G. avenacea*. PCR primers identified 30 isolates as *G. avenacea /F. arthrosporioides* (Table 4) and 15 of these produced curved, long and narrow spores typical of *G. avenacea*. In addition, there were 29 sporulating isolates, 4 of which did not amplify with *G. avenacea*-specific primers and 18 could not be characterized with molecular tools. Including 29 isolates that produced spores typical of *G. avenacea*, 59 isolates, similar to the isolate used in inoculations, were obtained in total. These 59 originated from different light treatment x storage combinations but were almost evenly split between light-outside storage and shade-freezer storage treatments. In the light-outside storage treatment, *G. avenacea* occurred more often in the seedling lower quarters. In the shade-freezer storage treatment, *G. avenacea* occurred more often in the top quarter and branches (Fig. 3, Table 4). The distribution of *G. avenacea* among different shoot parts seemed to be influenced by the extent of tissue damage from where the sample was taken (see Fig. 2). The number of seedlings, from which the isolation procedure resulted in *Gibberella avenacea* or isolates producing spores typical of *G. avenacea* was 13 and 5 in light seedlings stored outside or in the freezer, respectively, and 6 and 18 in shadow grown seedlings stored outside or the freezer, respectively.

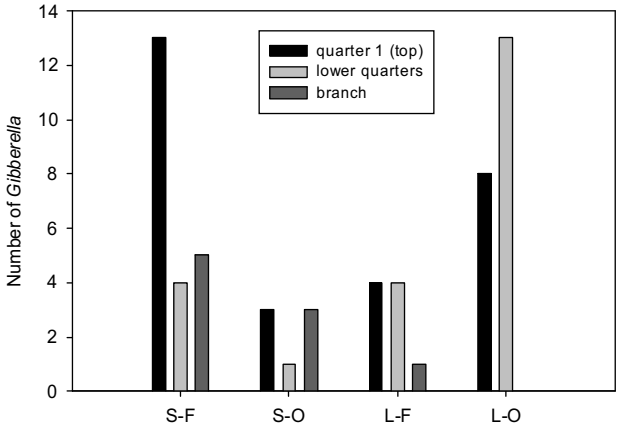

Figure 3. Number of isolates of G. avenacea confirmed or Fusarium-like spores from damaged needles of S-F, L-F, S-O and L-O seedlings, the needles from quarter 1(top), lower quarters or branches. S=shade, L=light, F=freezer, O=outdoors

Discussion and conclusions

The one aim of this study was to identify *Fusarium*-like isolates in Norway spruce seedlings suffering from a springtime needle disease associated with snow mold fungus, *Herpotrichia juniperi*. The species was proved to be *Gibberella avenacea* (syn. *Fusarium avenaceum* (Fr.) Sacc.). We successfully combined molecular techniques with traditional morphological measurements to help identify *G. avenacea* from a nursery in central Finland (Pielavesi). Species-specific PCR was also used to verify that *Gibberella* was present in isolates recovered from inoculated seedlings. Of the primers used, J1Af/r successfully detected *G. avenacea* (Turner et al. 1998, Yli-Mattila et al. 2004) and *F. arthrosporioides* (Paavanen-Huhtala 2000, Yli-Mattila et al. 2004). Primer pair Fa17f/r was originally designed to identify a group of *G. avenacea* (main group II: Paavanen-Huuhtanen, 2000) but it also amplified most isolates of *F. arthrosporioides* (Yli-Mattila et al. 2004). Since none of the isolates amplified with Fa17f/r, we believe most or all of our isolates represent *G. avenacea /F. arthrosporioides* rather than *G. avenacea* main group II.

Taxonomic concepts based on morphology maintain *G. avenacea* and *F. arthrosoprioides* as separate

Table 4. Isolation data of symptomatic needles from inoculated and control seedlings. Number (N) of isolations that produced mycelia, mycelial isolates (and %) in which *G. avenacea* was identified, producing *Gibberella*-like spores, producing spores and/ or confirmed *G. avenacea* (% of mycelia)

	Inoculated				Control			
	Top	Lower	Branch	TOTAL	Top	Lower	Branch	TOTAL
Grown in a shade, stored in freezer								
N mycelia	46	11	3	60	25	9	3	37
N *G. avenacea* confirmed	6	1	2	9	0	0	0	0
G. avenacea confirmed/mycelia: %	13.04	9.09	66.67	15.00	0	0	0	0
N *G. avenacea* confirmed or *Gibberella*-like spores	13	4	3	20	0	0	2	2
N *G. avenacea* confirmed or *Gibberella*-like spores/mycelia: %	28.26	36.36	100.00	33.33	0	0	0	0
Grown in a shade, stored outside								
N mycelia	22	12	7	41	16	20	5	39
N *G. avenacea* confirmed	2	1	2	5	0	0	0	0
G. avenacea confirmed /mycelia: %	9.09	8.33	28.57	12.20	0	0	0	0
N *G. avenacea* confirmed or *Gibberella*-like spores	3	1	3	7	0	0	0	0
N *G. avenacea* confirmed or *Gibberella*-like spores/mycelia: %	13.64	8.33	42.86	17.07	0	0	0	0
Grown in light, stored in freezer								
N mycelia	35	21	9	65	33	25	5	63
N *G. avenacea* confirmed	2	2	0	4	1	1	1	3
G. avenacea confirmed /mycelia: %	5.71	9.52	0	6.15	3.03	4.00	20.00	4.76
N *G. avenacea* confirmed or *Gibberella*-like spores	3	3	0	5	1	1	1	3
N *G. avenacea* confirmed or *Gibberella*-like spores/mycelia: %	5.71	14.29	0	7.69	3.03	4.00	20.00	4.76
Grown in light, stored outside								
N mycelia	15	45	4	64	19	46	4	69
N *G. avenacea* confirmed	3	6	0	9	0	0	0	0
G. avenacea confirmed /mycelia: %	20.00	13.33	0	14.06	0	0	0	0
N *G. avenacea* confirmed or *Gibberella*-like spores	8	13	0	21	0	0	0	0
N *G. avenacea* confirmed or *Gibberella*-like spores/mycelia: %	53.33	28.89	0	32.81	0	0	0	0

species (Booth 1971, Gerlach and Nirenberg 1982) or place *F. arthrosoprioides* as a subspecies of *G. avenacea* (Nelson et al.1983). However, these species are difficult to distinguish morphologically and molecular methods group them in a single clade (Yli-Mattila et al. 2004, Kristensen et al. 2005). Sequence data derived from the original isolates were identical with many public sequences identified as *Gibberella avenacea*, and which we believe to be conspecific with the pathogen isolated here. Furthermore, spore measurements also support this conclusion. According to Booth (1971), the length of 4-7 septate spores produced in sporodochia of *G. avenacea* is 40–80 x 3.5–4 µm, which are more similar to our measurements than 3-5 septate spores of *F. lateritium* (22–48 x 3.5–5.5 µm).

The other aim was to determine how environmental conditions during the growth period and winter storage affect seedlings and further the pathogenicity of *G. avenacea*. Although seedlings were asymptomatic at the end of the growing season, after winter storage there were dead spots on needles and dead needles in both inoculated and control seedlings.

Light intensity in shade during July–August was mainly half of that in open field (in light). In spite of lower growth and in shoot quarters 1-2 smaller number of needles, the seedlings grown in shade (both inoculated and control, freezer or stored outdoors) were generally less damaged in shoot quarters 2-4 than the light grown seedlings. Norway spruce is considered intermediate in shade tolerance with seedlings growing better in more open environments with just lateral shade (Stanescu and O'Hara 2006). Here in our study the better growth of seedlings grown in light was probably due to light intensity that was a 1.3–2.8 fold more µmol/m^2/s in light site than in shade (Xiaobing Dai 1996, Mortensen and Sandvik 1988).

Storage conditions in the first half of the winter of 2008/2009 were more stable in the freezer (in cardboard boxes) than outdoors, but in the second half snow cover increased the stability outdoors. In general, temperature was lower in freezer storage. Seedlings stored outdoor fared better than in the freezer over the winter of 2008/2009 if the dry weight percent of fresh needles and the length of new growth measured in the spring of 2009 are taken into account. This is in accordance with the earlier results (Petäistö and Laine 1999). After storage, the dry weight percent of needles was lower in seedlings stored in the freezer. Inoculation seemed to increase damage in freezer seedlings. Among other fungi, Venn (1983) detected *Gibberella* in moldy Norway spruce seedlings that had overwintered in cold storage. In his test, *Gibberella avenacea* was able to discolour detached needles at 15 °C, and within 14 days over 50% of needles were lethally affected (Venn 1983). In the present study, the *in vitro* growth rate of *G. avenacea* correlated positively with temperature and was highest at 25 °C. However, we note that the isolate grew at temperatures considerably lower than this.

It was also possible to isolate *G. avenacea* from damaged needles, especially from the top quarter (young needles) of shade seedlings stored in the freez-

er and from lower quarters (old needles) of light seedlings stored outdoors. According to a visual inspection, light seedlings were more damaged in lower shoot quarters. Poor health of needles might increase the success of *Fusarium* infection in light seedlings stored outside. Surface wetness can also enhance infection, which was slightly higher during the growing period for seedlings in light, most likely due to the need for increased irrigation. In shade seedlings stored in the freezer, successful infection might be manifest in slightly diminished health of top quarters where the relative humidity was somewhat higher. Freezer stored seedlings also had a lower needle dry weight percent than those stored outside, reaching a minimum in shade grown seedlings stored in the freezer. Freezer storage also decreased seedling vigor, which was also evident in slower growth the following spring. Low light intensity is reported to be a predisposing factor for foliage pathogens (Wall and Magasi 1976, Zhang et al. 1994, Zhang et al. 1995). Thus, the shade-freezer combination likely promoted infection and growth of *G. avenacea* on young needles. The pathogen used here was derived together *Herpotrichia nigra,* a species known to thrive in high humidity and at low temperatures (Gäumann et al. 1943, Hanso and Torva 1975).

In conclusion, this investigation shows that the *Fusarium*-like fungus isolated from Norway spruce nursery seedlings is *Gibberella avenacea*. The fungus could be reisolated more frequently from young needles of seedlings grown in the shade and overwintered in a freezer and from older needles of seedlings grown in light and stored outdoors. In general, light intensity and storage treatments affected seedling health. Young needles of both inoculated and control seedlings stored outdoors were generally healthier than seedlings stored in the freezer. In light, both inoculated and control seedlings had more damage on older needles than those grown in the shade, although shoot growth was superior in light.

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ИССЛЕДОВАНИЕ ВЛИЯНИЯ СВЕТА И УСЛОВИЙ ЗИМНЕГО СОДЕРЖАНИЯ НА СЕЯНЦЫ ЕЛИ ЕВРОПЕЙСКОЙ С УДЕЛЕНИЕМ ОСОБОГО ВНИМАНИЯ ЗАРАЖЕННОСТИ ГРИБОМ *GIBBERELLA AVENACEA*

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Резюме

После зимы 2006 года из сеянцев ели европейской в питомнике вместе с грибами рода *Herpotrichia* были выделены *Fusarium*-подобные грибы. Согласно последовательностям нуклеотидов внутренних транскрибируемых спейсеров (ITS) и морфологическим данным, наиболее распространенным *Fusarium*-подобным видом был вид *Gibberella avenacea***.** Полное (т. е. 100 %) прорастание *конидиоспор* наблюдалось в условиях *in vitro* при 0–25 °C, однако его скорость и рост ростковой трубочки зависели от температуры. В начале июля сеянцы переносили на открытый воздух и содержали в двух видах условий: в тени или под солнечными лучами. После этого половину сеянцев раз в месяц заражали спорами бесполого размножения *Gibberella avenacea* на протяжении четырех месяцев. После зимнего содержания в морозильной камере или на открытом воздухе на хвое зараженных и контрольных сеянцев наблюдали локализованные некрозы. Количество хвои и скорость роста были выше у сеянцев, находившихся на свету. В контрольной группе сеянцев скорость роста побегов и процент сухого веса хвои были выше у растений, содержавшихся на открытом воздухе по сравнению с сеянцами, перезимовавшими в морозильной камере. На верхней четверти побега хвоя была менее здоровой у сеянцев, содержавшихся в морозильной камере, в то время как хвоя на нижней четверти побегов у сеянцев, выращивавшихся в тени, оказалась более здоровой, чем у сеянцев, росших на свету. Повреждение, вызванное заражением, не было статистически достоверным.

Культуры *G. avenacea* получали почти полностью из зараженных сеянцев и идентифицировали с помощью анализа нуклеотидных последовательностей ITS и видоспецифической полимеразной цепной реакции (ПЦР). Число изолятов *G. avenacea* было наивысшим у сеянцев, росших в тени и содержавшихся в морозильной камере, а также у сеянцев, росших на свету и содержавшихся на открытом воздухе. У сеянцев, росших в тени и содержавшихся в морозильной камере, *G. avenacea* чаще всего выделяли из верхушек, в то время как у сеянцев, росших на свету и содержавшихся на открытом воздухе, этот гриб чаще всего обнаруживали на нижней четверти.

Ключевые слова: *Gibberella avenacea*, *Fusarium*, *Picea abies*, кассетные сеянцы, перезимовка, особенности содержания в питомнике, свет, лесовозобновление, лесное хозяйство Фенноскандии, фузариоз, фузариозная гниль