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Interaction of Prechilling, Temperature, Osmotic Stress, and Light in *Picea abies* Seed Germination

Kari Leinonen and Hannu Rita

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A multi-factor experimental approach and proportional odds model was used to study interactions between five environmental factors significant to Norway spruce seed germination: prechilling (at +4.5 °C), suboptimal temperatures (+12 and +16 °C), osmotically induced water stress (–0.3 Mpa and 0 Mpa), prolonged white light, and short-period far-red light. Temperature and osmotic stress interacted with one another in the germination of seeds: the effect of osmotic stress being stronger at +16 °C than at +12 °C. In natural conditions, this interaction may prevent germination early in the summer when soil dries and temperature increases. Prolonged white light prevented germination at low temperature and low osmotic potential. Inhibitory effect was less at higher temperatures and higher osmotic potential, as well as after prechilling. Short-period far-red light did not prevent germination of unchilled seeds in darkness. Prechilling tended to make seeds sensitive to short pulses of far-red light, an effect which depended on temperature: at +12 °C the effect on germination was promotive, but at +16 °C, inhibitory and partly reversible by white light. It seems that Norway spruce seeds may have adapted to germinate in canopy shade light rich in far-red. The seeds may also have evolved mechanisms to inhibit germination in prolonged light.

Keywords germination ecology, germination inhibition, far-red light, osmotic stress, *Picea abies*, stratification, prolonged white light.
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1 Introduction

Temperature and moisture are important environmental factors controlling the germination of Norway spruce seeds under field conditions (Mork 1933, Yli-Vakkuri 1961a, b, Bjør 1971, Leinonen et al. 1993). In Finland, Norway spruce seeds disperse in April and May on snow or cold moist soil (Heikinheimo 1932), enabling the seeds to undergo a few weeks' chilling (stratification) period during spring. The seeds dispersing under canopy shelter do not, however, germinate until late July or early August (Yli-Vakkuri 1961b). The germination of Norway spruce seeds is regulated within microhabitats by slowly increasing temperatures and decreasing soil moisture (Mork 1933, Nordén 1990, Kubin and Kemppainen 1991). From an ecological perspective, it is important to know how different environmental factors control the timing of Norway spruce seed germination in natural microenvironments.

Norway spruce is a shade-tolerant climax species which can regenerate under spruce forest and in small canopy gaps (Leinonen et al. 1989, Leemans 1991, Liu Quighong and Hytteborn 1991). Many Norway spruce seed lots do not require light for high germination capacity at optimum temperatures (Kamra 1967, Heit 1968, Safford 1974, Bergsten 1989, Leinonen et al. 1993). The literature offers insufficient evidence as to the response of Norway spruce seeds to light under suboptimal incubation conditions. In many species, the effect of light depends on the other environmental factors present (Hilhorst and Karssen 1989, Pons 1992). The effect of light on seeds is mediated by the pigment phytochrome, which exists in two forms – P_R and P_{FR} (e.g. Cone and Kendrick 1986, Attridge 1990). Seeds of many species require the presence of a particular threshold level of P_{FR} to germinate. Exposure of P_R to red light transforms it to P_{FR}. Exposure to light rich in far-red reverse most of the P_{FR} to P_R, resulting in P_{FR} levels below the threshold (e.g. Attridge 1990). Prolonged light, in contrast, may either stimulate or inhibit germination (e.g. Pons 1992). In addition, the phytochrome reaction can be affected (sensitized) by prechilling (VanderWoude 1989). As a climax species, Norway spruce seeds may have adapted to germinate in shade conditions. The photon flux density under spruce forest is reduced across the spectrum, but it is relatively rich in far-red range, because the canopy reduces more effectively the photosynthetically active part of spectrum (400–700 nm) than the longer (700–1000 nm) wavelengths (Smith 1982).

We wanted to test in the laboratory, which environmental factors would most effectively cause Norway spruce seed germination to vary in different natural microhabitats. We explored the any possible interactions between prechilling, osmotically induced water stress, suboptimal temperatures, and light in germination of Norway spruce seeds. The test temperatures +12 °C and +16 °C represent temperature conditions under spruce forest in the summer when the seeds germinate (Mork 1933, Kubin and Kemppainen 1991). Decreased osmotic potential (–0.3 Mpa) served to simulate natural moisture stress under spruce stands (c.f. Nordén 1990). We hypothesized that the effect of light would be more profound at suboptimal temperatures and under water stress than under standard laboratory germination test conditions (International Seed Testing Association 1985). Concerning light we wanted to know the effect of prolonged white light and short-period far-red light on germination. In addition, we wanted to know whether light responses are affected by prechilling.

2 Material and methods

2.1 Seed Material and Treatments

Seed source, extraction, and processing

Seeds were collected in October 1989 from ten open-pollinated Norway spruce (*Picea abies* (L.) Karst.) trees near the Forestry Field Station of the University of Helsinki, southern Finland (61°51'N, 24°20'E 160–170m a.s.l.); approximately 10 liters of cones were collected from each tree. The seeds were extracted in a ventilated oven at +35 °C for 72 hours. Wings were removed by hand, and seeds were stored airtight in a cold room (+4.5±0.5 °C) until used. In the present study seeds from one of those trees were selected for further testing after 17 months' storage. All the seeds tested were chosen at random by use of a soil divider. Empty, immature, and insect-damaged seeds were removed by X-ray analysis (Simak 1980); only seeds with fully developed embryo and megagametophyte were tested.

Experimental design

The experimental design was 2^5-factorial with five factors: temperature (T), osmotic potential (O), prolonged white light (W), short-period far-red light (FR), and prechilling (C). Each factor had two levels. A total of 3200 seeds were randomized into the resultant 32 different treatment combinations, 100 seeds in each.

Temperature treatments

The temperature response of the seed lots studied was tested three months after cone collection (Leinonen et al. 1993). Temperature levels +12 °C and +16 °C were selected on the basis of that 1993 study, because dark-germination was found to increase strongly – from about 15 % to 80 % – between these temperatures and because we were interested in this ecologically important temperature regime within which adaptation mechanisms were supposed to be most discernable. At optimum temperature +21 °C, the dark-germination of this seed lot was close to 100 % (Leinonen et al. 1993). The temperature treatment took place in two constant-temperature chambers maintained at +12 °C and +16 °C. The chambers were monitored continuously with a temperature recorder and electronic sensors. Maximum deviation from target temperature during the test was 0.3 °C.

Osmotic potential treatments

Osmotic potential levels were 0 Mpa and –0.3 MPa. Moisture stress was developed with polyethylene glycol (PEG 6000) according to the formula described by Michel and Kaufmann (1973). The concentration of PEG 6000 in distilled water was adjusted to provide an osmotic potential of –0.3 MPa at the two incubation temperatures. Deionized distilled water was used as a control (0 MPa).

Light treatments

Continuous white light (W) (24h light, 60 µmol $m^{-2}s^{-1}$ PAR) was given in growth chambers furnished with two fluorescent tubes (Philips 15 W TLD 80). The photon flux densities at 660 nm and 730 nm wavelengths were 0.562 µmol $m^{-2}s^{-1}$ and 0.255 µmol $m^{-2}s^{-1}$. The red/far-red ratio was 2.16. Dark control was achieved by closing the petri dishes inside aluminum molds.

Far-red light (FR, 730 nm) for 15 minutes was supplied to the seeds after 0, 3, 6, and 12 hours from the beginning of the test (c.f. Nyman 1963). Far-red light was obtained by filtering one 250W incandescent lamp (Airam LMK 250W) by water (10 cm) and double layers of red and blue cellophane filters, resulting in photon flux densities 0.8 µmol $m^{-2}s^{-1}$ (on 660 nm wave band) and 32 µmol $m^{-2}s^{-1}$ (on the 730 nm wave band) the red/far-red ratio being 0.0025 (c.f. Henry and Blazich 1990). The control seeds were not treated by FR.

All manipulations of imbibed seeds during stratification and germination tests were conducted in dim green safe light. This light was obtained by filtering two green fluorescent tubes (Philips 36W/17) with two layers of yellow (No. 101) and two layers of blue (No. 183) Cinemoid filters (LeeColotran Ltd.). The photon flux densities were measured with the Skye instrument SKR 110 as 0.002 µmol $m^{-2}s^{-1}$ and 0.000 µmol $m^{-2}s^{-1}$ at wavebands 660 and 730 nm, respectively. The manipulations of dry (8 % fresh weight basis) seeds were done in the laboratory under white light.

Prechilling

The seeds were prechilled in a dark, cold room at +4.5±0.5 °C for 32 days on germination paper in petri dishes moistened with distilled water. After prechilling, the seeds were germinated without subsequent drying (International Seed Testing Association 1985). The unchilled seeds were not imbibed in water before the start of the experi-

ment to ensure that the seeds would not escape from far-red control (c.f. Pons 1992).

Germination test

The seeds were germinated in 50 mm disposable polystyrene petri dishes lined with 10 sheets of filter paper (30 mm) and two sheets of filter paper (50 mm) moistened with 10 ml of test solution (either distilled water or PEG). The petri dishes were sealed with laboratory film in order to minimize evaporation and then placed at random into constant temperature chambers.

The emergence of the radicle (1 mm) showing positive geotropism was the criterion for germination. The germinated seeds were counted on the 7th, 14th, and 21st days from the beginning of the test. The viability of non-germinated seeds from different treatments was tested at +21 °C, with a photoperiod of 16/8 h (light/dark) for 14 days when almost all of the seeds were germinated.

2.2 Statistical Methods

The time needed for germination is a continuous variable, but we made the germination counts only on the 7th, 14th and 21st days of imbibition, in order to minimize disturbances during checking. The continuous response variable, germination time, was thus reduced to ordinal variable Y with the four possible values (categories):

Categories (j)
1 seeds germinating within 1–7 days
2 seeds germinating within 8–14 days
3 seeds germinating within 15–21 days
4 viable seeds remained ungerminated during 21 days.

The behavior of germination time Y can be characterized by probabilities

$$\pi_j(\mathbf{x}) = P(Y = j|\mathbf{x}) \quad (1)$$

in which j = 1, 2, 3, 4 that a single seed falls into germination category and the vector **x** specifies factor level. Because the response variable Y was measured on an ordinal scale, the cumulative germination probabilities can be expressed as

$$\gamma_j(\mathbf{x}) = P(Y \le j|\mathbf{x}) \quad (2)$$

for which j = 1, 2, and 3, the term $\gamma_4(\mathbf{x}) = 1$ as a definition of Y. The effects of the factors are modeled using the proportional odds model (McCullagh and Nelder 1989, p. 151–155), in which the cumulative probabilities $\gamma_j(\mathbf{x})$ are transformed into the logistic scale and modelled using parallel linear regression as follows:

$$\log\frac{\gamma_j(\mathbf{x})}{1-\gamma_j(\mathbf{x})} = \Theta_j - \beta'\mathbf{x}, \quad j = 1,2,3. \quad (3)$$

The parameters Θ_j characterize the effects of the counting dates on the logistic scale, i.e. the cut-off points used to subdivide the germination period into different time segments. As noted by Andersson (1984), the interpretation of the cut-point parameters is difficult and depends on how often germination counts are made.

The components of the coefficient β characterize the effects of the factors and their interactions. In the proportional odds model, there is only one regression coefficient β for each factor or interaction, which is independent of the choice of category (j). That is, the coefficients β are common for all three log odds $(\gamma_j(\mathbf{x})/(1-\gamma_j(\mathbf{x}))$ for categories (j = 1, 2, 3).

To simplify interpretation we used coded values 0 and 1 for factors. The value 0 corresponds to the lower level and the value 1 to the higher level of a specific factor. All data was thus related to the baseline, which was defined in this experiment as a treatment combination of +12 °C, –0.3 Mpa and darkness with no FR or prechilling treatment. The values of the coefficients are interpreted as odds ratios

$$\log\frac{odds(\gamma_j(1))}{odds(\gamma_j(0))} = \exp[\beta] \quad (4)$$

where β is the coefficient corresponding to the factor under study. Positive values of regression coefficients represent a tendency towards the earlier germination categories, i.e. shorter germination times, when the factor level increases from the low to the high level.

The models were fitted using the PR-procedure (polychotymous logistic regression) of BMDP (Moran et al. 1990) and the LOGISTIC procedure of SAS (SAS/STAT 1989); the parameter estimates were the same with both procedures. The parallelism of the regression lines (proportional odds assumption) was tested using the score test in SAS LOGISTIC. The same procedure was used to obtain the 95 % confidence intervals for the probabilities.

3 Results

The parameter estimates of the model that was found most adequately to describe the germination data are given in Table 1. The model includes all main effects, nine first-, five second-, and one third-order interaction term. The complexity of the model is not only an implication of the large sample size, because the odds ratio describing the effect of the only third-order interaction was high (15.7). The deviance for this model was insignificant (57.2 df = 73, p = 0.91), indicating a proper goodness of fit. The proportional odds assumption was not violated, as the score test was nonsignificant (39.5 df = 40, p = 0.49). As the sample size was large, this can be regarded as evidence of the validity of the proportional odds assumption. The observed and predicted values are presented in Table 2.

The predicted germination probabilities at the baseline were 0.00, 0.21 and 0.90 after 7, 14, and 21 days, respectively. The regression coefficients for temperature (β_T) and osmotic potential (β_O) were positive and significant (Table 1), repre-

Table 1. Parameters of proportional odds model. Abbreviations: T = temperature, O = osmotic potential, W = prolonged white light, FR = short-period far-red light, and C = prechilling. The interaction of the treatment effect were marked by ×. Wald statistic = coefficient/standard error and odds ratio = $e^{coefficient}$.

Term	Coefficient	Standard error	Wald-statistics	P-value	Odds ratio	95% confidence interval of odds ratio	
Θ_1	–12.082	1.048	–11.53	<0.001	0.000	0.000	0.000
Θ_2	–1.350	0.199	–6.79	<0.001	0.259	0.176	0.383
Θ_3	2.156	0.212	10.19	<0.001	8.634	5.704	13.060
β_T	4.348	0.342	12.70	<0.001	77.316	30.520	151.261
β_O	3.430	0.235	14.57	<0.001	30.880	19.467	48.984
β_C	0.278	0.277	1.00	0.316	1.321	0.767	2.275
β_W	–1.836	0.257	–7.14	<0.001	0.159	0.096	0.264
β_{FR}	0.219	0.253	0.87	0.392	1.245	0.758	2.044
$\beta_{T\times O}$	4.271	1.060	4.03	<0.001	71.557	8.963	571.294
$\beta_{T\times C}$	6.448	1.118	5.77	<0.001	631.249	70.63	5642.03
$\beta_{T\times W}$	0.941	0.362	2.60	<0.001	2.561	1.260	5.207
$\beta_{T\times FR}$	0.574	0.375	1.53	0.125	1.776	0.852	3.399
$\beta_{O\times C}$	0.713	0.394	1.81	0.070	2.041	0.942	4.420
$\beta_{O\times W}$	0.887	0.235	3.77	0.009	2.428	1.532	3.849
$\beta_{C\times W}$	1.150	0.369	3.12	0.002	3.159	1.534	6.506
$\beta_{C\times FR}$	1.219	0.379	3.21	0.001	3.383	1.609	7.114
$\beta_{W\times FR}$	0.550	0.342	1.61	0.107	1.733	0.887	3.386
$\beta_{T\times O\times C}$	–5.270	1.204	–4.38	<0.001	0.005	0.000	0.054
$\beta_{T\times C\times W}$	–0.046	0.532	–0.09	0.931	0.955	0.337	2.710
$\beta_{T\times C\times FR}$	–3.483	0.549	–6.34	<0.001	0.031	0.010	0.090
$\beta_{T\times W\times FR}$	–2.480	0.502	–4.94	<0.001	0.084	0.031	0.224
$\beta_{C\times W\times FR}$	–0.013	0.524	–0.02	0.980	0.987	0.354	2.756
$\beta_{T\times C\times W\times FR}$	2.755	0.760	3.62	<0.001	15.724	3.542	69.783

Deviance 57.156 d.f. 73, p-value = 0.914
Score test for propotional odds assumption 39.5 d.f. 40, p-value=0.493

Table 2. Cumulative proportions observed (obs) and cumulative probabilities predicted (pred) with 95% confidence intervals (c.i) for each outcome category. Abbreviations: T = temperature, O = osmotic potential, W = white light, FR = far-red light, and C = prechilling. Zero (0) = lower treatment level or no treatment and one (1) = higher treatment level or active treatment. Cell residuals exceeding the value 2.0 are marked with letter [a].

	Within 1–7 days			Within 8–14 days			Within 15 –21 days		
T,O,C,W,FR	Obs.	Pred.	95% c.i.	Obs.	Pred.	95% c.i.	Obs.	Pred.	95% c.i.
0 0 0 0 0	0.00	0.00	0.00–0.00	0.21	0.21	0.15–0.28	0.89	0.90	0.85–0.93
0 0 0 0 1	0.00	0.00	0.00–0.00	0.20	0.24	0.18–0.32	0.94	0.92	0.88–0.94
0 0 0 1 0	0.00	0.00	0.00–0.00	0.03	0.04	0.03–0.06	0.67	0.58	0.49–0.66
0 0 0 1 1	0.00	0.00	0.00–0.00	0.07	0.08	0.05–0.12	0.69	0.75	0.67–0.81
0 0 1 0 0	0.00	0.00	0.00–0.00	0.25	0.26	0.19–0.34	0.91	0.92	0.88–0.95
0 0 1 0 1	0.00	0.00	0.00–0.00	0.60	0.59	0.49–0.68	0.99	0.98	0.97–0.99
0 0 1 1 0	0.00	0.00	0.00–0.00	0.15	0.15	0.10–0.21	0.83	0.85	0.79–0.90
0 0 1 1 1	0.00	0.00	0.00–0.00	0.59	0.55	0.46–0.65	0.95	0.98	0.96–0.98
0 1 0 0 0	0.00	0.00	0.00–0.00	0.89	0.89	0.83–0.93	1.00	1.00	0.00–1.00
0 1 0 0 1	0.00	0.00	0.00–0.00	0.93	0.91	0.86–0.94	1.00	1.00	0.99–1.00
0 1 0 1 0	0.00	0.00	0.00–0.00	0.68	0.76	0.68–0.82	0.98	0.99	0.98–0.99
0 1 0 1 1	0.00	0.00	0.00–0.00	0.93	0.87	0.81–0.91	0.99	1.00	0.99–1.00
0 1 1 0 0	0.00	0.00	0.00–0.00	0.97	0.96	0.91–0.98	1.00	1.00	1.00–1.00
0 1 1 0 1	0.00	0.00	0.00–0.01	0.98	0.99	0.98–0.99	1.00	1.00	1.00–1.00
0 1 1 1 0	0.00	0.00	0.00–0.00	0.98	0.96	0.93–0.98	1.00	1.00	1.00–1.00
0 1 1 1 1	0.00	0.00	0.00–0.03	0.98	1.00	0.99–1.00	1.00	1.00	1.00–1.00
1 0 0 0 0	0.01	0.00[a]	0.00–0.00	0.98	0.95	0.92–0.97	1.00	1.00	1.00–1.00
1 0 0 0 1	0.00	0.00	0.00–0.01	0.95	0.98	0.96–0.99	1.00	1.00	1.00–1.00
1 0 0 1 0	0.00	0.00	0.00–0.00	0.84	0.89	0.84–0.93	1.00	1.00	0.99–1.00
1 0 0 1 1	0.00	0.00	0.00–0.00	0.77	0.72	0.64–0.79	0.98	0.99	0.98–1.00
1 0 1 0 0	0.23	0.27	0.20–0.35	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00
1 0 1 0 1	0.13	0.08[a]	0.05–0.12	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00
1 0 1 1 0	0.32	0.31	0.23–0.40	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00
1 0 1 1 1	0.16	0.19	0.13–0.27	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00
1 1 0 0 0	0.45	0.49	0.40–0.58	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00
1 1 0 0 1	0.71	0.68	0.59–0.76	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00
1 1 0 1 0	0.54	0.49	0.40–0.58	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00
1 1 0 1 1	0.19	0.24	0.17–0.31	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00
1 1 1 0 0	0.94	0.89	0.84–0.93	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00
1 1 1 0 1	0.61	0.66	0.57–0.74	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00
1 1 1 1 0	0.95	0.96	0.93–0.98	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00
1 1 1 1 1	0.93	0.93	0.88–0.96	1.00	1.00	1.00–1.00	1.00	1.00	1.00–1.00

senting a tendency towards earlier germination categories at higher treatment levels, relative to the baseline. The odds ratios $e^{\beta T}$ (77.3) and $e^{\beta O}$ (30.9) characterize the differences in cumulative germination probabilities between temperature and osmotic treatment levels 0 and 1. The large odds ratio for temperature indicates that raising the temperature by 4 °C improved germination more than did increasing the osmotic potential from –0.3 to 0 Mpa. The cumulative germination probabilities on the 7th, 14th, and 21st days at 95 % confidence intervals are shown in Fig. 1.

The interpretation of interaction coefficients is also based on the odds ratios. The value $e^{\beta T \times O}$ (71.6) indicates how the temperature odds ratio at the lower osmotic potential level has to be modified to obtain a comparable odds ratio at the higher osmotic level (Table 1). This significant positive interaction coefficient represents a strong trend towards earlier germination categories compared to what would be expected by the main effect model. Under these conditions germination increased rapidly, reaching 50 % germination during the first 7 days of imbibition; by 14 days, all the seeds had germinated (Fig. 1).

The effect of prechilling on germination depended on temperature. At +12 °C, prechilling did not improve germination significantly in darkness, nor did differences in osmotic levels as supported by the insignificant values for regression coefficients β_C and $\beta_{O\times C}$ (Table 1). At 16 °C, prechilled seeds germinated faster than unchilled ones at osmotic level –0.3MPa as indicated by the significant positive interaction coefficient $\beta_{T\times C}$; at 0MPa the effect of prechilling was not as profound due to the negative second-order interaction coefficient $\beta_{T\times O\times C}$, which negates part of the effect of $\beta_{T\times C}$. The effect of these factors on a probability scale is shown in Fig. 1.

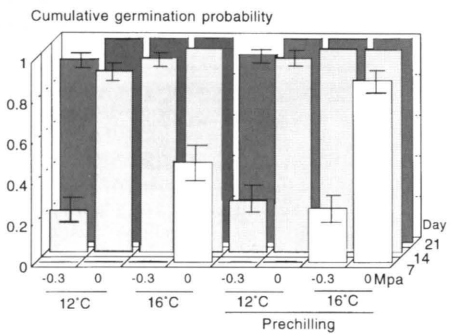

Fig. 1. The effect of osmotic potential, temperature and prechilling on cumulative germination probability of Norway spruce seeds in darkness. Small bars represent 95 % confidence bounds for predicted probability.

Germination of Norway spruce seeds was inhibited by prolonged white light at +12 °C and –0.3 MPa as shown by odds ratio ($e^{\beta W}$ = 0.159) which characterizes the change in effect when the light factor increased from 0 to 1 (from darkness to light). The cumulative germination probability after 21 days was 0.58 and 0.90 in light and darkness, respectively (Fig. 2a). The inhibitory effect of light decreased when either temperature or osmotic potential increased to a higher level (negative regression coefficients $\beta_{T\times W}$ and $\beta_{O\times W}$; Table 1). At +16 °C and 0 MPa the seeds germinated equally well in light and darkness (Fig. 2a).

Prechilled seeds were not inhibited at +12 °C and –0.3MPa by white light as strongly as were unchilled seeds, as indicated by the significant positive interaction coefficient $\beta_{C\times W}$ (Table 1). The cumulative germination probability of seeds in light after 21 days was increased from 0.58 to 0.85 by prechilling (Fig. 2a, b). Prechilled seeds germinated equally well in light and darkness at +12 °C and 0 MPa, and at +16 °C, the seeds germinated even faster in light than in darkness.

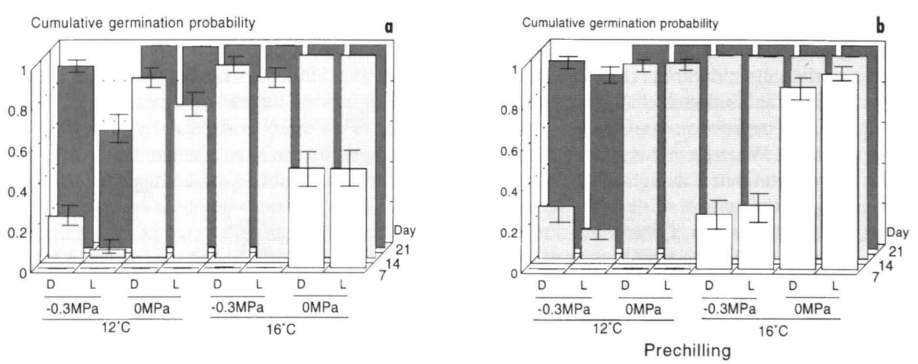

Fig. 2. The effect of prolonged white light on cumulative germination probability of Norway spruce seeds under different germination conditions. (a) unchilled seeds, (b) prechilled seeds. Small bars represent 95 % confidence bounds for predicted probability. D = dark, L = light.

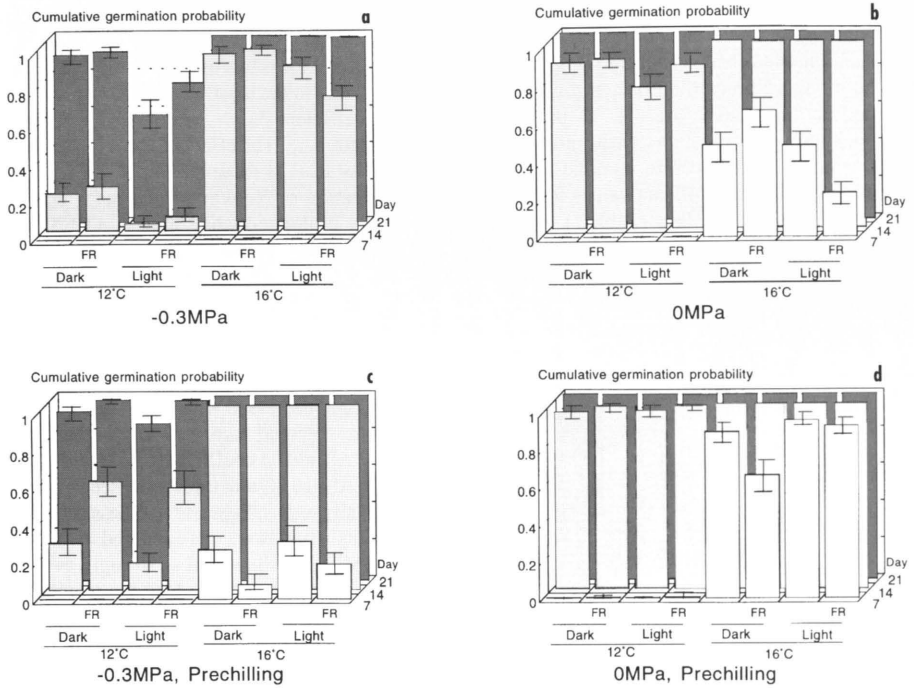

Fig. 3. The effect of short-period far-red light on cumulative germination probability of Norway spruce seeds under different germination conditions. (a) unchilled seeds at –0.3MPa, (b) unchilled seeds at 0 MPa, (c) prechilled seeds at –0.3 MPa, (d) prechilled seeds at 0 Mpa. Small bars represent 95 % confidence pounds for predicted probability. FR = far-red light.

Far-red light did not prevent unchilled seeds from germinating in darkness (FR+D). The lack of far-red light inhibition is characterized by the small positive regression coefficients β_{FR} and $\beta_{T\times FR}$ (Table 1). When far-red irradiated unchilled seeds were germinated in light (FR+W), there was no significant effect at +12 °C ($\beta_{W\times FR}$ was insignificant), but at +16 °C the effect was inhibitory, as indicated by the significant second-order interaction coefficient $\beta_{T\times W\times FR}$ (Fig. 3a, b).

Prechilling treatment sensitized the Norway spruce seeds to far-red light, but this was dependent upon temperature level. At +12 °C, far-red light promoted germination both in light and darkness (Fig. 3c, d). This effect is characterized by the significant positive interaction coefficient $\beta_{C\times FR}$. At +16 °C, the germination of prechilled seeds was inhibited by far-red light as indicated by the significant negative interaction coefficient $\beta_{T\times C\times FR}$. This effect was partially reversible by white light, because the positive third-order interaction coefficient $\beta_{T\times C\times W\times FR}$ negated the effect of $\beta_{T\times C\times FR}$.

4 Discussion

In this work a proportional odds model was used to compare sigmoid cumulative germination curves and to study the structure of interactions between different germination probabilities. Use

of this model eliminates the need to restrict data analysis to a single counting day, as is usually done when germination data is subjected to ANOVA. In addition, the estimates should be independent from counting days chosen. With regard to the latent quantitative variable, germination time, we believe that here there are none of the problems mentioned by Andersson (1984) concerning the dimensionality of the latent variable. In addition, the proportional odds assumption as to the existence of a common regression coefficient was not violated. Some odds ratios were large because germination curves were very different: for example, at the 7th day, when the germination percentages under different treatment combinations varied from 0 to 95. However, the only way to detect the complex interaction structure between the factors was to include all data in the same model.

The final germination percentages at +12 °C and +16 °C in darkness were higher than in the test done earlier for the same seed lots (Leinonen et al. 1993). The logistic germination probability curve presented in the previous work has shifted towards lower temperatures, and the shape of the curve has possibly changed during the storage of seeds at +4.5 °C for 14 months. It seems that during dry storage the seeds will decline in level of dormancy, because their temperature requirement has become less specific (Probert 1992). Dry after-ripening of seeds in storage is reported in many species and seed lots (Evenari 1965, Bewley and Black 1985, Côme and Corbineau 1989, Probert 1992). Such after-ripening has possibly changed the effects of other environmental factors studied (c.f. Taylorson 1991). According to Hilhorst and Karssen (1989), the previous environmental conditions of seed lots determine the number and magnitude of environmental factors that are required for germination.

The effect of seed-bed moisture on seed germination was simulated using osmotically induced water stress. Added osmotica controls the imbibition rate by decreasing the moisture gradient between the seed and the environment. The increased viscosity of the imbibing medium also decreases the diffusivity of water in the seed (Vertucci 1989). The osmotic potential of –0.3 MPa resulted in slower germination and decreased total germination at 21 days. Osmotic potentials ranging from –0.3 MPa to –1.5 MPa have also been shown to decrease the germination rate and total germination of a number of coniferous species (Barnett 1969, Larson and Shubert 1969, Djavanshir and Reid 1975, Dunlap and Barnett 1984, Thomas and Wein 1985b).

There was an interaction between temperature and osmotic potential with the effect of osmotic stress on germination being strongest at the highest temperature. This interaction may prevent germination early in the summer when soil dries and temperature increases delaying it until rainy periods occurring usually in late July or early August, in southern Finland (c.f. Yli-Vakkuri 1961a, b, Norden 1990, Kubin and Kemppainen 1991). Since these results were obtained by imbibing seeds in water solutions where the water diffusivity into seeds is controlled by temperature, seed permeability and medium viscosity, factors such as soil diffusivity and seed/soil contact were eliminated (Vertucci 1989).

The seed lot studied had high dark-germination at suboptimal temperature even under water stress. Thus, dark-germination of this seed lot was not affected as strongly by prechilling as is the germination of dormant, positively photoplastic (light demanding) species and seed lots (Toole 1973, Safford 1974, Farmer et al. 1983, Adkins et al. 1984, Leadem 1986, Caron et al. 1990, Young and Young 1992). The results from prechilling of Norway spruce under suboptimal conditions were similar to those obtained by Jensen et al. (1967) under standard test conditions (see International Seed Testing Association 1985) i.e. prechilling increased the germination rate of seeds, but did not affect their germination capacity. It is, however, evident that variation exists in this respect between Norway spruce seed lots, and between the same seed lots after different storage periods similarly as in other species (Côme and Corbineau 1989, Hilhorst and Karssen 1989, Caron et al. 1990).

The unchilled Norway spruce seeds did not need light for maximum germination at temperatures chosen (c.f. Heit 1968, Leinonen et al. 1993). Instead, white light inhibited germination of non-prechilled seeds, especially at low temperature under osmotic stress. According to Taylorson (1991), germination inhibition by white light is most common in seed populations which

have high dark germination, but not all species having high dark germination are inhibited by light. This high irradiance response (HIR, see e.g. Attridge 1990) caused by white or far-red light is reported for many tree species (Toole 1973). We found that far-red light interacted with white light, causing inhibition of Norway spruce seed germination at +16 °C. This interaction is consistent with the theory of phytochrome, in which HIR operates via cycling of phytochrome. The effect depends not only upon the duration of irradiation but also upon the fluence rate, the wavelength and the temperature at which the seeds are imbibed, but not the temperature of the radiation period (Attridge 1990).

We found that white light inhibited germination most strongly under osmotic stress. Bewley and Black (1985, p. 227) give examples of species inhibited by light at low osmotic potentials and suggested that HIR has an impact on the cell elongation of the radicle. Djavanshir and Reid (1975) found a decrease in radicle elongation of some *Pinus* species, when they were germinated in light under a different osmotic stress. Shelter from direct sunlight is more beneficial to establishment of some coniferous species than to others under field conditions or in nurseries (McDonald 1984, Thomas and Wein 1985a, b), and it is possible that Norway spruce may have evolved mechanisms to inhibit germination in open areas when the seeds are subjected to moisture stress. The adaptation to shelter conditions may be one reason why the results of direct sowing of Norway spruce in clear-cut area have been poor (Yli-Vakkuri 1961a, Kolström 1991).

Handling of dry (8 %) seeds was performed under normal laboratory light conditions. As shown by Vertucci et al. (1987) phytochrome conversion may occur in dry seeds. High germination in darkness may result from this pre-existence of a promotive form of phytochrome (P_{FR}) (Taylorson 1991). Far-red light for 15 minutes supplied to the Norway spruce seeds after 0, 3, 6 and 12 hours from the beginning of the imbibition could not inhibit the germination in darkness, as it does e.g. in Scots pine (*Pinus sylvestris* L.) seeds (Nyman 1963). The results at 16 °C indicate that prechilling tended to make seeds sensitive to short pulses of far-red light, and this effect was reversible by white light. The effect of short period far-red light was, however, relatively week also in prechilled seeds. Lack of strong far-red inhibition indicate that Norway spruce seeds have requirement for low P_{FR} levels.

In this work we identified potentially interesting interactions of ecologically important environmental factors in germination of Norway spruce seeds and related the findings to possible adaptive significance of the responses. High germination in darkness and lack of strong far-red inhibition indicated that Norway spruce seeds may have adapted to germinate in canopy shade light rich in far-red. In addition the seeds may have evolved mechanisms to inhibit germination in prolonged light, especially when the seeds are subjected to moisture stress. These results cannot, however, be generalized to species level before we have sufficient evidence on variation between seed lots in light responses. In addition to pretreatments and conditions during germination test, the actual response of Norway spruce seeds to light may also depend on light conditions during ripening, extraction and handling (c.f. Vertucci et al. 1987, Leopold and Vertucci 1989); furthermore, the level of dormancy may change during dry-storage (c.f. Probert 1992).

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Effect of Repeated Fertilizer Application on the Nutrient Status and Biomass Production of *Salix* 'Aquatica' Plantations on Cut-Away Peatland Areas

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Hytönen, J. 1995. Effect of repeated fertilizer application on the nutrient status and biomass production of *Salix* 'Aquatica' plantations on cut-away peatland areas. Silva Fennica 29(2): 107–116.

The effects of repeated fertilizer treatment on biomass production and nutrient status of willow (*Salix* 'Aquatica') plantations established on two cut-away peatland areas in western Finland were studied over a rotation period of three years. Comparisons were made between single fertilizer applications and repeated annual fertilization.

The annually repeated fertilizer application increased the amounts of acid ammonium acetate extractable phosphorus and potassium in the soil as well as the concentrations of foliar nitrogen, phosphorus and potassium compared to single application. Depending on the fertilizer treatment and application rate, annual fertilizer application resulted in over two times higher biomass production when compared to single fertilizer application over a three-year rotation period. The effect of phosphorus fertilizer application lasted longer than that of nitrogen. The optimum fertilization regime for biomass production requires that nitrogen fertilizer should be applied annually, but the effect of phosphorus can last at least over a rotation of three years. Potassium fertilizer treatment did not increase the yield in any of the experiments during the first three years. The leafless, above-ground yield of three-year-old, annually NP-fertilized willow plantations was 9.5 t ha^{-1} and the total biomass, including stems, leaves, roots and the stump, averaged 17 t ha^{-1}.

Keywords biomass production, fertilization, peatlands, *Salix*, fuelwood.
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