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Seed Moisture Content during Chilling and Heat Stress Effects after Chilling on the Germination of Common Alder and Downy Birch Seeds

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The effects of seed moisture content (MC) and heat treatment on the germination response of common alder (*Alnus glutinosa*) and downy birch (*Betula pubescens*) seeds were examined. Seeds of each species were adjusted to MC of 7% to 50% MC, then chilled for up to 36 weeks, after which they were allowed to germinate at 15°C with 8 hours lighting per day or 20 (dark)/ 30°C (light). Seed lot effects were evident, but treatment effects were consistent in each lot and species. The response to moist chilling treatments was larger at 15°C than at 20/30°C. Chilling had no effect on germination unless seed MC was >15%, but it was low also at 20% MC. The highest germination was achieved following 24–36 weeks chilling at the optimum or target MC (TMC) levels of about 30% in alder and 35% in birch. In a separate experiment, seeds were fully imbibed (FI) (~50% MC; standard method used in operational practice) or adjusted to TMC levels, after which some seeds of each treatment group were chilled to release dormancy. Following this, the seeds were dried back to TMC levels and then subjected to 60°C for up to 4 hours after which they were allowed germinate under the same conditions described above. Heat treatment damaged the prechilled FI seeds, but no damage occurred to the non-chilled seeds. However, heat stress stimulated germination in the non-chilled FI seeds of both species and the TMC seeds of alder.

Keywords *Alnus glutinosa, Betula pubescens*, germination temperature, stress, moist chilling
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1 Introduction

There has been a large increase in the planting of broadleaf species in Ireland, especially native broadleaf species such as common alder *Alnus glutinosa* (L.) Gaertn.) and downy birch (*Betula pubescens* Ehrh.). However, germination of the seeds of these species is often low/ unreliable, especially in bare-root nurseries. Seed [alder and birch 'seeds' are actually winged fruits (achenes) that contain a single seed without endosperm that is surrounded by a pericarp, but seed is used for convenience] factors, especially dormancy status, may be contributing to this problem.

Moist chilling (ca 0–5°C) for 4–8 weeks in fully imbibed (FI) state usually releases dormancy in alder and birch seeds (Suszka et al. 1996), but premature germination is likely to occur, probably mainly because of individual seed variation in dormancy intensity (Bewley 1997). Seeds of birch are also sensitive to photoperiod, but the light response diminishes during moist chilling (Vanhatalo et al. 1996, Ahola and Leinonen 1999). In large commercial bare-root nurseries, sowing operations are often delayed. Consequently there is a risk that the seeds will germinate before sowing, or they get damaged during sowing operations (Tanaka 1984, Jensen 1997a). However, chilling at a lower seed moisture content (MC) level than the FI state may be preferable because it greatly reduces the risk of premature germination, and in some cases increases germination after pretreatment (Gosling et al. 2003). Less water is needed to break dormancy than is required for germination (Jensen 1997a, Derkx 2000). Respiratory functions and dormancy release start at seed MC of 20–23% (Obroucheva and Antipova 2000, Smith et al. 2003) but lower levels are generally not effective (Gosling and Rigg 1990, Jensen 1997b). However, MC levels from 30–40% are effective in releasing seed dormancy during chilling in most broadleaf (Suszka et al. 1996, Derkx 2000, De Atrip and O'Reilly 2005) and conifer (Jones and Gosling 1994, Jinks and Jones 1996, Poulsen 1996, Jensen 1997b) species. Detailed studies of the effect of seed MC on germination have been carried out on a number of species, including two *Abies* species (Edwards 1986), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Gosling and Rigg 1990), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Gosling et al. 2003) and nordmann fir (*Abies nordmannia* (Stev.) Spach) (Jensen 1997a). De Atrip and O'Reilly (2005) provided some preliminary results on the effect of a few different seed MC levels during chilling on the germination of alder and birch seeds.

There are several other advantages (to those described above) to breaking dormancy at seed MC levels lower than the FI state, including the fact that it may improve seed vigour. Seed vigour measures the potential for rapid, uniform seed germination under a wide range of field conditions (Bray 1997). Seeds deteriorate more rapidly at high MC levels and high temperatures than at lower levels (McDonald 1999), leading to genetic and structural damage (Villiers 1974). The vigour of seeds can be assessed using a variety of methods, including physiological and biochemical tests. The assessment of vigour using some form of vigour test is the mostly commonly used approach (Bonner 1998). Accelerated ageing (AA) and controlled deterioration (CD) tests are probably the most commonly used ones. Most of these tests have been developed to evaluate the seeds of agricultural crops (Marcos-Filho 1998). The AA tests involve subjecting seeds to high temperatures and high relative humidity, factors that are known to cause rapid deterioration (Delouche and Baskin 1973). The CD test involves adjusting the initial seed MC to a specific level and then exposing the seeds to high temperatures (Matthews 1980). The loss of vigour usually precedes the loss of viability. De Atrip and O'Reilly (2006) showed that seeds that have been chilled at MC levels lower than the FI state were more resistant to freezing stress than the FI seeds. However, the response of pretreated seeds of these species to heat stress has not been evaluated previously.

The main objective of this study was to investigate the effect of seed MC during moist chilling on percentage germination and germination speed of alder and birch seeds. The second objective was to assess the effect of heat treatment (vigour test) on the germination of the seeds of both species after moist chilling or no chilling, but this experiment was restricted to seeds of two different seed MC levels only.

Table 1. Provenance details for seed lots of alder and birch used in study.

Species	Country Seed zone and latitude Origin Master certificate of provenance number	Year of seed ripening Category Type of basic material[a)]	Seed lot code[b)]
Alder	Southwest Scotland 01 (203) K; 56º28'N, 3º0'W Origin, unknown CP 163/02	2001 Source identified Seed source	AC-UK203- **B104**
	Mideastern England 01 (403) F; 52º22'N, 2º43'W Origin, unknown CP 162/02	2001 Source identified Seed source	AC-UK403- **B106**
Birch	Co. Laois, Ireland 00 (417); 53º02'N, 7º17'W Origin, indigenous n/a[c)]	2000 Selected Stand	BC-IELAO- **A20**
	Co. Cork, Ireland 00 (417); 51º54'N, 8º8'W Origin, indigenous n/a[c)]	2000 Source identified Seed source	BC-IECORK- **A62**

[a)] As per Seed Supplier's Document (EU Council Directive 1999/105/EC)
[b)] Unique identity code assigned by supplier; the abbreviated part (last four digits in bold text) is used in this paper for convenience
[c)] Not available; information not mandatory at that time

Table 2. Physical and germination (%) characteristics[a)] of the alder and birch seed lots used in the study.

Species	Seed lot identity code[b)]	Moisture content (%)	Purity (%)	1000 seed weight (g)	Viable seeds kg^{-1}	Germination	
						Non chilled	Pre-chilled
Alder	B104	11.6	90	1.44	443624	55.5	58.5
	B106	10.6	91	1.71	294149	58.5	65.5
Birch	A20	11.7	–[c)]	0.620	368000	–[c)]	–[c)]
	A62	13.5	–[c)]	0.453	302000	–[c)]	–[c)]

[a)] All treatments/ observations carried out according to International Seed Testing Association rules by the Dept. of Agriculture, Ireland, Abbotstown, Co. Dublin
[b)] Abbreviated code (see Table 1)
[c)] Because it is difficult to clean birch seeds, purity and percentage germination are not reported (as per ISTA guideleines)

2 Material and Methods

2.1 Seed Material

Two seed lots each of alder and birch, provided by the Coillte National Seed Centre (Carlow, Ireland), were used in this study (Table 1), which was carried out in 2003. The physical and germination characteristics of the seed lots, based upon the official test results, are given in Table 2. Since the birch seeds were destined for broadcast sowing in a bare-root nursery, the lots were not cleaned to a high standard. However, the birch seed lots were cleaned further for use in this study to reduce among-sample variation (especially due to presence of inert material) and to increase the number of seeds per kg. The seed MC of the seeds was determined soon after arrival in the laboratory, based upon four replicate samples (including impurities in birch) of approximately 0.5 g seeds per lot by drying seed at 103±2°C for 17±1 hours. The MC (fresh weight basis) of the seeds was about 11–13%, within the range reported on the official seed documents (see Table 2).

Table 3. Summary of freezing treatments applied to alder and birch seeds.

Treatment and treatment description	Number of levels
Experiment 1. Moisture content and moist chilling	
Seed lots	2
Seed moisture content (%) 7, 15, 20, 25, 30, 35, 40 and 50%	8
Moist chilling – 0, 6, 12, 24, and 36	5
Experiment 2. Heat treatment and seed moisture content	
Seed lots	2
Seed moisture content – FI or TMC	2
Heat treatment or no heat treatment	2

FI = fully imbibed; TMC = target moisture content (30% in alder, 35% in birch)

2.2 Experiment 1. Seed Moisture Content Treatments

The seeds of each species were adjusted to MC levels of 7, 15, 20, 25, 30, 35, 40 and 50%, for a total of eight MC levels. Since the original seed MC ranged from 10 to 14%, seeds had to be dried back to 7% MC; the seeds were spread evenly on trays and allowed to dry at room temperature (20–22°C) until they reached this MC (ca 24 hours). A known quantity of water was added to other seeds to adjust them to MC of 15 to 40%. The 50% MC level corresponded with the FI state (these seeds were allowed to absorb water freely), the method most commonly used in nursery operational practice. The MC of the seeds was also checked using the oven-drying method, as described above. A MC of 45% was not included in the experiment because it resulted in relatively high rates of premature germination during preliminary trials, as occurred also in the FI seeds. The seeds were then placed in loosely tied plastic bags in a refrigerator at 4 ± 1°C (in the dark) and stored for 0, 6, 12, 24, or 36 weeks (Table 3). The seed to air volume ratio was about 1:4. The bags were shaken once weekly to prevent an accumulation of water at the bottom of the bags. After they had been chilled, the seeds were allowed to germinate, as described below.

2.3 Experiment 2. Stress Treatments

This experiment was restricted to two seed MC levels per lot per species. Seeds were fully imbibed (standard method) or adjusted to a lower, target MC (TMC) level. The TMC treatment chosen resulted in the highest germination over longest periods of moist chilling in Experiment 1 (30% and 35% MC in alder and birch, respectively). After adjusting their MC, seeds received no chilling or were moist chilled (Table 3). The moist chilling period which delivered the highest percentage germination for that MC level was used in each case (see Results for Experiment 1), which corresponded to 12 and 24 weeks chilling for the FI and TMC seeds, respectively. Because seed MC level during heat treatment might confound the results, the FI seeds were dried (2 days at 4 ± 1°C) back to TMC levels before treatment. The FI seeds were spread out evenly on an open tray and mixed regularly during this period. However, since seeds needed time to adjust to their MC level, the minimum period of moist chilling that could be used was 4 days (4 days at TMC level; or 2 days at FI level followed by 2 days drying back to TMC level). These few days chilling were considered minimal in context of time scale studied, so they are referred to as zero days chilling. The seeds then were sealed in vials and then placed in a water bath at 60°C for 0, 1, 2 or 4 hours. After treatment, the vials were cooled for a few minutes in running cold tap water. The results of preliminary tests showed that this temperature provided good discrimination among treatments, whereas lower temperatures were less effective.

2.4 Germination Tests

The seeds were germinated in 12×8×5 cm transparent, rectangular plastic boxes (Hofstätter & Ebbesen A/S, model 500/50, Espergærde, Denmark), each containing one germination paper (Frisenette Aps, No. AGF 725, 11.5×7.5 cm, Ebeltoft, Denmark). The germination paper was kept continuously moist through a filterpaper wick to the water reservoir in the box (containing approximately 150 ml distilled water).

Germination was assessed in both experiments over a 42-day period at a constant 15°C with 8 hours lighting (32 μmol m^{-2} s^{-1}) per day and at 20 (dark)/ 30°C (light) and close to 100% relative humidity inside the boxes. Seed dormancy is not normally expressed in these species at the higher test temperature (De Atrip and O'Reilly 2005). Each germination cabinet (CMC Germination Cabinet 400L D/N-L, Glesborg, Denmark) contained four replications per treatment combination. A replicate was a germination box containing approximately 0.15 g (alder) and 0.05 g (birch) seeds, equivalent to 50 to 100 seeds in both species. On basis of the official germination results (which might underestimate viability), about 60% of the alder seeds were viable, but no official data of this kind were provided for birch (see Table 2). However, the results from a preliminary study, using the same seed lots, indicated that about 50% of the birch seeds were viable. The number of seeds that germinated prematurely was determined before the boxes were placed in the germinators; these values were excluded from the germination data. Thereafter, the number of seeds that germinated was recorded every 3 or 4 days. A seed was considered to have germinated in either species when the radicle protruded about 2 mm. Percentage germination and mean germination time (MGT) were calculated from these data. MGT was calculated as the mean number of days for the seeds to germinate (Jones and Gosling 1994).

2.5 Data Analyses

Because the effects on the germination responses were so large, the data were analysed separately for each species and germination temperature. The percentage germination (following arc-since square root transformation) and MGT (log transformed) data in each experiment were analysed according to a full factorial ANOVA design to test for the effects of seed lot, MC, moist chilling and heat treatment (Experiment 2 only) effects using the GLM procedure in SAS (SAS 1989). The data for MGT at 15°C in birch were analysed following an ANOVA for an unbalanced design (GLM procedure for type III sum of squares) because no seeds germinated in a few cases. Means were compared further using least significant means tests.

3 Results

Germination was much higher in alder than in birch, although treatment effects were generally consistent in each species. Since a high proportion of birch seeds may be empty or non viable (see Suszka et al. 1996), germination was considered acceptable when ≥20%.

3.1 Seed Lot Effects

Seed lot effects on germination and MGT were highly significant in both experiments. In Experiment 1 for example, as seed MC increased in alder, MGT declined, but this occurred more slowly in one lot than in the other lot. This pattern of response was reflected in the highly significant interaction between seed lot and MC (Table 4). Seed lot effects are not described further for this reason. In all cases treatment effects were consistent across seed lots, even where interaction effects were significant (and these were generally small relative to other effects).

3.2 Experiment 1. Seed Moisture Content

The effect of most treatments and their interactions on germination and MGT was highly significant in both species (Table 4). Seed MC effects were large at the low germination temperature, but were smaller at the high germination temperature (Figs. 1–4), especially in birch. In general,

Table 4. ANOVA of the effects of seed lot, seed moisture content and moist chilling duration on germination and mean germination time (MGT) of alder and birch seeds at 15ºC and 20/30ºC. Values in bold are significant at <0.05. The df are the same in all cases (second column), except as shown for birch MGT at 15°C.

Source of variation	Alder					Birch				
		Germination		MGT			Germination		MGT	
	df	F	P	F	P	df	F	P	F	P
15°C										
Seed lot (S)	1	9.8	**0.0019**	0.29	0.5937	1	205.1	**0.0001**	64.9	**0.0001**
Moisture content (M)	7	28.3	**0.0001**	340.6	**0.0001**	5	503.6	**0.0001**	129.8	**0.0001**
Chilling (C)	4	136.8	**0.0001**	434.7	**0.0001**	3	648.0	**0.0001**	101.2	**0.0001**
S×M	7	1.6	0.1472	0.5	0.8260	5	18.8	**0.0001**	2.9	**0.0156**
S×C	4	5.1	**0.0006**	1.4	0.2235	3	16.1	**0.0001**	0.2	0.8707
M×C	28	14.3	**0.0001**	36.3	**0.0001**	15	41.0	**0.0001**	12.7	**0.0001**
S×M×C	28	0.7	0.8531	1.6	0.0410	15	2.4	**0.0003**	0.8	0.7162
Error	240					144				
20/30°C										
Seed lot (S)	1	0.2	0.6623	5.84	0.9844	1	1.3	0.2486	96.8	**0.0001**
Moisture content (M)	7	9.9	**0.0001**	51.52	**0.0001**	7	10.7	**0.0001**	70.2	**0.0001**
Chilling (C)	4	22.1	**0.0001**	0.55	0.7970	4	118.1	**0.0001**	123.5	**0.0001**
S×M	7	1.1	0.3550	88.24	**0.0164**	7	0.3	0.9372	0.9	0.4804
S×C	4	0.8	0.5587	1.39	**0.0001**	4	0.1	0.9916	0.6	0.6521
M×C	28	10.9	**0.0001**	7.49	0.2370	28	3.9	**0.0001**	5.8	**0.0001**
S×M×C	28	0.5	0.9835	0.5	**0.0001**	28	0.1	0.9999	0.5	0.9935
Error	240					240				

germination was low or zero across the seed MC range for seeds that received no moist chilling. No non-chilled birch seeds germinated at 15°C, but some non-chilled alder seeds (<20%) germinated. Premature germination accounted for about 5% germination in seeds chilled at 35–40% MC and 10.3% in those chilled at 50% MC in alder; about 8% of the birch seeds germinated prematurely at 50% seed MC. None germinated prematurely over the test period following chilling at the other MC levels.

In alder, seed germination was generally low (with similar values) for seeds prechilled at 7 or 15% MC (Fig. 1). Seeds responded better than this to chilling at 20% MC, but germination was still low, especially for those given short chilling periods. Seed germination was high for chilling periods at 25% seed MC. The seed MC at which germination was high across most chilling periods in alder was 30–35% at the low germination temperature and 30% at the higher temperature. Germination was highest at these MC levels at both germination temperatures following 24 or 36 weeks chilling. The chilling duration required to achieve acceptable germination was shorter for seeds chilled at MC levels higher than 30–35% MC (e.g. seeds chilled at 40% MC) than at lower levels.

The response to seed MC in birch was consistent with the trend described for alder, but there were some important differences (Fig. 2). Percentage germination at 15°C was high following the longest chilling periods at 25–35% MC in alder compared with 30–35% MC in birch. Although seeds chilled for 24 weeks at 40% MC had high germination at 15°C, seeds chilled for longer periods at same MC had lower germination. Therefore, 35% seed MC was considered best, although this MC did not deliver significantly higher germination than the 30% MC treatment.

As expected, seeds germinated more quickly at the high temperature than at the low temperature. Temperature differences were small for seeds that received moist chilling at MC ≥ 30% (Figs 3, 4). MGT declined rapidly in both species for seed MC >20% during chilling. Germination was fastest for seeds chilled at ≥30% for 24 or 36 weeks.

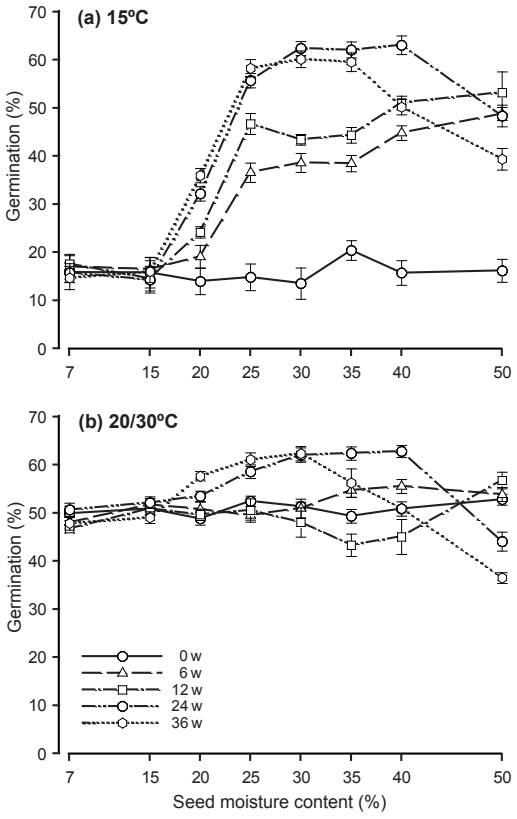

Fig. 1. Effect of seed moisture content and moist chilling duration (weeks, w) on percentage germination of alder seeds at 15°C (a)and 20/30ºC (b). The vertical lines are standard errors (some smaller than symbols).

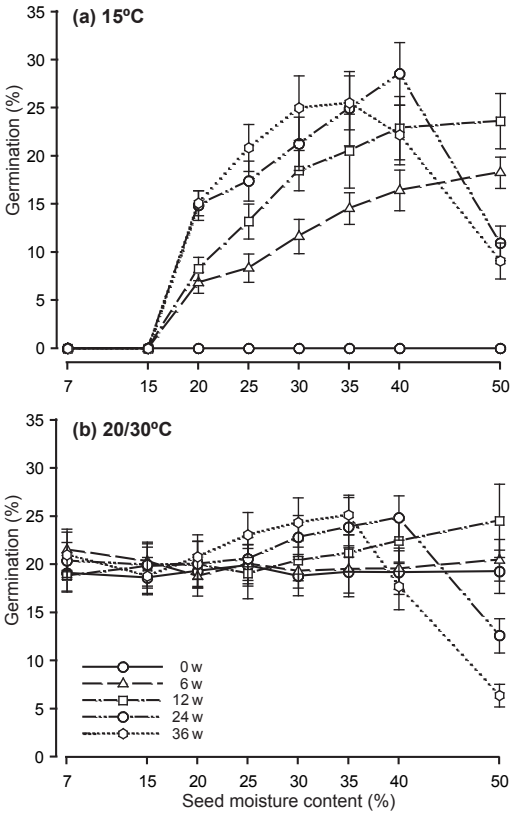

Fig. 2. Effect of seed moisture content and chilling duration (weeks, w) on percentage germination of birch seeds at 15°C (a) and 20/30ºC (b). The vertical lines are standard errors (some smaller than symbols).

3.3 Experiment 2. Heat Stress Treatments

The effect of both moist chilling and heat treatment (applied before the seeds were allowed to germinate) were expressed more strongly at 15°C than at 20/30°C (Table 5). Heat treatment reduced percentage germination in the chilled seeds of both species, but the effect was not significant for the TMC seeds. Heat treatment reduced germination from 54.1% to 35.9% at 15°C and from 57.0% to 31.0% at 20/30°C in the FI seeds of alder. In birch, heat treatment reduced germination from 24% to 11% at 15°C and from 24.3% to 10.9% at 20/30°C. In contrast, heat treatment significantly increased germination in the non-chilled FI seeds germinated at 15°C, from 11.5% to 51.9% in alder and 0% to 20.6% in birch. There was no effect for seeds tested at 20/30°C. There was a similar trend in the non-chilled TMC seeds, but the effect was smaller and was significant in alder only.

4 Discussion

4.1 Seed Moisture Content and Moist Chilling

Moist chilling had no effect on percentage germination unless seed MC was >15%. The moist chilling effect commenced at 20% MC, although

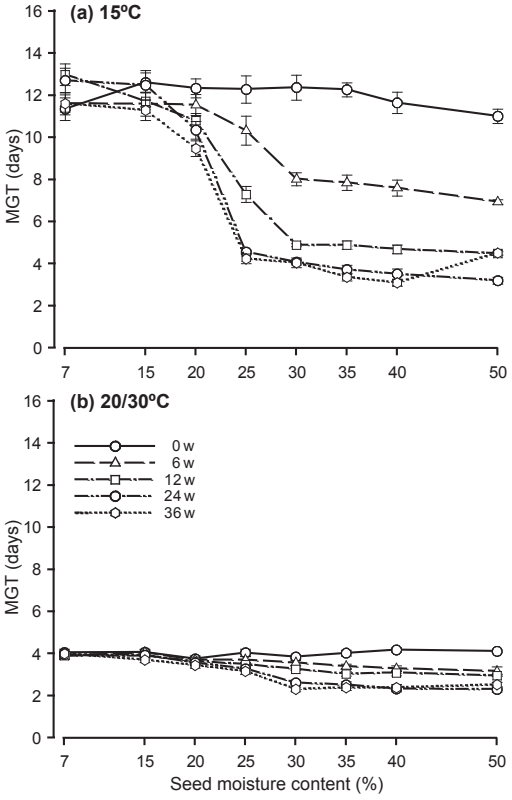

Fig. 3. Effect of seed moisture content and chilling duration (weeks, w) on mean germination time (MGT) of alder seeds at 15°C (a) and 20/30ºC (b). The vertical lines are standard errors (some smaller than symbols).

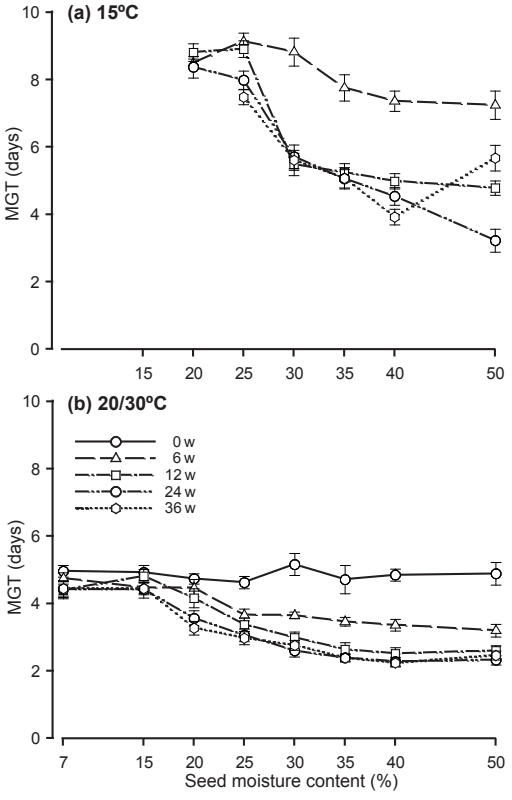

Fig. 4. Effect of seed moisture content and chilling duration (weeks, w) on mean germination time (MGT) of birch seeds at 15°C (a) and 20/30ºC (b). The vertical lines are standard errors (some smaller than symbols). Some treatments resulted in no germination, so MGT values could not be presented in all cases (including some full treatment response curves).

the maximum chilling period used was 36 weeks. It is possible that the 20% MC treatment might have resulted in higher germination if a longer chilling period than 36 weeks had been used. A MC of >25% (fresh weight) was needed to initiate dormancy release in white spruce (*Picea glauca* [Moench.] Voss.) (Downie et al. 1998), but the process commenced at 15% MC in both Sitka spruce (Gosling and Rigg 1990) and Douglas fir (Gosling et al. 2003), and 23% in nordman fir (*Abies nordmanniana*) (Jensen 1997a). The level of physiological activity in seeds changes at different MC levels, reflecting changes in the thermodynamic properties of water (Vertucci 1989). Seeds begin to respond to chilling and other cues as the water in the seed reaches the boundary between water binding regions 2 and 3 (Vertucci 1989), corresponding to about 20% MC (fresh weight). However, the relationship between seed MC and the different boundary hydration levels change with seed composition and structure. Moisture contents are lower in seeds that have high lipid content than in seeds that have high starch content (Vertucci and Leopald 1987) and the embryo and endosperm usually contain more water than other parts of the seed (Villela 1998). Differences in seed MC among individual seeds, exacerbated by the high content of inert

Table 5. Effect of heat treatment of unchilled or chilled seeds of alder and birch seeds on their subsequent germination at 15°C and 20/30°C. Seeds were heat treated after prechilling in fully imbibed (FI) state or at a lower target moisture content (TMC). The value for the heat treatment is significantly different from the non-treated control where indicated (*).

Seed pretreatment		Germination temperature			
		15°C		20/30°C	
MC	Chilled	Control	Treated	Control	Treated
Alder					
FI	No	11.5 (2.89)	51.9 (1.31)*	56.3 (3.10)	53.9 (1.43)
FI	Yes	54.1 (1.84)	35.9 (2.63)*	57.0 (1.96)	31.0 (3.21)*
TMC	No	14.1 (1.45)	29.6 (2.86)*	56.4 (2.19)	57.4 (1.71)
TMC	Yes	59.5 (2.23)	55.4 (3.06)	58.1 (1.62)	57.1 (1.98)
Birch					
FI	No	0	20.6 (2.22)	24.7 (2.15)	23.1 (2.64)
FI	Yes	24.0 (1.99)	11.0 (1.62)*	24.3 (2.06)	10.9 (1.47)*
TMC	No	0	0	22.6 (2.01)	23.7 (1.47)
TMC	Yes	27.5 (2.60)	24.9 (2.54)	26.3 (2.65)	22.7 (2.13)

material and dead seeds, may have contributed to the higher variation in treatment responses in birch than in alder.

The effect of seed MC on speed of germination was less complex than its effect on germination. Germination was rapid and about same at any MC level ≥25%, provided the seed received sufficient moist chilling. The longer periods of moist chilling may have helped to release dormancy more fully (without premature germination) than the shorter periods of chilling, thus perhaps increasing germination speed. Similar findings have been reported for the seeds of other tree species (Edwards 1986, Gosling and Rigg 1990, Jones and Gosling 1994, Jensen 1997a, Gosling et al. 2003).

The seed MC level which resulted in the highest percentage germination without premature germination over the longer periods of moist chilling was considered the TMC level, which was 30% in alder and 35% in birch (Figs. 1–4). The recommended TMC level should be interpreted cautiously because of the effect of the composition and structure of the seeds (as discussed above). Although germination was high also for seeds given the 40% MC treatment (and 24 weeks chilling at this MC resulted in the highest germination in birch), long periods (>24 weeks) of chilling at this MC generally reduced it. Premature germination was a major contributor to low germination for seeds chilled at 40% and 50% MC. The seeds of both of these species can be held at the TMC level for up to 48 weeks at chilling (ca 4°C) temperatures (De Atrip and O'Reilly 2005) or 60 weeks at –3°C (De Atrip and O'Reilly 2006) after moist chilling without greatly affecting post-treatment percentage germination. The TMC level for treating alder and birch seeds in this study was within the range reported for several other broadleaf species, including beech (30–32%) (*Fagus sylvatica* L.) (Muller and Bonnet-Masimbert 1989, Muller et al. 1999), Norway maple (36–40%) (*Acer platanoides* L.) (Derkx 2000) and several conifer (Jones and Gosling 1994, Jinks and Jones 1996, Poulsen 1996, Jensen 1997b) species, but lower than that (44–50%) recommended for samaras of sycamore (*Acer pseudoplatanus* L.) (Suszka et al. 1996).

The germination response to moist chilling and seed MC treatments was larger at 15°C than at 20/30°C, reflecting the strong expression of conditional dormancy (De Atrip and O'Reilly 2005). Nevertheless, dormancy was still expressed relatively strongly also at 20/30°C, especially in alder. From a practical viewpoint, these results suggest that both species should be prechilled at the TMC level to release dormancy, even if it is envisaged that they might be sown under close to ideal conditions (e.g. container nursery). Tanaka et al. (1991) reported that FI seeds of red alder (*Alnus rubra* Bong.) germinated faster at 20/30°C fol-

lowing moist chilling than untreated seeds, but percentage germination was similar in each case. The effect of MC levels lower than FI state was not investigated in that study.

4.2 Heat Stress

Heat treatment damaged the chilled FI seeds, whereas both the non-chilled FI and the chilled TMC seeds were undamaged (Table 5). The pattern of response of the seeds to storage/ freezing stresses (De Atrip and O'Reilly 2006) was similar to that described here for heat stress. In contrast to TMC seeds, FI seeds begin to germinate during moist chilling once dormancy has been released (De Atrip and O'Reilly 2005); stress is more likely to damage germinating seeds. Heat treatments are known to reduce the levels of starch, non-reducing and reducing sugars (Blanche et al. 1990), saturated and unsaturated fatty acids (Marquez-Millano et al. 1991) in tree seeds. There was a reduction in membrane integrity and degradation of storage lipids in Scots pine (*Pinus sylvestris* L.) seeds during long-term storage (up to 29 years) (Tammela et al. 2000). However, it is unlikely that such a rapid decline in reserves could occur over the short period (4 h) of heat treatment used in this study, but rapid deterioration due to other reasons may have been more likely. Heat treatments can damage plastids and mitochondria and other cellular organelles (Wang and Berjak 2000) and reduce membrane integrity (Basavarajappa et al. 1991).

Heat treatment stimulated germination in the non-chilled FI seeds of both species and the TMC seeds of alder (but the effect was smaller than for the FI seeds). A similar trend has been noted in response to priming (15–20°C) (Jones and Gosling 1994, Doody and O'Reilly 2005) and other heat (30–45°C for various periods) (AA) treatments (Blanche et al. 1988, Chaisurisri et al. 1993, Leinonen 1998, Wang and Berjak 2000) in the seeds of several other tree species. Heat treatment may provide a chilling-like effect, resulting in the breakdown of polymeric storage compounds (Blanche 1988). It is likely that the heat treatment used in this study stimulated dormancy-mediated responses, especially since germination was relatively high at 15°C. Dormant seeds of these species germinate poorly at 15°C (De Atrip and O'Reilly 2005). Chaisurisri et al. (1993) suggested that this type of stimulation was due to the increase in seed MC during treatment. However, the FI seeds responded much better to the heat treatment than the TMC seeds in this study, although all seeds were at TMC levels during treatment, supporting the view instead that dormancy-mediated processes were affected.

4.3 Seed Lot Effects

Although seed lot effects were significant, the response to MC and heat treatment was remarkably similar in each seed lot in each species. This also suggests that the TMC levels recommended might deliver similar results in other lots, but further testing is needed to confirm this. However, only two lots of each species were used and there was little difference in the quality of these lots, based upon the official seed test results (Table 2). The level of seed dormancy varies with species and seed lot/ provenance (Tanaka et al. 1991, Jones et al. 2002). The relatively northerly origin of the seed lots used (Table 1) may partially explain the deep dormancy levels reported in this study.

5 Conclusions and Recommendations

Seed germination in both species following chilling in the 25–40% seed MC range was higher than was achieved for those chilled in the FI state. Chilling had no effect on germination unless seed MC was >15% and was low for seeds chilled at 20% MC. The MC (or TMC level) which resulted in the highest germination, over the longest moist chilling periods, was about 30% in alder and 35% in birch. Seeds chilled at TMC levels germinated better than the non-chilled seeds at 20/30°C, suggesting that primary dormancy was present in addition to conditional dormancy.

Heat treatment damaged the chilled FI seeds, but the TMC seeds were not affected. Heat treatment stimulated germination in the non-chilled FI seeds of both species and the TMC seeds of alder.

This may be a useful pretreatment in operational practice if there is insufficient time to carry out moist chilling.

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