



HAL
open science

Growth and frost hardening of European aspen and backcross hybrid aspen as influenced by water and nitrogen

Nikula, Manninen, Pulkkinen

► **To cite this version:**

Nikula, Manninen, Pulkkinen. Growth and frost hardening of European aspen and backcross hybrid aspen as influenced by water and nitrogen. *Annals of Forest Science*, 2011, 68 (4), pp.737-745. 10.1007/s13595-011-0090-2 . hal-00930823

HAL Id: hal-00930823

<https://hal.science/hal-00930823>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Growth and frost hardening of European aspen and backcross hybrid aspen as influenced by water and nitrogen

Suvi Nikula · Sirkku Manninen · Pertti Pulkkinen

Received: 27 August 2010 / Accepted: 22 November 2010 / Published online: 31 May 2011
© INRA and Springer Science+Business Media B.V. 2011

Abstract

• **Introduction** The interactive effects of water and nitrogen (N) on frost hardiness are not well known in broad-leaved trees. Furthermore, new environmental conditions may favour naturally generated hybrids between native and introduced tree species over native species.

• **Methods** A greenhouse experiment with freezing tests was carried out to study how water (low, medium, high) and N (low, high) supply influence the growth, bud phenology and frost hardening of seven young European aspen (*Populus tremula*) and backcross hybrid aspen ($[P. tremula \times Populus tremuloides] \times P. tremula$) families.

• **Results** The native European aspen grew faster, whereas hybrid aspen \times aspen frost hardened faster and exhibited better overall frost hardiness and earlier budburst. Hybrid aspen \times aspen also showed intraspecific variation in frost hardiness. The two species showed similar responses to different water and N supplies, and both species were more affected by changes in N supply than in water supply. Higher N supply—especially when combined with drought—

delayed frost hardening, an effect that was more pronounced in European aspen.

• **Conclusions** The results suggest that backcross hybrid aspen may in some respects be better adapted to a range of environmental conditions than the native species.

Keywords Frost hardiness · Environmental change · *Populus* · Hybrid

1 Introduction

Together with the increasing use of introduced species in many parts of the world, the consequences of possible natural hybridization between introduced and native tree species have raised concern. Hybridization may increase competition and narrow the gene pool of native species, thus threatening their overall fitness (Vanden Broeck et al. 2005). European aspen (*Populus tremula*) is a widely distributed species native to Eurasia and of high ecological importance in terms of biodiversity and soil processes of boreal forests (Suominen et al. 2003; Kouki et al. 2004). It has been demonstrated that hybrid aspen, a man-made cross between European aspen and North American trembling aspen (*Populus tremuloides*) which is commercially cultivated in Fennoscandia and Baltic countries (Holm 2004; Rytter 2006), can hybridise with European aspen in nature (Suvanto et al. 2004). Furthermore, backcrosses between hybrid and European aspen may have some advantages over the pure native species concerning seed production and viability, as well as early competition (Suvanto et al. 2004; P. Pulkkinen, unpublished data).

Although the performance of secondary hybrids is generally considered inferior to that of the parent species (Burke and Arnold 2001), sometimes, especially in new

Handling Editor: Michael Tausz

S. Nikula (✉)
Department of Biosciences, University of Helsinki,
P.O. Box 56, 00014 Helsinki, Finland
e-mail: suvi.nikula@helsinki.fi

S. Manninen
Department of Environmental Sciences, University of Helsinki,
P.O. Box 56, 00014 Helsinki, Finland

P. Pulkkinen
Haapastensyrjä Breeding Station,
Finnish Forest Research Institute,
Haapastensyrjäntie 34,
12600 Läyliäinen, Finland

habitats and environmental conditions, secondary hybrids may be better adapted (Burke and Arnold 2001; Kimball et al. 2008). Climate change, together with increases in concentrations of many pollutants in our environment, such as nitrogen (N), creates novel environmental conditions and may thus promote the occurrence of hybrids. Predictions of future environmental change in Finland include a rise in annual precipitation by 12–22%, combined with a possible increase in heavy rains, but also periods of drought during the growing season (Jylhä et al. 2009). Globally, the annual average N deposition over forests has been estimated to about double by the end of the century (Lamarque et al. 2005).

Water and N are among the most important environmental factors regulating tree growth. Both factors also modify bud phenology and frost hardiness, which are vital traits for the adaptation of trees in cold climates. An increase in N availability usually improves the growth of trees, e.g., by increasing photosynthesis (Cooke et al. 2005), whereas drought stress often limits photosynthetic gas exchange and, consequently, growth (Ibrahim et al. 1997). Conflicting results have been reported on the role of water and N status on the frost hardiness of woody plants. N inputs have been observed to both increase (DeHayes et al. 1989; Rikala and Repo 1997) and decrease (Aronsson 1980; Hellegrén 1981) frost hardiness. Positive effects have been hypothesized to result from factors such as the accumulation of soluble carbohydrates, amino acids and proteins that increase frost hardiness and can act as cryoprotectants (Sheppard and Pfanz 2001). Negative effects can be mediated through delayed growth cessation and thereby frost hardening (van den Driessche 1991), and advanced budburst, which increases the risk of spring frost damage (Sheppard and Pfanz 2001). Increased N assimilation following high N uptake also requires carbon (C), which may deplete cryoprotectant carbohydrates (Thomas and Ahlers 1999). Mild water stress during the growing season can retard growth and promote terminal bud set and frost hardening. Conversely, prolonged periods of drought can impede frost hardening by restricting photosynthesis, resulting in decreased accumulation of carbohydrates (Colombo et al. 2001).

This study investigated how the availability of water and N influences the growth, bud phenology and frost hardening of young European aspen and backcross hybrid aspen seedlings. Gas exchange and leaf N and C concentrations were also measured to study differences within and between species. The effects of water and N on tree growth are well studied in commercially important species, which, in the genus *Populus*, include trembling aspen and various *Populus* hybrids. In contrast, limited research exists on the widely distributed European aspen. Furthermore, information on the interactive effects of water and N supplies on

the frost hardiness of broad-leaved trees is somewhat limited, with the majority of previous studies concentrating on evergreen coniferous species. We therefore focus especially on the effects upon frost hardening in the two species. The method employed was a controlled greenhouse experiment together with freezing tests. Previously, this technique has mainly been used for frost hardiness testing of conifers such as *Pinus sylvestris* and *Picea abies* (e.g. Andersson 1992; Pulkkinen 1993). The results from these early seedling tests have correlated fairly well with survival test results from field experiments on mature trees (Andersson 1992).

2 Materials and methods

2.1 Plant material

Seed material was collected from seven European aspen families and seven backcross hybrid aspen families, produced by crossing F₁ hybrid aspen females with European aspen males (referred to as hybrid aspen × aspen). The F₁ hybrid aspens were crosses between Southern Finnish *P. tremula* and Southern Canadian *P. tremuloides*. The seed trees of European aspen and F₁ hybrid aspen originated from a geographically small area in Southern Finland (60°30′–60°40′ N, 24°23′–24°42′ E), where they have been grown for over 30 years.

Seeds were sown between 30 October and 15 November 2006 and seedlings transplanted into Styrofoam boxes (59×39×11 cm) 10 days after sowing. Each box contained 40 seedling cavities (370 cm³ per cavity, 174 cavities per square metre) filled with pre-fertilized peat (N/P/K 14:4:20, Kekkilä horticultural peat, Finland). Before the start of the frost hardening period, the seedlings were grown under the mean day and night temperatures of +22°C and +15°C, respectively. The relative humidity was 73%, the day length 20 h and the mean photosynthetic photon flux density 150 μmol m⁻² s⁻¹ provided by artificial lighting (GE Lucalox XO PSL 400 W). During the experiment, the seedlings went through the whole growing season in terms of effective temperature sum (ETS) above 5°C, measured as degree days (d.d.). The average ETS, accumulated before the last freeze test, was 1485 d.d., which corresponds to that predicted for Southern Finland in about 2050 (Peltonen-Sainio et al. 2009).

2.2 Water and nitrogen treatments

The experiment was designed as a three water × two N full factorial experiment (i.e. six treatments). Each treatment consisted of eight replicates, each with five seedlings per family. Within a replicate, the positions of the families were

randomized, with the five seedlings per family forming one row in a Styrofoam box. Thus, one treatment included altogether 560 seedlings, with 40 seedlings per family.

Water and N applications commenced on 12 December 2006 and continued for 7 weeks, i.e. until the first freezing test. The three water levels—low (LW), medium (MW) and high (HW)—were chosen to simulate the amount of precipitation during the growing season in Southern Finland at present (320 mm, MW), a 50% reduction (160 mm, i.e. drought, LW) and a 50% increase (480 mm, HW). To achieve this, the seedlings were applied with 5 l m^{-2} (LW), 10 l m^{-2} (MW) and 15 l m^{-2} (HW) of water five times per week during the treatment period with a watering can. As we did not account for possible leachate, the water levels should be regarded as relative rather than as absolute amounts.

The two N levels were designed to provide the seedlings with 20 kg N ha^{-1} (low N, LN) and 120 kg N ha^{-1} (high N, HN) during the treatment period. The LN dose roughly corresponds to the average annual N deposition in Central Europe and is about twice the N deposition in Southern Finland (Lorenz et al. 2008). The HN dose is similar to the N fertilization level typically used in Finnish forest nurseries for 1-year-old seedlings (Juntunen and Rikala 2001). The fertilizers were applied with the irrigation water. All seedlings received a basic N fertilization (N/P/K 19:4:20, Taimi-Superex, Kekkila, Finland) four times during the experiment (5 kg N ha^{-1} on average biweekly). The total N of the basic fertilizer consisted of $\text{NH}_4^+\text{-N}$ (1.8%), $\text{NO}_3^-\text{-N}$ (7.2%) and urea (10.0%). The HN seedlings were additionally fertilized with a solution containing $\text{NH}_4^+\text{-N}$ (13.1%) and $\text{NO}_3^-\text{-N}$ (12.9%; N/P/K 26:0:1, Suomensalpietari, Kemira, Finland) six times ($16.7 \text{ kg N ha}^{-1}$ once a week). The position of the seedling boxes was changed three times during the treatments to avoid the possible effects of varying environmental conditions in different parts of the greenhouse.

2.3 Frost hardening period and freezing tests

On 15 January 2007, after 5 weeks of water and N applications, the environmental conditions in the greenhouse were changed to simulate a frost hardening period, which continued for a maximum of 9 weeks. This was done by decreasing the mean day and night temperatures by 2°C and 1.5°C , respectively, and the day length by 1.5 h per week. During the frost hardening period, one replicate from each water and N treatment was freeze-tested each week over a period of 7 weeks. Thus, the freeze test groups were identical, each containing five seedlings per family per treatment. The first group was freeze-tested on 30 January 2007 after a frost hardening period of 15 days and the last one after 64 days. The freezing tests were carried out in a

dark, air-cooled chamber. The seedlings were transferred to the chamber in their Styrofoam boxes and were first exposed to $+5^\circ\text{C}$ for 11 h. Then, the temperature was gradually decreased to -10°C , maintained there for 2 h, and gradually increased back to $+5^\circ\text{C}$. The average freezing and warming rates were 3°C h^{-1} .

During the freeze test period, the untested seedlings received irrigation according to their relative water treatments, albeit with differences between treatments being slightly diminished. The seedlings were supplied with 5 l m^{-2} (LW), 7.5 l m^{-2} (MW) and 10 l m^{-2} (HW) of water two to five times per week, with irrigation diminishing towards the end of the frost hardening period as plants gradually become dormant and use less water. Twenty-five days after the start of the frost hardening period, the seedlings of the last six groups to be freeze-tested received an extra N fertilization (N/P/K 19:4:20, Taimi-Superex) of 5 kg N ha^{-1} to ensure enough nutrients for growth after freezing.

After freezing, each seedling group was placed in a dark room (mean temperature, 2°C) and kept there until the end of March 2007. All the seedlings were then transferred for dehardening in a greenhouse without controlled lighting or heating (mean temperature, 16°C), where they were irrigated as required.

2.4 Measurements of gas exchange, leaf and soil chemistry, and growth

Stomatal conductance (g_s) and net photosynthesis (P_n) were measured from one leaf (leaf plastochron index, LPI, 5) from one randomly chosen seedling per family per treatment on 3–4 January 2007 using a portable Infra Red Gas Analyser (LCA-3, ADC Ltd., Hoddesdon, UK). No additional light source was used when conducting the measurements.

Leaf samples were collected from three European aspen families and three hybrid aspen \times aspen families (sown on the same date) on 9–11 January 2007. Three replicate samples per treatment were taken from each of these families, each sample containing one leaf (LPI 3–5) from every seedling of each family. C and N concentrations were determined from dried leaf samples (48 h at 60°C) by combustion (Vario MAX CN analyzer, Elementar Analysensysteme GmbH, Germany). The height of each seedling was measured on 22–25 January 2007. The value was further divided by the accumulated ETS to obtain the growth rate (millimetres per degree day), which was used as a measure of whole-plant vigour. Growth rate was expressed as millimetres per degree day to enable comparisons with studies that have been carried out in different temperatures. The height was also measured 1 day before freeze testing.

Soil samples were collected from each freeze test group 1 week after they had been freeze-tested. The soil samples were pooled samples from each treatment, containing equal amounts of soil from all the families within the treatment. The samples were dried (72 h at 60°C), sieved (ϕ 2 mm), milled and analysed for total N concentration by combustion (Vario MAX CN analyzer).

2.5 Scoring of bud phenology and freezing injury

One day before the last freeze testing, the existence of a terminal bud (indicating growth termination) was recorded on each seedling. During the dehardening period, between 13 April and 25 May 2007, budburst (indicating growth initiation) of the same seedlings was scored once or twice a week.

The relative frost hardiness level of the seedlings was determined by assessing the level of freezing injury, measured as the proportion of dead shoot on the seedlings. This was carried out between 14 and 25 May 2007, when it was ascertained that all living seedlings had initiated their growth (ETS of the new growing season > 700 d.d.), and involved recording the height of the topmost living bud (the highest starting point of growth, H_b). Using the height measurements recorded just before the freeze testing (height of seedling, H_s), a freezing injury percentage (FI) was calculated for each seedling as follows:

$$FI = ((H_s - H_b)/H_s) \times 100$$

An FI of 0% means an uninjured seedling and that of 100%, a seedling with no living aboveground shoot.

2.6 Statistical analyses

Analysis of variance (ANOVA) was applied to growth rate and leaf chemistry, and analysis of covariance (ANCOVA) to other variables to test the main effects and interactions of aspen species, water, N and time since the start of frost hardening (only for FI) on the variables measured. To eliminate the possible effect of different sowing dates on the variables, the growth rate (millimetres per degree day) of the seedlings was used as a covariate. To test the differences among families within each species and the family \times water and family \times N interactions, ANOVA (for growth rate and leaf chemistry) or ANCOVA (for other variables; growth rate as a covariate) with family as a random factor was employed. The Pearson or Spearman's correlation test was used to examine the relationships between FI and other variables. The data were log- or arcsin square root-transformed when necessary to obtain a normal distribution. The results were considered significant at $P < 0.05$. All statistical analyses were performed with SPSS 15.0 for Windows.

3 Results

The two aspen species differed in terms of growth rate, photosynthesis, leaf chemistry and FI (Table 1). However, their responses were rather similar with regard to water and N applications. In general, both species were more affected by changes in N supply than in water supply. Hybrid aspen \times aspen also showed intraspecific variation in FI.

3.1 Growth, gas exchange, and leaf N and C

European aspen grew faster than hybrid aspen \times aspen in all treatments (Fig. 1 and Table 1). The average height of the European aspen families ranged from 27 to 48 cm and that of the hybrid aspen \times aspen families from 18 to 45 cm at the end of the frost hardening period. The growth rate of both species was increased by HN (average +78%). Water supply also affected growth, with both LW and HW reducing the growth rate (-15% and -11%, respectively) compared with MW.

The g_s of the seedlings varied from 0.022 to 0.075 mol m⁻² s⁻¹ and P_n from 1.60 to 3.06 μ mol m⁻² s⁻¹ in the different treatments. On average, European aspen had slightly higher P_n (2.36 μ mol m⁻² s⁻¹) than hybrid aspen \times aspen (2.31 μ mol m⁻² s⁻¹). Higher N supply resulted in a higher g_s and P_n than LN in both species (average +154% and +59%, respectively; Table 1). Water treatment did not have a significant main effect on gas exchange, but interacted with N supply to affect P_n . In LN seedlings, LW and HW reduced P_n , whereas water treatment had no effect under HN.

Hybrid aspen \times aspen had higher leaf N and C concentrations (on average 1.8% and 41.0%, respectively) than European aspen (1.7% and 40.6%, respectively; Table 1). In both species, leaf N concentration was higher in HN than in LN (2.7% and 0.8%, respectively, averaged over species). Higher N supply also increased leaf C concentration and decreased leaf C/N ratio (+6% and -68%, respectively, averaged over species). Water treatments modified leaf N chemistry together with N supply. The leaf N concentration was highest and C/N ratio lowest in MW for LN seedlings, but overall highest in HN seedlings grown under drought (LWHN, W \times N interaction). The average soil total N concentration was 0.5% in all treatments (no statistical differences between treatments, data not shown).

3.2 Bud phenology and frost hardening

Higher N supply delayed the bud set of both species (Fig. 2a and Table 1). Under LN, the proportion of seedlings with terminal buds was similar in both species

Table 1 ANOVA/ANCOVA (growth rate as covariate) results (*P* values) for the main effects and interactions of aspen species (Sp), water (W), and nitrogen (N), as well as time since the start of frost

hardening (T, only for freezing injury) on growth rate, leaf chemistry, stomatal conductance (*g_s*), net photosynthesis (*P_n*), bud phenology and freezing injury of aspen

Factor	ANOVA				ANCOVA				
	Growth rate	Leaf N	Leaf C	Leaf C/N	<i>g_s</i> ^a	<i>P_n</i> ^a	Bud set ^b	Budburst ^b	Freezing injury ^c
Sp	<0.001*	0.041*	0.025*	0.085	0.993	0.008*	0.550	0.059	<0.001*
W	0.003*	<0.001*	0.784	<0.001*	0.740	0.281	0.274	0.073	0.012*
N	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.006*	<0.001*	<0.001*	0.005*
Sp × W	0.733	0.711	0.150	0.633	0.105	0.152	0.014*	0.070	0.900
W × N	0.165	0.001*	0.152	0.001*	0.387	0.002*	0.196	0.002*	0.010*
Sp × W × N	0.788	0.483	0.727	0.507	0.390	0.402	0.007*	0.333	0.277
T									<0.001*
Sp × T									0.001*
N × T									<0.001*
W × N × T									0.001*
Sp × W × N × T									0.048*

Non-significant (*P*>0.05) interaction terms and results on covariate are not included

^a Measured before the freeze tests

^b Measured from the seedlings that were freeze-tested after 64 days of frost hardening

^c Measured after the freeze tests

**P*<0.05

and in different water treatments. In contrast, the species differed in their response to water treatments under HN (species × W and species × W × N interactions). In European aspen, both LW and HW promoted bud set, whereas in hybrid aspen × aspen, HW delayed bud set compared with MW. Although the species did not differ in bud set when averaged over treatments (no significant main effect), under HN, hybrid aspen × aspen showed earlier bud set under MW and later bud set under HW when compared with European aspen.

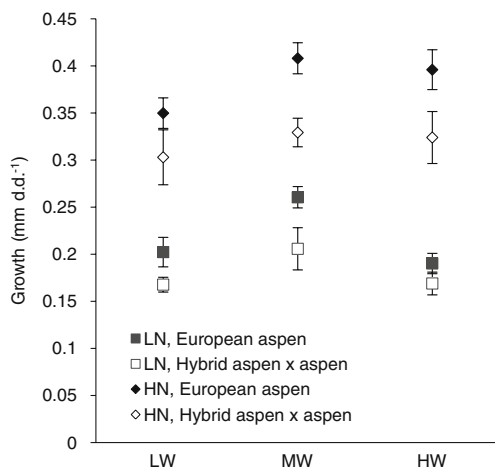


Fig. 1 Growth rate of European aspen and hybrid aspen × aspen under different water and nitrogen treatments (mean±SE, *n*=7). *L* low, *M* medium, *H* high

After the dehardening period, hybrid aspen × aspen tended to initiate growth on average 77 d.d. earlier than European aspen (Fig. 2b and Table 1). Higher N supply advanced budburst, but only under MW and HW (W × N interaction). Both aspen species responded similarly to water treatments under LN. Under HN, however, both LW and HW delayed the budburst of hybrid aspen × aspen, whereas in European aspen, LW delayed but HW advanced budburst compared with MW.

Hybrid aspen × aspen had a lower FI (Fig. 3 and Table 1) and the amount of freezing injury decreased faster than in European aspen as the frost hardening period progressed (species × time interaction), indicating a faster development of frost hardiness. In addition, significant variation among the hybrid aspen × aspen families was recorded in FI (*P*=0.037).

Higher N supply increased FI in both species, although mainly at the beginning of the frost hardening period (N × time interaction; Fig. 3 and Table 1). The HN seedlings showed more severe freezing injury when grown under LW than under MW or HW (main effect of W and W × N interaction). This effect was more apparent in European aspen, especially at the end of the frost hardening period (species × W × N × time interaction), whereas hybrid aspen × aspen was not affected by W or N treatments at that time (Fig. 3). When averaged over treatments per aspen family, higher FI was correlated with faster height growth rate (*r*=0.635, *P*=0.015). The correlations between FI and other measured variables were not significant (data not shown).

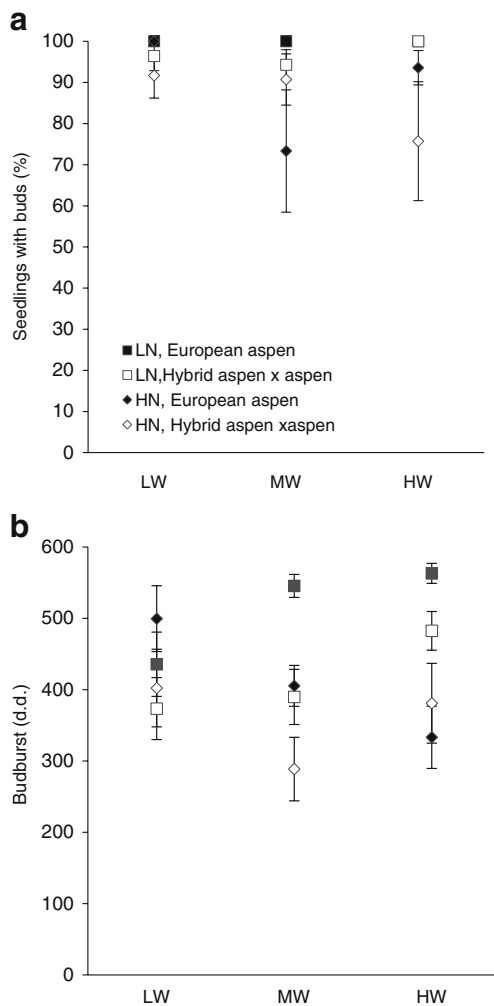


Fig. 2 Bud set (proportion of seedlings with terminal buds) (a) and timing of budburst (b) of European aspen and hybrid aspen \times aspen under different water and nitrogen treatments (mean \pm SE, $n=7$). *L* low, *M* medium, *H* high

4 Discussion

The freeze test technique employed here does not test frost hardiness to a particular sub-zero temperature, but gives an estimate of the development of frost hardiness under certain environmental conditions, as well as enabling a comparison of relative frost hardiness between the two aspen species tested. The freeze test mimics an abrupt autumn frost when snow cover is absent. Although a warming climate may postpone the occurrence of first autumn frosts, frost events early in autumn will still be possible in the future (Ruosteenoja et al. 2005).

In the present study, hybrid aspen \times aspen was more tolerant of frost and frost hardened faster than the native European aspen under all the treatments. Similar results have been obtained from a parallel experiment with other European aspen and backcross hybrid aspen families (P.

Pulkkinen, unpublished data). No interspecific differences in growth cessation, which could contribute to the observed difference in frost hardiness, were found at the end of the frost hardening period. As trees have been shown to be well adapted to local environmental conditions (Hurme et al. 1997), the *P. tremuloides* parental material of hybrid aspen \times aspen may partly account for its better frost hardiness. The climate in southern continental Canada, the origin of the *P. tremuloides* material, consists of lower daily mean and average minimum winter temperatures than in the more maritime Southern Finland. In contrast, southern ecotypes generally develop frost hardiness later than northern ones when grown in the same environment (Hurme et al. 1997; Lennartsson and Ögren 2002). Thus, the origin of *P. tremuloides* material, with more southern origin (about 45–55° N) than that of *P. tremula* (60° N), does not appear to unambiguously explain the difference between species in frost hardening. The higher leaf N and C

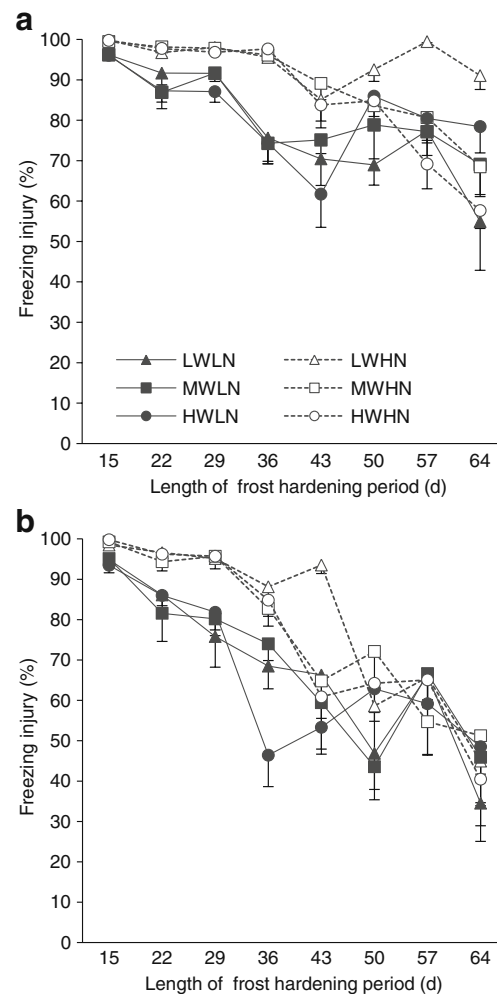


Fig. 3 Occurrence of freezing injury (proportion of dead shoot) in European aspen (a) and hybrid aspen \times aspen (b) under different water and nitrogen treatments in relation to the length of frost hardening period (mean \pm SE, $n=7$). *L* low, *M* medium, *H* high

concentrations of hybrid aspen \times aspen may also indicate a higher concentration of compounds related to increased frost hardiness, such as proteins and soluble sugars (cf. Sheppard and Pfan 2001), although confirmation for this was not found from the correlation analysis.

High N supply decreased tolerance to frost in both aspen species by delaying frost hardening, a phenomenon also reported for *Pinus halepensis* (Puértolas et al. 2005). The delayed formation of terminal buds under high N supply, as also observed for *Populus trichocarpa* (Sigurdsson 2001), suggests that this was at least partly mediated by an N-related prolongation of the vegetative period since frost hardiness commonly starts to gradually develop around the time of growth cessation (Hurme et al. 1997). However, the negative effect of high N supply was evident mainly at the beginning of the frost hardening period, implying that if the hardening period is long enough, N supply does not notably affect frost hardiness later in the autumn/winter. This is supported by observations in other studies (Jalkanen et al. 1998; Thomas and Ahlers 1999; Puértolas et al. 2005). The leaf N concentration in LN treatment (0.8%) was below the sufficient range suggested for *Populus* (Tullus et al. 2010), implying that nutrient deficiency may have improved the frost hardiness of LN seedlings by restricting growth, thus allowing more C to be available for the production of cryoprotectant carbohydrates (Jalkanen et al. 1998). Despite the similar soil N concentration in LN and HN treatments, the leaf N concentration was notably higher in HN seedlings (2.7%), corresponding to optimum concentration for the growth of hybrid aspen (Tullus et al. 2010).

High N supply combined with drought delayed frost hardening the most, especially in European aspen. Drought-stressed *Quercus* species have also been reported to manifest increased freezing damage, although the impact of high N supply was minor (Thomas and Ahlers 1999). Our results suggest that hybrid aspen \times aspen has better tolerance against autumn frosts than the native aspen species and that this difference may become more pronounced in environments with high N supply and drought periods, a situation likely in forthcoming decades (Lamarque et al. 2005; Jylhä et al. 2009).

Height growth rate was inversely related to frost hardiness, as observed for *Salix* (Lennartsson and Ögren 2002), implying a trade-off in investments between growth and frost hardening processes. European aspen grew faster than the backcross hybrid aspen in all treatments, similar to observations of backcross and parent generations of *Salix* (Fritz et al. 2006). Compared with F₁ hybrids, further hybrids of *Populus* are generally slower growing (Karim and Hawkins 1999). The higher P_n of European aspen may partly explain its faster growth. The relatively low g_s and P_n measured in this study (vs. Häikiö et al. 2007; Nikula et al. 2009) are likely explained by the

comparatively low irradiance in the greenhouse, which has been suggested to correspond to shade conditions in nature (Monclus et al. 2005).

Regarding growth, gas exchange, and leaf N and C concentrations, the two aspen species responded similarly to water and N treatments. As pioneer trees, *Populus* species respond readily to increases in N availability with enhanced leaf N concentration, photosynthetic rate and growth (Cooke et al. 2005; Häikiö et al. 2007), which was also observed here. Although pioneer species are often adapted to variable soil water conditions, drought and wet conditions have been reported to limit the growth of *Populus* (Ibrahim et al. 1997; Guidi and Labrecque 2010). The results of P_n indicate the interactive effects of water and N supply on aspen and support the hypothesis that high nutrient availability improves the drought tolerance of trees by maintaining physiological processes (Abrams 1988).

Although the growth rate measurements imply greater vigour of European aspen during the first growing season under greenhouse conditions, the earlier budburst of hybrid aspen \times aspen may allow more growth than in European aspen during subsequent growing seasons. This would agree well with observations on F₁ hybrid aspen: the faster growth of F₁ hybrid aspen compared with European aspen has been attributed particularly to its longer vegetative period (Yu et al. 2001). These phenological traits of hybrid aspen \times aspen may be even more important in the future as the warming climate will provide longer growing seasons (Jylhä et al. 2009).

Earlier growth initiation of hybrid aspen \times aspen was manifested in almost all the treatments, except for HWHN. In accordance with observations on *Quercus* (Thomas and Ahlers 1999), high N supply generally promoted the budburst of both aspen species. However, when grown under high N supply, the species differed in their bud phenology responses to high water supply. Wet conditions slightly altered the timing of the vegetative period of both species, although in different directions—by promoting the budburst and bud set of European aspen and delaying them in hybrid aspen \times aspen. In high-latitude environments, earlier timing of the vegetative period presumably supports growth more efficiently because of higher light intensity and longer days in spring compared with autumn (Peltonen-Sainio et al. 2009). In any case, earlier budburst can predispose trees to damage by spring frosts.

Although intraspecific variation is typically high in *Populus*, especially in F₂ generations (Karim and Hawkins 1999), we recorded variation among backcross hybrid aspen families only in frost hardiness; European aspen did not show significant variation in any of the measured traits. Intraspecific differences possibly become more evident as the trees age (Yu et al. 2001). The higher intraspecific variation in hybrid aspen \times aspen may confer better

potential to adapt to changing environmental conditions compared with European aspen (cf. Grulke 2010).

In conclusion, although the native European aspen grew faster than hybrid aspen \times aspen in all the treatments, the faster frost hardening, better overall frost hardiness and earlier budburst of hybrid aspen \times aspen imply that backcross hybrid aspen may have some advantages over the native species after their first growing season. However, earlier budburst can also be a disadvantage in the event of late spring frosts. In general, the two aspen species responded comparably to water and N treatments, suggesting that the interspecific differences in growth, frost hardiness and budburst are maintained under different environmental conditions. High N supply—especially when combined with drought during the growing season—delayed frost hardening, partly through an N-related prolongation of vegetative period. This effect was more pronounced in European aspen. The results suggest that backcross hybrid aspen may in some respects be better adapted to a range of environmental conditions than the native species.

Although aspen reproduces mainly vegetatively under present conditions, sexual reproduction through seedlings may be more common than is assumed (Suvanto and Latva-Karjanmaa 2005). Backcrosses probably have consequences for native species located near hybrid aspen plantations, the extent of which may increase with warming climate that enables the cultivation of hybrid aspen in more northern regions. Because the direction of crossing can notably affect the behaviour of the resultant cross (Suvanto et al. 2004), backcrosses with European aspen female and hybrid aspen male should also be investigated.

Acknowledgements We thank Raimo Jaatinen, Hiski Aro, Ana Juanes and other personnel from the Haapastensyrjä Breeding Station of the Finnish Forest Research Institute for helping with the experiment. Marjut Wallner, Department of Forest Sciences, University of Helsinki, is thanked for conducting the N and C analyses. Sally Ulich checked the language. The study was financially supported by the Finnish Forest Research Institute and the Helsinki University Environmental Research Centre.

References

- Abrams MD (1988) Sources of variation in osmotic potential with special reference to north American tree species. *For Sci* 34:1030–1046
- Andersson B (1992) Forecasting *Pinus sylvestris* field mortality by freezing tests: methods and applications. Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology, Umeå, 24 pp
- Aronsson A (1980) Frost hardiness in Scots pine (*Pinus sylvestris* L.). II. Hardiness during winter and spring in young trees of different mineral nutrient status. *Stud For Suec* 155:1–27
- Burke J, Arnold M (2001) Genetics and the fitness of hybrids. *Annu Rev Genet* 35:31–52
- Colombo SJ, Menzies MI, O'Reilly C (2001) Influence of nursery cultural practices on cold hardiness of coniferous forest tree seedlings. In: Bigras FJ, Colombo SJ (eds) *Conifer cold hardiness*. Kluwer, Dordrecht, pp 223–252
- Cooke JEK, Martin TA, Davis JM (2005) Short-term physiological and developmental responses to nitrogen availability in hybrid poplar. *New Phytol* 167:41–52
- DeHayes DH, Ingle MA, Waite CE (1989) Nitrogen-fertilization enhances cold tolerance of red spruce seedlings. *Can J For Res* 19:1037–1043
- Fritz RS, Hochwender CG, Albrechtsen BR, Czesak ME (2006) Fitness and genetic architecture of parent and hybrid willows in common gardens. *Evolution* 60:1215–1227
- Grulke NE (2010) Plasticity in physiological traits in conifers: implications for response to climate change in the western U.S. *Environ Pollut* 158:2032–2042
- Guidi W, Labrecque M (2010) Effects of high water supply on growth, water use, and nutrient allocation in willow and poplar grown in a 1-year pot trial. *Water Air Soil Pollut* 207:85–101
- Häikiö E, Freiwald V, Pasanen T, Beuker E, Holopainen T, Oksanen E (2007) Impacts of elevated ozone and nitrogen on growth and photosynthesis of European aspen (*Populus tremula*) and hybrid aspen (*P. tremula* \times *P. tremuloides*). *Can J For Res* 37:2326–2336
- Hellergren J (1981) Frost hardiness development in *Pinus sylvestris* seedlings in response to fertilization. *Physiol Plant* 52:297–301
- Holm S (2004) Haavan viljely Suomessa ja Virossa. *Metsätieteiden Aikakauskirja* 1/2004:117–118 (in Finnish)
- Hurme P, Repo T, Savolainen O, Pääkkönen T (1997) Climatic adaptation of bud set and frost hardiness in Scots pine (*Pinus sylvestris*). *Can J For Res* 27:716–723
- Ibrahim L, Proe MF, Cameron AD (1997) Main effects of nitrogen supply and drought stress upon whole-plant carbon allocation in poplar. *Can J For Res* 27:1413–1419
- Jalkanen RE, Redfern DB, Sheppard LJ (1998) Nutrient deficits increase frost hardiness in Sitka spruce (*Picea sitchensis*) needles. *For Ecol Manag* 107:191–201
- Juntunen M-L, Rikala R (2001) Fertilization practice in Finnish forest nurseries from the standpoint of environmental impact. *New For* 21:141–158
- Jylhä K, Ruosteenoja K, Räisänen J, Venäläinen A, Tuomenvirta H, Ruokola L, Saku S, Seitola T (2009) The changing climate in Finland: estimates for adaptation studies. ACCLIM project report 2009. Reports No. 4, Finnish Meteorological Institute, Helsinki, 114 pp (in Finnish with English abstract)
- Karim SA, Hawkins BJ (1999) Variation in response to nutrition in a three-generation pedigree of *Populus*. *Can J For Res* 29:1743–1750
- Kimball S, Campbell DR, Lessin C (2008) Differential performance of reciprocal hybrids in multiple environments. *J Ecol* 96:1306–1318
- Kouki J, Arnold K, Martikainen P (2004) Long-term persistence of aspen—a key host for many threatened species—is endangered in old-growth conservation areas in Finland. *J Nat Conserv* 12:41–52
- Lamarque JF, Kiehl JT, Brasseur GP, Butler T, Cameron-Smith P, Collins WD, Collins WJ, Granier C, Hauglustaine D, Hess PG, Holland EA, Horowitz L, Lawrence MG, McKenna D, Merilees P, Prather MJ, Rasch PJ, Rotman D, Shindell D, Thornton P (2005) Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: analysis of nitrogen deposition. *J Geophys Res Atmos* 110(D19):D19303
- Lennartsson M, Ögren E (2002) Causes of variation in cold hardiness among fast-growing willows (*Salix* spp.) with particular reference to their inherent rates of cold hardening. *Plant Cell Environ* 25:1279–1288

- Lorenz M, Nagel H-D, Granke O, Kraft P (2008) Critical loads and their exceedances at intensive forest monitoring sites in Europe. *Environ Pollut* 155:426–435
- Monclus R, Dreyer E, Delmotte FM, Villar M, Delay D, Boudouresque E, Petit J-M, Marron N, Brécher C, Brignolas F (2005) Productivity, leaf traits and carbon isotope discrimination in 29 *Populus deltoides* × *P. nigra* clones. *New Phytol* 167:53–62
- Nikula S, Percy K, Oksanen E, Holopainen T, Manninen S (2009) Effects of elevated ozone on growth and foliar traits of European and hybrid aspen. *Bor Environ Res* 14:29–47
- Peltonen-Sainio P, Jauhiainen L, Hakala K, Ojanen H (2009) Climate change and prolongation of growing season: changes in regional potential for field crop production in Finland. *Agric Food Sci* 18:171–190
- Puértolas J, Gil L, Pardos JA (2005) Effects of nitrogen fertilization and temperature on frost hardiness of Aleppo pine (*Pinus halepensis* Mill.) seedlings assessed by chlorophyll fluorescence. *Forestry* 78:501–511
- Pulkkinen P (1993) Frost hardiness development and lignification of young Norway spruce seedlings of southern and northern Finnish origin. *Silva Fenn* 27:47–54
- Rikala R, Repo T (1997) The effect of late summer fertilization on the frost hardening of second-year Scots pine seedlings. *New For* 14:33–44
- Ruosteenoja K, Jylhä K, Tuomenvirta H (2005) Climate scenarios for FINADAPT studies of climate change adaptation. FINADAPT Working Paper 15, Finnish Environment Institute Mimeographs 345, Helsinki, 32 pp
- Rytter L (2006) A management regime for hybrid aspen stands combining conventional forestry techniques with early biomass harvests to exploit their rapid early growth. *For Ecol Manag* 236:422–426
- Sheppard L, Pfanz H (2001) Impacts of air pollutants on cold hardiness. In: Bigras FJ, Colombo SJ (eds) *Conifer cold hardiness*. Kluwer, Dordrecht, pp 335–366
- Sigurdsson BD (2001) Elevated [CO₂] and nutrient status modified leaf phenology and growth rhythm of young *Populus trichocarpa* trees in a 3-year field study. *Trees* 15:403–413
- Suominen O, Edenius L, Ericsson G, de Dios VR (2003) Gastropod diversity in aspen stands in coastal northern Sweden. *For Ecol Manag* 175:403–412
- Suvanto LI, Latva-Karjanmaa TB (2005) Clone identification and clonal structure of the European aspen (*Populus tremula*). *Mol Ecol* 14:2851–2860
- Suvanto L, Stenvall N, Vares A, Pulkkinen P (2004) Hybridisaation geenivirta. *Metsätieteen Aikakauskirja* 1/2004:89–97 (in Finnish)
- Thomas FM, Ahlers U (1999) Effects of excess nitrogen on frost hardiness and freezing injury of above-ground tissue in young oaks (*Quercus petraea* and *Q. robur*). *New Phytol* 144:73–83
- Tullus A, Kanal A, Soo T, Tullus H (2010) The impact of available water content in previous agricultural soils on tree growth and nutritional status in young hybrid aspen plantations in Estonia. *Plant Soil* 333:129–145
- van den Driessche R (1991) Effects of nutrients on stock performance in the forest. In: van den Driessche R (ed) *Mineral nutrition of conifer seedlings*. CRC, Boca Raton, pp 229–260
- Vanden BA, Villar M, Van Bockstaele E, Van Slycken J (2005) Natural hybridization between cultivated poplars and their wild relatives: evidence and consequences for native poplar populations. *Ann For Sci* 62:601–613
- Yu Q, Tigerstedt PMA, Haapanen M (2001) Growth and phenology of hybrid aspen clones (*Populus tremula* L. × *Populus tremuloides* Michx.). *Silva Fenn* 35:15–25