

# Elevated temperature effects on germination and early growth of European aspen (*Populus tremula*), hybrid aspen (*P. tremula* × *P. tremuloides*) and their F<sub>2</sub>-hybrids

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**Abstract** This study aimed to understand the interaction between temperature and genotype in terms of the effect on early seedling development of European aspen (*Populus tremula*) and various F<sub>2</sub>-aspen hybrids. We evaluated the response of 16 different European aspen and F<sub>2</sub>-hybrid families on seed germination, survival rate and seedling height in one- and two-family trials under three different temperature regimes. In one-family trials, higher germination and higher survival rates were observed in higher temperature (C1700), leading to taller seedlings. European aspen × hybrid aspen individuals (Asp × Hyb) had a higher survival rate and taller seedlings than Asp, Hyb × Asp or Hyb × Hyb individuals. The difference between Asp × Hyb and Hyb × Asp was pronounced. Both growth conditions and genotype (i.e., hybrid cross) had strong effects on germination, survival rate and height of European aspen and F<sub>2</sub>-hybrid seedlings. However, the interaction of genotype and growth conditions also had a significant influence on survival rate and seedling height, but not on germination. Two-family trials involving European aspen and F<sub>2</sub>-hybrids led to significant negative effects on germination and survival rate and also

facilitation effects on seedling height. Similarly, genotype had strong effects on germination and survival rate. Different genotypes and traits affected survival rate and seedling height differently in different growth conditions. These differences were more pronounced in the warmest environment, i.e., 1,700 degree days. We conclude that introgression between F<sub>2</sub>-hybrids and local European aspen is likely in the current climate, and any warming will likely favor certain F<sub>2</sub>-hybrids, especially the most probable types (*P. tremula* × (*P. tremula* × *P. tremuloides*)).

**Keywords** Climate change · Temperature · Competition · Hybridization · Poplar · Fennoscandian forestry · Introgression

## Introduction

Plants belonging to the genus *Populus* (Salicaceae) are found throughout the northern hemisphere (Eckenwalder 1996). In addition to 22–85 species of poplar, aspen and cottonwoods, there are hundreds of hybrids, varieties and cultivars (Eckenwalder 1996). European aspen (*Populus tremula* L.) is one of the most widely distributed trees in the world, with a natural range that includes Lapland, North Africa, the British Isles, China and Japan (Yrjölä 2002).

In northern Europe, aspen has a fast growth rate (Hynynen et al. 2002) and is an important supply crop for the paper and timber industries (Yu et al. 2001a). Ecologically, aspen is an important forest species that supports a high biodiversity of organisms in its live and dead state (Kouki et al. 2004), and future climate models suggest it will gain a competitive advantage in certain locations (Santamaría and Diez 2005).

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Crosses between European aspen and North American quaking aspen (*Populus tremuloides* Michx.) have been known since the early twentieth century (Wettstein 1933). Resulting hybrid aspen grew rapidly and vigorously (Tullus et al. 2012), and many other crosses were subsequently made (Yu 2001). In response to its impressive growth rate, over 1,500 hectares of hybrid aspen were planted in Finland and Estonia during the 1990s (Holm 2004).

Hybrid aspen can produce viable seeds and is interfertile with European aspen (Koivuranta et al. 2012). Thus, the genetic integrity of this important native tree is threatened (Van den Broeck et al. 2004). However, the sexual reproduction of aspen may not be very frequent in nature (Worrell 1995; Latva-Karjanmaa et al. 2003). Generally, the performance of secondary hybrids is considered to be lower than that of either parent species [reviewed by Burke and Arnold (2001)]. However, secondary hybrids can be superior (Burke and Arnold 2001) under certain conditions in some habitats, as might occur as a consequence of climate change.

Interactions such as competition and facilitation are believed to be important at both the individual (Baraloto et al. 2005) and population scale (Tansley 1917; Callaway and Walker 1997). Furthermore, above- and below-ground interactions have been shown to affect tree growth and survival rate (Coomes and Grubb 1998; Coates et al. 2009). Interactive effects have been detected in early stages of development (Berkowiz et al. 1995; Kaelke et al. 2001) as well as in adult trees (Canham et al. 2004) where the proximity, density and size of neighboring plants have been implicated as the principal factors affecting the intensity of interaction (Weiner 1984; Bonan 1991). Although most studies have focused on interactions between species (e.g., Boyden et al. 2009), competition among genotypes is believed to affect yield (Sakai et al. 1967; Adams et al. 1973; Tauer 1975; Foster et al. 1998), microbe-mediated interactions are known to influence growth and survival rate (Packer and Clay 2004; Korkkama et al. 2007) and environmental factors are also believed to be involved (Maestre et al. 2005). Unfortunately, interactions between different hybrids and F<sub>2</sub>-hybrids have yet to be studied from this perspective.

Because hybrid aspen grows more rapidly and vigorously than local natives (Yu et al. 2001b; Hynynen et al. 2002), it is important to identify the stage(s) and situation(s) in which hybrids have a competitive advantage. Measuring relative performance during early stages is a priority, especially in the high seed/seedling densities (e.g., thousands m<sup>-2</sup>) that can occur for hybrid aspen (H. Aro, pers. comm). Future climate models predict that mean temperatures in Finland will increase 2–7 °C by 2100, likewise precipitation will rise by 8–37 % (Jylhä et al. 2004, 2009; Ruosteenoja et al. 2005). As a consequence,

the extended growing season coupled with relatively warm winters and increased precipitation will likely affect growth, hardiness and dormancy. The altered conditions experienced during early development stages may also affect hybrids as well as the interaction between native and hybrid aspens in natural situations.

In this study, we propose the following hypothesis: Interactions between aspens and their hybrids favor the spread of certain F<sub>2</sub>-hybrids under three different temperature conditions, and climate change will enhance the dispersal and establishment of hybrids into new areas and may also enhance more vigorous growth of the seedlings. We focused on early life stages and divided comparisons into one-family and two-family trials under three different environmental regimes. Seed germination rate, survival rate and seedling height after one growth season were analyzed. Our aims were to evaluate whether European aspen and their hybrids have different temperature optima during early development and to identify the influence of temperature on the interactions among aspen genotypes in situations of high seed/seedling density.

## Materials and methods

### Seed material

The seeds used in this study were from controlled aspen crossings made at the Haapastensyrjä field station of the Finnish Forest Research Institute in southern Finland (mean temperature sum 1,100 degree days [d.d]) (60°37'N, 24°26'E, 110 m a.s.l.) during April 2004. Prior to flowering, branches of randomly selected European and hybrid aspen were collected and transferred to a greenhouse.

Before starting the experimental trials, test tree branches received a 2-day pre-treatment to help force flowering (Koivuranta et al. 2012). Briefly, branches were placed in water-filled buckets in the greenhouse where the daytime temperature was 10 °C and fell to 8 °C at night. Branches and the greenhouse floor were watered 2–3 times per day to prevent withering. After 2 days, greenhouse temperatures were adjusted to 15 °C (day) and 12 °C (night) to stimulate male flowers, or 16 °C (day) and 12 °C (night) for female flowers.

Each female branch (genotype) was used in four crosses with two European aspen male and two hybrid aspen male trees (Table 1), thus the total number of different families produced was 16, consisting of four families of each cross type; European aspen × European aspen (Asp × Asp), European aspen × hybrid aspen (Asp × Hyb), hybrid aspen × European aspen (Hyb × Asp) and hybrid aspen × hybrid aspen (Hyb × Hyb). The temperature during pollination was ca. 13 °C and ca. 17 °C during seed

**Table 1** Seed numbers in two-family trials

Female	Male			
	Asp 1	Asp 2	Hyb 1	Hyb 2
Asp 1 <sup>a</sup>	(Asp1 × Asp1) (25 + 25) × 3 × 3 <sup>b</sup>	(Asp1 × Asp2) (25 + 25) × 3 × 3	(Asp1 × Hyb1) (25 + 25) × 3 × 3	(Asp1 × Hyb2) (25 + 25) × 3 × 3
Asp 2	(Asp2 × Asp1) (25 + 25) × 3 × 3	(Asp2 × Asp2) (25 + 25) × 3 × 3	(Asp2 × Hyb1) (25 + 25) × 3 × 3	(Asp2 × Hyb2) (25 + 25) × 3 × 3
Hyb 1	(Hyb1 × Asp1) (25 + 25) × 3 × 3	(Hyb1 × Asp2) (25 + 25) × 3 × 3	(Hyb1 × Hyb1) (25 + 25) × 3 × 3	(Hyb1 × Hyb2) (25 + 25) × 3 × 3
Hyb 2	(Hyb2 × Asp1) (25 + 25) × 3 × 3	(Hyb2 × Asp2) (25 + 25) × 3 × 3	(Hyb2 × Hyb1) (25 + 25) × 3 × 3	(Hyb2 × Hyb2) (25 + 25) × 3 × 3

<sup>a</sup> Asp, European aspen; Hyb, hybrid aspen

<sup>b</sup> The numbers indicate seed numbers of (Female + male) × treatments × replicates

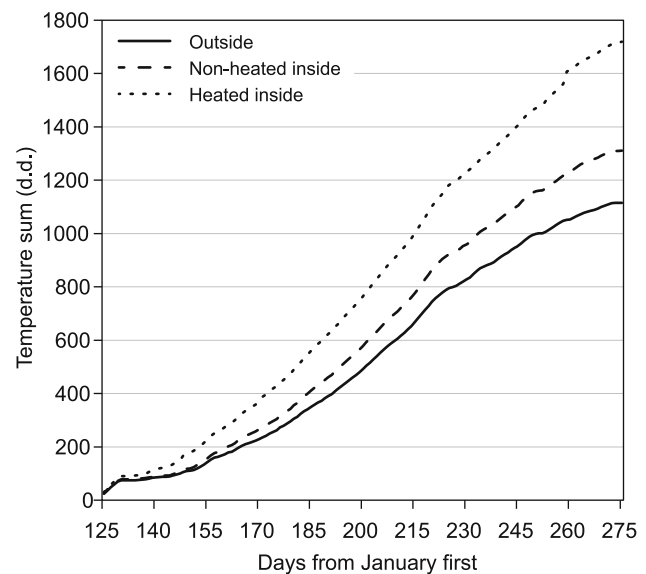
ripening to promote maturation. Catkins were gathered ca. 20 days after pollination when the seed hair was visible and mature seeds had started to shed. The seed material was cleaned as described in Latva-Karjanmaa et al. (2003).

### Experimental design

The study was conducted in three environments based on temperature sum during the growth period (Fig. 1): (1) a heated greenhouse with a total temperature sum of ca. 1,700 d.d. during the experiment, designated C1700; (2) a plastic greenhouse without any additional heating with a total temperature sum of ca. 1,300 d.d. during the experiment, designated C1300; and (3) an open nursery field without any cover or heating with temperature sum of ca. 1,100 d.d., during the experiment, designated C1100. The photoperiod and light intensity in the heated greenhouse were similar to ambient, but wavelengths below 400 nm and above 3,000 nm were ca. 20 % of ambient in the unheated plastic house.

Seeds were sown into three sets of growing boxes containing 16 pots (TAKO 1210, filled with pre-fertilized peat, N:P:K 13:4:2 Kekkilä) for each growth condition. Each pot had a surface area of 110 cm<sup>2</sup> and received 50 seeds at the start of the experiment. There were three replicates for each set, one of which was placed in each of the three different growth environments at the end of April 2004. Pots were moved randomly several times within each environment during the summer. Photoperiods and CO<sub>2</sub> levels during the growing season were similar among treatments and to the ambient. Irrigation was performed according to standard nursery practice.

This study consisted of experiments in which either a single or a mixture of two seed families were sown in each pot (i.e., Asp × Asp, Asp × Hyb, Hyb × Asp, and Hyb × Hyb), and all combinations were of different genotypes. In two-family trials, 25 seeds from European



**Fig. 1** Development of temperature sums in different growth conditions

aspen were sown with 25 seeds from one of the hybrid or other genotype families.

Seed germination was measured 3 weeks after sowing. After 5 months when seedlings had formed terminal buds, dead and live seedlings were counted to provide an estimate of survival rate of the germinated seedlings. Survival rate, of course, is dependent on germination rate but has been used here as a summary trait of the density effect. In addition, the height of live seedlings was measured. We calculated pot means and because we did not identify seeds of each family in the two-family trials, we constructed new variables to test the level of interaction. First, we generated expected (i.e., mean) values of seed germination, survival rate and seedling height for each family grown in one-family trials, i.e.,  $(\mu_1 + \mu_2)/2$ . Then, observed mean values of seed germination, survival rate and seedling height in two-family trials ( $\mu_3$ ) were tested against the expected values derived from

their growth in one-family trials. Differences between expected and observed means will be either positive (i.e., imply facilitation) or negative (i.e., imply competition). Performance comparisons among families were made only within the same growth environment.

$H_0$  states that  $(\mu_1 + \mu_2)/2 - \mu_3 = 0$ . Differences in seed germination, survival rate and mean seedling height between  $(\mu_1 + \mu_2)/2$  and  $\mu_3$  were evaluated with Student's  $t$  test, analysis of variance (ANOVA) where all factors were considered as fixed and Pearson's correlation coefficients. All statistical analyses were made using SYSTAT statistical program, version 13.

## Results

### One-family trial

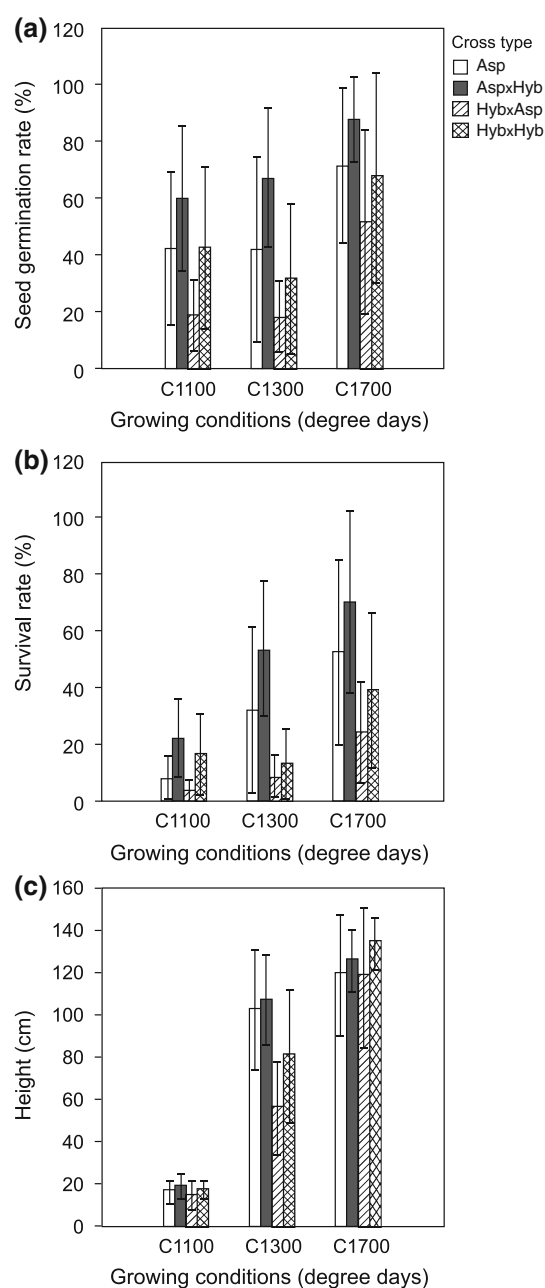
Over all crosses and growth environments for one-family trials, the mean value for seed germination rate was 46.4 % (SD = 29.6,  $N = 191$ ), survival rate 28.5 % (SD = 28.2,  $N = 144$ ) and seedling height 82.4 cm (SD = 54.3,  $N = 134$ ).

Mean germination rate was 69.5 % (SD = 30.0) in the warmest growth environment (C1700), which was significantly higher than in the cooler greenhouse (C1300); 39.7 % (SD = 29.5), and outside in the nursery field (C1100); 41.0 % (SD = 27.0). Seeds from the Asp  $\times$  Hyb cross had the highest germination rate in all growth environments, 71.6 % (SD = 23.9). Second highest in Asp was 51.9 % (SD = 30.7), third highest was in Hyb  $\times$  Hyb was 43.2 % (SD = 30.1) and the lowest in Hyb  $\times$  Asp was 30.5 % (SD = 24.2; Fig. 2a).

Mean seedling survival rate was highest in C1700, 46.3 % (SD = 31.4), second highest in C1300, 26.5 % (SD = 26.1) and only 12.5 % (SD = 12.6) in C1100 after one growing season (Fig. 2b). The highest survival rate was observed in Asp  $\times$  Hyb seedlings, 48.3 % (SD = 30.7), second highest in Asp  $\times$  Asp seedlings, 30.6 % (SD = 30.3), third in Hyb  $\times$  Hyb seedlings 22.7 % (SD = 21.6) and lowest in Hyb  $\times$  Asp seedlings 12.2 % (SD = 13.7).

The mean height of seedlings raised in C1700 was 131.6 cm (SD = 28.9) compared to 93.0 cm (SD = 35.1) in C1300 and 17.7 cm (SD = 5.8) in C1100. Mean seedling height in the warmest environment (C1700) was significantly taller than the field site (C1100). The tallest seedlings were of Asp  $\times$  Hyb, 87.8 cm (SD = 54.1), and Asp  $\times$  Asp, 86.5 cm (SD = 52.4). The shortest seedlings were of Hyb  $\times$  Asp, 72.6 cm (SD = 57.9; Fig. 2c).

The cross, growth conditions and their interactions all explained a significant component of the variation in survival rate and seedling height in the one-family trials (Table 2). In contrast, interaction did not explain a



**Fig. 2** Means and standard deviations of seed germination rate (a), survival rate (b) and seedling height (c) of European aspens and different F<sub>2</sub>-aspen hybrids in one-family trial under different conditions. Asp, European aspen; Asp  $\times$  Hyb, European aspen  $\times$  hybrid aspen; Hyb  $\times$  Asp, hybrid aspen  $\times$  European aspen; Hyb  $\times$  Hyb, hybrid aspen  $\times$  hybrid aspen

significant component of variation in germination rate. Interestingly, some families within a cross were associated with a significant amount of variation in germination and survival rate.

In all test conditions, there were significant positive correlations between germination and survival rate, but height was not significantly affected (Table 3). Survival

**Table 2** The effect of cross type and growth conditions on germination, survival rate and seedling height during the first growing season of European and F<sub>2</sub>-hybrid aspens in one-family trial

Source	Germination			Survival rate			Height		
	df	MS	p	df	MS	p	df	MS	p
Cross type	3	10,685	<0.001	3	8,352	<0.001	3	2,330	<0.05
Family (cross type)	12	4,225	<0.001	12	1,829	<0.001	12	759	ns
Growth condition	2	13,648	<0.001	2	13,801	<0.001	2	148,595	<0.001
Replication (growth condition)	6	456	ns	6	259	ns	6	181	ns
Cross type × growth condition	6	216	ns	6	986	<0.01	6	2,079	<0.01
Error	114	270		114	274		104	620	

**Table 3** Correlations between mean values of seed germination, survival rate and seedling height of European and F<sub>2</sub>-hybrid aspens in one-family trial

Growth condition (d.d.)	Variable	Survival rate			Height		
		r	N	p	r	N	p
1,700	Germination	0.702	48	<0.001	0.056	47	ns
	Survival rate				-0.093	47	ns
1,400	Germination	0.858	48	<0.001	-0.069	44	ns
	Survival rate				0.270	44	ns
1,250	Germination	0.699	48	<0.001	0.301	43	ns
	Survival rate		48		0.427	43	<0.01

rate showed a significant positive correlation with height only in the coolest condition (C1100; Table 3). In C1700, Asp × Hyb crosses showed a negative correlation ( $r = -0.81$ ,  $N = 12$ ,  $p < 0.01$ ) between germination and seedling height, and with Hyb × Hyb, the correlation was positive ( $0.744$ ,  $N = 12$ ,  $p < 0.01$ ). In C1300, Hyb × Asp showed a positive correlation with survival and height ( $r = 0.71$ ,  $N = 9$ ,  $p < 0.05$ ). In C1100, Hyb × Hyb had a positive correlation with germination, survival ( $r = 0.79$ ,  $N = 12$ ,  $p < 0.01$ ) and height ( $r = 0.83$ ,  $N = 11$ ,  $p < 0.01$ ). All other correlations between crosses and studied characteristics were not significant.

Two-family trials

In two-family competition trials, the overall mean germination rate was 47.8 % (SD = 23.8,  $N = 308$ ), survival rate 29.1 % (SD = 29.3,  $N = 233$ ) and seedling height 89.5 cm (SD = 56.8,  $N = 229$ ) after the first growing season. Observed mean germination and survival rate were significantly lower, whereas seedlings were significantly taller than the expected value of  $(\mu_1 + \mu_2)/2$  (Table 4).

In all conditions, germination rate was lower than expected with the greatest discrepancy of -9.3 % in C1100 (Table 4). Trials involving Asp/Asp × Hyb had a lower germination rate than expected in all trials (-4.7 %,

$p < 0.05$ ,  $t = -2.5$ ), while Asp/Hyb × Hyb was less so (-3.8 %,  $p < 0.05$ ,  $t = -2.2$ ). Across all competitive trial and condition combinations, Asp/Hyb × Asp and Asp/Hyb × Hyb grown in C1100, and Asp/Asp × Hyb in C1700 had significantly lower germination rates than expected (Table 4). Cross and family explained a significant component of variation in germination differences (Table 5).

Survival rate was significantly lower than expected in C1700 (-11.5 %) and C1100 (-8.2 %; Table 4). In all trials, Asp/Hyb × Asp (-13.6 %,  $p < 0.01$ ,  $t = -3.7$ ), Asp/Hyb × Hyb (-8.4 %,  $p < 0.001$ ,  $t = -4.6$ ) and Asp/Asp × Hyb (-5.8 %,  $p < 0.05$ ,  $t = -2.7$ ) had significantly lower survival rates than expected. Across all competitive trial and condition combinations, Asp/Asp × Hyb and Asp/Hyb × Hyb grown in C1100, and Asp/Hyb × Asp and Asp/Hyb × Hyb in C1700 had significantly lower survival rates than expected (Table 4). Growth condition was not a significant source of variation in survival difference (Table 5).

Seedlings were significantly taller than expected in C1100 and C1700, but shorter than expected in C1300 (Table 4) and in all Asp/Hyb × Asp trials (22.3 cm,  $p < 0.01$ ,  $t = 3.0$ ). Only one family had shorter seedlings than expected when grown in C1700. In other conditions, no clear pattern was apparent with respect to observed and

**Table 4** Difference between germination, survival rate and seedling height of European aspens (Asp) with other European aspens or F<sub>2</sub>-hybrid aspens (Hyb) in two-family trials compared to mean values measured from respective families in one-family trials after the first growing season

Growth condition (d.d.)	Cross type	Germination difference (%-units)				Survival rate difference (%-units)				Seedling height difference (cm)			
		Diff.	<i>N</i>	<i>t</i>	<i>p</i>	Diff.	<i>N</i>	<i>t</i>	<i>p</i>	Diff.	<i>N</i>	<i>t</i>	<i>p</i>
1,700	Asp/Asp	+7.0	6	+1.3	ns	+11.2	6	+1.1	ns	+23.1	6	+1.8	ns
1,700	Asp/Asp × Hyb	-12.9	24	-2.7	<0.05	-7.3	24	-1.9	ns	+19.9	24	+3.8	<0.01
1,700	Asp/Hyb × Asp	-16.5	12	-1.9	ns	-22.8	12	-3.7	<0.01	+42.4	12	+2.9	<0.05
1,700	Asp/Hyb × Hyb	-3.4	36	-1.1	ns	-13.8	36	-4.6	<0.001	+10.2	34	+1.8	ns
1,700	∑	-7.5	78	-3.0	<0.01	-11.5	77	-5.0	<0.001	+19.3	75	+4.9	<0.001
1,300	Asp/Asp	-2.5	6	-0.5	ns	-10.7	6	-1.8	ns	-26.4	6	-8.0	<0.001
1,300	Asp/Asp × Hyb	+3.6	24	+0.8	ns	-2.3	22	-0.5	ns	-9.9	22	-2.4	<0.05
1,300	Asp/Hyb × Asp	-12.3	12	+2.2	ns	-9.8	12	-1.5	ns	+14.2	9	+1.4	ns
1,300	Asp/Hyb × Hyb	+1.6	36	+0.5	ns	-0.2	33	-0.1	ns	-2.4	30	-0.6	ns
1,300	∑	-0.2	78	-0.1	ns	-3.3	73	-1.4	ns	-4.8	67	-1.6	ns
1,100	Asp/Asp	+9.8	6	+1.7	ns	+1.7	6	+0.6	ns	+1.9	6	+1.4	ns
1,100	Asp/Asp × Hyb	-7.6	24	-2.0	ns	-7.6	24	-2.3	<0.05	+4.0	20	+2.6	<0.05
1,100	Asp/Hyb × Asp	-13.3	12	-3.6	<0.01	-7.3	10	-1.3	ns	+3.9	9	+0.6	ns
1,100	Asp/Hyb × Hyb	-12.4	36	-3.6	<0.01	-10.7	34	-3.7	<0.01	+5.1	29	+2.1	<0.05
1,100	∑	-9.3	78	-4.2	<0.001	-8.2	74	-4.3	<0.001	+4.3	64	+2.9	<0.01
Total	∑	-5.7	234	-4.1	<0.001	-7.7	224	-6.1	<0.001	+6.8	206	+3.6	<0.001

**Table 5** The effect of cross types, families within the cross types and growth conditions on the difference of germination, survival rate and seedling height between observed and expected values during the first growing season of European and different F<sub>2</sub>-hybrid aspen

Source of variation	Germination difference			Survival rate difference			Height difference		
	<i>df</i>	MS	<i>p</i>	<i>df</i>	MS	<i>p</i>	<i>df</i>	MS	<i>p</i>
Cross type	3	1,550	<0.01	3	1,074	<0.01	3	1,451	ns
Family (cross type)	8	1,902	<0.001	8	1,411	<0.001	10	622	ns
Growth condition	2	238	ns	2	100	ns	2	10,848	<0.001
Replication × growth condition	6	387	ns	6	1,950	<0.001	6	877	ns
Cross type × growth condition	6	672	ns	6	626	<0.05	6	1,176	<0.05
Error	208	359		198	253		179	593	

expected seedling heights. Across all competitive trial and condition combinations, Asp/Asp × Hyb and Asp/Hyb × Hyb grown in C1100, and Asp/Asp × Hyb and Asp/Hyb × Asp in C1700 had significantly higher mean seedling height than expected (Table 4). Growth conditions and the interaction between cross type and growth condition were associated with a significant component of variation observed among seedling heights, whereas cross type alone was not (Table 5).

The effect of cross type and growth condition on seedling height difference was also evaluated by using germination, survival rate, germination difference and survival rate difference as covariates. Although the covariate had significant effects ( $p < 0.01$ – $0.05$ ), the decrease in explanation ratio for the ANOVA model used was rather small

(0.01–0.03), and cross, condition and their interaction explained a significant component of variation in observed and expected seedling heights.

Similarly, there were significant correlations between germination and survival in all growth conditions. However, the correlation became weaker in C1100 compared to C1700 and C1300. Germination and survival rate had significant negative correlations with seedling height in C1700 (Table 6). Germination and seedling height showed a significant negative correlation ( $r = -0.62$ ,  $p < 0.01$ ) in Asp/Asp × Hyb trials, likewise observed survival rate and seedling height in C1700 ( $r = -0.66$ ,  $p < 0.001$ ). Survival rate and seedling height showed significant negative correlations in Asp/Hyb × Asp ( $r = -0.64$ – $0.66$ ,  $p < 0.05$ ) and Asp/Hyb × Hyb ( $r = -0.39$ – $0.55$ ,  $p < 0.01$ – $0.05$ ).



**Table 6** Correlations among mean values of germination, survival rate and seedling height of European and F<sub>2</sub>-hybrid aspens raised in two-family trials

Growth condition (d.d.)	Variable	Survival rate			Height		
		<i>r</i>	<i>N</i>	<i>p</i>	<i>r</i>	<i>N</i>	<i>p</i>
1,700	Germination	0.669	77	<0.001	-0.544	77	<0.001
	Survival rate				-0.510	77	<0.001
1,300	Germination	0.919	77	<0.001	-0.066	77	ns
	Survival rate				-0.033	77	ns
1,100	Germination	0.434	78	<0.001	0.207	72	ns
	Survival rate				0.030	72	ns

There were no significant correlations among germination, survival rate or seedling height in either one-family or two-family trials of European aspen or F<sub>2</sub>-hybrids.

## Discussion

### Growth and early survival in one-family trials

Germination rates, survival rates and seedling heights were all highest in the warmest growth environment (C1700), and clearly different from seedlings grown in cooler conditions. Effects of the interaction between cross and condition on survival rate and seedling height differed among European aspen and the various hybrids. F<sub>2</sub>-hybrids had a lower survival rate and greater variation in seedling height in warmer conditions. Given this variation, certain F<sub>2</sub>-hybrids may gain an advantage over European aspen in a warmer climate, such as that predicted by climate modelers (IPCC 2007). Kimball et al. (2008) noticed that the performance of *Penstemon* reciprocal hybrids might be equal or even better than that of the parent species in some environments. The warmest growth condition in our study (C1700) is around 450 d.d warmer than the long-term average and close to the lower end of global warming scenarios without any increase in CO<sub>2</sub> levels in the near future (Jylhä et al. 2004, 2009). Even though there are clear differences among growth conditions, greenhouse seedlings are also sheltered from the effects of wind and diurnal variation in humidity.

The overall mean density after the first growing season was ca. 1,300 seedlings/m<sup>2</sup>. Similar densities of hybrid aspen have been recorded in natural situations when soil and conditions are suitable (H. Aro, pers. comm). Germination seemed to be positively correlated with survival rate in all conditions tested and, along with higher temperature, germination rate was not correlated with seedling height in one-family trials. These results suggest that germination rate may indicate survival probabilities of young seedlings.

However, a warmer climate would also enhance germination rate and lead to high seedling densities after one growing season.

Typically, correlations between survival rate and height growth were not significant even in the best conditions, with the only significant correlation being a positive one between survival rate and height in C1100. A strong negative correlation between survival rate and seedling height in European aspen/hybrid aspen trials indicates that competition had begun at this early stage.

The influence of the hybrid cross was also examined in this study, and our results showed that hybrids backcrossed to European aspen (i.e., Asp × Hyb) had, on average, the highest germination rate, the highest survival rate and the tallest seedlings after one growing season. On the other hand, respective backcrosses with the same hybrid genotype (Hyb × Asp) had the lowest germination and survival rate. The performance of the Asp × Asp and Hyb × Hyb F<sub>2</sub>-hybrids was usually between these two backcross hybrids. Secondary hybrids have been considered less viable than their parental species (Burke and Arnold 2001; Fritz et al. 2006), and growth of F<sub>2</sub>-hybrid poplar has been slower than either of its parental partners (Wu and Stettler 1994). The clear advantage of backcrossing hybrids to European aspen rather than to other hybrids seems to support the local adaptation theory, which suggests that in a backcross, the maternal parent should enhance adaptation to local conditions via her contribution of maternally inherited cytoplasmic genes (Fritsche and Kaltz 2000; Campbell and Waser 2007). Variation in the germination and survival rate was between crosses, families and growing conditions (Table 2), but within-cross variation of F<sub>2</sub>-hybrids was at a similar level in European aspens except for seedling height in C1100, where the variation was considerably higher. The trend seemed to be that in more favorable conditions, there is less variation between European aspens and F<sub>2</sub>-hybrids, although variation among F<sub>2</sub>-hybrids is known to be high (Karim and Hawkins 1999).

## Two-family trials

This study employed a similar method to Adams et al. (1973) and von Euler (1993). Our results showed that (1) interactions varied according to trait (also noted by Adams et al. 1973), condition (Foster et al. 1998) and cross (i.e., genotype; Sakai et al. 1967; Foster et al. 1998). The differences between non-competitive one-family trials and competitive two-family trials were clear (Table 4). Significant differences between one- and two-genotype trials have also been noticed in young *Picea abies* clones (Lundkvist et al. 1992), but differences have not always been apparent in *Pinus sylvestris* experiments (e.g., von Euler 1993).

Results from the two-family trials may be called complementary (i.e., the mixture equals the weighted mean of pure treatments), overyielding (i.e., the mixture exceeds the weighted mean) or underyielding (i.e., the mixture is lower than the weighted mean; Foster et al. 1998). According to our results, all of these responses can be found when the trial consists of not only different genotypes but also different growth conditions. All of these responses have been reported for different species and experiments, suggesting the results are family or clone specific (Foster et al. 1998). Planting site also affected the competition results of Eastern cottonwood clones (Foster et al. 1998).

Conditions and cross influenced the discrepancy between expected and observed results. Germination rate was lower than expected across all conditions. Thus, variation among conditions was rather limited, and the only crosses with complementary results were those including European aspen. The most pronounced decrease (i.e., underyield) in germination rate was in two-family trials when aspens were grown together with Hyb  $\times$  Asp. Differences among crosses and families within the crosses were the most important sources of variation in two-family trials.

Mean survival rate and seedling height of European aspen grown in ambient (1,100 d.d.) and the warmest conditions (1,700 d.d.) were similar to expected values, although conditions in C1700 were more similar to those experienced 3–4° to the south. It seems unlikely that native aspen in southern Finland is not fully adapted to these warmer or more southerly conditions, even though the response to higher temperatures was positive in all Asp and F<sub>2</sub>-hybrid genotypes. Although an overyielding survival rate was apparent in some competitive trials and seedling height in all trial combinations, there was no clear support for the theory that facilitation is the dominant interaction in stressful situations (Pugnaire and Luque 2001; Maestre et al. 2003). Both underyielding and overyielding results were obtained in the best (C1700) and worst (C1100) conditions, respectively. Interactions were most limited in the intermediate condition (C1300), and interactions involving

cross were also dependent on conditions. Thus, genotype/genotype  $\times$  environment interactions exist at least during the early stages of aspen seedling development.

In contrast to a similar field trial of Eastern cottonwood field (Foster et al. 1998), the performance of European aspens or F<sub>2</sub>-hybrids in one genotype trials did not seem to correlate with the observed results in mixed situations. Foster et al. (1998) noticed that the production of mixed clones was often related to clone productivity. Our experiment lasted only a single growing season, which may explain the seemingly conflicting results. However, according to Goldberg and Landa (1991), competitive interactions in plants are positively related to photosynthetic capacity and relative growth rates; thus, they should begin soon after germination and take place during the early stages of seedling growth.

In our experiments, F<sub>2</sub>-hybrids managed considerably well in both one- and two-family trials. In Asp/Asp trials, there were no significant differences between observed and expected values, and the only significant differences with respect to expected values were of lower seedling height in C1300 (Table 4). However, Asp/Hyb  $\times$  Hyb trials yielded a significantly lower germination rate and seedling height than Asp/Asp  $\times$  Hyb trials in C1700. Asp/Hyb  $\times$  Asp had lower germination and survival rates combined with taller seedlings. European aspen mixed with F<sub>2</sub>-backcross with European aspen revealed a moderate reduction in germination, a decrease in survival rate and moderate seedling height. Thus, both the Hyb  $\times$  Asp and Hyb  $\times$  Hyb yielded similar results when ran in trials with Asp. Generally, these differences were more pronounced in the warmest growth environment (C1700).

Substantial gene flow has been reported to occur between native and introduced poplars (Van den Broeck et al. 2004, 2005; Suvanto et al. 2004). Seed production of F<sub>1</sub>- and F<sub>2</sub>-hybrids is at least as high as that of either parent species (Schweitzer et al. 2002). Crosses of European aspen as a maternal partner and hybrid aspen as the paternal partner seem to yield the most productive offspring (Koivuranta et al. 2012). There is also some indication that increased temperatures experienced during pollination and seed maturation may favor hybrid seed production (Koivuranta et al. 2012). Our results tend to support the notion that introgression is a real risk if a sufficient density of mature hybrids exists in or near natural forests. Such situations already or will soon occur in northern Europe where recently over 4,500 ha of hybrid aspen plantations have been established (Holm 2004; Tullus et al. 2012). Moreover, future climate predictions (Jylhä et al. 2004) may enhance seed production of European aspens as well as certain F<sub>2</sub>-hybrids even though under the present conditions, naturally occurring seed-borne seedlings are uncommon (Latva-Karjanmaa et al.



2003; Tullus et al. 2012). The risk of introgression can also be considered from the perspective of Martinsen et al. (2001), in that different hybrids may increase genetic variation and thus hasten the adaptive response of poplar populations to a changing environment.

In conclusion, climate warming may promote the spread of certain hybrid aspen into the natural environment. Our study tested the performance of hybrid aspen in isolation and in competition with other hybrids and parental species in the first growth season. Our results indicate the following: (1) higher temperatures have positive effects on early growth, i.e., germination, survival rate and shoot growth; (2) hybrid aspen backcrossed to European aspen (Asp × Hyb) showed superior performance in germination, survival rate and shoot growth; (3) competition occurred in two-family trials in terms of lower germination and survival rates, especially in the warmest conditions, but not with respect to seedling height. Although different dihybrids were shorter and had lower survival than F<sub>1</sub>-hybrids or aspens, they survived and also grew taller in the warmest environment. We know that crosses between European aspen and hybrid aspen produce viable seed and that increased temperatures can favor seed production (Koivuranta et al. 2012). Thus, together with reasonable growth, survival rate and competitive ability, results of this study support the hypothesis that under natural conditions, gene flow from hybrid aspen plantations into local aspen stands is likely although the problem is rather limited as long as regeneration of aspen by seed will remain a rare event. In the future, warmer and wetter summers may create more favorable conditions, especially for aspen × hybrid aspen dihybrids.

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## References

- Adams WT, Roberds JH, Zobel BJ (1973) Intergenotypic interactions among families of loblolly pine (*Pinus taeda* L.). *Theor Appl Genet* 43:319–322
- Baraloto C, Goldberg DE, Bonal D (2005) Performance trade-offs among tropical tree seedlings in contrasting microhabitats. *Ecology* 86:2461–2472
- Berkowiz AR, Canham CD, Kelly VR (1995) Competition vs. facilitation of tree seedling growth and survival in early successional communities. *Ecology* 76:1156–1168
- Bonan GB (1991) Density effects on the size structure of annual plant populations—an indication of neighborhood competition. *Ann Bot* 68:341–347
- Boyden SB, Reich PB, Puettmann KL, Baker TR (2009) Effects of density and ontogeny on size and growth ranks of three competing tree species. *J Ecol* 97:277–288
- Burke JM, Arnold ML (2001) Genetics and the fitness of hybrids. *Annu Rev Genet* 35:31–52
- Callaway RM, Walker LR (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* 78:1958–1965
- Campbell DR, Waser NM (2007) Evolutionary dynamics of an *Ipomopsis* hybrid zone: confronting models with lifetime fitness data. *Am Nat* 169:298–311
- Canham CD, LePage PT, Coates KD (2004) A neighborhood analysis of canopy tree competition: effects of shading versus crowding. *Can J For Res* 34:778–787
- Coates KD, Canham CD, LePage PT (2009) Above- versus below-ground competitive effects and responses of a guild of temperate tree species. *J Ecol* 97:118–130
- Coomes DA, Grubb PJ (1998) Responses of juvenile trees to above- and below-ground competition in nutrient-starved Amazonian rain forest. *Ecology* 79:768–782
- Eckenwalder JE (1996) Systematics and evolution of *Populus*. In: Stettler RF, Bradshaw HD Jr, Heilman PE, Hinckey TM (eds) *Biology of Populus and its implications for management and conservation*. NRC Research Press, Ottawa, pp 7–32
- Foster GS, Rousseau RJ, Nance WL (1998) Eastern cottonwood clonal mixing study: intergenotypic competition effects. *For Ecol Manage* 112:9–22
- Fritsche F, Kaltz O (2000) In the *Prunella* (Lamiaceae) hybrid zone structured by an environmental gradient? Evidence from reciprocal transplant experiment. *Am J Bot* 87:995–1003
- 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
- Goldberg D, Landa K (1991) Competitive effect and response: hierarchies and correlated traits in the early stages of competition. *J Ecol* 79:1013–1030
- Holm S (2004) Haavanviljely Suomessa ja Virossa. *Metsätieteen aikakauskirja* 1(2004):117–118
- Hynynen J, Viherä-Aarnio A, Kasanen R (2002) Nuorten haapaviljelmien alkukehitys. *Metsätieteen aikakauskirja* 2(2002):89–98
- IPCC (2007) *Climatic change 2007: synthesis report*, summary for policymakers. IPCC Plenary XXVII, Valencia, Spain. [http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4\\_syr\\_spm.pdf](http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr_spm.pdf)
- Jylhä K, Tuomenvirta H, Ruosteenoja K (2004) Climate change projections for Finland during the 21st century. *Boreal Environ Res* 9:127–152
- Jylhä K, Ruosteenoja K, Räisänen J, Venäläinen A, Tuomenvirta H, Ruokolainen L, Saku S, Seitola T (2009) The changing climate in Finland: estimates for adaptation studies. [In Finnish with English abstract and figure and table captions]. ACCLIM project report 2009. Finnish Meteorological Institute Reports 4/2009, p 102
- Kaelke CM, Kruger EL, Reich PB (2001) Trade-offs in seedling survival, growth and physiology among hardwood species of contrasting successional status along a light-availability gradient. *Can J For Res* 31:1602–1616
- 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
- Koivuranta L, Latva-Karjanmaa T, Pulkkinen P (2012) The effect of temperature on seed quality and quantity in crosses between European (*Populus tremula*) and hybrid aspens (*P. tremula* × *P. tremuloides*). *Silva Fenn* 46:17–26
- Korkama T, Fritze H, Pakkanen A, Pennanen T (2007) Interactions between extraradical ectomycorrhizal mycelia, microbes

- associated with the mycelia and growth rate of Norway spruce (*Picea abies*) clones. *New Phytol* 173:798–807
- 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
- Latva-Karjanmaa T, Suvanto L, Leinonen K, Rita H (2003) Emergence and survival of *Populus tremula* seedlings under varying moisture conditions. *Can J For Res* 33:2081–2088
- Lundkvist K, Eriksson G, Norell L (1992) Performance of clonal mixtures and single-clone plots in young *Picea abies* trials. *Scand J For Res* 7:53–62
- Maestre FT, Cortina J, Bautista S, Bellot J (2003) Does *Pinus halepensis* facilitate the establishment of shrubs in Mediterranean semi-arid afforestations? *For Ecol Manage* 176:147–160
- Maestre FT, Valladares F, Reynolds JF (2005) Is the change of plant–plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *J Ecol* 93:748–757
- Martinsen GD, Whitham TG, Turek RJ, Keim P (2001) Hybrid populations selectively filter gene introgression between species. *Evolution* 55:1325–1335
- Packer A, Clay K (2004) Development of negative feedback during successive growth cycles of black cherry. *Proc R Soc Lond* 271:317–324
- Pugnaire FI, Luque MT (2001) Changes in plant interactions along a gradient of environmental stresses. *Oikos* 93:42–49
- 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, p 32
- Sakai K-I, Mukaide H, Tomita K (1967) Intraspecific competition in forest trees. *Silvae Genet* 17:1–5
- Santamaría O, Diez JJ (2005) Fungi in leaves, twigs and stem bark of *Populus tremula* from northern Spain. *For Pathol* 35:95–104
- Schweitzer JA, Martinsen GD, Whitham TG (2002) Cottonwood hybrids gain fitness traits of both parents: a mechanism for their long-term persistence? *Am J Bot* 89:981–990
- Suvanto L, Stenvall N, Vares A, Pulkkinen P (2004) Hybridisaavan geenivirta. *Metsätieteen aikakauskirja* 1:89–97
- Tansley AG (1917) On competition between *Gallium saxatile* L. & *Gallium sylvestre* Poll. on different types of soil. *J Ecol* 67:1047–1064
- Tauer CG (1975) Competition between selected black cottonwood genotypes. *Silvae Genet* 24:44–49
- Tullus A, Rytter R, Tullus T, Weih M, Tullus H (2012) Short-rotation forestry with hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) in northern Europe. *Scand J For Res* 27:10–29
- Van den Broeck A, Storme V, Cottrell J, Boerjan W, Van Bockstaele E, Quataert P, Van Slycken J (2004) Gene flow between cultivated poplars and native black poplar (*Populus nigra* L.): a case study along the river Meuse on the Dutch-Belgian border. *For Ecol Manage* 197:307–310
- Van den Broeck A, Villar M, Van Bockstaele E, Van Slycken J (2005) Natural hybridization between cultivated poplars and their wild relatives: evidence and consequences of native poplar populations. *Ann For Sci* 62:601–613
- von Euler F (1993) Plot design effects on phenotypic variability and dynamics of field competition between six *Pinus sylvestris* L. varieties. *Scand J For Res* 8:163–173
- Weiner J (1984) Asymmetric competition in plant populations. *Tr Ecol Evol* 5:360–364
- Wettstein W (1933) Die Kreuzungsmethode und die Beschreibung von F1 Bastarden bei *Populus*. *Z. Züchtung. A. Pflanzenzüchtung* 18:97–626
- Worrell R (1995) European aspen (*Populus tremula* L.)—a review with particular reference to Scotland. 2. Values, silviculture and utilization. *Forestry* 68:231–243
- Wu R, Stettler RF (1994) Quantitative genetics of growth and development in *Populus*. 1. A 3-generation comparison of tree architecture during the first 2 years of growth. *Theor Appl Genet* 89:1046–1054
- Yrjölä T (2002) Forest management guidelines and practices in Finland, Sweden and Norway. European Forest Institute. Internal Report No. 11
- Yu Q (2001) Selection and propagation of hybrid aspen clones for growth and fibre quality. University of Helsinki, Faculty of Agriculture and Forestry, Department of Applied Biology, Finland, p 41
- Yu Q, Pulkkinen P, Rautio M, Haapanen M, Alén R, Stener LG, Beuker E, Tigerstedt PMA (2001a) Genetic control of wood physicochemical properties, growth, and phenology in hybrid aspen clones. *Can J For Res* 31:1348–1356
- Yu Q, Tigerstedt PMA, Haapanen M (2001b) Growth and phenology of hybrid aspen clones (*Populus tremula* L. × *Populus tremuloides* Michx.). *Silva Fenn* 35:15–25