Epigenetic memory effects in Norway spruce: are they present after the age of two years?

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Abstract

Short-term trials on cultivated soil were planted with families of Norway spruce that had shown epigenetic memory effects in early tests up to age two years. Measurements and assessments were made of phenology traits, tree heights and stem defects until age 16 years in these trials. The memory effects of the temperature conditions during embryo development and seed maturation were confirmed for the timing of bud flush and for start and cessation of shoot elongation at age six years. The mean differences in timing of these events caused by temperature treatments were on average less than two days. They were considerably larger for families with strong effects on terminal bud set at the end of the first growing season. The memory effects did not result in a prolonged shoot growth period, nor did they affect height growth. Interaction effects expressed in adaptive traits between factorial treatments of temperature and daylength during seed production were large in the short-term trial and were still present at age nine years. The results presented demonstrate that strong memory effects observed in early tests may also be expressed in phenology traits for at least the next five growing seasons.

Key words: *Picea abies*, epigenetic memory effects, short-term tests, phenology, height growth.

Introduction

Epigenetic regulation of phenotypic traits in forest tree species caused by the maternal environment has recently attracted considerable attention (Bräutigam et al.2013, Sow et al. 2018). In Norway spruce (*Picea abies (L.) Karst*), many studies have shown that the temperature conditions and photoperiod during the reproductive phase can influence progeny performance, see review by Yakovlev et al. (2012). These memory effects were initially observed after flowering and seed production in seed orchards containing grafts of selected plus trees translocated to localities with warmer climatic conditions. When seedlings from seed lots produced in these orchards were grown in trials with comparable materials from natural stands of the origins of the plus trees, they performed differently for traits measured at the end of the first growth season, such as terminal bud set and frost hardiness in artificial freezing tests (Bjørnstad 1981; Johnsen 1989a). Similar differences in other traits were later observed in trials containing trees of ages up to 17 years from seed (Johnsen 1989b; Skrøppa 1994; Edvardsen et al. 1996; Skrøppa et al. 2007). Traits that are affected include the timing of dehardening and bud burst in the spring, leader shoot growth cessation in the summer, and bud set and cold acclimation in the autumn. These events occur early or late in the growing season in progeny with identical genetic background, depending on, respectively, cool or warm temperatures during female reproduction. Recently, it has been demonstrated that the temperature conditions during seed production in forest stands also influence both terminal bud set at the end of the first growing season and bud flush and growth cessations up to seven years later (Solvin and Steffenrem 2019). A memory effect with similar implications was reported by Gömöry et al. (2015): Norway spruce seedlings that grew the first year at warm conditions consistently flushed later the third growing season than those grown under lower temperatures. Other conifer species for which similar capabilities have been demonstrated are *Pinus contorta* (Wei et al. 2001), the *Picea glauca x engelmanni* complex (Webber et al. 2005) and *Pinus sylvestris* (Bose et al. 2020),

The memory effects of the maternal environment observed in the early tests can be explained by several mechanisms: properties of the seed (cytoplasmatic or somatic), directional selection through the reproductive phase or by epigenetic causes. In Norway spruce, the first mechanism is less likely as an early test trait such as bud set was found to be independent of seed weight (Skrøppa 1988) and the memory effects were still present after plant regeneration through somatic embryogenesis (Kvaalen & Johnsen 2008). The second mechanism can hardly explain the observed results, as discussed by Johnsen et al. (2005b). Epigenetic effects are heritable changes in gene expression that do not results from changes in DNA sequence (Bräutigam et al. 2013) and can be caused by several molecular mechanisms (Yakovlev et al. 2012). In Norway spruce, changes in methylation or chromatin remodelling are considered the more likely mechanisms behind the epigenetic memory and transcriptional changes related to different embryogenesis temperatures have been shown (Yakovlev et al. 2010, 2011). The significant family variation in how strongly the epigenetic memory is displayed may indicate that it is heritable (Yakovlev et al. 2012).

Several of the epigenetic memory effects in Norway spruce were observed in early tests (Johnsen et al 1995, 1996, 2005a, 2005b, Skrøppa et al. 2010) where the effects were expressed on traits measured during the first and second growing season. Later, seedlings from these trials were planted in short-term tests with duration up to age 16 years where measurements were made of phenology traits, tree heights and stem defects. Analyses of data sets with measurements from these trials will provide information about the duration and implications of the memory effects observed in the early tests. This study presents results

from six series of such short-term tests. The main objectives are to investigate whether the epigenetic memory effects in Norway spruce observed at the very early age will be present, will diminish or will disappear, in later growing seasons, and how they influence the variation in phenology traits which are important for adaptation of Norway spruce in the boreal forest.

Materials and methods

In six series of trials with Norway spruce seedlings from seed lots produced under different environmental conditions were tested in early tests from which evidence was established of memory effects related to the environmental conditions (treatments) influencing the maternal parent during the reproductive process (Table 1) (Johnsen et al. 1995; 1996; 2005a; Skrøppa et al. 2010; Yakovlev et al. 2011). The materials from these tests were later planted in short-term trials, most of these on cultivated soil at Hogsmark Experimental Farm, Ås (59°40'N, 85 m asl). The short-term trials were generally planted at one meter spacing in single tree plots randomized in blocks, the number varying from 20 to 40, and some were measured up to age 16 years.

   In all trials bud burst (flushing) was scored in the spring one or several years according to the Krutzsch scale (Krutzsch 1973), in which a high score means early shoot development. Measurements were made of tree heights every third or fourth year and assessments were made of ramicorn branches, occurring with steep branch angles, and double leader(s). The occurrence of 20 mm or longer lammas shoots on the main leader was also assessed. In one of the series, weekly measurements were made of shoot extension during the growth period. All measurements or assessments will be denoted by age or growing season from seed.

   All statistical analyses were made in SAS (SAS Institute 2003). The percentages of trees with lammas shoots, ramicorn branches or double stems were calculated for each family and treatment, and binary observations were transformed to normal scores before statistical analyses were made. Flushing scores were also similarly transformed, however, mean scores for families or treatments are reported in tables or figures. Analyses of variance were made by SAS Proc Mixed or Proc GLM. The general model used for such analyses were

$$Y_{ijkl} = \mu + T_i + F_j + TF_{ij} + B_k + E_{ijkl}$$

where T_i is the fixed effect of treatment i, F_j is the random effect of family j, TF_{ij} is the interaction between treatment i and family j, B_k is the random effect of block k and E_{ijkl} is the random error. In one series with two-factor treatments, the model was extended to main effects of the individual treatments, their interaction and interactions with families.

Table 1. Genetic materials and traits measured in early and short-term tests in six series of trials testing epigenetic response in Norway spruce.

Series	Treatment (environment) during embryogenesis	Trait measured in early tests	Materials in short-term trials	Traits measured in short-term trials and age of measurements reported	
1	Greenhouse (warm) Outdoors seed orchard (cold)	Height Autumn frost hardiness	11 pairs of identical full-sib families	Bud flush Timing of shoot elongation Lammas shoots Ramicorn branches Height	4,7 6 6,9 6,9 9
2	March and May crosses in greenhouse May crosses inside and outside greenhouse	Autumn frost hardiness	4 pairs of identical full-sib families 5 pairs of identical full-sib families	Bud set Bud flush Lammas shoots Height	1 5,9 9 16
3	Long and short days and high and low temperatures in a phytotron	Bud set Bud burst Timing of shoot elongation	3 full-sib families in four treatment combinations	Bud flush Lammas shoots Height	5,9 9 9
4	Greenhouse (warm) Outdoors (cold)	Bud set	13 pairs of identical full-sib families	Bud set Bud flush Lammas shoots Double leaders Ramicorn branches Height	1 6 6,13 6,13 6,13 13
5	Provenance transfer	Bud set	14 open pollinated families of Harz origin 12 open pollinated families of Norwegian origin Harz and Norwegian provenance	Bud flush Lammas growth Height	4,5 9 9
6	Provenance transfer	Bud set	7 open pollinated families of Austrian origin 6 open pollinated families of Norwegian origin Austrian and Norwegian provenance	Bud flush Lammas growth Height	4,5 9 9

Series 1

Identical crosses were performed in 1989 in a greenhouse seed orchard at Biri Nursery and Seed Improvement Centre (60°57'N, 150 m asl). and in an outdoor seed orchard at Huse 61°13'N, 180 m asl), as reported by Johnsen et al. (1995). Pollination took place 17 days earlier in the greenhouse than in the seed orchard. The potted grafts were moved outdoors when the seed cones were no longer receptive. Twelve full-sib family pairs from these crosses were grown in a phytotron and were tested for height and autumn frost hardiness at the end of their first growing season.

Seed lots of 11 of the pairs of full-sib families were germinated in June 1992 in the greenhouse at Hogsmark Experimental Farm, and two-year old seedlings were planted in 1994 in a trial on cultivated soil at Hogsmark and on a forest site at Berg (60°50'N, 270 m asl).

Assessments of bud flush were made at age six years from seed at Berg. At Hogsmark, assessments of bud flush were made at the beginning of growing seasons four and five, shoot extension was measured during seven weeks from May 30 to July 1 in growing season six and with a final shoot measurement on July 17. Lammas growth was assessed at ages six, seven,

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eight and nine years and ramicorn branches and double leaders at ages six and nine years. Height was measured at ages six and nine years.

Shoot elongation curves were fitted for each tree that developed a leader without damage, and estimates were found for the day of growth start, the day of growth cessation and the length of the shoot growth period by the method described by Skrøppa and Magnussen (1993).

Series 2

Pollinations were made in the spring of 1993 inside the heated greenhouse at Biri Nursery on potted grafts of one set of Norway spruce clones both in March and in May (Experiment 3, Johnsen et al. 1996). Another set of clones were pollinated in May both inside and outside the greenhouse. Flower induction treatments were made on all grafts the previous year. They were moved out from the greenhouse on June 11. Pairs of identical full-sib families were produced from both sets of crosses. The three crossing environments represented quite large differences in accumulated heat sums at the early stage of development of the embryo. Seedlings were tested in artificial freezing tests at the end of the first growing season (Johnsen et al. 1996).

Two sets of seed lots, each with five full-sib family pairs with sufficient amounts of seeds left from the 1993 crosses, were germinated in 2002 in the greenhouse at Hogsmark Experimental Farm, and terminal bud set of the seedlings was assessed at the end of the first growing season. A trial with these families was planted in a short-term test at Hogsmark in 2004, and the following measurements and assessment were made in the trial: bud flush growing seasons five (two assessments) and nine, height and lammas growth at ages six, nine and sixteen years.

Series 3

In each of eight phytotron rooms in the Phytotron at the University of Oslo three genetically independent crosses were made on potted grafts of Norway spruce, and mature cones were collected after four months (Johnsen et al. 2005a). Four of the rooms were programmed with northern (66°N) daylengths (long day; LD) and four with southern (52°N) daylengths (short days; SD). The rooms were programmed to high and low temperatures (HT and LT) in a factorial way with the LD and SD treatments. Day and night temperatures and light intensity during mornings and evenings were programmed to coincide with natural periods of light and darkness at the respective latitudes (66°N and 55°N) (Johnsen et al. 2005a).

Seedlings from the three full-sib families from seed production in each of the four environments were grown in early tests in three environments where measurements were made during the second growing season: in the phytotron, in the greenhouse and outdoors in the nursery at Hogsmark. Bud burst was recorded, and shoot extensions was measured weekly until terminal buds were formed.

Seedlings from the three full-sib families in the four combinations of light and temperature treatments were in 2004 planted in a short-term trial on cultivated soil at Hogsmark in single tree plots at 1 m spacing in 21 replicates. In this trial bud flush was assessed on three dates in the spring of growing seasons five and once in season nine. Lammas growth, ramicorn branches and double leaders were assessed at the end of growing season nine and height was measured at age six and nine years.

Series 4

Controlled crosses were made in the spring of 2004 on Norway spruce grafts growing in large pots in the greenhouse at Biri Nursery. A set of grafts were kept inside the greenhouse (warm environment) until the cones were collected and another set of grafts from the same clones were moved outdoor (natural cold environment) where the cones matured (Yakovlev et al. 2011). Thirteen pairs of identical full-sib families from these crosses were germinated in 2005 in the greenhouse at Hogsmark and terminal bud set was recorded twice at the end of the first growing season. In the spring of 2006, seedlings from the 13 family pairs were planted in a short-term test at Hogsmark. In this trial the following assessments and measurements were made: bud flush during the fourth and sixth growing season, height at ages four, six and thirteen years, and lammas growth, ramicorn branches and double leaders at the last two occasions.

Series 5

Cones were collected in 1998 in plantations with Norway spruce at two sites in Trøndelag, Central Norway; Steinkjer (lat. 64°01′N, 320 m asl) and Verdal (lat. 63°50′ N, 120 m asl), from trees of both local Norwegian origin and of Harz, Germany (Skrøppa et al. 2010). The plantation at Steinkjer was a provenance trial planted with seedlings of the same seed lots that were used in the Verdal plantation. Seeds from open-pollinated families from both sites, from a provenance seed lot from Harz (lat. 51°40′ N, 700 m asl) and from the local Steinkjer provenance were germinated and grown in the greenhouse at Hogsmark, and terminal bud set was assessed at the end of the first growing season.

A short-term trial with the seedlings from the early test was planted at Hogsmark in the spring of 2003. The following measurements and assessment were made in the trial: bud flush at the beginning of growing seasons four and five and, height, lammas growth, ramicorn branches and double leaders at the end of growing season nine.

Series 6

Cones were collected in 1998 in Hurdal, southern Norway (lat. 60°20′ N, alt. 450 m) from seven trees Norway spruce trees of Austrian origin (lat. 47° N, alt. 1000 m) and six trees of local southern Norwegian origin. Seeds from the open-pollinated families, a local Norwegian provenance and a provenance from Austria (lat. 47° N, alt. 900 m) were germinated in the greenhouse at Hogsmark, seedlings were cultivated, and terminal bud set was assessed at the end of the first growing season (Skrøppa et al. 2010).

A short-term trial containing the seedlings from the early test was planted at Hogsmark in the spring of 2003. The two trials in Series 5 and 6 were located side by side and the same measurements and assessments were made in both trials.

Results

Series 1

At the end of the first growing season in the early test no significant difference was found between indoor (Biri) and outdoor (Huse) progenies for height. In the freezing tests the progenies from the greenhouse seed orchard were more susceptible to frost damage than their full sibs from the outdoor seed orchard (Johnsen et al. 1995).

The mean values of the 11 families for the traits measured in the short-term test at Hogsmark are shown in Table 2 for the two crossing environment (treatments), and the means of days of initiation and cessation of shoot elongation for each full-sib family pair are shown in Figure 1.

Table 2. Means of families in the Hogsmark trial from seed produced in two environments, Biri greenhouse and Huse seed orchard. The shoot elongation parameters were estimated from measurements made in growing season six. Bud flush is the mean of ages four and seven and lammas growth the mean of assessment made in four years. Days are numbered from May 1.

Crossing environment	Bud flush score	Day of growth start age 6	Day of growth cessation age 6	Length of shoot elongation period	Lammas shoots %	Ramicorn branches age 9 %	Height age 9 cm
Biri – warm	2.2	33.6	62.0	28.4	13.2	5.3	206
Huse – cold	2.6	31.8	60.1	28.3	9.3	4.8	207

Significant differences were present for bud flush scores between treatments and among families (p<0.006) both at Hogsmark and Berg, and no interactions were present between treatment and families. The days of shoot growth initiation and growth cessation showed significant differences (p<0.001) both between treatments and among families, but no treatment x family interactions (p>0.75). The mean values presented in Figure 1 show that the differences in all family pairs varied but were in the same direction for the timing of the shoot elongation period. There were no significant differences between treatments for the length of the shoot elongation period (p=0.84), but differences among families (p=0.0007) and no treatment x family interactions (p=0.74).
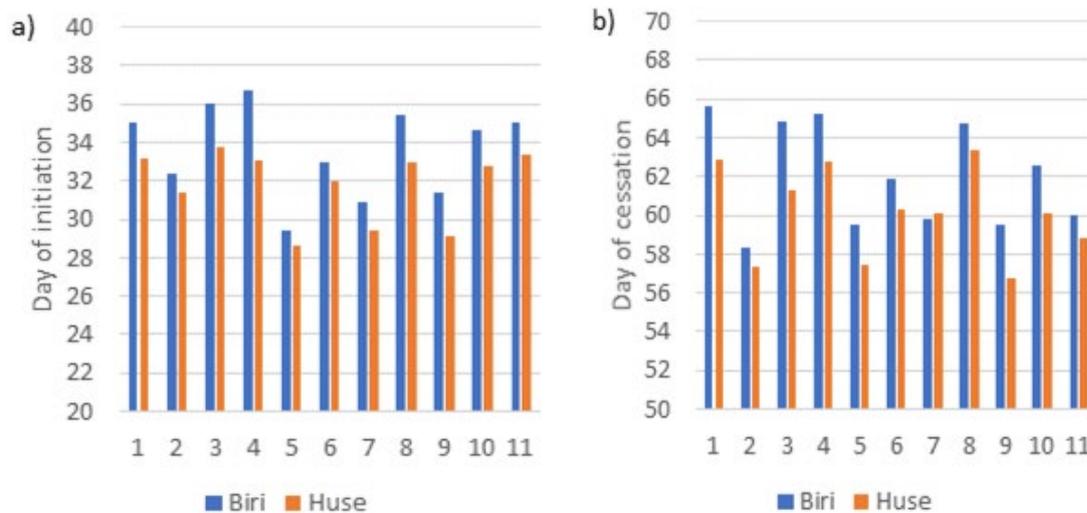
Figure 1. Means of days of initiation (a) and cessation (b) of shoot elongation at age 6 years for 11 pairs of full-sib families from seeds produced at Biri and Huse. Days are numbered from May 1.

No significant differences were present between treatments for height at ages six and nine years, but there were significant differences between families (p<0.0001) and no treatment x family interactions. The difference in lammas growth percentages between Biri and Huse was not significant, but the Biri families had 3 to 5 percent higher level of lammas growth in all four years.

These results show that trees from seeds produced in the warm environment in Biri have on average a delayed start of shoot elongation and delayed growth cessation of 1.8 to 1.9 days compared with their full-sibs from the outdoor Huse seed orchard, they have a slightly higher frequency of trees with lammas growth. The length of the shoot elongation period was similar for the two treatments and so was total tree height at age nine years.

Series 2

The crossing environment significantly affected the frost hardiness of the progenies in the early tests; the seedlings from the early spring crosses, from seeds produced under higher accumulated temperature sum from early embryo development heat sums, were less hardy than their full-sibs from the May crosses (Johnsen et al. 1996). Similar results, but with smaller differences, were obtained from the other set of pairs of full-sib families.

At the end of the first growing season in the nursery test significant differences (p<0.01) were present between the two environments for terminal bud set of the family pairs both from crosses made inside the greenhouse in March (high heat sum) and in May (lower heat sum) and inside and outside greenhouse crosses in May. Families from seed produced under high temperature sum had the latest bud set (Table 3). In the short-term test bud flush was assessed twice (June 1 and June 7) in the fifth growing season with mean score values 4.0 and 5.3, which tells that 0.2 unit corresponds to one day. At the beginning of growing season five this was the difference between the families in the early and late inside crosses (p=0.05), while the difference between the late inside and late outdoor crosses corresponds to three days (p=0.03). In both cases the families from high temperature during embryo development were the latest to flush. However, at age nine years there were no differences

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between the environments in the timing of flushing in any of the groups of crosses. No differences were present for lammas shoots, ramicorn branches, double leaders, or height at different ages.

Table 3. Means of four family pairs from crosses made inside the greenhouse in March and in May and five family pairs from crosses made inside and outside the greenhouse in May.

Environment	Bud set age 1 %	Bud flush score age 5	Bud flush score age 9	Lammas shoots age 9 %	Height age 16 cm
March crosses	45.3	3.8	4.3	28.3	710
May crosses	75.5	4.0	4.3	30.7	697
Inside greenhouse	45.0	3.7	4.4	23.2	699
Outside greenhouse	55.6	4.3	4.5	16.4	707

Series 3

During the second growth season in the early test an interactive memory effect of day length and temperature was expressed in the traits measured (Johnsen et al 2005a). Progenies from high temperature and short days (HT-SD), and from low temperatures and long days (LT-LD), initiated shoot growth later in spring, ceased growth later in summer, grew taller and were less frost hardy in the autumn than their full siblings from low temperatures and short days, and from high temperatures and long days.

Bud flush scores were in growing season five made on May 23, June 1 and June 7, with means scores 2.1, 3.7 and 5.6. Three years later the mean score was 3.8 on May 31. In the analysis of variance made on the transformed values there were not for any of the scoring dates significant differences between the temperature treatments (high and low) nor between the daylength treatments (short and long) (p>0.10). However, the temperature x daylength interaction was strongly significant (p<0.0001). No family times treatment interactions were present.

The means of the three families for the four combinations of temperature and day length are shown for bud flush, lammas shoots and height in Figure 2. It shows that similar epigenetic effects with interaction between day length and temperature that were found at the early age, are still present for the timing of bud flush. Progenies from HT-SD and LT-LD flush significantly later than those from LT-SD and HT-LD. The difference in the timing of bud flush between the earliest and latest treatment combination that was 3-4 days at age two years was as large as 7-8 days at the beginning of growing season nine.

For lammas shoots the only significant effect was the temperature x daylength interaction (p=0.03). For heights at age nine years there were no significant main nor interaction effects (p>0.28). However, the mean heights of the treatments HT-SD and LT-LD deviated in the same direction from the total, as did the mean heights of treatments HT-LD and LT-SD. The treatments with the latest bud flush produced the tallest trees with the lowest frequency of lammas shoots.
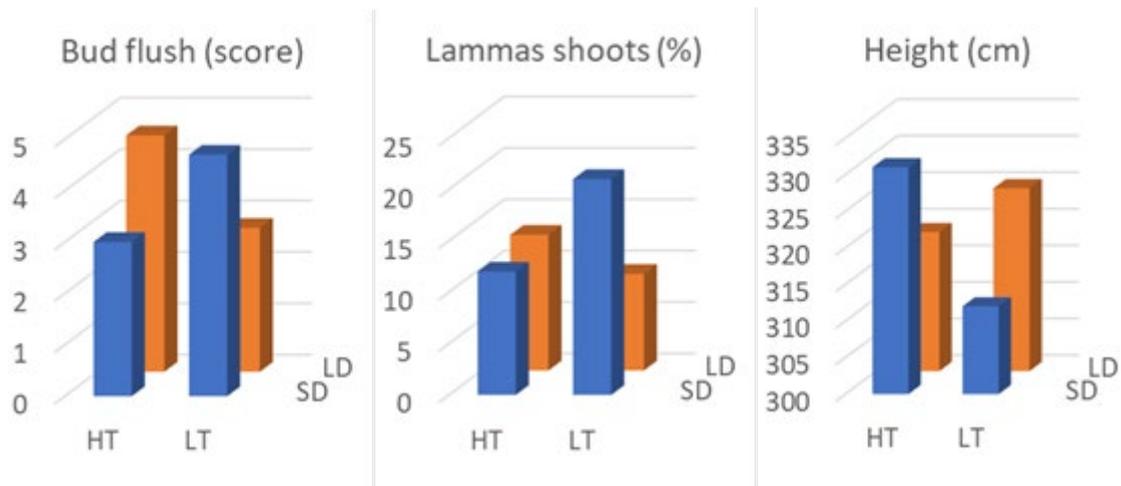
Figure 2. Mean scores for bud flush, lammas growth percentages and tree heights at age nine years for the combinations of high and low temperature (HT, LT) and day length (SD, LD) during seed production in the phytotron.

Series 4

The seedlings from seed produced in the warm environment in the greenhouse had a considerably later bud set (p<0.001) than their full sibs from the cooler outdoor environment (Table 4). The seedlings from the warm treatment had on average a bud flush approximately one day later than those from the cold treatment in growing season six (p=0.03). The other traits had quite similar and not significantly different means for the two treatments.

Among families, large and highly significant variation (p<0.0001) was present for most traits; range 2.9 to 6.5 for bud flush (age 6), 482 to 594 cm for height and 7.9 to 44.2 % for lammas shoots (age 13), calculated as means of the two treatments.

Table 4. Means of families from the two treatments (indoor/outdoor or warm/cold seed production). Lammas shoots, ramicorn branches and double leaders are the means of assessments made at ages six and thirteen.

Treatment	Bud set age 1	Bud flush score age 6	Lammas shoots	Ramicorn branches	Double leaders	Height age 13 cm
	%		%	%	%	
Warm	43.0	3.5	23.2	20.2	8.6	532
Cold	70.0	3.7	24.4	23.2	5.9	526

Table 5 presents results from six families that showed a range of variation from 3 to 36 % in bud set percentage between the two treatments (Yakovlev et al. 2011) at the end of the first growing season. The two families with the largest difference in bud set also had the largest difference in bud flush scores at the beginning of growing season six (Table 5). For families 5 and 6 this corresponds to three and five days, respectively. Similar differences between the warm and cold environment were not present for height and the other assessment traits.

Table 5. Differences in bud set percentage score and family means for bud flush score for six families with varying differences in bud set percentage.

Trait Environment	Family 1	Family 2	Family 3	Family 4	Family 5	Family 6
Bud set difference %	3	7	15	24	31	36
Bud flush score age 6:						
Warm - greenhouse	4.4	2.7	5.0	1.9	2.6	2.4
Cold – outside	4.6	3.2	4.7	2.2	3.2	3.3

Series 5

In the early test, the seedlings from the families of Harz mother trees producing seeds in Norway had on average a bud set more like the families of local Norwegian origin producing seeds at the same site than did the provenance of the same Central European origin (Skrøppa et al. 2010). The families from seed lots produced at Steinkjer, in a more severe climate, set buds earlier than those from Verdal.

In the short-term trial the Verdal families had an earlier bud flush than the families of Harz origin from seed produced at Verdal. No other differences were present for bud flush (Table 6). The Harz families had lower percentages of trees with lammas growth than the Norwegian families from both seed locations. A higher frequency of trees with lammas growth was present on families from Verdal (altitude 120 m) compared with families from Steinkjer (altitude 350 m). The trees of the Harz families were significantly taller (p<0.0001) than those of Norwegian origin from both sites and performed like the Harz provenance. No differences were present for the frequencies of trees with ramicorn branches and double leaders (not presented).

Table 6. Means of Harz and Norwegian families produced at Steinkjer and Verdal and control provenance Harz for bud flush, lammas growth and height. Bud flush is the mean score of growing seasons four and five.

Origin of families and seed location	Bud flush score age 4 and 5	Lammas growth age 9 %	Height age 9 cm
Harz Steinkjer	3.8	10.8	278
Norway Steinkjer	3.8	1.0	200
Harz Verdal	3.8	20.4	294
Norway Verdal	4.3	5.0	249
Harz provenance	3.6	3.2	297
Steinkjer provenance	3.8	0.0	183

Series 6

The Austrian families producing their seeds in South Norway had on average a bud set percentage like that of the Norwegian control provenance from altitude 400 m, considerably earlier than the seedlings of the Austrian provenance (Skrøppa et al. 2010).

The trees of the Austrian families had an earlier bud flush than the trees of the Austrian provenance (p=0.002) and they were not significantly different (p=0.07) from the Norwegian families from the same locality (Table 7). The frequencies of lammas shoots for

the two types of families were similar and both had considerably lower lammas shoot frequencies than the Austrian provenance. The Austrian families were on average slightly taller than the Norwegian families and had lower mean height than the Austrian provenance (p=0.05). No differences were found for the frequencies of trees with ramicorn branches and double leaders (not shown).

Table 7. Means of Austrian and Norwegian families produced at Hurdal and control provenance from altitude 900 m, Austria, and 400 m, Norway. Bud flush is the mean score of growing seasons four and five.

Origin of families and seed location	Bud flush score age 4 and 5	Lammas growth age 9 %	Height age 9 cm
Austria Hurdal	4.4	2.0	247
Norwegian Hurdal	4.1	1.2	231
Austria provenance	3.7	37.0	270
Norwegian provenance	3.5	0	242

Discussion

Some of the earlier studies showing epigenetic memory effects in Norway spruce demonstrated such effects both in early tests and at a later age; for instance, Johnsen (1989a, 1989b) with families up to age seven years from seed; Skrøppa et al. (2007) with both families and seed orchard seed lots up to age 12 years; Solvin and Steffenrem (2019) with provenances up to age eight years. However, none of these studies performed trials with genetic materials produced under controlled climatic conditions during reproduction, and only Johnsen (1989b) followed the performance of individual families from the early to short-term tests.

The results from Series 1 show that the memory effects expressed by frost hardiness at the end of the first growing season were expressed also for the timing of the shoot elongation period at age six years. This effect was clearly expressed by all tested families. However, the average difference between the two treatments in the timing of the shoot elongation was shorter than two days. In Series 2, the less autumn hardy seedlings from the warm maternal environment with a late bud set had in growing season five a slightly later bud flush than those from the cold environment. However, this effect was not present at age nine years. In Series 4, the large difference between the warm and cold treatment in bud set the first growing season was followed by a small average difference of approximately one day in bud flush in the beginning of growing season six. For individual families, those with a large difference in bud set also showed large differences in bud flush at age six years. The results from Series 4 shown in Table 5 show that families that have a strong response in bud set percentage at the end of the first growing season also may have the largest differences in bud flush several years later. Therefore, a differential response of the maternal parents, expressed in the next generation, is most likely. The same was shown by Dewan et al (2018) in full-sib families with of European black poplar from seeds produced under warm and cold conditions. They observed that the seedlings from the warmer maternal environment displayed the latest bud burst and earliest bud set, but only in one out of the three crossings made.

Except for the results from Series 6 and some indications in Series 3, no clear memory effects were found for tree heights. Such effects were demonstrated by Johnsen (1989b) for height of families at age seven years and by Solvin (2020) who found a 2 % height increase at age four years per each 100 degree-days increase in accumulated temperature sum until seed maturation, based on seed lots of the same provenances harvested different years in natural stands. Johnsen (1989b) also found memory effects causing higher frequencies of lammas growth. Parts of the materials in these studies were produced after open pollination in natural stands and more environmental factors may contribute to the differences found.

The results of the current study show that the timing and the duration of the shoot elongation period are differently affected by the temperature conditions during seed production. This is clearly demonstrated in Series 1 in which all families showed effects both for the day of growth start and the day of growth cessation. The length of the shoot elongation period, however, was the same within each family pair regardless of seed maturation either in the warm or cold environment. This may explain why no, or small effects, were found for tree heights. These results correspond with those reported by Solvin and Steffenrem (2019) and Solvin (2020) who found that temperature sums during seed maturation affected height only at very young age. They stated that possible reasons could be the changing growth pattern from free growth to predetermined growth during the early years. Other causes could be that the epigenetic modifications are temporal and are removed gradually, or that environmental factors become more important for height growth at later ages.

The seedlings from the two-factor seed production experiment with two levels of temperature and day length (Series 3) showed even stronger interactions between the treatments for bud flush at age nine years as they did in the same directions for the phenology traits and frost hardiness in the early tests (Johnsen et al. 2005a). Some interaction was also present for the frequency of lammas growth but were not significant for tree heights. This interaction and the non-additive effect of temperature in long days is hard to explain, as discussed by Johnsen et al. (2005a).

Implications of seed production in the northern environment on mother trees that had been transferred from Central Europe were investigated in Series 5 and 6 for traits measured during growing seasons four and five. In the first series, no specific differences were observed for bud flush, which was to be expected since Harz provenances generally have a bud flush like that of Norwegian provenances (Skrøppa unpublished). The Harz families had a higher frequency of trees with lammas growth than those of local origin and they had heights like those of the Harz provenance. Thus, the effects observed for bud set in the early test by Skrøppa et al. (2010) was not confirmed for other traits for this material at ages four and five years. In the other series, the off-spring from Austrian mother trees performed more like the families from seed of the local Norwegian parents both for bud flush, lammas growth and tree heights. Thus, the memory effects found for terminal bud set in the early test were confirmed for this material.

The memory effects observed in the early tests were expressed in phenology traits for at least the next five growing seasons, but with different responses among progenies from the maternal parents. The timing of the shoot growth period at the young age is important for survival and damage of Norway spruce seedlings when exposed to early summer frost (Hannerz et al. 1999). At exposed sites the late bud flush will be beneficial for offspring from maternal parents with large memory effects. The effects will also have implications under climate change conditions since spruce seedlings from seeds produced in a warmer climate with higher temperatures during seed maturation will have a delayed phenology with later bud flush. This may counteract the effects of climate warming which are expected to advance

bud flush in the future (Langvall 2011). Implications are also present for Norway spruce breeding and deployment of reproductive materials from seed years with differences in temperature conditions during seed maturation (Yakovlev et al. 2012, Solvin and Steffenrem 2019).

The molecular mechanism behind the memory effects has been explored by Yakovlev et al. (2011) who did transcriptional analyses of seedlings from the six family pairs tested in Series 4. In subtracted cDNA libraries significant differences in transcriptomes were found between the warm and cold reproduction environment. Eight ESTs were identified showing differential expression in progeny from the two environments which were correlated with the family differences in terminal bud set. The greatest difference in transcription level was found in the families with the largest difference in terminal bud set. This shows the genetic basis for the memory effects corresponds with the differential responses of the maternal parents observed in phenotypic traits.

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