PUBLIZIERBARER ENDBERICHT

A) Projektdaten

Kurztitel:	AdaptTree
Langtitel:	Bedeutung epigenetischer Variation für die natürliche Anpassung von Bäumen an den Klimawandel
Programm inkl. Jahr:	ACRP 2. Ausschreibung
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Schlagwörter:	forestry; forest seed production; epigenetic; forest tree nursery; seed orchard; forest genetics; *Picea abies*; *Larix decidua*, *Pinus sylvestris*; adaptation
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Projektübersicht

1 Kurzfassung

Die natürliche Anpassungsfähigkeit von Bäumen an sich ändernde Klimabedingungen erfolgt langfristig über genetische Prozesse und die natürliche Migration von Baumarten. Allerdings ging man bisher davon aus, dass die rasch voranschreitenden Veränderungen des Klimawandels innerhalb von ein bis zwei Baumgenerationen eine natürliche Anpassung nahezu unmöglich machen. Neuesten Studien zeigen, dass die Genexpression nicht ausschließlich vom genetischen Code selbst, sondern auch von Umwelt- bzw. Klimafaktoren gesteuert wird. Zudem können diese Steuerungsmechanismen vererbt werden und erlauben eine vergleichsweise rasche Anpassung von einer Generation zur Nächsten. Der Bedeutung dieser als epigenetische Variation bezeichneten Steuerungsmechanismen für die natürliche Anpassung von Bäumen an den Klimawandel widmete sich das Projekt AdaptTree.

Ziel von AdaptTree war, den Einfluss natürlicher Schwankungen der Klimabedingungen zum Zeitpunkt der Baumblüte und Samenreifung auf die adaptiven Eigenschaften der jungen Bäume und deren Anpassung an warme/trockene Klimabedingungen zu untersuchen. Für diese Fragestellung wurde Forstsaatgut der wichtigsten heimischen Nadelbaumarten Fichte (*Picea abies*), Weißkiefer (*Pinus sylvestris*) und Lärche (*Larix decidua*) aus unterschiedlichen Reifejahren aber von denselben Erntebeständen getestet. Die Reifejahre jedes Erntebestandes unterschieden sich signifikant in den Wetterbedingungen zur Blüte und Samenreifung. Am BFW wurde dieses Saatgut 2011 in einem Forstgartenversuch mit zwei unterschiedlichen Niederschlagsvarianten (Variante 1: 100% Niederschlag Wien-Mariabrunn; Variante 2: 50% Niederschlag Wien-Mariabrunn) angebaut und die Sämlinge bis 2013 betreut. Während des laufenden Versuches wurden zahlreiche Merkmale aufgenommen (Keimverlauf, Austrieb und Knospenabschluss) und ein- und zweijährige Sämlinge für quantitative Messungen von Wuchsparametern (Größe, Gewicht, Wurzelmasse, etc.) entnommen. Im dritten Untersuchungsjahr wurden zusätzliche Untersuchungen zur Trockenstresstoleranz und Frostempfindlichkeit durchgeführt. Für die statistische Auswertung kamen parametrische und nicht-parametrische Vergleiche zwischen den Reifejahren der verschiedenen Herkünfte zum Einsatz. Da sich die in die Kategorien „warm-trocken" und „kalt-feucht" eingeteilten Reifejahre zwischen den einzelnen Herkünften aber stark in den Klimabedingungen unterschieden, wurden zusätzlich allgemeine lineare Modelle eingesetzt, bei der die Klimabedingungen der Saatgutjahre als kontinuierliche Kovariablen verwendet wurden.

Die Projektergebnisse bestätigen die zentrale Hypothese und zeigen, dass die Klimabedingungen zur Bestäubung bzw. Samenreifung einen signifikanten Einfluss auf die phänotypischen Eigenschaften und die Vitalität der jungen Bäume haben. Dabei wurden die größten Effekte für typische Produktivitätsparameter (z.B. Sämlingsgewicht oder Höhe) gefunden. Die Klimabedingungen zur Samenreifung beeinflussten aber auch diejenigen Eigenschaften, die für das Überleben bei Wassermangel wichtig sind (z.B. der Anteil der Wurzellänge). Bei allen drei Baumarten wuchsen aus Samen, die in warm-trockenen Jahren gereift sind, kleinere und leichtere Pflanzen. In der Variante mit 100% Niederschlag zeigten diese Sämlinge einen größeren Wurzelanteil (für Fichte und Kiefer). Zudem fanden sich signifikante Unterschiede zwischen den beiden Niederschlagsvarianten: Sämlinge in der 100% - Variante waren größer und schwerer und hatten einen geringeren Wurzelanteil. Die Analyse der Sämlingseigenschaften mit linearen Modellen bestätigte den signifikanten Einfluss der Klimavariablen im Reifejahr auf die Eigenschaften der jungen Bäume. Zudem wurden signifikante Interaktionen zwischen den Behandlungsvarianten und dem Klima des Reifejahres beobachtet. Dabei zeigte sich, dass Sämlinge, deren Samen aus warm-trockenen Jahren stammen, eine geringere phänotypische Plastizität aufweisen als Sämlinge, deren Samen in kühl-feuchteren Jahren gereift sind. Für einige Wuchsparameter war die Plastizität der Sämlinge aus kühl-feuchten Jahren bis zu 10-mal höher als die aus warm-trockenen Jahren. Zusätzlich zur Analyse der quantitativen Merkmale bestätigten die beiden Trockenstressexperimente die Bedeutung der epigenetischen Anpassung. In beiden Experimenten wurden signifikante Unterschiede in der Trockenreaktion zwischen den Reifejahren der jeweiligen Herkünfte gefunden.

Alle vorliegenden Ergebnisse zeigen eindeutig, dass die klimatischen Bedingungen während der Baumblüte und Samenreifung eine signifikanten Einfluss auf phänotypische und physiologische Eigenschaften der jungen Bäume haben, und Sämlinge die aus Saatgut aus warm-trockenen Jahren aufgewachsen sind, eine „Vor"-Anpassung an potentielle Trockenstressbedingungen aufweisen. Allerdings weisen derartige Sämlinge auch eine geringere Produktivität und Plastizität aus, so dass diese nicht uneingeschränkt für alle Anbauregionen empfohlen werden können.

Schlussendlich zeigen die Untersuchungen, dass die klimatische Variation zwischen Reifejahren die potentielle klimatische Nische von Baumpopulationen erweitern und damit deren Überleben sichern kann. Da die so erworbenen Eigenschaften an die nächste Generation weitergeben werden, leistet sie einen wichtigen Beitrag zur Anpassung von Baumpopulationen an den Klimawandel. In der hier vorgelegten Studie konnte nur Saatgut, das in der letzten Dekade produziert wurde miteinander verglichen werden. Daher sind die klimatischen Unterschiede zwischen den Reifejahren vergleichsweise gering. Zeitreihen von Klimamessstationen belegen, dass die natürliche Klimavariation zwischen Reifejahren weitaus größer ist und diese im Klimawandel vermutlich sogar zunehmen wird. Das bedeutet, dass über einen längeren Zeitraum betrachtet auch größere phänotypische Unterschiede zwischen den Nachkommen derselben Saatguterntebeständen zu erwarten sind. Die hier vorgelegte Pilotstudie zur epigenetischen Anpassung von Bäumen im Klimawandel zeigt deutlich, welche Potentiale derartige natürliche Prozesse im Anpassungsprozess von Wäldern spielen und eröffnet neue Möglichkeiten der Saatguterzeugung und des Saatgutmanagement. Aufgrund der vergleichsweise geringen Anzahl geprüfter Herkünfte und Reifejahrbedingungen ist die Studie allerdings nicht geeignet, spezifische Modelle zum Einfluss des Reifejahrklimas auf das Überleben und die Produktivität von Waldbaumarten abzuleiten. Für die Entwicklung von konkreten Anpassungsoptionen wären solche Modelle wünschenswert und sollten daher in zukünftigen Forschungsprojekten bearbeitet werden.

2 Executive Summary

Climate conditions are among the most important determinants of tree species' distribution ranges and thus climate change is considered to have severe effects on the forests' crucial protective, ecological, economical and social functions. Tree growth and vitality strongly depend on the genetic variation and local adaptations of present populations to past and current climate conditions. Recent studies also provide evidence for environmental effects on the expression of various adaptive traits mainly through epigenetic imprinting. The present study aimed at testing for effects of interannual climate variation on the quantitative variation of seedling traits related to the growth performance and vitality under environmental stress conditions.

The natural variation of climate conditions during pollination and seed development throughout different seed maturation years of populations from Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and European larch (*Larix decidua*) represent the environmental trigger that is hypothesized to have affected the epigenetic imprinting of the developing seeds. In a two year nursery trial with two different precipitation treatments various growth traits were characterised (e.g. shoot dry weight, root dry weight, total weight, height, stem diameter, root length) together with the phenological behaviour (e.g. timing of bud burst, growth cessation, bud set) and specific tests for the resistance to drought and frost. The final analysis of these traits will help to estimate the impact of weather conditions during pollination and seed development on rapid adaptation of tree populations to future climates. In total, 17 different traits were measured on one- and two year old seedlings. For the statistical analysis, independent T-tests as well as general linear models were employed to test for differences between maturation years, treatments and provenances. In addition, the effect of the seed maturation year climate on the phenotypic plasticity of the seedlings was quantified.

The analysis showed that the climate conditions of the maturation year have significant effects on seedling growth and vitality. The strongest effects were found for typical growth traits, i.e. seedling weight, length or root collar diameter. However, also traits which are considered important for the survival under water stress conditions – mainly root characters – showed significant differences. For all three species, seeds that originate from hot-dry years were found to result in smaller and lighter seedlings. When planted within the full precipitation treatment also a

higher ratio of the root length to the total seedling length was found for these species. Besides the differences between the maturation years, the two precipitation treatments also resulted in strong differences among the seedlings: here, seedlings in the full precipitation treatment were found to be larger and heavier, but were also less slender and the ratio of the root length to the total length was smaller. Although these treatment effects were found for both groups of maturation years, also significant interactions between the treatment and the climate of the maturation years were found, suggesting differences in the plastic response of the seedlings to the treatment conditions. A more detailed analysis of phenotypic plasticity revealed that in Norway spruce and Scots pine, seeds that originate from hot-dry years have significantly less plasticity than seedlings from cold-humid years. For single traits, plasticity can be up to 10 times higher for seedlings from cold-humid years. In addition to the quantitative analysis, two drought experiments provide further evidence for epigenetic adaptation. In both experiments, significant differences were found between the maturation years for some provenances. Also, differences in chlorophyll fluorescence, a measure of the inhibition of the photosystem II by severe plant stress, were found.

Overall, the project provides strong evidence for effects of the maturation year climate on the expression of phenotypic and physiological seedling traits: seeds matured in hot-dry years seem to be preadapted to potential water stress conditions. This preadaptation has positive effects for the survival within stressful environments, but also negative effects for growth potential within more optimal growing conditions.

The analysis suggests that the pollination/maturation climate is able to extend the climatic amplitude of tree populations across generations and thus might shape the next tree generation towards better resistance to warmer climate conditions. Within the present study seeds from about the last decade could be tested and thus the tested seeds originate from a limited number of maturation years and a limited range of potential maturation climates. In the long-term – in particular due to climate change – a much higher variation in maturation climate can be expected and therefore also higher phenotypic variations of tree seedlings and the developing trees. The results of the present project call for a stronger consideration of the maturation year climate within ecological and genetic studies on local adaptation of tree species. Further studies should also aim at weighting effects of genetic vs. epigenetic variation on the adaptive performance of forest trees. Due to the restricted numbers of provenances and maturation years in the present study it was not possible to develop predictive models between the maturation year climate and the adaptive performance of trees. Thus, a repeated analysis with more provenances/maturation years of each species would be required to link the maturation climate with the adaptive characters of trees and thus to develop recommendations for seed planting regions. Such links could also be established by reanalysis of available datasets using the climate conditions of the maturation years as covariables.

3 Hintergrund und Zielsetzung

Predicted climate change scenarios will affect the ecological conditions of forests with severe effects on the forests' crucial protective, ecological, economical and social functions. To counteract loss of forest functions, the regeneration strategy (including artificial regeneration with material adapted to the projected climatic conditions) and changes in nursery and forest tree breeding have been identified as highly important within the current report of the European Forest Institute to the European commission (EFI 2008). Tree species and their characteristics are subject to genetic variation and evolutionary change. The expressions of traits that are important for the survival within given environmental and climatic conditions are often genetically controlled. Within the last decade, it has clearly been shown, that the environmental conditions during pollination, zygotic embryogenesis and seed development have major effects on the trait expression of the next tree generation. Such effects are described as epigenetic patterns, because they cannot be explained by changes in DNA sequences. However, although it is widely accepted that epigenetic phenomena are heritable and explain a significant part of quantitative trait variation within plants (including tree species), it has rarely been tested if the climatic variation *in situ*, i.e. within a populations natural environment affect epigenetic patterns and thus the adaptive performance of the growing offspring. If we are able to demonstrate such a climatic adjustment of trees within an *in situ*

experiment, this would allow the development of new forest adaptation measures. Adaptive genetic or epigenetic variation can be identified experimentally via long-term common garden experiments in strongly differing climates (e.g. Rehfeldt et al. 2001) or by seedling tests in controlled environments, where traits can be measured with little environmental error. Seedling tests measure the performance of trees only at the juvenile stage. However, within the seedling stage intraspecific competition in natural populations is highest and thus natural selection is strongest. Therefore, the adaptive performance of seedlings is thought to contribute significantly to the genetic variation of the following generations. The objectives of the present projects were:

- Characterisation of epigenetic effects in seed harvests that were produced at the same seed stands or seed orchards, but under strongly different climatic conditions at the time of pollination and seed maturation within the frame of a nursery experiment for three native tree species: Norway spruce, European larch, and Scots pine.
- To test for the first time, if pollination/seed maturation under a warm and dry environment preconditions the offspring to a better resistance against stress factors associated with high temperatures/low precipitation (e.g. drought)
- To correlate the measured seedling traits with the environmental conditions during pollination and seed maturation
- To compare the extent of epigenetic behaviour of different coniferous tree species
- To calculate the phenotypic response of the seed lots to two different nursery treatments (A – water deficit, B- sufficient water)
- To develop potential management measures in order to utilize epigenetic processes for faster adaptation to climate change

4 Projektinhalt und Ergebnisse

Project structure

The project is structured in three Workpackages: WP1: Preparatory phase; WP2: Experiment & Trait analysis; and WP3: Data analysis and Project reporting.
The aim of WP 1 was the establishment of the nursery trial with two different irrigation treatments and the final selection of seed provenances maturated at least within two contrasting climate conditions. Within the central WP2 a nursery trial with two different treatments (A-water deficit treatment; B-optimal water treatment) has been established and maintained for two vegetation periods. On the raised seedlings, a variety of growth traits (e.g. shoot dry weight, root dry weight, total weight, height, stem diameter, root length) and the phenological behaviour (e.g. timing of bud burst, growth cessation, bud set) were determined. WP3 aimed at the statistical analysis of the measured traits in order to understand the effects of interannual variation in weather conditions during pollination and seed maturation on the trait expression.

WP 1: Preparatory phase

The database of the seed storage facilities of the Department of Forest Genetics at the BFW was screened for provenances that were harvested at least two times within the last ten years. This information were merged with climate data of neighbouring ZAMG climate stations in order to identify seed provenances that were harvested both in years with higher temperatures and lower precipitation from April to September and in years with lower temperatures and higher precipitation during these pollination and seed development period of Norway spruce (*Picea abies*), European larch (*Larix decidua*) and Scots pine (*Pinus sylvestris*). 10 provenances were found to be suitable for the experiment, of which for 9 provenances seeds were available from two maturation years, and for one provenance seeds were available from 3 maturation years. This resulted in 21 seed lots to be tested (Table 1, Fig. 2).

Table 1: List of tested provenances and maturation years. The different colors mark the contrasting weather conditions in the maturation years: hot-dry years are orange and cold-humid years are green. (TKG: 1000-seed weight)

Seed lot	Species	Register number	Seed year	TKG
1	Norway spruce	Fi 102(4.1/tm:600-800m)	2003	8,03g
2	Norway spruce	Fi 102(4.1/tm:600-800m)	2009	6,68g
3	Norway spruce	Fi 28(9.2/mm:750-1000m)	2003	9,45g
4	Norway spruce	Fi 28(9.2/mm:750-1000m)	2009	9,03g
5	Norway spruce	Fi 8(9.2/tm:500-750m)	2003	8,83g
6	Norway spruce	Fi 8(9.2/tm:500-750m)	2006	7,13g
7*	European larch	Lä P3(4.2/sm-tm)	2001	7,63g
8*	European larch	Lä P3(4.2/sm-tm)	2006	5,83g
9	European larch	Lä P6(4.1, 4.2/tm)	2000	6,98g
10	European larch	Lä P6(4.1, 4.2/tm)	2006	6,15g
11	European larch	Lä P7(4.2/sm)	2000	5,78g
12	European larch	Lä P7(4.2/sm)	2006	6,00g
13	European larch	Lä P11(8.1,9.1,9.2/sm)	2003	6,27g
14	European larch	Lä P11(8.1,9.1,9.2/sm)	2006	5,95g
15	European larch	Lä P11(8.1,9.1,9.2/sm)	2009	6,15g
16	Scots pine	W.Ki P4(2.1,4.1,4.2/mm)	2000	7,60g
17	Scots pine	W.Ki P4(2.1,4.1,4.2/mm)	2008	7,85g
18	Scots pine	W.Kie P5(4.2,5.1/tm)	2000	8,23g
19	Scots pine	W.Kie P5(4.2,5.1/tm)	2002	8,35g
20	Scots pine	W.Kie P6(9.2/tm)	2000	8,30g
21	Scots pine	W.Kie P6(9.2/tm)	2006	7,13g

* - the two maturation years of this provenance were reclassified according to the improved climate data (for details see 2.4.4.3.)

WP 2: Experiment & Trait analysis

To measure the adaptive performance of the seed lots, a nursery experiment was installed which included two different precipitation treatments: one with 100% precipitation and the second with 50% precipitation. In our construction, we used V-shaped aluminum profiles for the dewatering of the water deficit treatment. The profiles were fixed exactly in the distance of the V-profile basis, leading to a theoretical precipitation reduction of 50%. In order to create similar shading for the 100% precipitation treatment, the same V-profiles were used in the opposite direction above the plant beds (see Fig. 1). The precipitation reduction and the seed beds were installed in April 2011. Seeds were sown on May 16 and 17, 2011. Starting in spring 2011, the technical care of the experiment included the manual weed control, the protection against mice, birds, ants and fungi, and - if necessary – an additive irrigation. Additive irrigation was necessary because the seed beds only contain 25-30 cm soil layer, which may dry out completely in periods without rain. The additive irrigation scheme followed the two treatment systems: seed beds with the normal water treatment received the double amount of water than the reduced water treatment by controlling for the irrigation time of a fixed irrigation system. Together with the precipitation reduction, the additive irrigation resulted in significantly lower soil water content in the 50% precipitation treatment then in the 100% treatment.

At the end of the first and the second vegetation period, one-, respectively two-year old seedlings (between 20-24 seedlings per provenance) were removed for measurements of quantitative traits. The phenological monitoring started directly after sowing and included observations of germination, bud set (in late summer and autumn) and bud burst (in spring). In preparation of the drought experiments, one-year old seedlings were removed after one vegetation period in March 2012 and planted in seedling containers (10 x 10 cm). After acclimatization of the seedlings two drought experiments were undertaken, one in spring 2013 and the other in Summer 2013. The frost hardiness of the seedlings was tested on needles from two year old seedlings in February 2013 for Norway spruce and Scots pine and in April 2013 for European Larch using the relative electrolyte leakage method (REL). Further details to the experiment can be found in section B6 (Methodik).
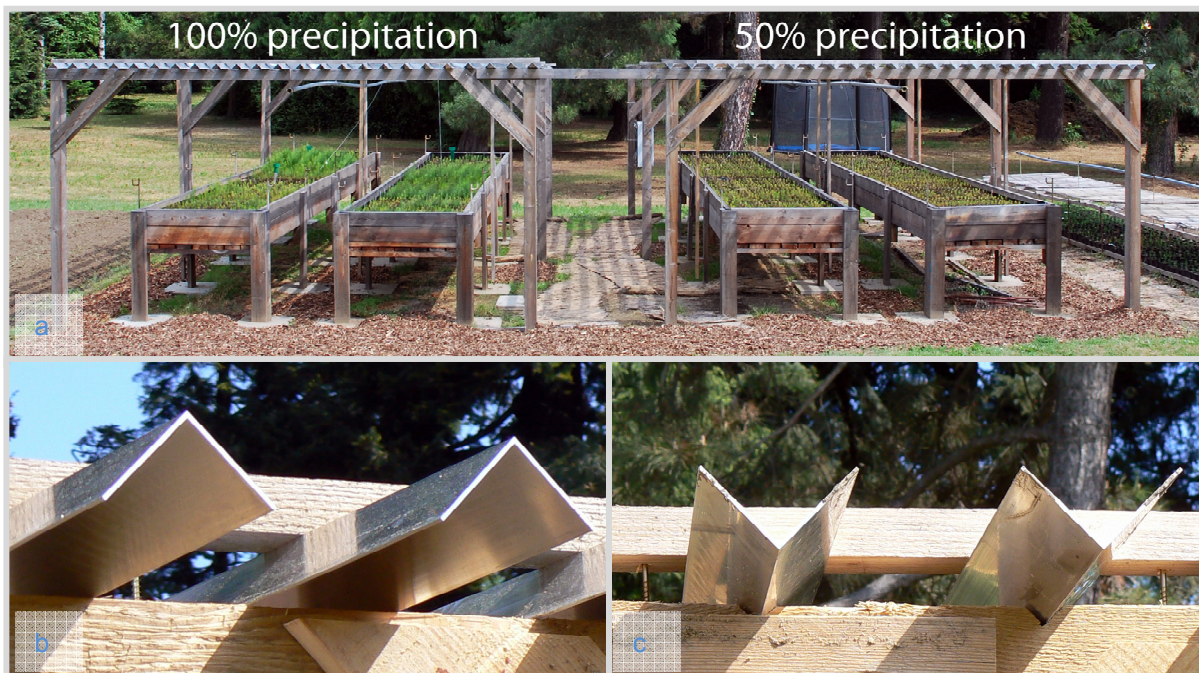
Fig. 1. Experimental Setup: In four elevated seed beds, 21 seed lots of coniferous trees were sown. Bed 1 and 2 (from left) are the „normal precipitation regime", whereas bed 3 and 4 receive only 50% of precipitation (a). b and c show the construction of the V-shaped aluminum profiles for the normal (b) and the reduction (c) treatment respectively.

WP 3: Data analysis and Project reporting

All quantitative and phenological traits were analyzed by series of independent t-tests between the trait means as well as by general linear models (Fig. 2) in order to test explicitly for the effects of the pollination and seed maturation climate on seedling performance for each single trait and on the three first components of the trait spectrum as revealed with principal component analysis.
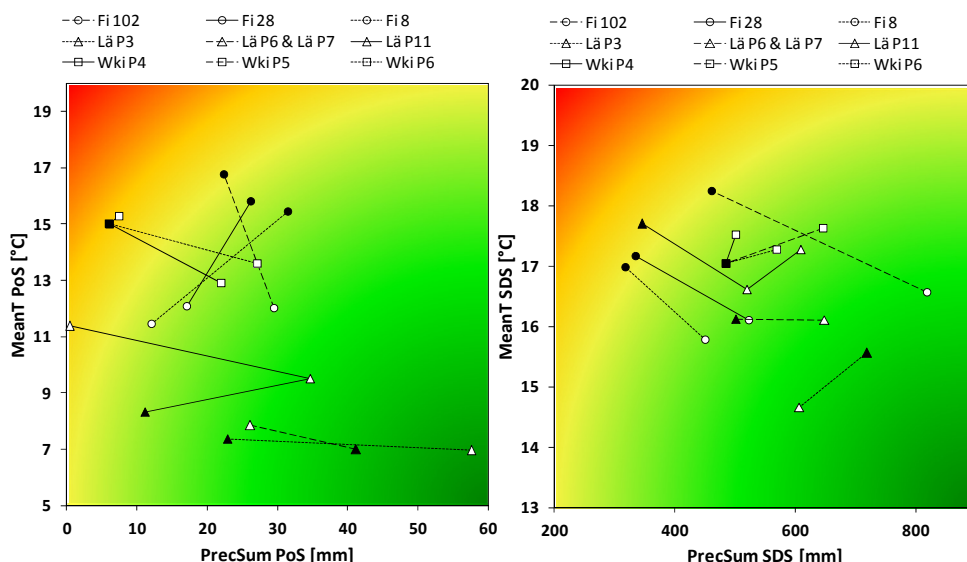
Fig. 2. Comparison of the climate conditions during pollination (left) and seed development (right) for each of the provenances' maturation years. It shows that not all provenances experienced the same climatic differences among the selected years and thus the putative environmental trigger for phenotypic variation might be inconsistent among provenances. Also, putative effects during pollination season (PoS) and seed developmental season (SDS) can be different. The classification of maturation years according to Li's moisture index LI2_SDS is marked by symbol filling: black filled - hot-dry years; white filled - cold-humid years. To account for the differences among the categorized maturation years, general linear models were applied, where the climate conditions were considered as continuous variables.

Results

From the 21 cultivated seed lots, 19 resulted in high and sufficient numbers of seedlings. Three seed lots provided only sporadic germination: in two of them (Norway Spruce Fi 8(9.2/tm) – seed years 2006 and European larch Lä P7 (4.2/sm) - seed year 2000) the number was below 10 seedlings/seed bed. These seed lots could not be used for statistical analysis. Although also within the third seed lot (Scots pine W.Kie P4 (2.1/mm) – year 2000) germination was low, sufficient seedlings remained for the phenological observations and for the drought experiments, but not for the full analysis of all quantitative traits.

Quantitative trait variation among maturation years

A comparison of the various traits for individual tree species shows that seedlings grown out of seeds from hot-dry years differ in many aspects from seedlings grown of seeds from cold-humid years. Fig. 3 shows the trait variation of four selected traits. For all species, seeds from hot-dry years resulted in lighter, smaller and thinner seedlings when grown in the full precipitation treatment. These differences are significant across provenances for Norway spruce (Fig. 3) as well as for several individual provenances (Table 2). However, the ratio of the root length to the total seedling length is higher when seeds were produced within hot-dry years (across provenances: significant effects for spruce and pine under full precipitation and for larch under half precipitation). When grown under the 50% precipitation treatment, the situation for spruce seedlings differed: now seeds from hot-dry years produced slightly larger and thicker seedlings. A series of t-tests was used to test for differences between the two maturation years of each single provenance for each single trait. These tests (Table 2) confirm that across all species and provenances and for many traits, highly significant differences between the maturation years exist at both environmental treatments. The most pronounced differences were found for traits of growth performance (total weight, root collar diameter, etc.). Differences in root length are also significant for many provenances.
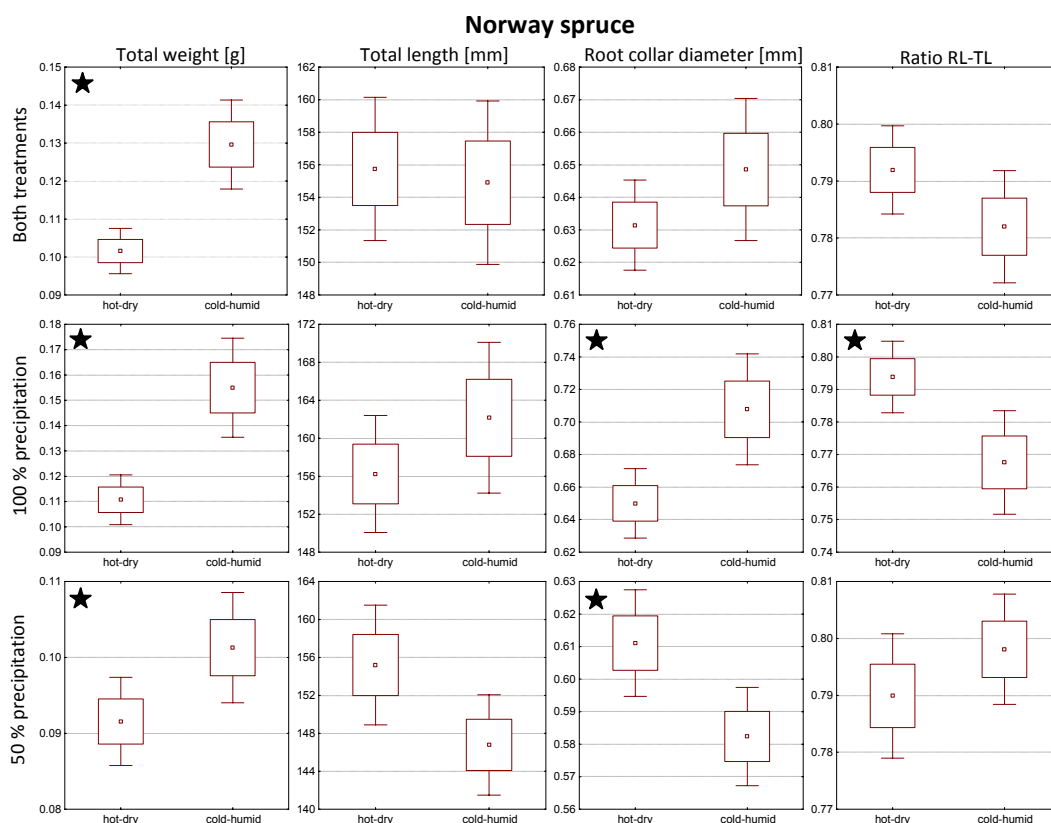

Fig. 3. Boxplot for four selected quantitative traits showing the variation between the two maturation years for 1-year old seedlings. Asterisks mark significant differences.

Fig. 3. Continued. Boxplot for four selected quantitative traits showing the variation between the two maturation years for 1-year old seedlings. Asterisks mark significant differences.
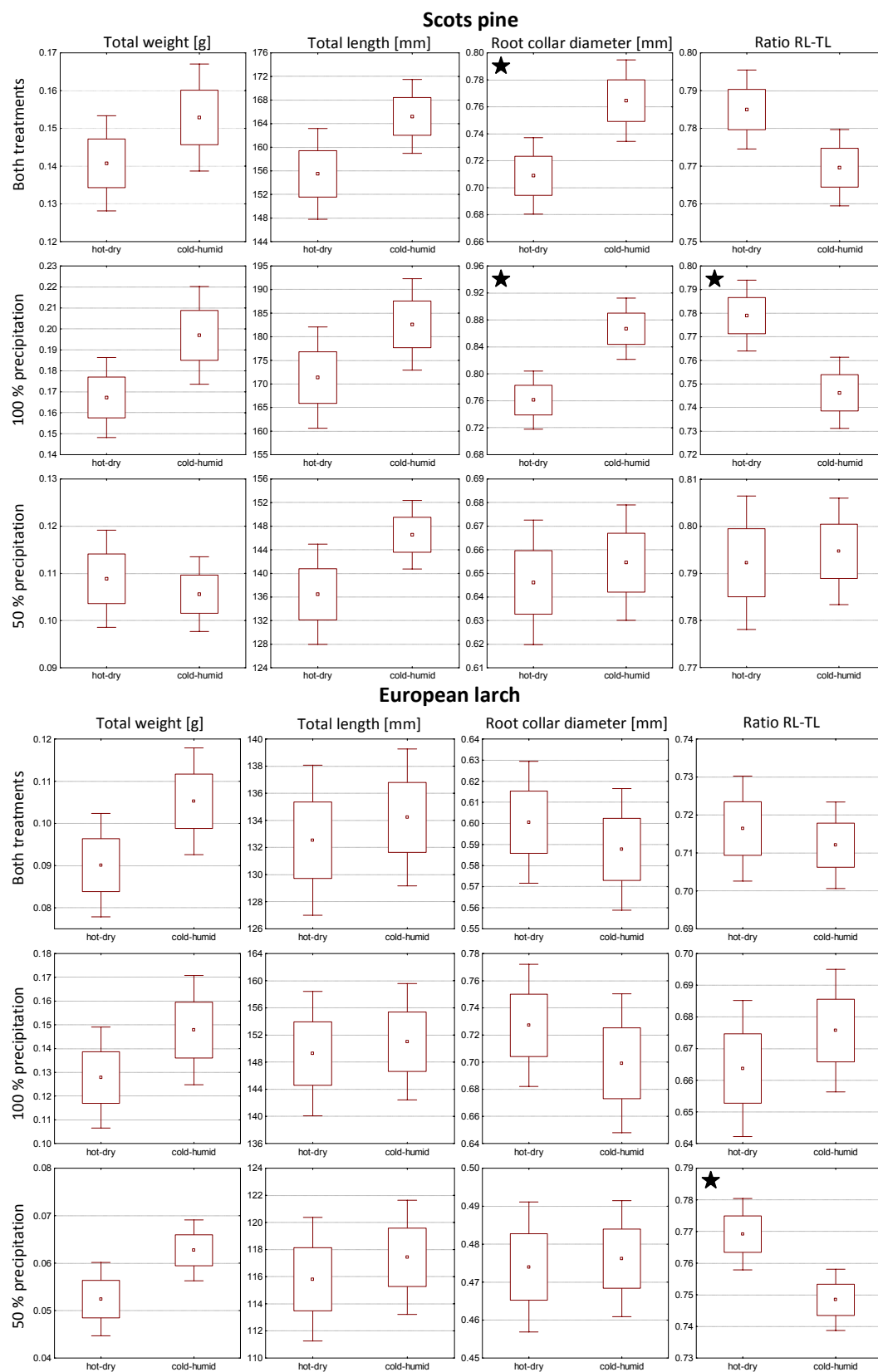
Table. 2. Results of series of t-test between hot-dry and cold-humid maturation years for each provenance and trait for Norway spruce. P-values below 0.05 are given in red.

Species	Norway spruce			
Provenance	Fi 102		Fi 28	
Treatment	Full	Reduced	Full	Reduced
Total weight	0.0000	0.0092	0.2590	0.1442
Needle weight	0.0002	0.0004	0.7365	0.0002
Root collar diameter (RCD)	0.0001	0.0225	0.1338	0.0163
Total length (TL)	0.0000	0.9309	0.6132	0.0002
Slenderness (Ratio RCD-TL)	0.9633	0.1934	0.6788	0.1217
Stem area with needles (SN)	0.0000	0.0587	0.9387	0.0000
Ratio SN-TL	0.0001	0.0753	0.7405	0.0162
Height (above ground)	0.0000	0.0018	0.2196	0.0000
Root length (RL)	0.0098	0.5724	0.8115	0.0052
Ratio RL-TL	0.0149	0.0216	0.6865	0.6534
Lenght of the 1st longest side root	0.0775	0.2016	0.8064	0.0005
Lenght of the 2st longest side root	0.1972	0.1127	0.5755	0.1419
Lenght of the 3st longest side root	0.2809	0.3883	0.8175	0.0115
Number of side roots longer than 2cm	0.3532	0.4093	0.0406	0.0962

Species	Europaen Larch							
Provenance	Lä P11_1		Lä P11_2		Lä P3		Lä P6	
Treatment	Full	Reduced	Full	Reduced	Full	Reduced	Full	Reduced
Total weight	0.5627	0.0007	0.4452	0.0018	0.0151	0.1204	0.0330	0.7882
Needle weight	0.7898	0.0000	0.0090	0.0000	0.0476	0.0002	0.0898	0.3203
Root collar diameter (RCD)	0.1940	0.0005	0.0013	0.9463	0.0478	0.0356	0.0443	0.6583
Total length (TL)	0.1654	0.0141	0.2029	0.0355	0.0228	0.7089	0.3628	0.0205
Slenderness (Ratio RCD-TL)	0.9149	0.7915	0.0048	0.0727	0.4453	0.2128	0.3362	0.0925
Stem area with needles (SN)	0.0003	0.0204	0.0140	0.0007	0.0041	0.0001	0.0036	0.4126
Ratio SN-TL	0.0000	0.5281	0.0399	0.0772	0.0315	0.0006	0.0029	0.0286
Height (above ground)	0.0002	0.0900	0.0210	0.0008	0.0058	0.0001	0.0033	0.6866
Root length (RL)	0.1331	0.0329	0.8400	0.1745	0.3860	0.6928	0.1845	0.0161
Ratio RL-TL	0.0000	0.4838	0.0929	0.3833	0.0797	0.0071	0.0011	0.0259
Lenght of the 1st longest side root	0.0004	0.0187	0.0562	0.0495	0.1737	0.9281	0.4007	0.1631
Lenght of the 2st longest side root	0.1829	0.1265	0.3054	0.5517	0.1948	0.2942	0.6570	0.9556
Lenght of the 3st longest side root	0.9189	0.4332	0.3223	0.1438	0.1441	0.2003	0.8236	0.5797
Number of side roots longer than 2cm	0.0079	0.0048	0.0118	0.0029	0.2925	0.3917	0.1146	0.2771

Species	Scots pine			
Provenance	Wkie P5		Wkie P6	
Treatment	Full	Reduced	Full	Reduced
Total weight	0.0007	0.4465	0.0011	0.6309
Needle weight	0.2894	0.9681	0.0518	0.1105
Root collar diameter (RCD)	0.7403	0.0668	0.0163	0.7130
Total length (TL)	0.0907	0.0403	0.1853	0.1355
Slenderness (Ratio RCD-TL)	0.1363	0.0013	0.2284	0.2731
Stem area with needles (SN)	0.0261	0.0001	0.0000	0.0000
Ratio SN-TL	0.0359	0.0000	0.0000	0.0000
Height (above ground)	0.1585	0.1726	0.0001	0.0000
Root length (RL)	0.1430	0.0203	0.8011	0.8488
Ratio RL-TL	0.9866	0.0012	0.0000	0.0003
Lenght of the 1st longest side root	0.6809	0.0039	0.3925	0.3587
Lenght of the 2st longest side root	0.2385	0.0148	0.0148	0.9000
Lenght of the 3st longest side root	0.0688	0.1024	0.0308	0.5368
Number of side roots longer than 2cm	0.3162	0.0010	0.6710	0.4126

Additional comparisons were made between seedlings grown under different precipitation treatments. These test series show that for all species under full precipitation seedlings are heavier and larger as well as have longer roots and side roots than seedlings in the 50% precipitation treatment. These differences are highly significant for the majority of traits for all three tree species. However, across all species two traits were found to be higher in the 50% treatment: this is the ratio of the root length to the total seedling length (RL-TL) and the slenderness, i.e. the relation between the total length and the root collar diameter. Thus, RL-TL and the slenderness can be considered as the plastic adaptation of seedlings to limited water resources.

Quantitative trait variation – general linear model analysis

Since the series of independent t-tests among maturation years did not reveal clear patterns of seedling performance across provenances and species (Table 2) and because the climate conditions during pollination and seed development varied among provenances within both groups of maturation years, i.e. within the hot-dry years as well as within the cold-humid years (Fig. 2), further analysis of the quantitative traits was performed with general linear models (GLM).

The GLMs confirmed the independent t-tests: for all three species, significant effects of the climate during pollination and seed development on various quantitative traits as well as for the traits principal components were found (Table 3a-c). For all species, the climate conditions resulted in stronger and more significant effects on seedlings that were grown under the full precipitation treatment. Also more significant effects were found for traits of growth performance (e.g. weight, length, or PC1) than for climate resistance (i.e. the ratio of root length to total length, PC2 and PC3). However, for spruce and larch, the temperature of the pollination period (for larch) or seed development period (for spruce) had also significant effects on climate resistance traits at the 50 % precipitation treatment. From the three climate parameters, temperature during pollination and seed development seemed to have the strongest effects on the performance of seedlings at both treatments.

Table 3: Results of the GLM analysis: the tables 3a-c provide the p-values of the linear models for all tested effects and interactions. Consider that only the significant effects (after sequential Bonferroni correction) of the continuous climate variables are being marked by red filling.

Table 3a: Norway spruce

50 % Precipitation

		Temperature (T_RPo)				Precipitation (PrecS_RPo)				Moisture Index (LI_RPo)			
		T_RPo	HK	Wdh	HK*T_RPo	PrecS_RPo	HK	Wdh	HK*PrecS_RPo	LI_RPo	HK	Wdh	HK*LI_RPo
Climate during pollination	Total weight	0,09751	0,81565	0,88219	0,13182	0,21011	0,50923	0,88137	0,11275	0,30386	0,38400	0,87055	0,11669
	Root collar diameter (RCD)	0,00148	0,77447	0,00002	0,05635	0,01956	0,10481	0,00002	0,03292	0,00924	0,27233	0,00002	0,02513
	Total length (TL)	0,10747	0,83526	0,19407	0,25650	0,06208	0,91671	0,20271	0,42636	0,05722	0,73951	0,21302	0,70952
	Height (above ground)	0,00076	0,00000	0,00011	0,60117	0,00000	0,00000	0,00015	0,04970	0,00826	0,00000	0,00019	0,00078
	Root length (RL)	0,31733	0,50343	0,56483	0,20611	0,45636	0,36755	0,57111	0,23273	0,14785	0,66188	0,58338	0,29930
	Ratio RL-TL	0,18122	0,00000	0,02506	0,06215	0,00202	0,00006	0,02810	0,00645	0,66549	0,00001	0,02944	0,00053
	PC1	0,00024	0,00042	0,00022	0,24810	0,00000	0,12877	0,00027	0,02994	0,00202	0,00224	0,00031	0,00185
	PC2	0,65115	0,07462	0,37243	0,01851	0,37748	0,19522	0,37657	0,00863	0,69419	0,20639	0,36887	0,00592
	PC3	0,94946	0,83727	0,87564	0,85379	0,80712	0,87243	0,87810	0,80414	0,96638	0,85990	0,87872	0,76333

		Temperature (T_SDS)				Precipitation (PrecS_SDS)				Moisture Index (LI2_SDS)			
		T_SDS	HK	Wdh	HK*T_SDS	PrecS_SDS	HK	Wdh	HK*PrecS_SDS	LI2_SDS	HK	Wdh	HK*LI2_SDS
Climate during seed development	Total weight	0,25326	0,18106	0,81060	0,41190	0,05727	0,66135	0,81667	0,33081	0,77297	0,23599	0,86992	0,11738
	Root collar diameter (RCD)	0,79788	0,00444	0,00002	0,11825	0,02187	0,06780	0,00002	0,08240	0,45700	0,01262	0,00002	0,02506
	Total length (TL)	0,18697	0,43775	0,22253	0,30402	0,38908	0,22641	0,22458	0,38827	0,22404	0,24930	0,21342	0,72287
	Height (above ground)	0,01058	0,00000	0,00011	0,00000	0,63722	0,00000	0,00013	0,00000	0,00000	0,00842	0,00019	0,00065
	Root length (RL)	0,06524	0,73420	0,62058	0,90517	0,42709	0,18833	0,61870	0,79127	0,85585	0,23465	0,58395	0,30299
	Ratio RL-TL	0,00137	0,01139	0,01971	0,00004	0,54348	0,00014	0,02140	0,00003	0,00001	0,08488	0,02944	0,00048
	PC1	0,23922	0,01305	0,00019	0,00001	0,49946	0,00724	0,00021	0,00001	0,00016	0,02697	0,00031	0,00165
	PC2	0,00887	0,93579	0,30268	0,05563	0,10594	0,16380	0,31052	0,03323	0,25832	0,36150	0,36831	0,00589
	PC3	0,69242	0,93727	0,86792	0,74773	0,92691	0,87320	0,86987	0,73889	0,66695	0,94323	0,87869	0,76201

100 % Precipitation

		Temperature (T_RPo)				Precipitation (PrecS_RPo)				Moisture Index (LI_RPo)			
		T_RPo	HK	Wdh	HK*T_RPo	PrecS_RPo	HK	Wdh	HK*PrecS_RPo	LI_RPo	HK	Wdh	HK*LI_RPo
Climate during pollination	Total weight	0,00006	0,00016	0,00072	0,32627	0,00000	0,13714	0,00062	0,02209	0,00069	0,00357	0,00065	0,00049
	Root collar diameter (RCD)	0,00000	0,01586	0,02173	0,66697	0,00000	0,06070	0,01942	0,68311	0,00000	0,00164	0,01877	0,20086
	Total length (TL)	0,00200	0,12855	0,00075	0,97093	0,00063	0,50293	0,00069	0,57613	0,00656	0,08920	0,00068	0,24762
	Height (above ground)	0,00000	0,00001	0,00479	0,42296	0,00000	0,04645	0,00372	0,42894	0,00000	0,00002	0,00344	0,01517
	Root length (RL)	0,11998	0,56283	0,00457	0,78259	0,18251	0,41120	0,00462	0,73502	0,22074	0,35508	0,00473	0,70474
	Ratio RL-TL	0,00041	0,00208	0,59012	0,62986	0,00001	0,19526	0,54789	0,47304	0,00058	0,00846	0,53629	0,05162
	PC1	0,00000	0,00112	0,00108	0,74205	0,00000	0,40848	0,00087	0,32731	0,00000	0,00233	0,00083	0,02149
	PC2	0,00173	0,03298	0,77282	0,47173	0,00023	0,61654	0,79458	0,11922	0,00683	0,10344	0,78638	0,01977
	PC3	0,18979	0,53147	0,03867	0,16302	0,20831	0,66300	0,03990	0,10872	0,48037	0,42570	0,04295	0,08874

		Temperature (T_SDS)				Precipitation (PrecS_SDS)				Moisture Index (LI2_SDS)			
		T_SDS	HK	Wdh	HK*T_SDS	PrecS_SDS	HK	Wdh	HK*PrecS_SDS	LI2_SDS	HK	Wdh	HK*LI2_SDS
Climate during seed development	Total weight	0,38828	0,00750	0,00176	0,00000	0,36543	0,00834	0,00155	0,00000	0,00033	0,00826	0,00066	0,00042
	Root collar diameter (RCD)	0,36954	0,00000	0,02761	0,00295	0,16287	0,00000	0,02588	0,00418	0,01775	0,00000	0,01879	0,18954
	Total length (TL)	0,97635	0,00905	0,00101	0,02770	0,44127	0,01134	0,00096	0,03160	0,08099	0,01058	0,00068	0,23871
	Height (above ground)	0,57603	0,00003	0,00809	0,00000	0,63229	0,00001	0,00702	0,00000	0,00000	0,00008	0,00344	0,01280
	Root length (RL)	0,79738	0,16098	0,00503	0,75478	0,27669	0,28221	0,00502	0,73734	0,96686	0,16906	0,00473	0,70401
	Ratio RL-TL	0,48435	0,03687	0,69148	0,00002	0,53614	0,01931	0,66411	0,00004	0,00035	0,01506	0,53663	0,04635
	PC1	0,60166	0,00008	0,00190	0,00000	0,72303	0,00005	0,00167	0,00001	0,00018	0,00004	0,00084	0,01887
	PC2	0,74921	0,05065	0,64232	0,00096	0,48047	0,06138	0,66056	0,00095	0,02132	0,03988	0,78538	0,01839
	PC3	0,18657	0,31347	0,05294	0,23590	0,10517	0,57563	0,05241	0,19019	0,78711	0,34429	0,04310	0,08855

Table 3b European larch

50 % Precipitation

		Temperature (T_RPo)				Precipitation (PrecS_RPo)				Moisture Index (LI_RPo)			
		T_RPo	HK	Wdh	HK*T_RPo	PrecS_RPo	HK	Wdh	HK*PrecS_RPo	LI_RPo	HK	Wdh	HK*LI_RPo
Climate during pollination	Total weight	0,09751	0,81565	0,88219	0,13182	0,21011	0,50923	0,88137	0,11275	0,30386	0,38400	0,87055	0,11669
	Root collar diameter (RCD)	0,00148	0,77447	0,00002	0,05635	0,01956	0,10481	0,00002	0,03292	0,00924	0,27233	0,00002	0,02513
	Total length (TL)	0,10747	0,83526	0,19407	0,25650	0,06208	0,91671	0,20271	0,42636	0,05722	0,73951	0,21302	0,70952
	Height (above ground)	0,00076	0,00000	0,00011	0,60117	0,00000	0,00000	0,00015	0,04970	0,00826	0,00000	0,00019	0,00078
	Root length (RL)	0,31733	0,50343	0,56483	0,20611	0,45636	0,36755	0,57111	0,23273	0,14785	0,66188	0,58338	0,29930
	Ratio RL-TL	0,18122	0,00000	0,02506	0,06215	0,00202	0,00006	0,02810	0,00645	0,66549	0,00001	0,02944	0,00053
	PC1	0,00024	0,00042	0,00022	0,24810	0,00000	0,12877	0,00027	0,02994	0,00202	0,00224	0,00031	0,00185
	PC2	0,65115	0,07462	0,37243	0,01851	0,37748	0,19522	0,37657	0,00863	0,69419	0,20639	0,36887	0,00592
	PC3	0,94946	0,83727	0,87564	0,85379	0,80712	0,87243	0,87810	0,80414	0,96638	0,85990	0,87872	0,76333

		Temperature (T_SDS)				Precipitation (PrecS_SDS)				Moisture Index (LI2_SDS)			
		T_SDS	HK	Wdh	HK*T_SDS	PrecS_SDS	HK	Wdh	HK*PrecS_SDS	LI2_SDS	HK	Wdh	HK*LI2_SDS
Climate during seed development	Total weight	0,25326	0,18106	0,81060	0,41190	0,05727	0,66135	0,81667	0,33081	0,77297	0,23599	0,86992	0,11738
	Root collar diameter (RCD)	0,79788	0,00444	0,00002	0,11825	0,02187	0,06780	0,00002	0,08240	0,45700	0,01262	0,00002	0,02506
	Total length (TL)	0,18697	0,43775	0,22253	0,30402	0,38908	0,22641	0,22458	0,38827	0,22404	0,24930	0,21342	0,72287
	Height (above ground)	0,01058	0,00000	0,00011	0,00000	0,63722	0,00000	0,00013	0,00000	0,00000	0,00842	0,00019	0,00065
	Root length (RL)	0,06524	0,73420	0,62058	0,90517	0,42709	0,18833	0,79127		0,85585	0,23465	0,58338	0,30299
	Ratio RL-TL	0,00137	0,01139	0,01971	0,00004	0,54348	0,00014	0,02140	0,00003	0,00001	0,08488	0,02944	0,00048
	PC1	0,23922	0,01305	0,00019	0,00001	0,49946	0,00724	0,00021	0,00001	0,00016	0,02697	0,00031	0,00165
	PC2	0,00887	0,93579	0,30268	0,05563	0,10594	0,16380	0,31052	0,03323	0,25832	0,36150	0,36831	0,00589
	PC3	0,69242	0,93727	0,86792	0,74773	0,92691	0,87320	0,86987	0,73889	0,66695	0,94323	0,87869	0,76201

100 % Precipitation

		Temperature (T_RPo)				Precipitation (PrecS_RPo)				Moisture Index (U_RPo)			
		T_RPo	HK	Wdh	HK*T_RPo	PrecS_RPo	HK	Wdh	HK*PrecS_RPo	LI_RPo	HK	Wdh	HK*LI_RPo
Climate during pollination	Total weight	0,00006	0,00016	0,00072	0,32627	0,00000	0,13714	0,00062	0,00155	0,00069	0,00357	0,00065	0,00049
	Root collar diameter (RCD)	0,00000	0,01586	0,02173	0,66697	0,00000	0,06070	0,01942	0,68311	0,00000	0,00164	0,01877	0,20086
	Total length (TL)	0,00200	0,12855	0,00075	0,97093	0,00063	0,50293	0,00069	0,57613	0,00656	0,08920	0,00068	0,24762
	Height (above ground)	0,00000	0,00001	0,00479	0,42296	0,00000	0,04645	0,00372	0,42894	0,00000	0,00002	0,00344	0,01517
	Root length (RL)	0,11998	0,56283	0,00457	0,78259	0,18251	0,41120	0,00462	0,73502	0,22074	0,35508	0,00473	0,70474
	Ratio RL-TL	0,00041	0,00208	0,59012	0,62986	0,00001	0,19526	0,54789	0,47304	0,00058	0,00846	0,53629	0,05162
	PC1	0,00000	0,00112	0,00108	0,74205	0,00000	0,40848	0,00087	0,32731		0,00233	0,00083	0,02149
	PC2	0,00173	0,03298	0,77282	0,47173	0,00023	0,61654	0,79458	0,11922	0,00683	0,10344	0,78638	0,01977
	PC3	0,18979	0,53147	0,03867	0,16302	0,20831	0,66300	0,03990	0,10872	0,48037	0,42570	0,04295	0,08874

		Temperature (T_SDS)				Precipitation (PrecS_SDS)				Moisture Index (LI2_SDS)			
		T_SDS	HK	Wdh	HK*T_SDS	PrecS_SDS	HK	Wdh	HK*PrecS_SDS	LI2_SDS	HK	Wdh	HK*LI2_SDS
Climate during seed development	Total weight	0,38828	0,00750	0,00176	0,00000	0,36543	0,00834	0,00155	0,00000	0,00033	0,00826	0,00066	0,00042
	Root collar diameter (RCD)	0,36954	0,00000	0,02761	0,00295	0,16287	0,00000	0,02588	0,00418	0,01775	0,00000	0,01879	0,18954
	Total length (TL)	0,97635	0,00905	0,00101	0,02770	0,44127	0,01134		0,03160	0,08099	0,01058	0,00068	0,23871
	Height (above ground)	0,57603	0,00003	0,00809	0,00000	0,63229	0,00001	0,00702	0,00000	0,00000	0,00008	0,00344	0,01280
	Root length (RL)	0,79738	0,16098	0,00503	0,75478	0,27669	0,28221	0,00502	0,73734	0,96686	0,16906	0,00473	0,70401
	Ratio RL-TL	0,48435	0,03687	0,69148	0,00002	0,53614	0,01931	0,66411	0,00004	0,00035	0,01506	0,53663	0,04635
	PC1	0,60166	0,00008	0,00190	0,00000	0,72303	0,00005	0,00167	0,00001	0,00018	0,00004	0,00084	0,01887
	PC2	0,74921	0,05065	0,64232	0,00096	0,48047	0,06138	0,66056	0,00095	0,02132	0,03988	0,78538	0,01839
	PC3	0,18657	0,31347	0,05294	0,23590	0,10517	0,57563	0,05241	0,19019	0,78711	0,34429	0,04310	0,08855

Table 3c. Scots pine

50 % Precipitation

		Temperature (T_RPo)				Precipitation (PrecS_RPo)				Moisture Index (LI_RPo)			
		T_RPo	HK	Wdh	HK*T_RPo	PrecS_RPo	HK	Wdh	HK*PrecS_RPo	LI_RPo	HK	Wdh	HK*LI_RPo
Climate during pollination	Total weight	0,00289	0,00026	0,43678	0,65037	0,02550	0,00006	0,41497	0,68100	0,00027	0,00243	0,42534	0,65447
	Root collar diameter (RCD)	0,00000	0,05635	0,73419	0,61229	0,00000	0,02057	0,81600	0,25479	0,00000	0,40376	0,76279	0,70941
	Total length (TL)	0,59381	0,36040	0,24445	0,32454	0,16311	0,72266	0,28597	0,93860	0,52421	0,58626	0,26754	0,73475
	Height (above ground)	0,00058	0,35562	0,76883	0,00076	0,76671	0,06839	0,69992	0,04710	0,05434	0,49504	0,73673	0,01635
	Root length (RL)	0,77041	0,55306	0,19942	0,70717	0,12370	0,90908	0,22338	0,92782	0,25006	0,74007	0,21182	0,99338
	Ratio RL-TL	0,00703	0,99268	0,20418	0,27436	0,19202	0,31396	0,19566	0,18450	0,02708	0,89739	0,20015	0,17533
	PC1	0,32138	0,05935	0,17354	0,02816	0,00557	0,83167	0,22236	0,34388	0,01468	0,95127	0,19915	0,12714
	PC2	0,26739	0,69034	0,03026	0,89524	0,19215	0,77893	0,03251	0,99532	0,14737	0,90554	0,03142	0,98103
	PC3	0,00094	0,66836	0,65983	0,91875	0,03739	0,05455	0,65712	0,91393	0,00193	0,50509	0,65521	0,91978

		Temperature (T_SDS)				Precipitation (PrecS_SDS)				Moisture Index (LI2_SDS)			
		T_SDS	HK	Wdh	HK*T_SDS	PrecS_SDS	HK	Wdh	HK*PrecS_SDS	LI2_SDS	HK	Wdh	HK*LI2_SDS
Climate during seed development	Total weight	0,00152	0,00031	0,39981	0,76546	0,00231	0,00028	0,45323	0,53617	0,00059	0,00051	0,43684	0,83954
	Root collar diameter (RCD)	0,00000	0,00932	0,81657	0,27356	0,02334	0,00002	0,72559	0,44906	0,00007	0,00058	0,70913	0,29300
	Total length (TL)	0,15047	0,17035	0,29612	0,74260	0,27993	0,61638	0,22539	0,08296	0,13362	0,25412	0,23825	0,17322
	Height (above ground)	0,67828	0,00446	0,65047	0,69188	0,68389	0,01907	0,80430	0,00000	0,78375	0,00202	0,75642	0,02070
	Root length (RL)	0,10804	0,52170	0,22770	0,55897	0,30552	0,75424	0,18726	0,38159	0,13595	0,61369	0,19350	0,39576
	Ratio RL-TL	0,13791	0,29860	0,18686	0,51441	0,63999	0,12678	0,21071	0,17302	0,37655	0,12786	0,20240	0,43315
	PC1	0,00139	0,17428	0,23352	0,97027	0,02261	0,67758	0,16017	0,00353	0,00156	0,49724	0,16879	0,03141
	PC2	0,08670	0,98380	0,03301	0,93423	0,23828	0,74700	0,02951	0,78564	0,11162	0,95669	0,03008	0,80122
	PC3	0,01106	0,14156	0,64521	0,99175	0,21322	0,01335	0,67338	0,80226	0,05133	0,03860	0,65369	0,88501

100 % Precipitation

		Temperature (T_RPo)				Precipitation (PrecS_RPo)				Moisture Index (LI_RPo)			
		T_RPo	HK	Wdh	HK*T_RPo	PrecS_RPo	HK	Wdh	HK*PrecS_RPo	LI_RPo	HK	Wdh	HK*LI_RPo
Climate during pollination	Total weight	0,00000	0,00000	0,00000	0,00034	0,00000	0,00000	0,00000	0,00056	0,00000	0,00000	0,00000	0,00040
	Root collar diameter (RCD)	0,00000	0,00000	0,00000	0,00009	0,00001	0,00000	0,00000	0,00072	0,00001	0,00000	0,00000	0,00054
	Total length (TL)	0,01633	0,00372	0,00000	0,01480	0,00332	0,01074	0,00000	0,04329	0,02209	0,00298	0,00000	0,03463
	Height (above ground)	0,01559	0,00000	0,00000	0,00000	0,00039	0,00000	0,00000	0,03571	0,00000	0,00000	0,00000	0,00000
	Root length (RL)	0,13539	0,92767	0,00307	0,80136	0,19819	0,78869	0,00380	0,52753	0,10746	0,93685	0,00359	0,63209
	Ratio RL-TL	0,65190	0,00000	0,00000	0,00001	0,14430	0,00000	0,00000	0,00001	0,76140	0,00000	0,00000	0,00001
	PC1	0,00003	0,00000	0,00000	0,00018	0,00000	0,00000	0,00000	0,00046	0,00004	0,00000	0,00000	0,00036
	PC2	0,28698	0,00094	0,00200	0,01675	0,63368	0,00077	0,00246	0,04751	0,08348	0,00258	0,00255	0,04932
	PC3	0,00312	0,16280	0,10317	0,98053	0,04997	0,01969	0,11082	0,97194	0,00213	0,23517	0,11025	0,97745

|		Temperature (T_SDS)				Precipitation (PrecS_SDS)				Moisture Index (LI2_SDS)			
		T_SDS	HK	Wdh	HK*T_SDS	PrecS_SDS	HK	Wdh	HK*PrecS_SDS	LI2_SDS	HK	Wdh	HK*LI2_SDS
Climate during seed development	Total weight	0,00000	0,00109	0,00000	0,00117	0,00105	0,00000	0,00000	0,00031	0,00000	0,00000	0,00000	0,00103
	Root collar diameter (RCD)	0,00001	0,00000	0,00140	0,17768	0,00000	0,00000	0,00005	0,00028	0,00000	0,00000	0,00018	
	Total length (TL)	0,00149	0,02076	0,00000	0,03501	0,71595	0,00027	0,00000	0,01081	0,03636	0,00241	0,00000	0,00990
	Height (above ground)	0,00002	0,00001	0,00000	0,00000	0,60926	0,00000	0,00000	0,00000	0,00000	0,00209	0,00000	0,00000
	Root length (RL)	0,29247	0,75428	0,00382	0,59076	0,92145	0,46950	0,00357	0,77261	0,65924	0,51905	0,00370	0,80515
	Ratio RL-TL	0,01934	0,00000	0,00000	0,00000	0,90190	0,00000	0,00000	0,00000	0,10252	0,00000	0,00000	0,00000
	PC1	0,00000	0,00081	0,00000	0,00041	0,02315	0,00000	0,00000	0,00013	0,00000	0,00000	0,00000	0,00020
	PC2	0,43217	0,00083	0,00227	0,01711	0,08807	0,00214	0,00222	0,00703	0,25493	0,00111	0,00227	0,00516
	PC3	0,00310	0,17325	0,10910	0,87702	0,01816	0,03771	0,10750	0,94746	0,00530	0,10151	0,10924	0,89556
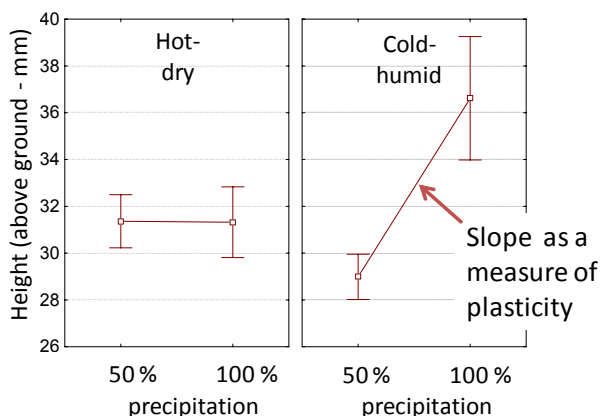
Fig. 4. Example (Norway spruce) for the differences in the plasticity among the seeds/seedlings from different maturation years. The slope of the linear regression has been used as measure of plasticity.

Quantitative trait variation – plasticity

Plasticity is the capacity of an (epi)-genotype to exhibit variable phenotypes in different environments. In the present analysis, two different approaches were used to test for phenotypic plasticity and differences in plasticity between seedlings from different maturation years. With the first approach - an extended linear model, we found for many traits significant differences between the two precipitation treatments and – again for many but not all traits – significant interactions between the climate conditions within the maturation years and the treatment. These significant interactions confirm that seedlings are able to produce different phenotypes, i.e. as measured by different trait means, under the two precipitation treatments. In the second approach, we tested for differences in the plasticity of seedlings from hot-dry and cold-humid maturation years.

Using linear regression to estimate the slope of the phenotypic response to the precipitation treatment (Fig. 4), highly significant differences were found (Table 4). For Norway spruce and Scots pine, for the majority of traits, seedlings from cold-humid maturation years were found to exhibit significantly larger plasticity than seedlings from hot-dry years (Table 4). For European larch, the differences in plasticity were small and significant only for root length (Table 4). For Scots pine, all root traits (root length, length of the 1st, 2nd, and 3rd side root, etc.) showed higher plasticity if seedling were from hot-dry years. However, except the length of the 3rd side root none of these differences were significant.

Table 4: Comparison of the trait plasticity between seed lots from cold-humid and hot-dry maturation years. The plasticity was calculated from the slope of the linear regression on the treatments precipitation regime (see Fig. 4). Bold values indicate the maturation years with the significantly higher plasticity.

Trait	Norway spruce		European larch		Scots pine	
	cold-humid	hot-dry	cold-humid	hot-dry	cold-humid	hot-dry
Total weight	**0.0011***	0.0004	0.0020	0.0020	**0.0018***	0.0012
Needle weight	**0.0006***	0.0002	0.0009	0.0008	**0.0008***	0.0006
Root collar diameter	**0.0025***	0.0008	0.0052	0.0057	**0.0042***	0.0023
Total length	**0.3076***	0.0209	0.7431	0.9020	0.7212	0.6978
Slenderness RCD-TL Ratio	0.3558	0.2273	-0.5101	-0.5167	0.3093	0.4053
Stem area with needles	**0.1905***	0.0390	0.6068	0.5905	**0.3363***	0.1759
Ratio SN-TL	**0.0011***	0.0002	0.0023	0.0028	**0.0013***	0.0007
Height (above ground)	**0.1527***	0.0008	0.5553	0.5409	**0.3425**	0.1869
Root length	0.1549	0.0217	0.1877	**0.3611***	0.3787	0.5109
Ratio RL-TL	**0.0006***	0.0001	-0.0016	-0.0019	**0.0010***	0.0003
Lenght of the 1st longest side root	**0.3480***	0.0602	0.1682	0.2287	0.0031	0.1182
Lenght of the 2st longest side root	0.1943	0.1108	0.1559	0.1804	0.0040	0.1152
Lenght of the 3st longest side root	**0.3157***	0.1593	0.1527	0.1469	**0.0115***	0.1469
Sum of the 3 longest side roots	**0.8580***	0.3304	0.4768	0.5560	0.0045	0.3803
Number of side root longer than 2 cm	**0.0216***	0.0068	0.0172	0.0199	0.0037	0.0077

Drought resistance experiments

Besides the two precipitation treatments, special tests for drought resistance were carried out on 2-year old seedlings. For the preparation of this experiment, 1-year old seedlings were removed out of the nursery beds in March 2012 and transferred to individual plant containers (size: 11 cm). In 2013, two drought tests were carried out: one in spring (15.4.2013 to 21.5.2013) to test for effects of drought during bud flush and a second in midsummer (10.7.2013 to 24.7.2013) to test for effects of summer drought. In both experiments, seedlings from each seed lot were relocated to a greenhouse, where they grew without any irrigation. All seedlings were periodically monitored and the withering was determined by assigning individual plants to one of five phenotypic phases from 1 - „green needles, shoots not sagging " to 5 „needles drop at contact". After each experiment was finished and the majority of seedlings were dead, the size of the seedlings was measured. During the spring experiment also the bud flush was monitored and during the summer experiment physiological measures of the chlorophyll fluorescence were made. The size of the seedlings and the needle enfolding (in spring) were used as covariables of drought response. The chlorophyll fluorescence and mainly the ratio of FV/FM – which gives the maximum quantum yield of primary photochemistry and can be used as a proper stress indicator for plant vitality – is being used as an additional parameter of drought resistance. We analysed drought response on species level, provenance level and maturation year level, both for the drought experiment in spring and in summer:

> A) Bud break in spring 2013 (as covariable of drought response)
> B) Tree height after drought experiments (as covariable of drought response)
> C) Drought response in spring and summer (measures as phenotypic phase)
> D) Chlorophyll fluorescence in the summer drought experiment

Bud break and drought response were recorded at phenotypic states at different monitoring days. For comparions we selected those monitoring days, where the highest intraspecific diversity was observed. For that purpose Shannon diversity indices (SDI) for each species were calculated and levels with maximum SDI were selected. For analyses between species, the level with highest maximum SDI across species was selected.

> This procedure provided the following response variables:

Bb	Bud break index in spring (measurement with max. species-specific Shannon diversity)
DrSp	Drought response index in spring (measurement with max. species-specific Shannon diversity)
DrSu	Drought response index in summer (measurement with max. species-specific Shannon diversity)
HtSp	Tree height after spring drought experiment
HtSu	Tree height after summer drought experiment
CFSu	Chlorophyll fluorescence in summer

> *A) Bud break in spring 2013*

Bud break indices (evaluated only in spring) differ significantly between the three species (Kruskal-Wallis rank sum test, $p<0.001$). Within each species significant differences in bud break across different provenances were found: pine (Kruskal-Wallis, $p=0.002$), spruce (Kruskal-Wallis, $p=0.035$) and larch (Kruskal-Wallis, $p<0.001$). However, significant differences between seed lots from hot-dry and cold-humid maturation years were not found for any provenance (Mann-Whitney U test). In all species, bud break indices correlate with tree height significantly (see Table 5).

> *B) Tree height*

In both, the spring and summer experiment, the three species responded differently to the cut of irrigation (Anova, $p<0.001$). However, there were no significant differences in mean tree heights across different provenances within each species.

In three provenances, significant differences between seed lots from hot-dry and cold-humid maturation years were found: spruce „Fi 28" in spring (t-test, $p<0.001$), spruce „Fi 102" in summer (t-test, $p=0.028$) and pine „Wkie P6" in summer (t-test, $p=0.033$). For all other

provenances, no significant differences in tree height between different maturation years were found after spring or summer drought experiment.

C) Drought response

Drought response was evaluated in two drought experiments in spring and summer. The response index was corrected for the effect of tree height separately for each experimental run and species. Due to seasonal differences in drought response for each species, we evaluated the two experimental runs separately. In both, the spring and summer experiment, the three species responded differently to the cut of irrigation (Kruskal-Wallis rank sum test, p<0.001). Moreover, significant differences in drought response across different provenances within each species were found. In the spring experiment, rates of withering differed significantly between provenances of pine (Kruskal-Wallis, p=0.03) and spruce (Kruskal-Wallis, p=0.033), but not of larch. In the summer drought experiment, drought response of again pine (Kruskal-Wallis, p=0.047) and spruce (Kruskal-Wallis, p<0.001) differed significantly, but not larch. Significant differences between seed lots from hot-dry and cold-humid maturation years were found for some provenances of spruce and larch and pine. As drought response is correlated with tree height, a species-specific linear regression model was used to correct for tree heights and seed lots were tested for differences in the resulting residuals of the linear fit to heights. This approach allows for detection of seed year effects independent from tree height effect. Thereafter, for the spring drought experiment we found significant differences in larch „Lä P6" and „Lä P11" (for both seed lots from dry years compared to the one from a moist year) and pine „Wkie P6". In the summer drought experiment we found significant differences among the maturation years of the spruce provenances „Fi 102" and „Fi 28" (see table 6).
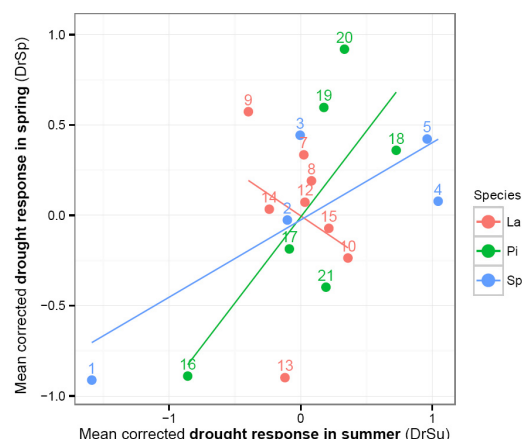
Fig. 5. Drought response in summer and spring correlate significantly for pooled provenances (adj. R²=0.267, p-value=0.014), but not for each species separately (La: adj. R²=-0.08, p-value=0.51; Pi: adj. R²=0.45, p-value=0.09; Sp: adj. R²=0.57, p-value=0.085). Labels above points show provenance IDs.

Table 5. Spearman rank-correlation measure r (r), adjusted coefficient of determination (adj. R²) and p-value from a linear regression model for measurements of tree height in spring (HtSp) and summer (HtSu), bud break index (Bb), drought response in spring (DrSp) and summer (DrSu) and corrected drought response in spring (DrSp (Corr.)

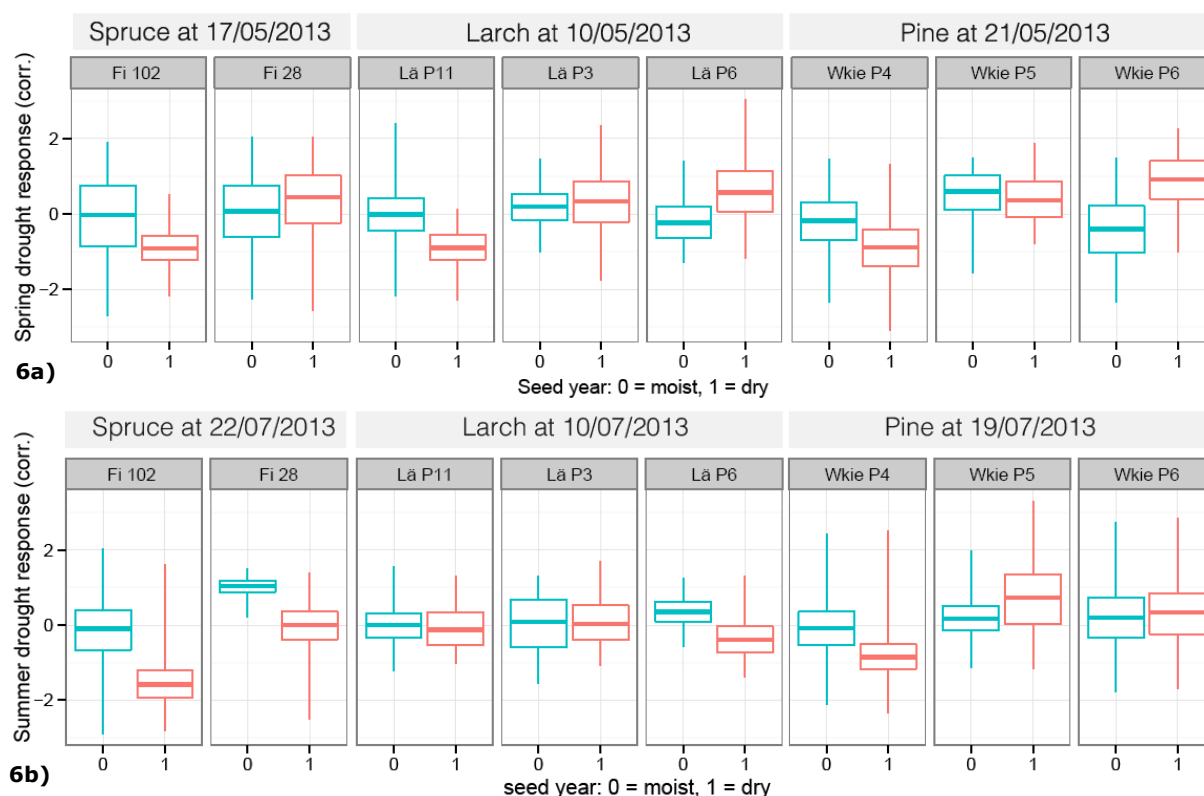| Species	Spruce			Larch			Pine		
x, y	r	adj. R²	p-value	r	adj. R²	p-value	r	adj. R²	p-value
HtSp, Bb	-0,32	0,05	0,0318	-0,59	0,34	0,0000	-0,33	0,10	0,0009
HtSp, DrSp	0,19	0,03	0,0682	0,61	0,37	0,0000	0,42	0,18	0,0000
HtSu, DrSu	0,39	0,11	0,0007	0,64	0,40	0,0000	0,41	0,15	0,0001
Bb, DrSp (Corr.)	0,37	0,17	0,0001	0,07	0,00	0,2338	0,05	-0,01	0,5831

Fig. 6. Spring (6a) and summer (6b) drought response at the date when Shannon diversity index was highest for each species. Drought response was corrected for the tree height effect.

Table 6. P-values of t-tests between seed lots from hot-dry and cold-humid years for all tested provenances. Bb = Bud break, DrSp = Drought response in spring drought experiment (height corrected), DrSu = Drought response in summer drought experiment (height corrected).

Species	Spruce		Larch				Pine		
Provenance	Fi 102	Fi 28	Lä P11_1	Lä P11_2	Lä P3	Lä P6	Wkie P4	Wkie P5	Wkie P6
Bb	1,000	0,072	0,080	0,094	0,545	1,000	0,082	0,806	0,215
DrSp	0,065	0,456	0,031	0,008	0,682	0,027	0,075	0,497	0,005
DrSu	0,000	0,000	0,729	0,297	0,899	0,003	0,010	0,170	0,722

D) Chlorophyll fluorescence

PAM (pulse amplitude modulation) fluorescence is versatile method to measure photosynthetic activity and to study plant health (Misra et al. 2012). With this method, the maximum quantum efficiency of PSII photochemistry is calculated as: $Fv/Fm = (Fm-Fo) / Fm$, where Fm is the fluorescence maximum at light adapted state, Fo ist the ground fluorescence in darkness and Fv is the variable fluorescence.The ratio Fv/Fm is used as the maximum quantum yield of primary PSII photochemistry and in a healthy plant it is ranging from 0.78–0.84. Under severe stress, an inhibition of PSII can be detected in vivo as a decrease in the chlorophyll fluorescence ratio Fv/Fm (Misra et al. 2012).

Figure 7 shows the correlation between the fluorescence measure Fv/Fm, measured on July 10th and 17th, and the results of the visual screening for drought response (DrSu). For larch, highest correlation was obtained on the July 10th, whereas on the July 17th most larch trees were already dead and fluorescence could not be measured. Pine and spruce reveal higher correlation on the 17th, indicating that plants visually assessed as already strongly desiccated, still seemed to be capable of photosynthetic activity. Individual t-tests (Table 8, Fig. 8) for effects of the maturation year on Fv/Fm revealed significant differences for two provenances on July 10th: Fi 28 and Lä P6. Interestingly, Fi28 had a higher Fv/Fm ratio in seedlings from the cold-humid years, although these

seedlings showed significantly more symptoms for drought than the seedlings from hot-dry years. One explanation for these somehow contradicting results could be that seedlings from hot-dry years are able to reduce physiological functions including photosynthetic activity and thus could withstand droughts with less visual symptoms. In the latter assessment and thus after prolonged drought also the seedlings from the hot-dry year showed similar reduction in the maximum quantum yield of primary PSII. In the larch provenance Lä P6, both measures of drought resistance, the Fv/Fm ratio and the visual assessment provided the same result and showed the seedlings from hot-dry years were less effected by drought than the seedlings form cold-humid years.
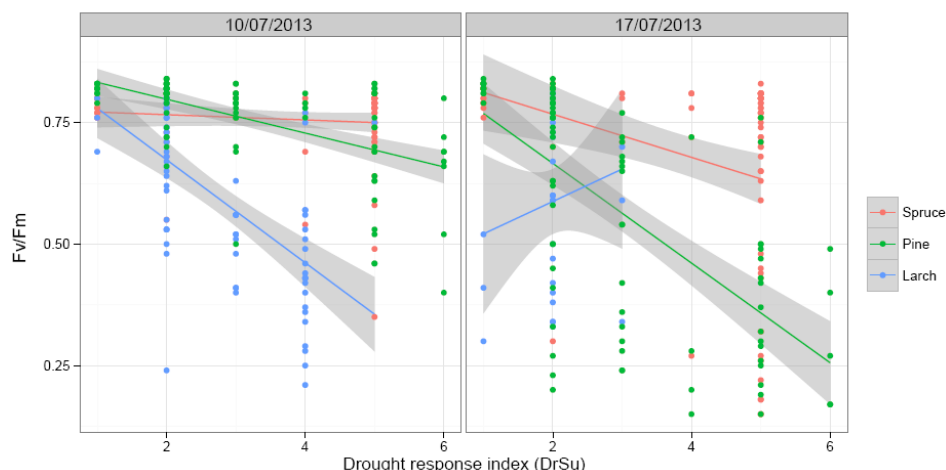
Fig. 7. Correlation between Fv/Fm, measured on July 10th and 17th, and phenotypic drought response index from dates with highest species-specific Shannon diversity indices. On 17th, many larch trees were already dead and fluorescence could not be measured for those individuals.
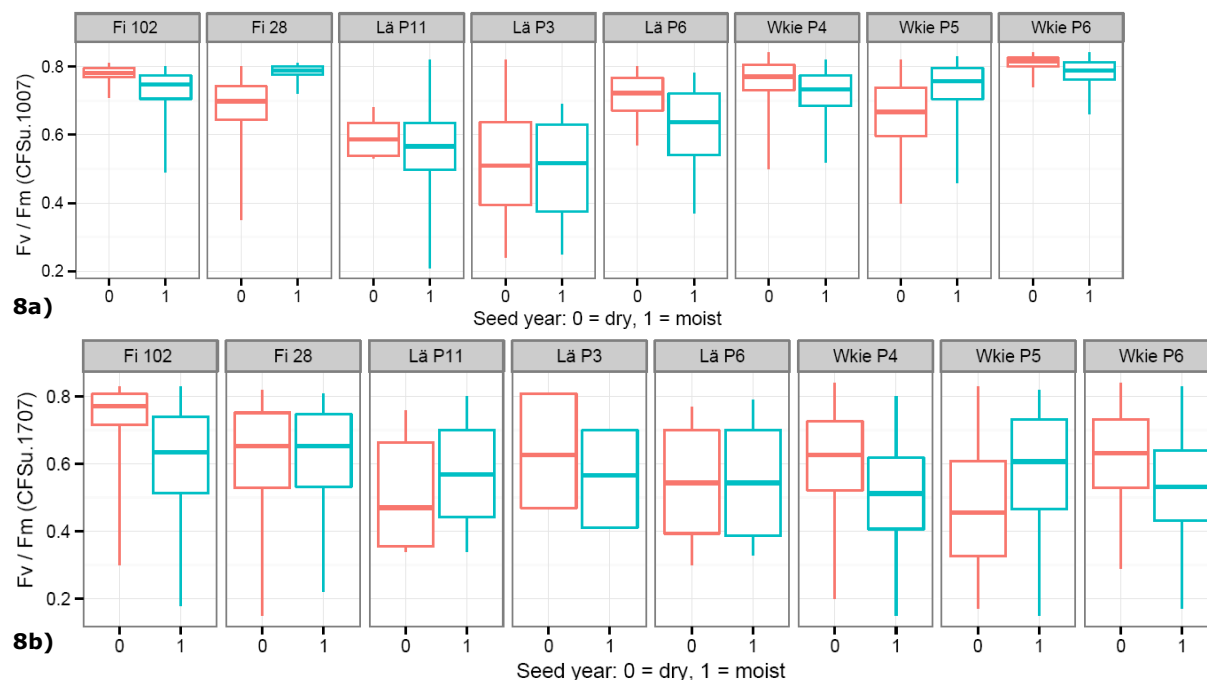
Fig. 8. Chlorophyll fluorescence (Fv / Fm) measured on 10/7 (8a) and 17/7 (8b).

Table 7. Spearman rank-correlation measure r (r), adjusted coefficient of determination (adj. R²) and p-value from a linear regression model for measurements of (visual) drought response in summer (DrSu and the chlorophyll fluorescence measure Fv/Fm on July 10th (CF107) and July 17th (CF1707). All variables were corrected for tree height effects.

Species	Spruce			Larch			Pine		
x, y	r	adj. R²	p-value	r	adj. R²	p-value	r	adj. R²	p-value
DrSu, CF1007	0,05	-0,01	0,8587	-0,55	0,23	0,0000	-0,47	0,21	0,0000
DrSu, CF1707	-0,23	0,04	0,0303	0,13	-0,02	0,5241	-0,59	0,35	0,0000

Table 8. P-values from t-tests for chlorophyll fluorescence (Fv / Fm) among seed years (dry and moist) for all provenances and measurements on July 10th and July 17th.

Species	Spruce		Larch				Pine		
Provenance	Fi 102	Fi 28	Lä P11_1	Lä P11_2	Lä P3	Lä P6	Wkie P4	Wkie P5	Wkie P6
CFSu.1007 (corr.)	0,298	0,007	0,619	0,804	0,908	0,007	0,443	0,098	0,264
CFSu.1707 (corr.)	0,327	0,665	0,214	NA	0,767	0,696	0,298	0,341	0,714

Frost resistance experiment

In order to test if the climate of the maturation year might also have negative effects on the performance of seedlings under extreme winter conditions, the frost injury of the shoots respectively the needles has been tested by the relative electrolyte leakage method (REL). The freezing experiment was undertaken in February 2013 for Norway spruce and Scots pine and at the end of April 2013 for European Larch when new needles were available.

Tree species responded very differently to the frost experiment. While the young larch needles revealed high percentages of frost damage already at 4°C and almost 100% damage at -40°C, spruce and pine provenances showed less frost sensitivity on their winter needles. Spruce and pine provenances had stable damage measures until -14°C, then had increased frost damage (Fig. 9). In all provenances (except Lä P7) needles from seed beds with full precipitation were more sensitive to frost than needles from seed beds with reduced precipitation. Due to rain a few days before the frost experiment, plants in all beds had sufficient water supply at the time of needle sampling. However, plants with previously moister conditions might have contained more water and were therefore more susceptible to frost damage. Besides the differences among treatments, no significant differences between seedlings from different maturation years were found.
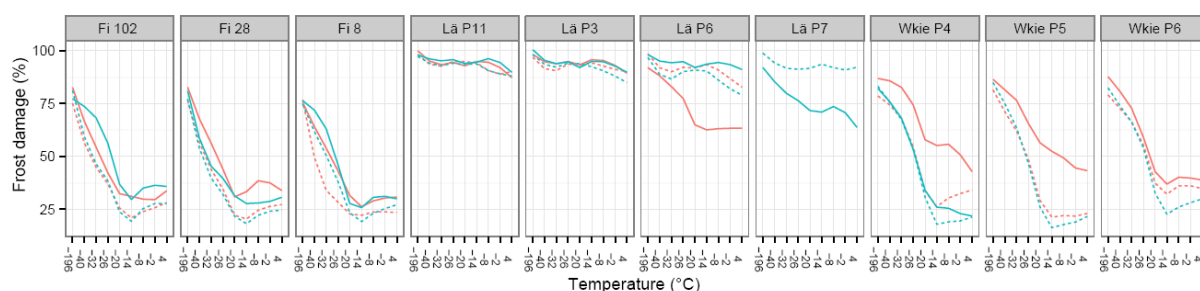

Fig. 9. Frost damage in needles with different temperature treatments. Full lines are needles from seed bed with full precipitation, dotted lines are needles with reduced precipitation. Red lines are needles from dry seed development years, blue lines are needles from moist seed development years. Lines are generated by local regression (loess smoothing).

5 Schlussfolgerungen und Empfehlungen

Understanding epigenetic marks in the plant genome (e.g. Becker et al. 2011, Schmitz et al. 2013) and the effects of these marks on the variation and heritability of quantitative traits (e.g. Johannes et al. 2009, Cortijo et al. 2014) as well as on the plasticity of such traits within variable environments (Zhang et al. 2013) has been a vital research object within the last decade and increasingly within the timeframe of this research project. The absolute majority of plant epigenetic studies focus on the model plant *Arabidopsis thaliana*, for which isogenic lines with methylation deficiencies are well established (e.g. Johannes et al. 2009) and now provide a basis for deciphering effects of genetic variation from epigenetic, i.e. methlylation patterns (Zhang et al. 2013, Cortijo et al. 2014). The present study differs in many aspects from the experimental approaches: first, instead of using a model plant species our focus is on coniferous forest trees for which by far less genomic resources are available and for which crosses of well-defined phenotypes under controlled environmental conditions are not possible within short term research projects; second, our focus was not only to quantify the adaptive genetic variation, but moreover, to test for directed effects of the pollination-, respectively seed developmental environment on trait means and plasticity. In particular, our focus was to test whether warm and relatively dry maturation years result in seeds that develop into seedling (and later trees) which are more suitable to grow under limited water resources.

Our nursery test and the final analysis of the trial data provide strong evidence for the hypothesized directed effects of the seeds maturation environment on the adaptive genetic variation. For all three tree species tested, we found smaller and lighter seedlings if the seed matured in "hot-dry" years as compared to seeds from "cold-humid" years. Also, we found that the share of the seedling roots on the total seedling size was higher for seeds matured in hot-dry years. Since the reduced precipitation treatment also resulted – via plastic seedling response – into smaller seedlings with longer roots and a higher root portion, these data suggest that the pollination/maturation climate helped to preadapt and shape the next tree generation towards better resistance to warmer climate conditions. The observed effects of the seed maturation climate on the performance of the progenies can be well compared to the so called "priming" effect, which describes the phenomenon that plants that experience some kind of biotic (e.g. pest attacs) or abiotic stresses for the first time will make the plants more resistant to future stress exposures (e.g. Bruce et al. 2007). The advantage of "primed" plants is that they can show a stronger or faster response to the respective stress factor and thus an enhanced protection. However, while such "priming" concerns the memory of the respective plant (e.g. Goh et al., 2003) and can also be inherited to the next generation, our data show that stress effects during seed maturation have an effect on the offspring generation. In addition to the significant effects of the maturation climate on trait means, we also found significant effects on the plasticity of the seedlings for Norway spruce and Scots pine. For these two species, seeds from cold-humid maturation years resulted in seedlings with higher plasticity in comparison to seeds matured under hot-dry conditions. Similar effects of epigenetic variation on phenotypic plasticity have already been described for *Arabidopsis thaliana* (Zhang et al. 2013). Our results suggest that seeds matured in hot-dry years are preactivated to potential water stress conditions: this has positive effects for the survival within stressful environments, but also negative effects for growth potential within "better" and more optimal growing conditions.

The results of the present project can be transferred into the following lessons:
1. In forest ecosystems with naturally regeneration trees, our results suggest that present tree populations (across generations) within a given forests might have a higher climatic amplitude than previously thought. Even if the adult trees might not be able to cope the ongoing environmental changes, their offspring generation will likely show better resistance to abiotic stress than their parent trees if the seeds matured under climatic conditions similar to the future. Thus, high mortality of the adult tree population might not necessarily mean that the respective populations (i.e. their offspring) are not able to survive future conditions.
2. Within naturally regenerating forests, seedlings that germinated out of the seeds from different maturation years will likely show high variation in adaptive traits depending on the climate of the maturation year. In dependence of the current and future climate conditions, selection

among these seedlings will favour either the more productive seedling from cold-humid years or – under severe stress conditions – the seedlings from hot-dry years. Thus, forest managers should favour natural regeneration out of many (climatic) different maturation years in order to increase the adaptive plasticity of the next tree generation.

3. If forests are regenerated artificially, forest and nursery managers should be aware of the maturation year effect. Generally, the choice of the appropriate provenance and maturation year will make reforestation decisions even more difficult. For drought prone regions (e.g. Eastern Austria, lower elevations) and forest habitats (e.g. south exposed, thin soil layer), seeds from cold-humid maturation years should rather be avoided. On the other hand, for regions where no drought effects are expected, seeds form hot-dry maturation years are only second choice, because such seedlings would seriously limit the productivity of the forest plantation.

4. Seed harvest should be accompanied by assessments (via available weather service data) of the climate conditions in the maturation year. This information would be required to identify potential seed plantation areas. However, the correlations between the seed harvests maturation climate and the adaptive performance of the seedlings drawn within the present pilot study from only 3-4 provenances is not valid enough to allow exact recommendations. Thus, a repeated analysis with more provenances/maturation years of each species is strongly required for prognostic models of potential planting regions. Also, already available datasets (e.g. Holzer 1978) could be reanalysed by considering the climate conditions of the maturation years. Another implementation could be the mixture of seeds from different maturation years – however, such a step would also require changes of the legal rules for reproductive material.

Although the project results do not allow predictive models of the adaptive performance of seedlings with respect to the seed maturation years so far, the overall practical importance should not be underestimated, because Austrian nurseries produce between 25 and 30 million tree seedlings every year. Tree seed production and seed harvest are highly variable among years. Also the maturation year climate varies considerably. For example, Figure 10 gives the total amount of raw unprocessed (including cones, wings, etc.) seed material harvested in Austria between 1997 and 2011. It shows the high variation among years (from 0 kg up to 115 tons) and also demonstrates that singles seed years can dominate for more than a decade. If we consider, that the very productive seed years 2003 was one of the "hottest" years of the younger climate history (with a long drought period in many parts of Austria and Europe), it is very likely that such conditions are able to shape the adaptive performance and productivity of future forest. Thus, a thorough understanding of how these conditions shape our forests and how to use seeds of such years to adapt our forest is strongly required.
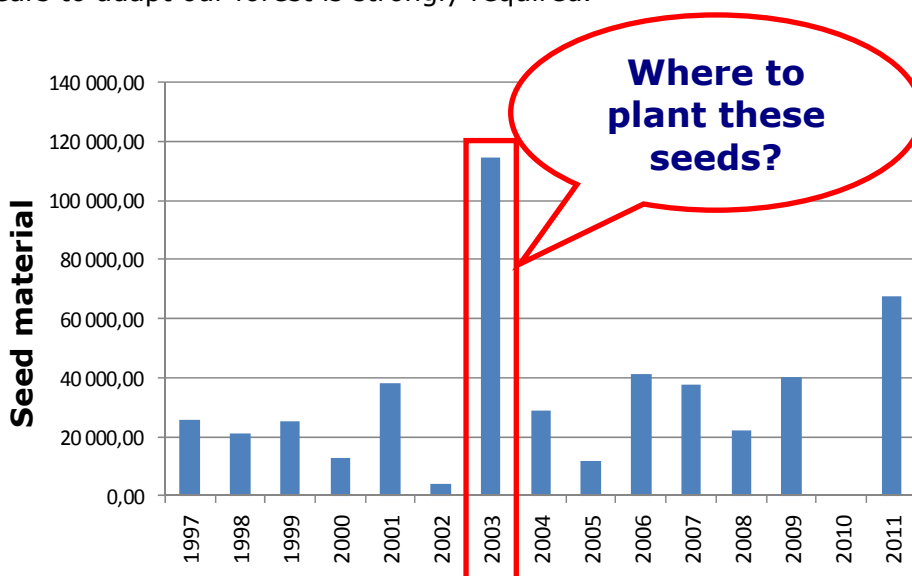

Figure 10. Total amount of raw unprocessed seeds harvested from 1997 to 2011 in Austria for all tree species.

The present experiment could only test conifer seed materials from the decade 2000-2003, because tree seeds from older harvests of the same seed stands were not available and if they would have been available, they might have shown reduced vitality or effects of long term storage. Thus, the seeds tested originate only from a limited number of maturation years and from a limited range of potential climate conditions. Figure 11 shows the climate variation of the larch provenance Lä P11 for the last 40 years. It demonstrates that the climate variation throughout that period is much larger than the climate variation among the tested seed lots of this provenance. Therefore, our study covers only a small part of the potential climate variations that a population experiences and thus suggest that the phenotypic variation we observed is likely also limited. In the long-term – in particular due to climate change – we can expect much higher climate variation and resulting out of this, higher phenotypic variations of tree seedlings and the developing trees.
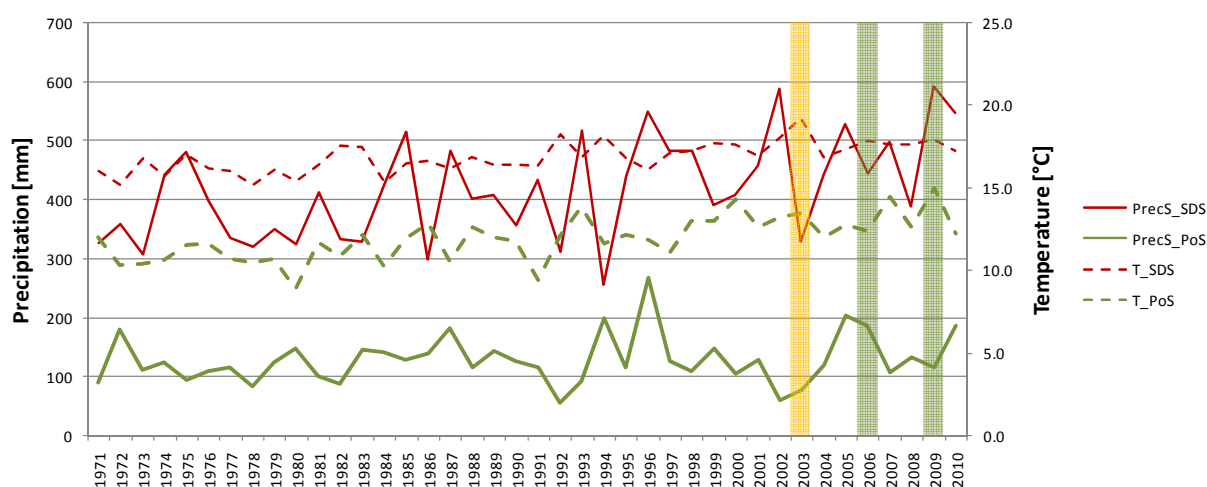

Figure 11. Climate variation for the larch provenance Lä P11. The marked years 2003 (yellow – "hot-dry") and 2006/2009 (green – "cold-humid") depict the two maturation years from which seed material where available and has been used for the present experiment. The graphs show, the climate variation between these three years only covers a very small part of the climate variation observed within the last 40 years. PrecS_SDS – Precipitation sum across the seed development period (SDS); PrecS_PoS - Precipitation sum across the pollination period (PoS); T_SDS – Mean temperature within SDS; T_PoS – Mean temperature within PoS.

6 Methodik

Seed material

Precondition for the realisation of AdaptTree was the availability of seed material from multiple harvests that were undertaken in years with contrasting climate conditions during pollination and seed maturation. The Department of Forest Genetics at the BFW maintains extensive seed storage facilities for the legal control of forest reproductive material. In the present project, we screened the database of this seed bank to identify seed stands (i.e. provenances) that were harvested at least two times within the last ten years. Thereafter, we merged this information with climate data of neighbouring ZAMG climate stations and identified seed provenances that were harvested both in years with higher temperatures and lower precipitation from April to September and in years with lower temperatures and higher precipitation during these months. April to September represent the pollination and seed development period of the three selected tree species Norway spruce (*Picea abies*), European larch (*Larix decidua*), and Scots pine (*Pinus sylvestris*). After testing the viability of the available seeds and quantifying the amounts of seed available, 10 provenances were found to be suited for the experiment. From 9 provenances seeds were available from two maturation years and for one provenance seeds were available from 3 maturation years. In total, this resulted in 21 seed lots to be tested (Fig. 12).
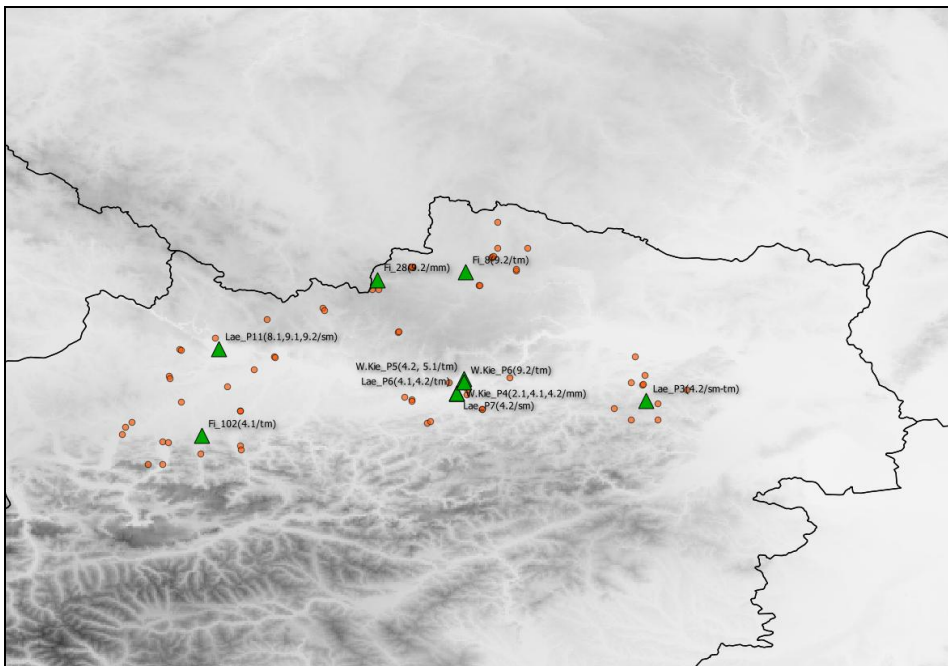

Fig. 12. Geographic location of the provenances (green triangle) and the surrounding climate station from which the weather conditions during pollination and seed maturation were obtained. See Table S6 for full list of climate stations.

Nursery experiment

After the seed lots were finally selected, nursery beds and precipitation reduction has been installed. The aim of the precipitation reduction was to provide growing conditions under two different environments for the seedlings in order to quantify the differential behavior of seedlings as a measure for drought sensitivity. In our construction, we used V-shaped aluminum profiles for the dewatering of the water deficit treatment. The profiles were fixed exactly in the distance of the V-profile basis, leading to a theoretical precipitation reduction of 50%. However, besides the precipitation reduction, the aluminum profiles also shade the nursery bed. In order to create

similar shading for the "normal" treatment, the same V-profiles were used in the opposite direction above the plant beds.

The precipitation reduction and the seed beds were installed in April 2011. In order to allow a simple handling during cultivation, measurements and maintenance and to protect the beds from mice and moles, elevated seed beds were constructed. In total, four seed beds were built, two of which contain the reduced precipitation and two with normal precipitation patterns. This design allowed repeating the sawing of each seed lot in each of the treatments and to estimate effects of the within treatment variation. On each seed bed, with a total plantation area of 6x1m 63 seed rows in a distance of 9.2 cm were marked, and each seed lot was sown in three neighbouring rows. The order of seed lots within the single raised beds randomised. Seeds were sown on May 16 and 17, 2011.

Starting in spring 2011, the technical care of the experiment included the manual weed control, the protection against mice, birds, ants and fungi, and - if necessary – an additive irrigation. Additive irrigation was necessary because the seed beds only contain 25-30 cm soil layer, which may dry out completely in periods without rain. However, we were interested in the survival of the seedlings until the end of the second vegetation period at least. Also, during germination (approximately until the end of June 2011), the seed beds were regularly irrigated, to guaranty the germination of the seeds and the initial seedling growth. The additive irrigation scheme followed the two treatment systems: seed beds with the normal water treatment received the double amount of water than the reduced water treatment by controlling for the irrigation time of a fixed irrigation system. Together with the precipitation reduction, the additive irrigation resulted in significantly lower soil water content in the 50% precipitation treatment then in the 100% treatment. In spring 2012, seed beds were fertilized in order to avoid undesirable interactions between nutrient limitation and water supply.

Trait measurement and data analysis

At the end of the first and the second vegetation period, one-, respectively two-year old seedlings (between 20-24 seedlings per provenance) were removed for measurements of quantitative traits (see Table 9).

The phenological monitoring started directly after sowing and included observations of germination, bud set (in late summer and autumn) and bud burst (in spring).

In preparation of the drought experiments, one-year old seedlings were removed after one vegetation period in March 2012 and planted in seedling containers (10 x 10 cm). After acclimatization of the seedlings two drought experiments were undertaken, one in spring 2013 and the other in Summer 2013.

The frost hardiness of the seedlings was tested on needles from two year old seedlings in February 2013 for Norway spruce and Scots pine and in April 2013 for European Larch using the relative electrolyte leakage method (REL) according to Strimbeck et al. (2007). For this method, needles from the current year (2-10) were sampled from 10 individuals of each provenances/treatment and cut into pieces of approximately 0.5 cm. The 10 individuals were combined into a mixed sample and subsequently divided into nine subsamples subjected to different temperature levels for 1 h (+4°C, -2°C, -8°C, -14°C, -20°C, -26°C, -32°C, -40°C, -196°C (liquid N)) using a controlled environment chamber (Binder climate chamber MK 53)

All quantitative and phenological traits were analysed in two steps: First, for each single trait, comparisons between the trait means of the two maturation years of each seed lot were inferred by series of independent t-tests. Secondly, general linear models were used to test for the effects of the pollination and seed maturation climate on seedling performance for each single trait and on the three first components of the trait spectrum as revealed with principal component analysis. Since the climate conditions during pollination and seed development of the two groups of maturation years varied among the different provenances these linear models allow an uncategorial assessment of climate effects by considering the absolute climatic differences among maturation years and provenances as well. Fig. 2 gives the average conditions of temperature and precipitation during the periods of pollination and seed development for each provenance within the analysed 2-3 maturation years: For example, the spruce provenance Fi 102 received higher amounts of precipitation during seed development (right figure) in its "hot-dry" maturation year

(black-filled symbol) then the provenance Fi8 in its "cold-humid" maturation year (white-filled symbol). The analysis of the quantitative data in linear models allows to regognize these differences among maturation years and provenances. In the linear models, each tree species was treated separately.

Pollen records from a pollen monitoring network (Litschauer et al., 2012, Geburek et al., 2012) were used to identify the exact pollination season within the respective maturation years. For these pollination seasons and for the subsequent seed development seasons, climate data (mean temperature, precipitations sum, and Lang's moisture index) were obtained from the nearest ZAMG climate stations (Fig. 12). Norway spruce and Scots pine flowered from approximately end of April until mid of May and thus the assignment of maturation years into hot-dry and humid-cold years followed the original classification. However, European larch already flowered from late March until early April and thus the climate variables for the pollination season and the seed developmental season resulted into a reclassification of the maturation years of the larch provenance P3 according to Lang's moisture index[1] LI2_SDS. For all three species, the exact climate conditions in the maturation years were used a continuous variables within general linear models and thus helped to consider the true climate variation.

Table 9: List of the measured quantitative parameters (including the measurement units) throughout the seedling experiment

	2011		2012		2013	
	Trait	N	Trait	N	Trait	N
Quantitative traits						
Total weight (g)	TW	20	TW	24		
Needle weight (g)	NW	20	NW	24		
Root collar diameter (mm)	RCD	20	RCD	24		
Total length (mm)	TL	20	TL	24		
Slenderness RCD-TL Ratio	RCD-TL	20	Rat_TL-RCD	24		
Stem area with needles (mm)	SN	20	SN	24		
Ratio SN-TL	SN-TL	20	Rat_SN-TL	24		
Height (above ground) (mm)	H	20	H	24		
Root length (mm)	RL	20	RL	24		
Ratio RL-TL	RL-TL	20	Rat_RL-TL	24		
Lenght of the 1st longest side root (mm)	SR1_L	20	-	24		
Lenght of the 2st longest side root (mm)	SR2_L	20	-	24		
Lenght of the 3st longest side root (mm)	SR3_L	20	-	24		
Sum of the 3 longest side roots (mm)	SR1-3_L	20	-	24		
Number of side root longer than 2 cm (2011), and 5 cm (2012)	N_SR2cm	20	N_SR5cm	24		
Root branching type (1-3)	RootTyp	20	RootTyp	24		
Stem branching type (1-3)	StemTyp	20	StemTyp	24		
Phenological traits						
Germination velocity and rate	GS	103				
Bud set	BS	20		24		
Bud burst (Needle unfolding)			BB	24	BB	24
Specific tests						
Frost hardiness					FrDS	10 (mixed)
Drought resistence spring					DRSp	16
Drought resistence summer					DRSu	14

Two types of linear models were tested: first, we tested for differences between provenances and maturation years individually for each treatment. Here, the provenance and the repetition were considered as categorical variables and the climate data as continuous variables. These models also allowed for interactions between climate variables and provenances. The second models tested for differences between treatments and for the plasticity of the seed lots. Here, treatment, provenance, and repetition were considered as categorical variables and climate as continuous variable. The model also allowed for the following interactions: provenance x climate, treatment x provenance as well as treatment x climate. The latter was of highest interest, because if such interaction were found to be significant, they can be interpreted as a plastic response of the

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[1] Lang's moisture index (see Ingerpuu & Kupper 2007) has been modified to account for the short and variable length of the pollination and seed developmental seasons: $LI\_PoS=1000*(P_{days}/N_{days})*(P_{sum}/N_{days})/T_{mean}$; $LI2\_SDS=P_{days}*P_{sum}/e^{(Tmean/4)}$. Both indices were used as continuous variables in the GLM model, but only LI2_SDS was used for the classification of maturation years into hot-dry and humid-cold years.

(epi)-genotypes to the treatment conditions. To quantify the amount of plasticity and to compare the plasticity of the maturation years, linear regression models were applied, where the treatments (50% and 100% precipitation) were used as independent variable and the trait means as dependent variables. The slope of each regression model is a measure of plasticity and the 95% confidence limits of slope were used to compare the maturation years. Due to multiple testing for different traits on the same climate variables, the p-values of the obtained models were corrected by sequential Bonferonni correction.

7 Arbeits- und Zeitplan

	Workpackages	Actions	2011				2012				2013				2014	
			1.Qu.	2.Qu.	3.Qu.	4.Qu.	1.Qu.	2.Qu.	3.Qu.	4.Qu.	1.Qu.	2.Qu.	3.Qu.	4.Qu.	1.Qu.	2.Qu.
1	Preparatory phase	• Testing the viability of the seed lots in vitro in the seed laboratory	X													
		• Construction of the precipitation reduction according to Fig. 2	X													
		• Set-up of the seed beds and sawing	X													
2	Experiment & Trait analysis	• Realisation of the nursery trial		X	X	X	X	X	X	X						
		• Technical care for the seedlings of the differnet seed lots		X	X	X	X	X	X	X						
		• Phenological monitoring		X	X	X	X	X	X	X		X				
		• Measurements of various seedling traits at the end of the first and second vegetation		X	X	X	X	X	X	X	X	X				
		• Tests for drought and frost resistance									X	X				
3	Data analysis & Project reporting	• Statistical analysis of the phenological data, the quantitative measurements and the results of the drought and freezing test									X	X	X	X	X	X
		• Preparation of scientific publications									X	X	X	X	X	X
		• Development of adaptation measures on basis of the result									X	X	X	X	X	X
		• Writing of the final report									X	X	X	X	X	X

██ - Activity finished

Diese Projektbeschreibung wurde von der Fördernehmerin/dem Fördernehmer erstellt. Für die Richtigkeit, Vollständigkeit und Aktualität der Inhalte übernimmt der Klima- und Energiefonds keine Haftung.

8 Publikationen und Disseminierungsaktivitäten

Poster presentations:
- Schüler, S., Franner, T. Thalmayr, T. 2012. **Natürliche Anpassung von Bäumen an den Klimawandel: welche Rolle spielt die epigenetische Variation des Klimaresponse von Sämlingen?** 13. Österreichischer Klimatag, Universität für Bodenkultur, Wien. 14 – 15 Juni 2012.
- Schüler, S. **Annual climate variation shapes adaptive seedling characters - A contribution to the adaptation of trees to future climates?** Poster to be presented at the 24th IUFRO World Congress, Salt Lake City, 5 – 11 October 2014.

Oral presentations:
- Schüler, S., Kapeller, S. (2013) **Effects of weather conditions during seed maturation on the adaptive performance of seedlings - A contribution to the adaptation of trees to future climates** ClimTree 2013: International Conference on Climate Change and Tree Responses in Central European Forests. Zurich, Switzerland, 1 – 5 September 2013
- Schüler, S., Kapeller, S., Franner, T. Thalmayr, T. (2014) **Zur Anpassung von Waldbäumen an den Klimawandel oder wie das Wetter während Blüte und Samenreifung das Wachstum und die Stresstoleranz der nachfolgenden Baumgeneration beeinflusst.** 15. Österreichischer Klimatag, Universität Innsbruck. 2 - 4 April 2014.

Dissemination activities:

- Schüler, S., **AdaptTree - Die Bedeutung epigenetischer Variation für die natürliche Anpassung von Bäumen an den Klimawandel.** In ACRP in Essence. Berichte zur Klimafolgenforschung 2014 Forstwirtschaft. S.21-26.

Scientific publications in preparation:
- Schüler, S. (in preparation) **Interannual climate variation affects the plasticity and variation of adaptive traits in coniferous trees: a chance for rapid adaptation to global warming.**
- Schüler, S., Kapeller, S. (in preparation) **Drought resistance of coniferous trees as influenced by provenance and the climate of the seed maturation year.**

PhD-thesis:
Some results of the project will be part of the PhD- thesis of Stefan Kapeller ("Adaptive genetic diversity of forest trees in climate change" – draft title) to be submitted 2015 to the BOKU University of Natural Resources and Applied Life Sciences Vienna.

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