Impact of genetic variation and long-term limited water availability on the ecophysiology of young Sitka spruce (*Picea sitchensis* (Bong.) Carr.)

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Abstract

Future limited water availability may reduce the potential of tree improvement to increase timber yields. We investigated ecophysiological variation between full-sibling families of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) growing under contrasting water availability conditions: control (optimal) water availability and limited water availability. One-year old seedlings of nine improved families plus an unimproved seed lot were grown in pots in a greenhouse and the two water availability treatments imposed via drip irrigation. Whole plant water use varied between families. Stomatal conductance and the light-saturated quantum yield of photosystem II at times differed between families, but not consistently. Certain families showed considerably greater increases in Electron Transport Rate with increasing photosynthetically active radiation. Limited water availability resulted in reduced branch water potential, leaf stomatal conductance, and transpiration per unit leaf area, and increased whole plant water use efficiency, in all genetic material. The responses of plant water use and leaf carbon isotope composition to water-limitation, were, however, initially influenced by variation in vigour between families – with conservative growth in some material slowing the decline in substrate moisture content. As the duration of water deficit extended, these variables showed a more uniform response across families. Between-family variation in physiological mechanisms of drought tolerance was not detected. Thus, for Sitka spruce, assessing juvenile material may not allow selection to prevent reductions in productivity associated with long-term sub-optimal growing conditions, but screening for conservative growth (within families as well as between families) may be beneficial where survival of relatively short-term water-limitation is the primary concern.
Introduction

Globally, water is the key limiting factor for forest tree growth (Alfaro et al. 2014). The severity and duration of this limitation will be exacerbated by climate change. Prediction of future forest productivity and capacity to cope with changing climatic conditions requires a better understanding of ecophysiological responses to drought (Hartl-Meier et al. 2015). Tree improvement programmes can increase forest productivity, but the impact of drought or limited water availability on new material being developed in such programmes must be considered (Attia et al. 2015).

Sitka spruce [Picea sitchensis (Bong.) Carr.] is the main forestry plantation species in Ireland and the UK, and is of some commercial value in a few other European countries (Lee et al. 2013). The Irish Sitka spruce improvement programme has focused on selecting genetic material with good quality (straight stems, relatively little branching) and also vigorous growth (without reduction in wood density), giving higher yields (Thompson 2013). The mild, wet, climate in Ireland is particularly suited to planting Sitka spruce, an oceanic species growing in its native range only in areas with over 1,000 mm rainfall per annum (Lee et al. 2013). Ireland, however, is projected to become warmer, with more damaging extreme events (IPCC-WGI 2013), and, by the 2080s, a 40% reduction in summer rainfall in the south-east (Sweeney et al. 2008). In Scotland, low annual rainfall in eastern parts already limits the planting of Sitka spruce (Cameron 2015), and stem crack and tree death have been reported as a result of recent very dry summers (Green et al. 2008). The more vigorous material produced as a result of genetic improvement programmes may be expected to need more water, and may therefore suffer more than current unimproved planting stock under drier conditions. Both theoretical (Mäkelä et al. 1996) and experimental (Bourne et al. 2015, Zhou et al. 2014) approaches suggest greater sensitivity to water-limitation in trees adapted to
mesic environments compared with those from xeric environments. Experimental investigations of the response of Sitka spruce to limited water availability have, however, been confined to the very short (Black et al. 2005) or medium term (Townend 1993) withholding of water. It has been long-known that plant responses to short-term rapid imposition of stress can differ drastically from responses to a slow decline in water availability, maintained over the long-term (e.g. Bray 1993). The objective of this work was to explore the impacts of interaction of water availability and genetics on the ecophysiology of young Sitka spruce, by comparing genetically diverse seedlings from year two to four of growth under slowly imposed limited water availability, compared to optimal water availability.

Materials and methods

Experimental design

The experiment used a two-factor design, with the factors being genetic background and water availability. With respect to genetic background (hereafter referred to as ‘family’), nine full-sibling families (Table 1) of Sitka spruce [Picea sitchensis (Bong.) Carr.], and an unimproved seed lot (collected from natural stands growing in the Forks region, and referred to here as the Washington control, WC), were compared. The full-seedling families were developed within the Irish Sitka spruce breeding programme, and were classified as vigorous, intermediate, or slow-growing on the basis of their heights at the start of the experiment. They will be referred to according to their allocated vigour code e.g. V1 was the tallest family. With respect to water availability, control and limited water availability were compared. In the control (optimal) water availability treatment, all transpired water was replaced. In the limited water availability treatment, seedlings were provided with less water than they transpired, creating a water deficit. Family and water availability were allocated.
randomly to each of 100 plots. Thus, each combination of family \(\times\) water availability treatment was replicated in five plots.

**Plant material and growing conditions**

Seeds representing each full-sibling family and the WC were germinated at the same time in seed trays in a greenhouse in spring 2011. In July 2012, seedlings were planted in 2 L pots in a peat:sand mix (2:1), with controlled release fertiliser (Osmocote Exact Patterned Release Fertiliser, 5.5 g L\(^{-1}\)) incorporated during potting, and transferred to a greenhouse at the National Tree Improvement Centre in Kilmacurra, Co. Wicklow (52°56'3'' N, 6°9'10'' W), 58 km south of Dublin. They were arranged in plots of nine pots on greenhouse benches, with each plot consisting of seedlings (one per pot) of one family. Seedlings were potted up to 10 L pots prior to spring 2014. To allow for the larger pots and wider branches, plant numbers were reduced to four seedlings per plot at this stage. One seedling per plot (initially the central seedling) was selected each year for ecophysiological measurements.

**Whole plant water use**

The growing substrate in each pot was covered with black plastic, to prevent evaporation from the substrate. To determine whole plant water use, each selected pot was weighed two days in a row (one hour after irrigation the first day, to ensure no transient water deficit in the control irrigation treatment, yet allow sufficient time for excess water to run through the pores in the base of the pot, and before irrigation the second day). In parallel, wet leaf depression was measured with a sensor and meter (Evaposensor and Evapometer, respectively, Skye Instruments Ltd., Powys, UK) placed within the canopy. This sensor measures accumulated °C h which is proportional to plant transpiration: 1 °C h equates to a difference in temperature between the wet and dry artificial leaves on the sensor of 1°C for a
period of 1 h. Plant water use divided by the accumulated °C h over the same duration
provided water use per °C h for each selected tree. Multiplying this calibration factor by the
accumulated °C h each day (the Evaposensor logged data continuously throughout the
experiment) allowed daily water use in mL to be estimated for the plant. The relationship
between water use and °C h however increases as a plant grows: therefore the calibration
factor for each selected tree was determined, as above, at frequent intervals during each
growing season. Using the calibration factors and the Evaposensor readings of daily
accumulated °C h, water use was estimated for each selected tree for every day of the
experiment – an approach used in several previous studies (Davies et al. 2016, Grant et al.
2012b, Šurbanovski et al. 2013). Total whole plant water use within a year was estimated as
the sum of all the daily water use values between terminal bud flush (when needles emerged
from the terminal bud) and harvest.

**Irrigation**

A drip irrigation system was installed with two irrigation valves, one controlling either water
availability treatment (optimal vs. limited); each pot was supplied with water via a pressure-
compensated drip nozzle. Plant water use per °C h (see above) of the greatest water using
individual at any given time was used as an irrigation factor for the control irrigation
treatment: it was multiplied by the accumulated °C h since the last irrigation event to give
actual water use since the last irrigation. This value was then divided by the irrigation output
of the nozzles per second, to determine the duration of irrigation required to replace water
used by these highest water users. This value was inserted into a timer (MCI-16, Heron
Electric Company Ltd., Apopka, FL, USA), which automatically triggered the irrigation
system daily at 11 h. While lower water-users will have received more water than they
transpired, the excess water ran through the base of the pot and therefore there was no risk of
For the water-limited treatment, the average water use of WC trees growing under control irrigation per °C h was multiplied by 0.3 to obtain the required irrigation factor. This was multiplied by the accumulated °C h since the last irrigation event and divided by the irrigation output of the nozzles per second, to determine the duration of irrigation required to replace 30% of the water used by WC under control irrigation, and this value input into the timer, to control the valve used to irrigate water-limited trees. Thus, in the water-limited treatment, irrigation was applied to match 30% of that lost in transpiration by the well-watered Washington control – a severe limitation to ensure any differences that exist between families in response to water-limitation would be detected. As a result, the WC seedlings growing in the water-limited treatment received irrigation to match 30% crop evapotranspiration, whereas more vigorous material with greater transpiration received a lower percentage of its crop evapotranspiration. This reflects that limited rainfall in forests results in a greater water deficit for greater water users.

Substrate moisture content

Volumetric moisture content (θ) of the growing media was measured with a hand-held soil moisture sensor and meter (Theta Probe ML2x and Theta Meter HH1, respectively, Delta-T Devices Ltd, Cambridge, UK), in the pot of each selected seedling, at frequent intervals during each growing season.

Stomatal conductance and leaf transpiration

Stomatal conductance ($g_s$) of a group of needles sufficient to cover the aperture of a porometer (Leaf Porometer, Decagon Devices, Pullman, WA, USA) was measured for each selected seedling at frequent intervals. The porometer’s sensor head was placed over the group of needles. The porometer is appropriate for measurement of narrow leaves, requiring
no recalculation of area, as the entire aperture is covered. However, due to the shortness of Sitka spruce needles, it was only possible to use this procedure on needles at the tips of branches. Exposed first order branches near the top of the section of the stem that developed the previous year were always used; one branch was measured per seedling, to allow 100 plots to be assessed within a reasonable duration. Measurements were conducted around solar noon.

Transpiration per unit leaf area ($E$) was measured with an infra-red gas analyser (IRGA) in September 2013. The purpose of this was to validate porometric and gravimetric measurements, and was not repeated due to the difficulty of estimating leaf area in the chamber for Sitka spruce. Branch tips were placed in an IRGA chamber (CIRAS1, PP Systems, Amesbury, MA). Gas exchange was measured under ambient light and air temperature. Before removing the branch tip, the branch within the chamber head was photographed through the Perspex chamber cover. Photos were printed and the diameter of the circular area which contains the measured leaves and is exposed to light during recording of gas exchange variables was measured. The leaves in the photos were cut out and their area measured with a leaf area meter (LI-3000, LiCOR Inc., Lincoln, Nebraska, USA). The results were calibrated against the known area of the circular measurement area of the chamber, to determine actual leaf area in the chamber. This leaf area was input into the CIRAS software to recalculate gas-exchange variables including $E$.

**Water potential**

Water potential ($\Psi$) of one branch (selected as for $g_s$) per seedling was measured at solar noon once or twice per year using a pressure chamber (SKPM 1400, Skye Instruments Ltd.).

**Leaf chlorophyll a fluorescence**
Maximal photochemical efficiency of photosystem (PS) II (Fv/Fm, where Fv is variable fluorescence, and Fm is maximum fluorescence in the dark-adapted state) was measured around midday on a few occasions during each summer, using a fluorometer (Mini-PAM, H. Walz, Effeltrich, Germany) in groups of needles (as for gs) which had been dark-adapted in a leaf clip. Fv = Fm – F0, where F0 is basal fluorescence. F0 was measured using weak light (<< 0.15 μmol m⁻² s⁻¹), and Fm was determined following a 0.8 s saturating pulse. Light-saturated quantum yield of PS II (ΦPSII), where

\[ Φ_{PSII} = (F_{M}' - F_{i}) / F_{M}' \] (Genty et al. 1989),

and Fm’ is the maximal fluorescence of the illuminated sample and Fi is the momentary fluorescence level of the sample measured before the saturating pulse, was measured at intervals during each summer following 5 s of actinic light of 460 μmol m⁻² s⁻¹ (to remove variability due to fluctuating cloud cover).

Rapid light curves were conducted in October 2012, by increasing incident PAR through six increments from 87 to 658 μmol m⁻² s⁻¹. For the light curves, leaves were exposed to each PAR level for 1 min prior to measurement of ΦPSII. Electron transport rate (ETR) was calculated as

\[ ETR = Φ_{PSII} × 0.5 × 0.84, \]

where 84% of incident light on a leaf is assumed to be absorbed and 50% of energy is assumed to be partitioned to photosystem II (Maxwell and Johnson 2000). Light-saturation was considered to have occurred when further increases in PAR did not result in a significant increase in ETR.
In December 2013 and 2014, several needles near the tips of each of three exposed first order branches from the previous year were plucked and brought back to the laboratory in 1.5 ml Eppendorf tubes, dried at air temperature, and milled to a fine powder in a ball mill (MM2 Pulveriser Mixer Mill, Retsch, Haan, Germany) for 5 minutes at 70 rpm. Samples (1 mg) were analysed for carbon content and for carbon isotope composition, $\delta^{13}C$, by continuous-flow isotope-ratio mass spectrometry (OEA Laboratories Ltd., Callington, Cornwall, UK), where

$$\delta = \frac{[^{13}\text{C}]_{\text{leaf sample}}}{[^{13}\text{C}]_{\text{standard}}} - 1.$$

Additionally, in 2013, oxygen isotope composition ($\delta^{18}O$) of the samples from the two families showing highest and lowest $\delta^{13}C$ in the well-watered treatment was compared across the two water availability treatments. Pee Dee Belemnite, and Vienna Standard Mean Oceanic Water are used as the standard references in calculation of $\delta^{13}C$ and $\delta^{18}O$, respectively.

Seedling heights were measured at the start of the experiment (August 2012), and annually thereafter. The selected seedlings were harvested annually. Roots were washed, and total dry mass obtained after drying the entire seedling in an oven at 80°C for 48 h prior to weighing. The increase in biomass over a growing season of an individual was estimated by subtracting the average total dry mass at the end of the previous year for that family $\times$ water availability.
Whole plant water use efficiency (WUE) was calculated as the estimated increase in biomass divided by the estimated water use over the same duration.

Data analyses

Effects of genetic origin (‘family’) and water availability, or an interaction between these two factors, were assessed using ANOVA. Analysis of variance of repeated measures (ANOVAR) was used where measurements were repeated along the season, to take into account that the same plants were measured throughout. Where Mauchly's test returned $P > 0.05$, sphericity was assumed; otherwise Greenhouse-Geisser corrections were used to determine the significance of within-subjects effects. Analysis of Co-Variance of repeated measures (ANCOVAR) was used to determine whether certain results were significant, taking into account variation in another variable e.g. initial height. The significance of correlations between different variables was determined by Pearson correlation analysis. Data were analysed using SPSS software (version 20, IBM).

Results

Water use

Whole plant water use was significantly reduced for water-limited compared to control seedlings as early as four days after the start of the treatments in 2012, and continued to fall over the rest of that growing season (Table 2, Figure 1). While on average the difference between well-watered and water-limited seedlings was not as large at the start of the next growing season (2013), for most families water use of water-limited seedlings relative to that of well-watered seedlings declined, albeit more gradually, through 2013. In May, water use
of some families such as S was relatively unaffected by water-limitation, whereas water use of water-limited V4 was only 21% of that of its well-watered siblings (Figure 1). Over the season as a whole, the effect of water availability was highly significant (Table 2), irrespective of family. In 2014, on average over the season, water use of S was not significantly reduced by water-limitation, whereas water-limitation significantly reduced water use of all other improved families and the Washington control.

Family impacted on water use throughout the experiment (Table 2), interacting with date throughout and also with water availability in both 2012 and 2014. At the start of the experiment, water use of S was relatively low (Figure 1), but differences between families were less clear in September 2012 or during 2013. In 2014, under control irrigation water use of V4 was high relative to S and WC.

Transpiration per unit leaf area ($E$) was only measured once, in September 2013, using an IRGA. A highly significant impact of water availability was found (Table 3), with transpiration per unit leaf area more than halved in water-limited seedlings (Table 4). No effect of family and no interaction between family and water availability was detected, suggesting that differences between families in whole plant water use at this stage related entirely to differences in plant size (Table 1, Figure 2A, B). Indeed, in 2013 when height at the start of the year was used as a covariate, analysis of whole-plant water use over the year no longer showed a significant effect of family (Table 5), although there was a significant interaction of family and water availability. In 2014, using height at the start of the year as a covariate in the analysis of whole-plant water use during the year, only water availability, date, and the interaction of water availability and date were significant.

Under control irrigation, estimated total water use was strongly positively correlated with total dry mass at the end of the year, in both 2013 and 2014 (Figure 2C). When water-limited, however, no significant correlation was found in 2013, and the correlation in 2014...
was weak (Figure 2D). Whole plant water use efficiency (WUE) in terms of biomass produced per unit of water transpired was significantly increased as a result of water-limitation (Tables 3, Figure 2E, F), in both 2013 and 2014. In 2013 WUE was doubled as a result of water-limitation. The following year, the increase in WUE due to water-limitation was not as great, but still considerable. Family also significantly affected WUE (Table 3). S showed relatively low WUE both years, but between-family variation did not consistently correspond with differences in vigour.

Substrate drying

Substrate θ fell rapidly in water-limited pots of families such as V3 (Figure 3). On the other hand, for S and I2, substrate θ in pots in the water-limited treatment remained similar to that in well-watered pots through 2012, as a result of relatively low water use (see above). Even for S, however, substrate θ fell during 2013 (Figure 3), and remained low in all pots under water-limitation through 2014 (data not shown). Substrate θ on average over 2012 showed a significant interaction between family and water availability, which was lost in 2013, but re-emerged during the final year of the experiment (Table 2).

Stomatal conductance and water potential

Leaf gs differed significantly between families in 2012 (Table 2, Figure 4A), with V4 showing the highest and S showing the lowest gs on average, but the pattern varied over time, as shown by a significant interaction between family and date. Relatively low gs in leaves of S reflects the relatively low water use of this family – although the small size of S trees (Table 1) will have had a greater impact on whole plant water use. V4, in contrast, showed relatively high gs. This variation in gs between families was not seen the following two years.
(Table 2), whereas variation in whole plant water use was maintained (Table 2, Figure 1), reflecting that the initial divergence in plant size was maintained (and, indeed, accentuated) as the seedlings grew (Figure 2A, B).

Water availability did not significantly impact on leaf g, in 2012 (another discrepancy between leaf g, and whole plant water use; Table 2), but by June 2013 g, was significantly and considerably reduced in response to water-limitation – a situation that was maintained through the 2013 and 2014 growing seasons (Table 2, Figure 4B). Results from porometry throughout 2013 were consistent with results for E measured in September 2013 – in both cases only water availability had a significant impact, not family. Midday branch Ψ was significantly reduced (Tables 3, 4) in the water-limited treatment by April 2013, falling gradually through June and July that year.

329 **Chlorophyll a fluorescence**

Fv/Fm was not significantly reduced by water-limitation (data not shown). On average over eight measurements from early June until late September 2013, ΦPSII showed a significant, but mild, reduction in water-limited seedlings (Table 2, Figure 5A). The magnitude of the difference between treatments increased the following year, when there was also a significant impact of family, but no interaction of family and water availability (Table 2, Figure 5B). On average, V3 showed the highest ΦPSII during 2014. WC showed the lowest ΦPSII during 2014, on average – though not significantly lower than S.

Repeated measures analysis of rapid light response curves in October 2012 indicated a significant interaction of PAR and family (Table 6, Figure 6) on ETR. S was saturated at lower PAR (292 μmol m⁻² s⁻¹) than the Washington control and other improved families. V1, V2, V3, and V4 were not saturated until 658 μmol m⁻² s⁻¹, while the Washington control and remaining improved families showed light-saturation at 439 μmol m⁻² s⁻¹. Those families
which did not achieve light-saturation until the highest measured level were also those, along with V6, that showed the highest ETRs at all levels from 130 μmol m$^{-2}$ s$^{-1}$ upwards. From this PAR upwards, ETR of S was significantly lower than that of the Washington control or any other family.

**Leaf isotope composition**

In needles produced during 2013 and harvested at the end of the year, $\delta^{13}C$ showed a significant interaction between family and water availability (Table 3, Figure 7A). Water-limitation led to an increase in $\delta^{13}C$ of leaves harvested at the end of 2013, for all families except S. The largest increases (of greater than 3.5‰) were for families V1, V6 and V5. In the water-limited treatment, $\delta^{13}C$ of needles from S was significantly lower than that from all families except I2 and the Washington control. In contrast, there was no interaction between family and water availability with respect to leaves that developed during 2014, with water-limitation leading to a significant increase in $\delta^{13}C$ irrespective of family (Tables 3, 4).

In both years, $\delta^{13}C$ showed a hyperbolic decay relationship with average g$_s$ measured over the growing season [$\delta^{13}C = -31.736 + 590.016/(40.576 + g_s), F = 79.851$ in 2013; $\delta^{13}C = -32.591 + 506.313/(17.158 + g_s), F = 35.166$ in 2014; Figure 7B]. $\delta^{18}O$ was significantly increased in the water-limited treatment in 2013 (Tables 3, 4) and $\delta^{13}C$ showed an increase as $\delta^{18}O$ increased [$\delta^{13}C = 0.125(\delta^{18}O)^2 - 5.31\delta^{18}O + 24.173, F = 32.282$; Figure 7C]. There was no correlation between $\delta^{13}C$ and total dry mass at the end of 2013 under control irrigation, and the following year the correlation was weak (Figure 7D). In the water-limited treatment, in contrast, $\delta^{13}C$ correlated significantly with total dry mass both years (Figure 7E).

**Discussion**
**Between-family variation in ecophysiology**

That initial size varied between families might lead to an expectation that differences in growth are the result of variation in photosynthetic carbon gain – which would be influenced by $g_s$ and $\Phi_{PSII}$. However, $g_s$ only showed differences between families early in the experiment, and there is no evidence of variation between families in transpiration on a leaf area basis – only at the whole plant level, which relates to variation in plant size. Although light curves conducted in 2012 showed clearly contrasting ETR between families, $\Phi_{PSII}$ varied between families only in the final year (2014). The shortest family at the start of the experiment, S, showed relatively low $g_s$ in 2012, relatively low $\Phi_{PSII}$ in 2014, and relatively low ETR even at low PAR. V1, V2, V3, and V4, superior families in terms of height at the start of the experiment, in contrast showed efficiency in using high PAR. This capacity is likely to have been of limited benefit in the greenhouse, where PAR was never very high. In the early stages of afforestation, however, enhanced capacity to use relatively high PAR may be of considerable advantage for maximising use of resources and allowing rapid growth.

Differences between families in $\delta^{13}$C in 2013 were reflected to some extent in whole plant water use efficiency, but whole plant WUE does not always closely reflect $\delta^{13}$C due to within-canopy variation (Medrano et al. 2015). Higher $\delta^{13}$C did not result in water saving under control irrigation, since it was associated with larger trees, which used more water. Differences between families in $\delta^{13}$C were not maintained in 2014.

It is worth noting that considerable variation was observed between individuals within families. Other work from this group (Donnelly et al. 2016) has shown variation in a range of traits between clones derived from the same parental crosses. Individuals with desirable ecophysiological traits could be cloned, and used in future afforestation. Future research
could investigate contrasting individuals, rather than families, potentially revealing more distinct performance strategies.

**Ecophysiological responses to water-limited**

In response to declining water availability, Sitka spruce seedlings showed a reduction in $g_s$ and whole-plant water use. Reduced $g_s$ led, presumably, to reduced photosynthesis and hence growth. Reduced leader growth combined with reduced branch growth for most families will have reduced total leaf area and thus transpiring surface area, controlling the intensity of stress, such that $\Psi$ fell only gradually.

Increased $\delta^{13}C$ as a result of water-limitation implies an increase in photosynthetic water use efficiency (Farquhar and Richards 1984), as often seen in response to reduced $g_s$ during water stress (e.g. Grant et al. 2012a, 2012b). Correlations both years between $\delta^{13}C$ and total dry mass suggest that isotopic signatures were influenced by variation in plant size, which influenced water demand, as seen in the differing water use rates between families when well-watered. The absence of an interaction between family and water availability for $\delta^{13}C$ in 2014 suggests that while earlier in the experiment the impact of water-limitation was strongly dependent on vigour (as evidenced by larger plants actually using less water when water-limited in 2013, implying a greater reduction of transpiration), as the duration of stress extended, the leaf physiology of all seedlings was more similarly affected. Increased $\delta^{13}C$ in response to water-limitation appears to have been the result of reduced transpiration due to reduced $g_s$, accompanied by increased $\delta^{18}O$, rather than any change in photosynthetic capacity (Barbour and Farquhar 2000). Photoinhibition was not detected. In *P. glauca*, $F_v/F_M$ was found to decline only at water potentials of less than $-1$ MPa (Bigras 2005). In the current study, although photochemistry may have been down-regulated in response to long-term water stress, as suggested by reduced $\Phi_{PSII}$ (which would result in reduced ETR), it is
unlikely that photosynthetic capacity would have been severely impaired. Increased δ¹³C as a result of reduced transpiration also agrees with the increased whole plant water use efficiency detected both years.

Responses in the current study in which a deficit was slowly imposed (as occurs under limited rainfall in forests), differed from those conducted on the same species when water was completely withheld (Black et al. 2005, Townend 1993). For example, the decline in Fv/FM in response to drastically low Ψ found by Black et al. (2005) was not seen here, when Ψ declined gradually – consistent with drought alone not usually resulting in photoinhibition (see Grant and Incoll 2005, Grant et al. 2015 for discussion), and with a reduction in Fv/FM in *P. abies* only being detected when dehydration was relatively advanced (Ditmarová et al. 2010). A complete absence of available soil water is unlikely to occur in Sitka spruce forests, but limited availability, as investigated here, is increasingly likely. Studies of this nature, investigating the impact of relatively long-term, slowly imposed, and relatively mild stress are therefore increasingly important.

**Concluding remarks**

Uniform responses to the severe water-limitation imposed in the current research, in terms of declining gs and Ψ, and a lack of interaction between family and water availability for most traits, suggest that different strategies to cope with water shortage do not exist within the selected material. Lack of variation in the mechanism of response to water-limitation may reflect the limited climatic range of Sitka spruce, in which it is unlikely that resistance to drought will have evolved in the seed material that forms the basis of current tree improvement programmes. Although Bigras (2005) found genotypic variation in response to drought stress in *P. glauca*, a recent study of *P. abies* indicates little between-family variation in response to drought, with all families tending to show a decline in traits such as gs.
Chmura et al. 2016), just as in the current study. In general, heritable mechanisms of resistance to water-limitation might be expected in species that have evolved under drought conditions (Aranda et al. 2015, Grivet et al. 2011, Théroux Rancourt et al. 2015). With species such as Sitka spruce, the absence of such a selection pressure in the past may represent a challenge for current efforts to breed for drought tolerance. It should therefore be acknowledged that while improvement programmes can lead to substantial gains in productivity under optimal conditions (Näsholm et al. 2014), it is unlikely that such gains can be maintained where water availability is limited. Further research is needed, however, into the potential of material of contrasting vigour to recover from water-limitation, and into responses to fluctuating conditions, such as predicted in areas where Sitka spruce is of economic importance. Moreover, more mature material may respond differently, due to different structure and water storage capacity, and should therefore also be investigated.

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Tables

Table 1 Vigour classes and codes assigned to the full-sibling families used in the experiment, which were ranked according to their height at the start of the experiment in August 2012, and total dry mass of harvested seedlings at the end of 2012. Percentage gain of each family over the unimproved (Washington control – WC) material at the start of the experiment is provided in parenthesis. Families are named as female parent × male parent.

<table>
<thead>
<tr>
<th>Family</th>
<th>Height (cm)</th>
<th>Vigour class</th>
<th>Vigour code</th>
<th>Total dry mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 × 190</td>
<td>52.59 ± 1.08 f (+63%)</td>
<td>V1</td>
<td>V1</td>
<td>13.19 ± 1.03 cde</td>
</tr>
<tr>
<td>577 × 500</td>
<td>51.64 ± 1.23 f (+60%)</td>
<td>V2</td>
<td>V2</td>
<td>15.46 ± 2.76 de</td>
</tr>
<tr>
<td>286 × 500</td>
<td>49.15 ± 2.02 e (+53%)</td>
<td>V3</td>
<td>V3</td>
<td>17.09 ± 1.26 e</td>
</tr>
<tr>
<td>574 × 500</td>
<td>48.65 ± 0.63 e (+51%)</td>
<td>V4</td>
<td>V4</td>
<td>16.67 ± 2.01 de</td>
</tr>
<tr>
<td>286 × 519</td>
<td>48.29 ± 1.59 e (+50%)</td>
<td>V5</td>
<td>V5</td>
<td>12.61 ± 1.25 cde</td>
</tr>
<tr>
<td>574 × 542</td>
<td>46.93 ± 1.31 e (+46%)</td>
<td>V6</td>
<td>V6</td>
<td>15.11 ± 2.17 de</td>
</tr>
<tr>
<td>577 × 542</td>
<td>41.31 ± 0.80 d (+28%)</td>
<td>I1</td>
<td>I1</td>
<td>12.03 ± 2.38 cd</td>
</tr>
<tr>
<td>500 × 574</td>
<td>35.03 ± 0.97 c (+9%)</td>
<td>I2</td>
<td>I2</td>
<td>9.87 ± 1.33 bc</td>
</tr>
<tr>
<td>WC</td>
<td>32.22 ± 0.64 b</td>
<td>WC</td>
<td>WC</td>
<td>6.12 ± 0.92 ab</td>
</tr>
<tr>
<td>577 × 519</td>
<td>23.52 ± 0.50 a (–27%)</td>
<td>S</td>
<td>S</td>
<td>3.61 ± 0.25 a</td>
</tr>
</tbody>
</table>

Data are means ± SE of 10 plots, each plot consisting of 9 individuals, except for total dry mass, for which data are means ± SE of 10 individuals. Means with different letters are significantly different to each other (LSD following ANOVA). Water availability did not impact on total dry mass at the end of 2012. Parents 190 and 286 are of Washington provenance, parents 500 and 519 are a mix of Washington (75%) and Queen Charlotte Islands, British Columbia, Canada (25%) provenances, 542 is of unknown provenance, and 574 and 577 are of unknown Irish provenance.
Table 2 ANOVAR results for diverse variables, measured at intervals throughout a season, showing analysis of repeated measures on different dates on the same individuals (within-subjects effects) and analysis of the average responses over those different dates (between-subjects effects).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Year</th>
<th>Date</th>
<th>Date × Family</th>
<th>Date × Water</th>
<th>Date × Family × Water</th>
<th>Error df</th>
<th>Within-subjects effects</th>
<th>Between-subjects effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant water use</td>
<td>2012</td>
<td>14127.378*** (1.012)</td>
<td>6.554*** (9.111)</td>
<td>86.562*** (1.012)</td>
<td>2.876** (9.111)</td>
<td>77.949</td>
<td>5.780*** (9 df)</td>
<td>140.711*** (1 df)</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>6611.003*** (1.020)</td>
<td>2.337* (9.183)</td>
<td>155.438*** (1.020)</td>
<td>ns</td>
<td>81.625</td>
<td>2.139* (1 df)</td>
<td>338.726*** (9 df)</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>205.228*** (1.450)</td>
<td>5.405*** (13.053)</td>
<td>115.758*** (1.450)</td>
<td>3.819*** (13.053)</td>
<td>107.326</td>
<td>5.722*** (1 df)</td>
<td>194.411*** (9 df)</td>
</tr>
<tr>
<td>θ</td>
<td>2012</td>
<td>78.101*** (3.561)</td>
<td>2.011** (32.047)</td>
<td>33.689*** (3.561)</td>
<td>1.823* (32.047)</td>
<td>284.859</td>
<td>4.622*** (3 df)</td>
<td>95.342*** (1 df)</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>32.617*** (3.139)</td>
<td>ns</td>
<td>5.213** (3.139)</td>
<td>1.834* (28.252)</td>
<td>251.127</td>
<td>2.858** (1 df)</td>
<td>153.037*** (9 df)</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>44.880*** (6.311)</td>
<td>1.616* (56.802)</td>
<td>84.701*** (6.311)</td>
<td>1.521* (56.802)</td>
<td>504.903</td>
<td>4.972*** (1 df)</td>
<td>274.616*** (9 df)</td>
</tr>
<tr>
<td>gs</td>
<td>2012</td>
<td>13.534*** (3.512)</td>
<td>1.724* (31.609)</td>
<td>ns</td>
<td>ns</td>
<td>273.948</td>
<td>3.160** (1 df)</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>16.321*** (2.001)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>160.113</td>
<td>ns</td>
<td>46.627*** (1 df)</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>45.124*** (3.407)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>272.570</td>
<td>ns</td>
<td>65.972*** (1 df)</td>
</tr>
<tr>
<td>ΦPSII</td>
<td>2013</td>
<td>44.809*** (5.456)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>420.135</td>
<td>ns</td>
<td>7.080* (1 df)</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>8.888*** (4.197)</td>
<td>ns</td>
<td>2.967* (4.197)</td>
<td>ns</td>
<td>310.548</td>
<td>3.379** (3 df)</td>
<td>18.811*** (1 df)</td>
</tr>
</tbody>
</table>

Where effects are significant, F-values are provided, followed by significance indicated as *, P < 0.05; ** P < 0.01; *** P < 0.001. ns indicates not significant. Degrees of freedom for within-subjects effects vary and are provided in parenthesis. 'Water' refers to Water availability.
Table 3. ANOVA results for diverse variables, measured at single time-points

<table>
<thead>
<tr>
<th>Variable</th>
<th>Date</th>
<th>Family</th>
<th>Water</th>
<th>Family x Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(9 df)</td>
<td>(1 df)</td>
<td>(9 df)</td>
</tr>
<tr>
<td>E</td>
<td>Sept 2013</td>
<td>ns</td>
<td>16.295***</td>
<td>ns</td>
</tr>
<tr>
<td>WUE</td>
<td>2013</td>
<td>3.997***</td>
<td>75.197***</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>2.248*</td>
<td>69.499***</td>
<td>ns</td>
</tr>
<tr>
<td>Ψ</td>
<td>April 2013</td>
<td>ns</td>
<td>5.760*</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>June 2013</td>
<td>ns</td>
<td>4.278*</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>July 2014</td>
<td>ns</td>
<td>19.605***</td>
<td>ns</td>
</tr>
<tr>
<td>δ¹³C</td>
<td>December 2013</td>
<td>ns</td>
<td>72.864***</td>
<td>2.117*</td>
</tr>
<tr>
<td>δ¹⁸O</td>
<td>December 2013</td>
<td>ns</td>
<td>35.449***</td>
<td>ns</td>
</tr>
</tbody>
</table>

Where effects are significant, F-values are provided, followed by significance indicated as *, P < 0.05; ***, P < 0.001. ns indicates not significant. ‘Water’ refers to Water availability.
Table 4. Mean ecophysiological values for Sitka spruce grown under control-irrigation vs. water-limitation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Date</th>
<th>Control irrigation</th>
<th>Water-limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$ (mol m$^{-2}$ s$^{-1}$)</td>
<td>Sept 2013</td>
<td>0.64 ± 0.06</td>
<td>0.31 ± 0.05</td>
</tr>
<tr>
<td>$\Psi$ (MPa)</td>
<td>April 2013</td>
<td>−0.83 ± 0.04</td>
<td>−0.96 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>June 2013</td>
<td>−0.90 ± 0.04</td>
<td>−1.02 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>July 2014</td>
<td>−1.00 ± 0.04</td>
<td>−1.38 ± 0.04</td>
</tr>
<tr>
<td>$\delta^{13}$C (%)</td>
<td>December 2014</td>
<td>−30.25 ± 0.18</td>
<td>−27.65 ± 0.19</td>
</tr>
<tr>
<td>$\delta^{18}$O (%)</td>
<td>December 2013</td>
<td>26.9 ± 0.3</td>
<td>28.4 ± 0.1</td>
</tr>
</tbody>
</table>

Data are means ± SE, $n = 50$, except $\Psi$ in June 2013, when $n = 24-30$, and $\delta^{18}$O, for which $n = 6-10$, pooled across all families, because family did not have a significant effect on these variables, and there was no interaction.
Table 5. ANCOVAR results for whole plant water use, showing analysis of repeated measures on different dates on the same individuals (within-subjects effects) and analysis of the average responses over those different dates (between-subjects effects).

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Year</th>
<th>Date</th>
<th>Date × Family</th>
<th>Date × Water</th>
<th>Date × Family × Water</th>
<th>Family</th>
<th>Water</th>
<th>Family × Water</th>
<th>Error</th>
<th>Error</th>
<th>F value</th>
<th>Error</th>
<th>Error</th>
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</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height August</td>
<td>2012</td>
<td>ns</td>
<td>1.912**</td>
<td>32.051***</td>
<td>ns</td>
<td>241.163</td>
<td>3.824**</td>
<td>210.504***</td>
<td>ns</td>
<td>76</td>
<td>28.559</td>
<td>3.173</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height start</td>
<td>2013</td>
<td>ns</td>
<td>ns</td>
<td>20.393***</td>
<td>1.574*</td>
<td>318.563</td>
<td>ns</td>
<td>149.902***</td>
<td>2.629*</td>
<td>79</td>
<td>4.032</td>
<td>36.292</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height start</td>
<td>2014</td>
<td>ns</td>
<td>11.799***</td>
<td>39.153***</td>
<td>ns</td>
<td>126.401</td>
<td>ns</td>
<td>85.800***</td>
<td>ns</td>
<td>73</td>
<td>1.732</td>
<td>1.732</td>
<td>1</td>
</tr>
</tbody>
</table>

Where effects are significant, $F$-values are provided, followed by significance indicated as *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. ns indicates not significant. Degrees of freedom for within-subjects effects are provided in parenthesis. 'Water' refers to Water availability.
Table 6. ANOVAR results for ETR measured during rapid light curves, showing analysis of repeated measures at different levels of PAR on the same individuals (within-subjects effects) and analysis of the average responses over those different levels of PAR (between-subjects effects)

<table>
<thead>
<tr>
<th>Within-subjects effects</th>
<th>Between-subjects effects</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td></td>
<td></td>
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<tr>
<td>PAR</td>
<td></td>
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<tr>
<td>PAR × PAR</td>
<td></td>
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<tr>
<td>PAR × Family</td>
<td></td>
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<tr>
<td>Family × Family</td>
<td></td>
</tr>
<tr>
<td>Water × Family</td>
<td></td>
</tr>
<tr>
<td>Water × Water Family</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PAR</th>
<th>PAR × PAR</th>
<th>PAR ×</th>
<th>Family</th>
<th>Water</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

Where effects are significant, F-values are provided, followed by significance indicated as *, P < 0.05; **, P < 0.01; ***, P < 0.001. ns indicates not significant. Degrees of freedom for within-subjects effects are provided in parenthesis. 'Water' refers to Water availability.
**Figure 1.** Whole plant water use of well-watered (left) and water-limited (right) seedlings of two contrasting families (one vigorous and one slow-growing) and the Washington control during growing seasons 2012-2014. Note different scales in different years. Data are means ± SE, n = 5 individuals per family.
Figure 2. Total dry mass (A, B), the relationship between total water use and total dry mass (C, D), and water use efficiency (WUE; E, F) of Sitka spruce from different families under control irrigation (A, C, E) or water-limitation (B, D, F) at the end of 2013 (closed bars/symbols) and 2014 (open bars/symbols). Data in A, B, E, and F are means of the 5 selected trees per family x water availability. In C and D, lines represent linear regressions through the data; each symbol represents an individual tree. Note different scales between water availability treatments. Significance of correlations (n = 50) is indicated by **, P < 0.01; *** P < 0.001.
Figure 3. The decline in volumetric moisture content in the growing substrate for water-limited seedlings of improved families (V1, V2 and V3 shown in A, V4, V5 and V6 shown in B, and I1, I2 and S shown in C), and the Washington Control (shown in each graph, for comparison), during 2012 and 2013. Data are means ± SE, n = 5 individuals per family.
Figure 4. Stomatal conductance ($g_s$) of seedlings from two families of contrasting vigour and the Washington control in 2012 (A) and of seedlings subjected to control irrigation or water-limitation in 2013 and 2014 (B). Data are means ± SE of 10 individuals, pooled over the two water availability treatments (A) or of 50 individuals, pooled over all families (B). In 2012 there was no significant effect of water availability, and in 2013 and 2014 no significant effect of family.
Figure 5. The quantum yield of photosystem II ($\Phi_{\text{PSII}}$) of seedlings subjected to control irrigation or water-limitation in 2013 (A) and of seedlings from two families of contrasting vigour (one vigorous and one slow-growing) and the Washington control in 2014 (B) subjected to control irrigation (left) or water-limitation (right). Data are means ± SE of 50 individuals, pooled over all families (A) or of 5 individuals (B). In 2013 there was no significant effect of family.
Figure 6. Electron transport rates (ETR) of Sitka spruce seedlings from improved families (V1, V2 and V3 shown in A, V4, V5 and V6 shown in B, and I1, I2 and S shown in C), and the Washington Control (shown in each graph, for comparison) in response to different levels of photosynthetically active radiation (PAR). Data are means ± SE of 10 individuals, pooled over two water availability treatments, as there was no significant effect of water availability.
Figure 7. Carbon isotope composition (δ¹³C) of needles from different families under control irrigation or water-limitation produced during 2013 and harvested at the end of the year (A), and the relationship between δ¹³C and average stomatal conductance (gₛ) over the growing season (B), oxygen isotope composition (δ¹⁸O) (C), and total dry mass of seedlings grown under control irrigation (D) or water-limitation (E) in 2013 (closed symbols) or 2014 (open symbols). Data are means ± SE, n = 5 (A) or represent individual trees (B-E). In (A), means with different letters are significantly different from each other (P < 0.05). Lines represent hyperbolic decay curves (B; n = 100), the best-fit curve (C; n = 36), or linear regressions (D-E, n = 50). Note different scales in D and E. Significance of regressions and correlations is indicated by **, P < 0.01; ***, P < 0.001.