

## Unrestricted quality of seeds in European broad-leaved tree species growing at the cold boundary of their distribution

C. Kollas\*, Y. Vitasse, C. F. Randin, G. Hoch and C. Körner

Institute of Botany, University of Basel, Schönbeinstrasse 6, 4056 Basel, Switzerland

\*For correspondence. E-mail [chris.kollas@unibas.ch](mailto:chris.kollas@unibas.ch)

Received: 14 July 2011 Returned for revision: 21 September 2011 Accepted: 31 October 2011 Published electronically: 7 December 2011

- **Background and Aims** The low-temperature range limit of tree species may be determined by their ability to produce and disperse viable seeds. Biological processes such as flowering, pollen transfer, pollen tube growth, fertilization, embryogenesis and seed maturation are expected to be affected by cold temperatures. The aim of this study was to assess the quality of seeds of nine broad-leaved tree species close to their elevational limit.
- **Methods** We studied nine, mostly widely distributed, European broad-leaved tree species in the genera *Acer*, *Fagus*, *Fraxinus*, *Ilex*, *Laburnum*, *Quercus*, *Sorbus* and *Tilia*. For each species, seeds were collected from stands close to optimal growth conditions (low elevation) and from marginal stands (highest elevation), replicated in two regions in the Swiss Alps. Measurements included seed weight, seed size, storage tissue quality, seed viability and germination success.
- **Key Results** All species examined produced a lot of viable seeds at their current high-elevation range limit during a summer ranked 'normal' by long-term temperature records. Low- and high-elevation seed sources showed hardly any trait differences. The concentration of non-structural carbohydrates tended to be higher at high elevation. Additionally, in one species, *Sorbus aucuparia*, all measured traits showed significantly higher seed quality in high-elevation seed sources.
- **Conclusions** For the broad-leaved tree taxa studied, the results are not in agreement with the hypothesis of reduced quality of seeds in trees at their high-elevation range limits. Under the current climatic conditions, seed quality does not constitute a serious constraint in the reproduction of these broad-leaved tree species at their high-elevation limit.

**Key words:** Rosaceae, Fagaceae, Aceraceae, Oleaceae, Tiliaceae, Aquifoliaceae, Fabaceae, seed morphology, elevation, germination, carbohydrates, Alps.

### INTRODUCTION

Niche boundaries of species are often controlled by adverse environmental effects (Austin and Smith, 1989; Guisan and Zimmermann, 2000). Such range limits can be identified in the landscape by searching for 'outpost' individuals or using archive data (Jalas and Suominen, 1988). However, when using such range limits for predictive purposes, it is essential that individuals found at the periphery of a species range reproduce successfully, rather than reflecting historical long-distance seed dispersal events only, as was shown for *Tilia cordata* at the northern species limit in the United Kingdom (Pigott and Huntley, 1978).

Broad-leaved tree taxa such as *Fagus*, *Quercus*, *Fraxinus*, *Sorbus*, *Acer*, *Tilia*, *Ilex* and *Laburnum* reach their upper elevational limits between 1100 and 2200 m in the Swiss Alps (Aeschmann *et al.*, 2004). Among those species, only *Sorbus aucuparia* is able to grow at the tree-line (a life-form limit) at around 2200 m. While the worldwide tree-line phenomenon is comparatively well understood (Körner, 2007; Hoch and Körner, 2011), the species-specific range limits for non-tree-line-forming taxa still need functional explanations. Among many other decisive factors for the current range limit, the ability to produce viable seed is crucial for tree populations to remain viable or even to expand the range limit.

Many factors can constrain the production of viable seeds in cold environments such as the upper-elevational limits. These include successful flowering and pollen transfer (Kudo, 1996), pollen tube growth (Pigott and Huntley, 1981), fertilization, embryogenesis and seed maturation into a viable propagule (for details of predispersal hazards, see Fenner, 1985). Even if trees succeed in producing seeds in such cold environments, the quality of the mature seeds may still be inferior compared with those produced in warmer conditions. In particular, the length of the growing season might be too short to achieve seed maturation (Grenier and Sirois, 2009). Important seed traits encompass seed size, seed weight, quality of seed storage tissue (endosperm or storage cotyledons), viability and germination success. The quality of storage tissue is reflected in the concentration of nitrogen (protein) and non-structural carbohydrates (NSC) or lipids.

Seed mass tends to increase with increasing elevation in herbaceous taxa (Körner, 2003; Pluess *et al.*, 2005), but no general pattern has been detected for trees. For example, seed mass had been reported to decline with elevation in *Sorbus aucuparia*, *Pinus sylvestris* and *Picea abies* (Engler, 1913; Barclay and Crawford, 1984; Oleksyn *et al.*, 1998), whereas the opposite was found for *Betula pubescens* (Holm, 1994).

A sufficient supply of C- and N-reserves is a prerequisite for successful seedling establishment (e.g. Naegle *et al.*, 2005). Tissue concentrations of NSC and nitrogen may either be adaptive or be the result of accumulation due to restricted structural growth as was reported for plants growing at high elevation (e.g. Körner, 1989; Hoch and Körner, 2011). To our knowledge, the present study is the first to investigate the influence of elevation on storage carbohydrates in seeds of deciduous tree species.

Once well supplied with reserves, seeds may be viable but may still show restricted germination. Both viability and germination capacity have been reported to decline with increasing elevation/latitude of the seed source, e.g. for *Pinus* (Engler, 1913), *Picea* (Sirois, 2000), *Nothofagus* (Cuevas, 2000) and *Betula* (Holm, 1994; Sveinbjörnsson *et al.*, 1996). An exception to this pattern is the (sub-) tropical woody species *Polylepis australis*, which has been shown to increase germination capacity with elevation of seed source (Marcora *et al.*, 2008).

So far, no comparative study focusing on tree seed response to elevation has included a wider range of tree taxa. Here we explored seed traits in a suite of major European broad-leaved tree species at their current high-elevation limit in the Alps. We quantified the quality of their seeds by the above criteria at high and low elevation in two regions in the Swiss Alps.

We predicted that due to the lower temperatures and shorter growing season there would be a decline in regenerative performance, as seeds from the high-elevation limit for tree species would (1) show reduced quality and (2) exhibit reduced germination success as compared with lower-elevation seed sources.

## MATERIALS AND METHODS

### Species and seed sources studied

We selected nine broad-leaved tree species with a wide distributional range in Europe: *Acer pseudoplatanus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Ilex aquifolium*, *Laburnum alpinum*, *Quercus petraea*, *Sorbus aria*, *Sorbus aucuparia* and *Tilia platyphyllos*.

To identify the uppermost elevational limit of the nine selected species in the Swiss Alps, species occurrence data were extracted from a spatial-temporal, multi-species vegetation database of Switzerland based on the Second Swiss National Forest Inventory (Brändli, 2010) and the forest plots database (Wohlgemuth, 1992). Considering the 10 % highest occurrences of these species in Switzerland, two distinct regions were identified: near Martigny (46°6'N, 7°4'E) in the western part of the Swiss Alps (called hereafter West, covering about 750 km<sup>2</sup>) and the Upper Rhine region near Chur (46°51'N, 9°32'E) in the eastern part (called hereafter East, covering an area of about 650 km<sup>2</sup>; see Fig. 1). For each species high- and low-elevation mother trees were selected in these two regions (West and East). Hereafter, the term provenance refers to seeds sampled in one region and one elevation. The West and East areas were considered as replicates. Seed sources of two species deviate from that design: seeds of *L. alpinum* were obtained in the western region only (West High and West Low) because this species does not occur in the eastern region. Furthermore seeds of *I. aquifolium* were collected in between these two former regions (where it is absent) at Niederhorn (Kanton Bern),

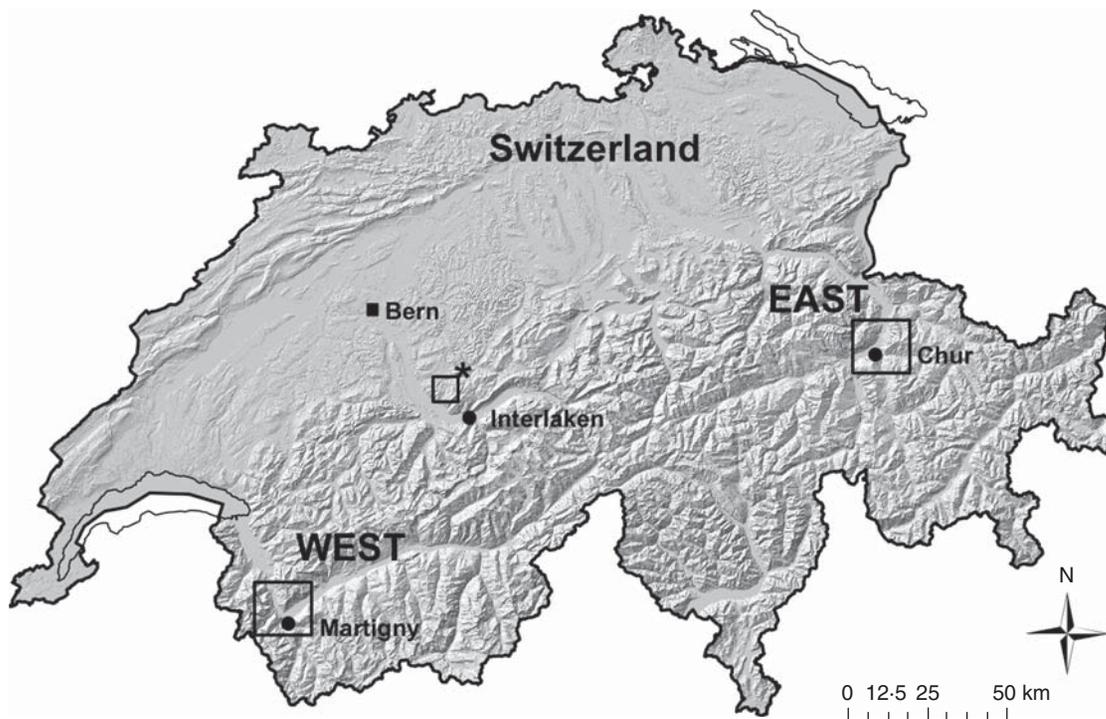


FIG. 1. Locations of the two areas (West and East) selected as seed sources of eight broad-leaved tree species. \* Sampling area of *Ilex aquifolium*.

TABLE 1. Mean elevations (with difference in brackets) for the four sampled provenances (West Low, West High, East Low, East High) for each selected species, with type (cold, warm, unstratified) and length (in months) of seed stratification

Species	West Low–High (m)	East Low–High (m)	Type of stratification/length (months)
<i>A. pseudoplatanus</i>	400–1760 (1360)	590–1430 (840)	Cold/2
<i>F. sylvatica</i>	540–1240 (700)	610–1280 (670)	Cold/2
<i>F. excelsior</i>	420–1440 (1020)	570–1230 (660)	Warm, cold/4,4
<i>I. aquifolium</i>	630–1120 (490)	–	Cold/4
<i>L. alpinum</i>	690–1770 (1080)	–	Unstratified
<i>Q. petraea</i>	550–1080 (530)	580–1130 (550)	Unstratified
<i>S. aria</i>	570–1750 (1180)	620–1290 (670)	Cold/5
<i>S. aucuparia</i>	950–2060 (1110)	930–1870 (930)	Cold/5
<i>T. platyphyllos</i>	440–1190 (750)	600–1200 (600)	Cold/6

TABLE 2. Location, long-term temperature/precipitation records and records of the seed sampling year of the two regions (seed sources)

Region	Co-ordinates	Elevation (m a.s.l.)	Temperature <sub>6–8</sub> * (°C) [min–max]	Precipitation <sub>6–8</sub> † (mm a <sup>-1</sup> ) [min–max]
West	46°19'N 6°58'E	381	19.4 [17.7–21.4]	266 [212–575]
	46°7'N 7°30'E	1825	12.4 [10.7–15.1]	152 [152–447]
East	46°51'N 9°32'E	556	19.2 [17.2–21.9]	280 [223–484]
	46°47'N 9°41'E	1840	11.5 [10.0–14.5]	391 [348–637]

Lowest and highest seasonal means of temperature and precipitation sums during the period 1991–2010 are shown in square brackets.

\* Temperature<sub>6–8</sub>, mean summer temperature from June to August 2009.

† Precipitation<sub>6–8</sub>, sum of precipitation from June to August 2009.

where this species reaches the highest elevation in Switzerland (see Fig. 1 and Table 1).

Seeds were collected in 2009 from June to November, first at low and later at high elevation, according to their maturation stage. Over the last 20 years, 2009 was a normal year in terms of temperature and slightly drier than average (Table 2). Due to snowmelt, seasons commonly start with high soil moisture and critical water shortage becomes effective only late in the season if at all. For each selected provenance and species, about 500 seeds were collected from each of five trees randomly sampled with a minimal distance of 50 m from each other and within 50 m of elevation. Since seed traits have been shown to be variable even within an individual tree crown (Salisbury, 1976), samples were taken from as many branches and positions within the tree as possible and were pooled per tree. Immediately after collection, seeds were cleaned and aborted, predated or malformed seeds were removed. Seeds from fleshy fruits were separated from the fruit pulp by rinsing squashed fruits with tap water over a sieve. All cleaned seeds were allowed to after-ripen and dry at room temperature (approx. +22 °C). Here and subsequently, the term 'seed' refers to an embryo plus endosperm and seed coat, whereas the seed plus dispersal appendages and pericarp is termed 'diaspore'.

#### Morphological and physiological seed traits

Prior to measurements, the following parts were removed from the diaspores: wings and pericarp in *F. excelsior*, *A. pseudoplatanus* and *T. platyphyllos*, the pod in *L. alpinum* and the pericarp in *Q. petraea* and *F. sylvatica*. A subsample

of 20 healthy seeds of each individual was analysed for the following morphological traits. Seed dry mass ( $\pm 0.01$  mg) was obtained after drying at 80 °C for 48 h. The size of dry seed was determined by scanning (300 dpi) and calculating the projected area (software WinSEEDLE Version 2001a). Seed viability was tested applying the tetrazolium test (The International Seed Testing Association, 2006) on a different subsample of 20 seeds per tree and evaluating stained living tissue visually.

NSC (low molecular weight sugars, such as glucose, fructose and sucrose, plus starch) were analysed using the method of Wong (1990) as described in detail in Hoch *et al.* (2002). All NSC concentrations are given on a percentage dry matter basis. Total nitrogen concentration was determined in dry and powdered samples with a CHN elemental analyser (Vario EL III; Elementar Analysensysteme, Hanau, Germany).

#### Germination trials

Each species was represented by 20 mother trees, ten from each region (East and West), five from high elevation and five from low elevation. For the germination experiment, seeds were stratified by species-specific treatments according to Burkart (2000) (see Table 1). According to these recommendations seeds were mixed with a moist substrate (50 % turf, 50 % sand) in perforated plastic bags. The bags were then stored either outdoors in closed plastic boxes (cold stratification) under roofed (shaded, weather-proof) conditions or indoors in the office (warm stratification; Basel, 47°33'N, 7°36'E). Mean temperature in the cold stratified boxes was 2.8 °C during the stratification period (December 2009 to

TABLE 3. Differences in seed traits between low and high populations of nine broad-leaved tree species in the Swiss Alps

	Seed size	Seed weight	NSC	Nitrogen	Viability	Germination capacity	Germination rate
<i>A. pseudoplatanus</i>	0.193	0.160	< <b>0.001</b> / +	<b>0.053</b> / +	0.689	<b>0.003</b> / +	0.552
<i>F. sylvatica</i>	0.068	0.066	< <b>0.001</b> / +	0.458	0.470	/	/
<i>F. excelsior</i>	0.525	0.116	0.619	0.912	0.036	<b>0.033</b> /–	0.098
<i>I. aquifolium</i>	0.560	0.840	0.192	<b>0.011</b> /–	0.763	/	/
<i>L. alpinum</i>	0.906	<b>0.026</b> / +	0.075	0.439	0.272	0.152	<b>0.035</b> / +
<i>Q. petraea</i>	0.162	0.093	0.390	<b>0.016</b> / +	/	/	/
<i>S. aria</i>	0.160	0.179	0.332	< <b>0.001</b> /–	0.051	<b>0.050</b> /–	0.162
<i>S. aucuparia</i>	<b>0.002</b> / +	< <b>0.001</b> / +	< <b>0.001</b> / +	< <b>0.001</b> /–	<b>0.039</b> / +	/	/
<i>T. platyphyllos</i>	0.697	<b>0.048</b> /–	<b>0.008</b> /–	<b>0.014</b> /–	0.342	/	/
Overall*	0.266	0.098	< <b>0.001</b>	<b>0.050</b>	0.294	0.795	0.777

*P*-values of the factor elevation in the generalized linear model ( $P < 0.05$  in bold) are given along with the direction of influence: +, higher trait value at high elevation; –, lower trait value at high elevation; /, no data (low germination or unsuitability of tetrazolium test).

\**P*-values obtained from generalized linear mixed effects model across all species (species stated as random effect; see Materials and Methods for details).

March 2010; absolute minimum  $-6.3$  °C). The substrate in the bags was checked regularly to be moist. Seeds of *Q. petraea* were sown in pots and placed outside (unroofed) 1 week after seed collection (Burkart, 2000).

Seeds of *S. aucuparia*, *S. aria*, *A. pseudoplatanus* and *F. sylvatica* were transferred to an unheated, open-window greenhouse and sown on 1 March 2010. Seeds of *F. excelsior*, *T. platyphyllos*, *I. aquifolium* and *L. alpinum* were transferred to the greenhouse and sown on 1 April to account for their known longer dormancy. One hundred ripe seeds per mother tree were randomly chosen and evenly spaced ( $3 \times 4$  cm) in gridded germination trays. They were covered with a layer of substrate corresponding to the diameter of the seed. The substrate was a mix of 30 % pumice stone, 20 % bark compost, 20 % quartz sand, 16 % cocofibre, 10 % turf and 4 % clay. Trays were put on tables and watered regularly with tap water. Every 2 weeks all trays were randomized within the greenhouse to avoid position effects. Daily mean temperatures at seedbed level varied between 9 and 15 °C in March and April and between 10 and 22 °C in May. Daily mean soil temperatures at seed depth oscillated between 10 and 20 °C over the whole germination experiment. The greenhouse was moderately heated overnight during the first two weeks of March.

All seed trays were checked for germinating seeds every 2–3 d. Germination was defined as the first emergence of cotyledons. Germination capacity was defined as the final percentage of germinated seeds, and germination rate ( $t_{50}$ ) was defined as the time in days required to reach 50 % of final germination percentage.

#### Data analysis

We assessed general trends in seed traits across species using generalized linear mixed models. We considered region and elevation nested in region as fixed effects, species as random effect and mother tree as the replicated unit [function lme in package nlme (Pinheiro *et al.*, 2011) within the R statistical software (R Development Core Team, 2011)].

In a second step, the effect of elevation on seed traits was analysed species by species. The corresponding generalized linear models used region and elevation nested in region as linear predictors and Gaussian error distributions. For all

traits and analysis, residuals conformed to the assumptions of normality and homoscedasticity, except for seed-weight and seed-size data which were therefore log-transformed prior to analyses.

Pearson's correlation coefficients were calculated between all traits within species. Furthermore species were ranked by their mean NSC and nitrogen concentrations to evaluate whether these traits are related to species ecological indices (e.g. dispersal type, shade tolerance) as listed by Brzeziecki and Kienast (1994).

## RESULTS

### Seed size and seed weight

Adult individuals growing at the upper elevational limit of a given species all produced plentiful seeds of good quality (see below). Across the species studied, neither seed size nor seed weight varied consistently between low- and high-elevation provenances (Table 3). For example, seed size of *S. aucuparia* from the low-elevation provenances was significantly smaller than from high-elevation provenances. None of the other species showed significant differences in seed size, but the seed weight of both *L. alpinum* and *S. aucuparia* was heavier at high elevation, whereas the seed weight of *T. platyphyllos* was lighter at high elevation. The other five species investigated did not show significant differences in seed weight between high and low provenances.

### Seed carbon-reserves and total nitrogen concentrations

Elevation effects on NSC and nitrogen were inconsistent: in three species (*A. pseudoplatanus*, *F. sylvatica* and *S. aucuparia*) NSC concentrations increased with elevation, in *T. platyphyllos* concentrations decreased and no difference was detected in the other species (Table 3). Both of the *Sorbus* species and *T. platyphyllos* and *I. aquifolium* showed lower seed nitrogen concentrations at higher elevation, whereas in *A. pseudoplatanus* and *Q. petraea* nitrogen concentrations increased with elevation. Neither NSC nor nitrogen concentration was found to correlate with other seed traits, hence there was no relationship between concentrations of NSC or nitrogen and seed viability. NSC and nitrogen

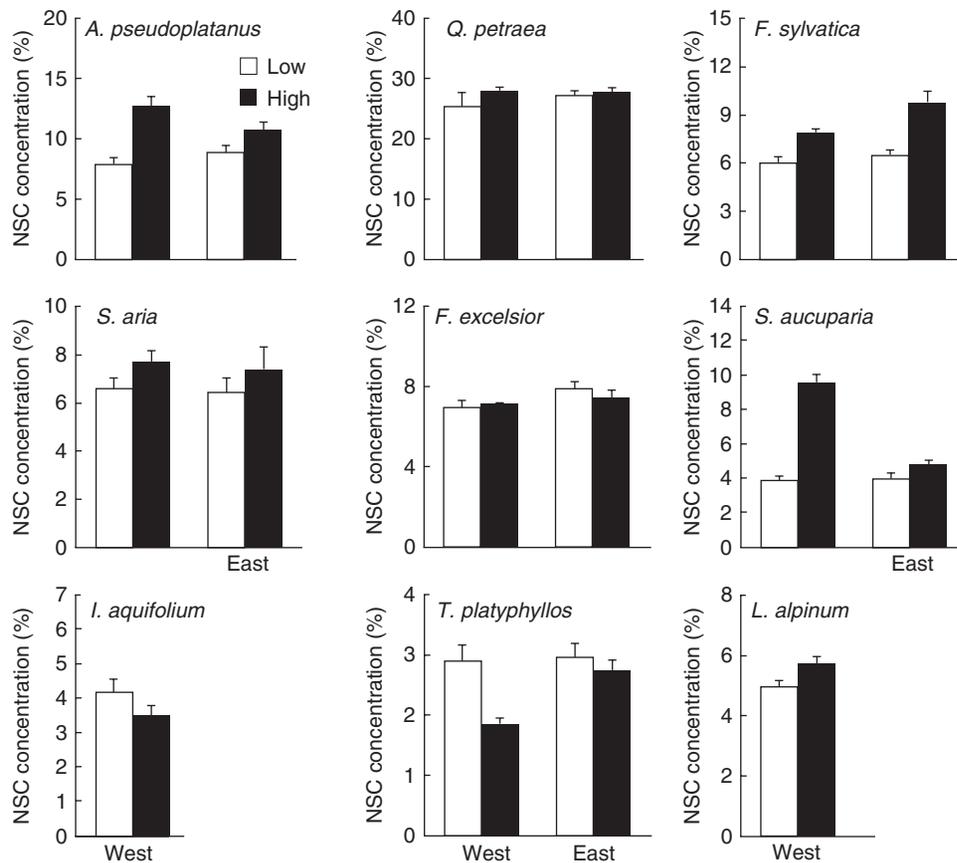


FIG. 2. NSC concentration in seeds of nine broad-leaved tree species from low and high elevation in both selected regions (West Low, West High, East Low, East High). Means and standard errors are shown ( $n = 5$  trees per elevation, region and species, with 20 seeds per tree; for statistics see Table 3). Note the different scales for each species.

concentration did not correlate with any of the species ecological indices such as species dispersal type, shade tolerance, frequency of seed crop, temperature index, frost resistance, drought resistance and nitrate index as defined by Brzeziecki and Kienast (1994).

#### Viability and germination

Seed from high-elevation provenances showed high viability and germination success. The tetrazolium test revealed no effect of elevation on seed viability for seven of the tested species, while for seeds of *Q. petraea* the test was unsuitable (The International Seed Testing Association, 2006). *Sorbus aucuparia* turned out to be the only species in which the fraction of viable seeds was significantly higher in high-elevation seed sources (Table 3).

Despite the positive viability tests, five species (*T. platyphyllos*, both Fagaceae species, *S. aucuparia* and *I. aquifolium*) showed very poor germination irrespective of source elevation. Hence, no statistics for germination can be provided for these species. For the remaining four species, both germination traits (capacity and rate) showed no consistent elevation effect (Table 3). In seeds of *A. pseudoplatanus* germination capacity was significantly higher for high-elevation sources, whereas seeds of *S. aria* and *F. excelsior* showed the

opposite trend and *L. alpinum* showed no trend with elevation. Seeds of *L. alpinum* obtained from high elevations germinated significantly slower than seeds of low-elevation provenances.

In summary, all seed traits studied did not show consistent differences between low and high seed provenances in each of the nine investigated species, except for *Sorbus aucuparia* with seed from high elevation exhibiting an overall higher quality (seed weight, seed size, NSC, viability; Table 3 and Fig. 3). For concentrations of NSC we found a trend for higher concentrations in high elevation seed sources (Table 3 and Fig. 2).

## DISCUSSION

At their uppermost elevational limit, the reproductive success of trees may strongly depend on seed quality. Tests of seed quality and germination of seeds from low- and high-elevation provenances of nine broad-leaved tree species revealed no indication that adult trees from high elevations of any of the tested species have difficulties producing viable seed. Elevational differences in traits across species were inconsistent. The seed-failure hypothesis for trees at their upper elevational limit can therefore be rejected for the set of species explored here under the current climatic conditions.

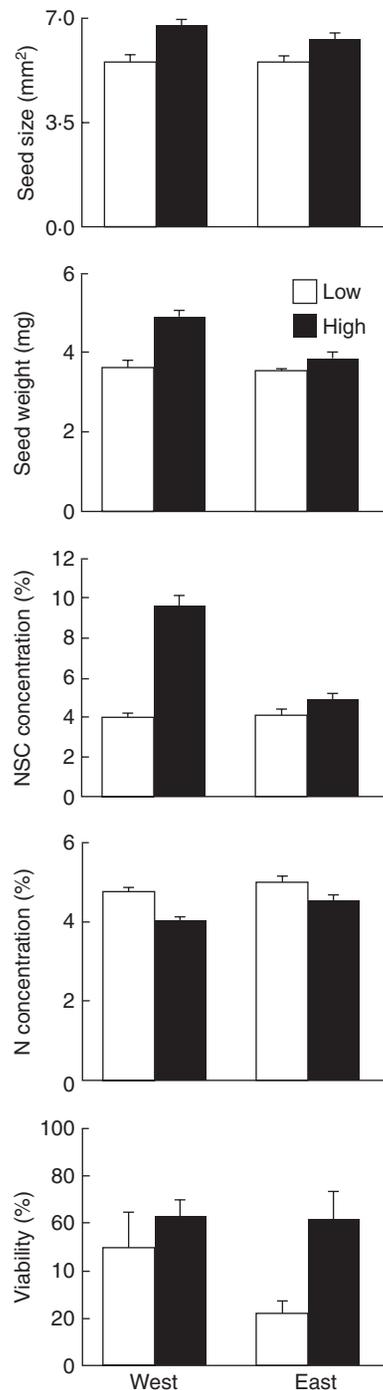


FIG. 3. Differences in seed size, seed weight, nitrogen concentration, NSC concentration and seed viability between low and high provenances of *S. aucuparia* in the two selected regions (West Low, West High, East Low, East High). Means and standard errors are shown ( $n = 5$  trees per elevation and region, with 20 seeds per tree; for statistics see Table 3).

Summer temperatures during the year of seed collection (2009) were 12.4/11.5 °C (June to August, West/East) at 1825/1840 m a.s.l., indicating that this year was not out of the temperature range for the last 20 years for both regions (Table 2). The slightly reduced precipitation during June to

August is unlikely to have affected trees in this mountainous terrain, but it is possible that trees at the lowest elevation were affected by the drier conditions during the final part of seed maturation.

#### Seed size and seed weight

Since the growing season and the period available for seed maturation get shorter the higher the elevation, we expected lower seed weights at the tree species' limits. Surprisingly, seed weight did not show significant differences between high- and low-elevation provenances in six out of nine of the species studied, and the three remaining species showed opposite trends (two increasing, one decreasing). The existing literature is similarly inconsistent in this respect. Some studies showed no change in seed mass with elevation or latitude, e.g. for *Sorbus torminalis* in Croatia (Orsanic, 2009) and for *Nothofagus* spp. in New Zealand (Ledgard and Cath, 1973), while seed weight in *Betula pubescens* was reported to increase with elevation (Holm, 1994). A strong decrease of seed weight in *S. aucuparia* along an elevational gradient from 0 to 600 m a.s.l. in Scotland found by Barclay and Crawford (1984) contrasts with our observations. However, this may reflect a collection bias because the Scottish *Sorbus* seeds were collected on the same day; hence the decrease in seed weight with elevation might reflect incomplete maturation at high elevation. Overall, our data support the notion that seed weight is a conservative trait (Fenner, 1985). It is well known that species retain seed size irrespective of plant size (homeostasis effect) over a wide range of life conditions (Harper *et al.*, 1970), with strong theoretical support (Smith and Fretwell, 1974). Plants vary the number rather than the size of their seeds. Based on the optimization theory, the mother plant should invest in fewer seeds if reserves for reproduction are scarce. Hence we would expect high-elevation trees to produce fewer seeds than trees at low elevation if there were resource limitations. This is hard to assess for isolated tall trees in closed mountain forest, but from our experience during the seed harvesting campaign, there was no obvious decline in seed numbers with elevation in the deciduous species. The seed quality traits (NSC, nitrogen), however, did not indicate any resource limitation at the upper distribution limits. The data for *S. aucuparia* may reflect the smaller stature of this species, causing the lower-elevation individuals to be suppressed by tall trees in the montane forest, whereas the individuals at the tree-line profited from full sun exposure and hence tended to produce larger, heavier, more viable seeds with higher NSC and lower nitrogen concentration.

#### Seed carbon-reserves and total nitrogen concentrations

The majority of the investigated species exhibited high concentrations of NSC and nitrogen in seeds from the uppermost trees and revealed no indication of carbon shortage or nutrient starvation. In fact, most of the species even tended to have higher NSC concentrations in seeds from high elevation (Fig. 2), matching general trends of mobile carbon charging in high elevation-tree tissues (e.g. Hoch *et al.*, 2003; Shi *et al.*, 2008). In the case of seeds, we cannot exclude ecotypic differentiation for higher quality of seed near the upper tree

species limit, although the isolation of these outpost trees and the presumed gene-flow from lower elevation make this unlikely. These trends may equally well reflect the consequences of retarded growth rate of trees at otherwise hardly constrained CO<sub>2</sub> uptake, with the inevitable consequence of high abundance of non-structural carbon compounds (growth-limitation hypothesis; Körner, 1998, 2003). It remains open whether seedlings take advantage from that trend of higher NSC reserves at our high-elevation sites. The fact that seed size and weight do not decline with elevation in most of the nine species studied indicates that seed growth is not constrained at the current tree-species limit, perhaps because it is confined to the warmest part of the season. We attribute the missing correlation between germination rate and NSC concentrations (in otherwise viable seeds) to developmental constraints (seed dormancy). Unlike NSC, nitrogen did not show a consistent trend with elevation across species. Hence, if there were elevational reductions in tree nutrient supply, these are not reflected in seeds' nitrogen concentrations, which was also found in leaves (Körner, 1989; Birman and Körner, 2009).

#### Viability and germination

As with our first hypothesis of lower seed quality at high elevations, our second hypothesis predicting lower germination success in seeds sourced from the upper range limit, is not supported by the current study. However, five of the species studied germinated poorly (across all provenances) and could thus not be taken into account for the germination analyses. Fungal infections (in *Q. petraea* and *S. aucuparia*) and insufficient time to complete the needed seed coat decay (in *I. aquifolium*) were likely explanations for the very low germination success, while we have no explanation for the low germination success in *F. sylvatica*. For *T. platyphyllos* it is known from nurseries that it is generally hard to raise plants from seed.

As seen in our results, trends in germination capacity have been previously reported to be species dependent. An increase of germination capacity with increasing latitude was found in *Betula papyrifera* (Bevington, 1986), whereas a decreasing capacity with elevation or latitude was found in *Abies guatemalensis*, *Pinus sylvestris*, *Picea mariana*, *Nothofagus pumilio* and *Betula* spp. (Engler, 1913; Holm, 1994; Sveinbjörnsson et al., 1996; Cuevas, 2000; Sirois, 2000; Strandby Andersen et al., 2008). The lack of an overall decline in germination capacity with increased elevation observed here, might relate to the monotone life conditions far below the tree-line. Like the overall germination success, the rate (speed) of germination (analysed as the date by which 50% of the final germination was reached) did not show a consistent elevational trend across the four species that germinated well. Only one species (*L. alpinum*) showed significantly slower germination in seeds from higher elevation. Since all germination trials were conducted under 'common garden' conditions at low elevation, *in situ* germination behaviour remains to be explored, yet the observed seedling success of high-elevation provenances (Y. Vitasse et al., unpubl. res.) suggests that the seed traits observed here scale to reproductive fitness.

#### Conclusions

In summary the present study revealed two major outcomes.

(1) High-elevation seed sources exhibit high quality, similar to those produced by trees growing under close-to-optimal conditions (lower elevation). In addition, seeds of high-elevation provenances of *S. aucuparia* (at the tree-line) exhibit an even higher quality than their lower-elevation counterparts, indicating that life conditions at the cold boundary of deciduous tree growth do not constrain seed development.

(2) Consequently, as the seed traits were largely unchanged from the optimum stands to the low-temperature limits of the tree species investigated, the notion is supported that reproduction at the upper-elevation species limit is not limited by seed quality. Since proper seed formation and germination success constitute only one chapter of the demographic novel (Moles and Westoby, 2006), extended studies of population dynamics are needed to complement the current results (Lenoir et al., 2009). Based on our ongoing work (Y. Vitasse et al., unpubl. res.) and the data presented here, there is successful tree recruitment above the current deciduous species limits in Switzerland. These results are consistent with the general rise in elevation of species-abundance peaks in trees (Lenoir et al., 2009).

#### ACKNOWLEDGEMENTS

We thank Niklaus Zimmermann and Thomas Wohlgenuth for their advice on high-elevation broad-leaved tree positions in Switzerland and Anton Burkart for his advice on seed treatments. We also thank Pascale Flury and Lea Steinle for field assistance and the Technologiezentrum Witterswil for providing their large greenhouse for the germination trials. This work was supported by the European Research Council (233399) and carried out as part of the TREELIM project.

#### LITERATURE CITED

- Aeschmann D, Lauber K, Moser DM, Theurillat JP. 2004. *Flora alpina*. Paris: Belin.
- Austin MP, Smith TM. 1989. A new model for the continuum concept. *Vegetatio* 83: 35–47.
- Barclay AM, Crawford RMM. 1984. Seedling emergence in the rowan (*Sorbus aucuparia*) from an altitudinal gradient. *Journal of Ecology* 72: 627–636.
- Bevington J. 1986. Geographic differences in the seed germination of paper birch (*Betula papyrifera*). *American Journal of Botany* 73: 564–573.
- Birmann K, Körner C. 2009. Nitrogen status of conifer needles at the alpine tree-line. *Plant Ecology & Diversity* 2: 233–241.
- Brändli U-B. 2010. *Swiss National Forest Inventory – results of the third inventory 2004–2006*. Bern: Paul Haupt.
- Brzeziecki B, Kienast F. 1994. Classifying the life-history strategies of trees on the basis of the Grimian model. *Forest Ecology and Management* 69: 167–187.
- Burkart A. 2000. *Kulturblätter. Angaben zur Samenernte, Klengung, Samenlagerung, Samenausbeute und zur Anzucht von Baum- und Straucharten*. Birmensdorf: Swiss Federal Institute for Forest, Snow and Landscape Research, WSL.
- Cuevas JG. 2000. Tree recruitment at the *Nothofagus pumilio* alpine timberline in Tierra del Fuego, Chile. *Journal of Ecology* 88: 840–855.
- Engler A. 1913. Einfluss der Provenienz des Samens auf die Eigenschaften der forstlichen Holzgewächse. *Mitteilungen der Schweizerischen Centralanstalt für das forstliche Versuchswesen* 10: 189–386.
- Fenner M. 1985. *Seed ecology*. London: Chapman and Hall.

- Grenier M, Sirois L. 2009.** Reproductive development and seed ripening in *Betula papyrifera* along an altitudinal thermal gradient in eastern Appalachia (Canada). *Botany* **87**: 492–500.
- Guisan A, Zimmermann NE. 2000.** Predictive habitat distribution models in ecology. *Ecological Modelling* **135**: 147–186.
- Harper JL, Lovell PH, Moore KG. 1970.** The shapes and sizes of seeds. *Annual Review of Ecology, Evolution, and Systematics* **1**: 327–356.
- Hoch G, Körner C. 2011.** Global patterns of mobile carbon stores in trees at the high-elevation tree line. *Global Ecology and Biogeography*, in press. <http://dx.doi.org/10.1111/j.1466-8238.2011.00731.x>.
- Hoch G, Popp M, Körner C. 2002.** Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss tree-line. *Oikos* **98**: 361–374.
- Hoch G, Richter A, Körner C. 2003.** Non-structural carbon compounds in temperate forest trees. *Plant, Cell & Environment* **26**: 1067–1081.
- Holm SO. 1994.** Reproductive patterns of *Betula pendula* and *B. pubescens* Coll. along a regional altitudinal gradient in northern Sweden. *Ecography* **17**: 60–72.
- Jalas J, Suominen J. 1988.** *Atlas florae Europaeae: distribution of vascular plants in Europe*. Cambridge: Cambridge University Press.
- Körner C. 1989.** The nutritional status of plants from high altitudes – a worldwide comparison. *Oecologia* **81**: 379–391.
- Körner C. 1998.** A re-assessment of high-elevation tree-line positions and their explanation. *Oecologia* **115**: 445–459.
- Körner C. 2003.** *Alpine plant life*. Berlin: Springer.
- Körner C. 2007.** Climatic tree-lines: conventions, global patterns, causes. *Erdkunde* **61**: 316–324.
- Kudo G. 1996.** Effects of snowmelt timing on reproductive phenology and pollination process of alpine plants. *Memoirs of National Institute of Polar Research Special Issue* **51**: 71–82.
- Ledgard NJ, Cath PW. 1973.** Seed of New Zealand *Nothofagus* species: studies of seed weight, viability, shape and the effect of varying stratification periods. *New Zealand Journal of Forestry* **28**: 150–162.
- Lenoir J, Gégout J-C, Pierrat J-C, Bontemps J-D, Dhôte J-F. 2009.** Differences between tree species seedling and adult altitudinal distribution in mountain forests during the recent warm period (1986–2006). *Ecography* **32**: 765–777.
- Marcora P, Hensen I, Renison D, Seltsmann P, Wesche K. 2008.** The performance of *Polylepis australis* trees along their entire altitudinal range: implications of climate change for their conservation. *Diversity and Distributions* **14**: 630–636.
- Moles AT, Westoby M. 2006.** Seed size and plant strategy across the whole life cycle. *Oikos* **113**: 91–105.
- Naegle ER, Burton JW, Carter TE, Ruffly TW. 2005.** Influence of seed nitrogen content on seedling growth and recovery from nitrogen stress. *Plant and Soil* **271**: 329–340.
- Oleksyn J, Modrzynski J, Tjoelker MG, Zytowskiak R, Reich PB, Karolewski P. 1998.** Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. *Functional Ecology* **12**: 573–590.
- Orsanic M. 2009.** Variability of morphological and biological characteristics of wild service tree (*Sorbus torminalis* (L.) Crantz) fruits and seeds from different altitudes. *Periodicum Biologorum* **111**: 495–504.
- Pigott CD, Huntley JP. 1978.** Factors controlling distribution of *Tilia cordata* at northern limits of its geographical range. 1. Distribution in northwest England. *New Phytologist* **81**: 429–441.
- Pigott CD, Huntley JP. 1981.** Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. 3. Nature and causes of seed sterility. *New Phytologist* **87**: 817–839.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team. 2011.** nlme: linear and nonlinear mixed effects models. R package version 3-1-102.
- Pluess AR, Schutz W, Stöcklin J. 2005.** Seed weight increases with altitude in the Swiss Alps between related species but not among populations of individual species. *Oecologia* **144**: 55–61.
- R Development Core Team. 2011.** *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>.
- Salisbury E. 1976.** Exceptional fruitfulness and its biological significance. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **193**: 455–460.
- Shi P, Körner C, Hoch G. 2008.** A test of the growth-limitation theory for alpine tree line formation in evergreen and deciduous taxa of the eastern Himalayas. *Functional Ecology* **22**: 213–220.
- Sirois L. 2000.** Spatiotemporal variation in black spruce cone and seed crops along a boreal forest-tree line transect. *Canadian Journal of Forest Research* **30**: 900–909.
- Smith CC, Fretwell SD. 1974.** The optimal balance between size and number of offspring. *The American Naturalist* **108**: 499–506.
- Strandby Andersen U, Prado Cordova JP, Bräuner Nielsen U, Kollmann J. 2008.** Provenance variation in germination and seedling growth of *Abies guatemalensis* Rehder. *Forest Ecology and Management* **255**: 1831–1840.
- Sveinbjörnsson B, Kauhanen H, Nordell O. 1996.** Tree-line ecology of mountain birch in the Torneträsk Area. *Ecological Bulletins* **45**: 65–70.
- The International Seed Testing Association. 2006.** *International rules for seed testing*. Bassersdorf: The International Seed Testing Association (ISTA).
- Wohlgemuth T. 1992.** Die vegetationskundliche Datenbank. *Schweizerische Zeitschrift für das Forstwesen* **143**: 22–36.
- Wong SC. 1990.** Elevated atmospheric partial-pressure of CO<sub>2</sub> and plant growth. 2. Non-structural carbohydrate content in cotton plants and its effect on growth parameters. *Photosynthesis Research* **23**: 171–180.