LE STUDIUM
Loire Valley
Institute for Advanced Studies

LE STUDIUM Multidisciplinary Journal
ISSN 2669-7808
www.lestudium-ias.com

FELLOWSHIP FINAL REPORT

Larix decidua altitudinal reciprocal transplant experiment in the French Alps. A preliminary analysis

Cuauhtémoc Sáenz-Romero[1,2,3], Luc E. Pâques[2], Philippe Rozenberg[2*]

[1] Le Studium Loire Valley Institute for Advanced Studies, 45000 Orléans, France.
[2] L'Unité Mixte de Recherche Biologie intégrée pour la valorisation de la diversité des arbres et de la forêt (UMR BioForA 0588), l'Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE), 45075, Orléans, France.
[3] Instituto de Investigaciones sobre los Recursos Naturales, Universidad Michoacana de San Nicolás de Hidalgo (UMSNH). 58330. Morelia, Michoacán, Mexico.
* Corresponding author: philippe.rozenberg@inrae.fr

REPORT INFO

Fellow: **Cuauhtémoc Sáenz-Romero.**
From: Universidad Michoacana de San Nicolás de Hidalgo, Mexico.
Host laboratory in region Centre-Val de Loire: UMR BioForA 0588, INRAE, Orleans.
Host scientist: **Philippe Rozenberg.**
Period of residence in region Centre-Val de Loire: March – July 2024.

Keywords :
Larix decidua, reciprocal transplants, elevational gradients, climatic change, assisted migration, clonal growth

ABSTRACT

The ongoing acceleration of climatic change makes it even more urgent to understand how tree seed sources (provenances) respond when growing in climates different from those they are naturally adapted to, either when planted on warmer or on colder sites than the climate that occur at their native distribution site. We evaluated four years of growth, bud phenology and survival of a Larix decidua clonal elevational reciprocal transplant trial in the French Alps, at Villard-St-Pancrace, close to Briançon (LN 44.9°; LE 6.65°). The experiment has four experimental sites, distributed along a north-faced Alpine steep-slope, at contrasting elevations: 2,400, 2,000, 1,700 and 1,350 m a.s.l. On each site, 4 sets of 30 clones were reciprocally planted, with each set originating from adult trees selected in natural forest plots at nearly the same elevations (2,300, 2,000, 1,700 and 1,350 m a.s.l). Results indicate that: (a) Plot populations have lower survival rates when relocated to environmental extremes within the mountain range, whether to colder sites at higher elevations or to warmer sites at lower elevations. (b) Growth also decreases when they are moved to colder (higher elevation) sites, although in general it increases when they are moved to warmer (lower elevation) sites. (c) Such growth pattern might be in part explained by the phenology of the leader bud elongation: by the end of spring, leader buds have already started to elongate at lower elevations, meanwhile they are still in full dormancy at the highest elevational site.

1- Introduction

Altitudinal reciprocal transplants experimentation of forest tree species along environmental gradients on mountain slopes offer the opportunity to test the survival and growth response of genotypes when exposed to climatic conditions different from those of their original seed source (Cruzado-Vargas et al 2021). That makes possible the simultaneous testing of two contrasting research questions: (a) How far can we move species and provenances towards colder sites, with the goal that (if they successfully establish), they will be adapted to warmer climates in the future due to the ongoing climatic change? And (b) What would be the performance of species and provenances under projected warmer climates, if we move them toward sites warmer today than that of their seed sources?

The current acceleration of climatic change (Hansen et al 2023) makes it even more urgent to address these two questions, in order to make informed decisions and adapt forest management to decrease the anticipated

Cuauhtémoc Sáenz-Romero, Luc E. Pâques, Philippe Rozenberg. *Larix decidua* altitudinal reciprocal transplant experiment in the French Alps. A preliminary analysis, *LE STUDIUM Multidisciplinary Journal*, **2024**, 8, 61-67
https://doi.org/10.34846/le-studium.287.03.fr.07-2024

negative impacts of climatic change, particularly the more frequent and hotter droughts (Hammond et al 2022). One of the most important challenges posed by climatic change is derived from an incontrovertible fact: although natural migration moves population of forest tree species toward higher elevation in forest mountains (Lenoir et al 2008), such displacement is happening at a much slower speed than what is needed, given the speed at which the suitable climatic habitat for forest tree populations is shifting to higher altitudes (Jump et al 2006, 2009; Peñuelas et al 2007). Such disparity is at the heart of the forest management and stresses the importance to innovate.

A management measure proposed to accommodate the expected impacts of climatic change on forest tree populations is to conduct assisted migration: the shifting of forest tree provenances to sites where the expected future climate is that for which these trees have evolved (Sáenz-Romero et al 2021). However, such action has intrinsic risks: projected future climate does not occur yet today and is partially unknown. If the shifting is too small or excessive, the seedlings will be probably damaged or killed by hydric stress or frost damage (respectively). Then, such climatic transfer needs to be done within an acceptable adaptive range of a provenance. That requires understanding the phenotypic plasticity of the trees: the ability of a genotype to adjust its phenotype when growing under different environmental conditions during their life span (Arnold et al 2019; Escobar-Sandoval et al 2021).

For the present work, *Larix decidua* adult trees from natural populations distributed along an elevational gradient in the French Alps were collected at 4 elevations and grafted. An altitudinal reciprocal transplant clonal trial was planted in four field sites at four contrasting altitudes on a close by mountain range transect. The objective was to construct a climate response function for survival and growth, using the differences between seed sources and planting sites climates.

The objectives were to assess the impact of colder climates at higher elevation, as well as of warmer climates at lower elevations and, in doing so, to measure the viability of conducting assisted migration in the present day. The goal is (a) To eventually realign the populations with the climate to which they are adapted, which is expected to happen at higher elevation in the future; (b) Measure the impact of growing at sites warmer than their original habitat, to evaluate the anticipated impact of climatic change on natural populations; (c) Determine the degree of local adaptation in natural populations by the way of quantifying the genetic differentiation among populations for quantitative traits, such as seedling height growth

2- Experimental details

On June 2024 we evaluated four years of growth (seedling length as a proxy of seedling height), bud phenology and survival of a clonal elevational reciprocal transplant trial in the French Alps. The tests are located at Villard-St-Pancrace, close to Briançon (latitude: 44.9° N; longitude: 6.65° E) in the French Alps. The experiment has four experimental sites, distributed along a north-faced Alpine steep-slope, at contrasting elevations: 2,400, 2,000, 1,700 and 1,350 m a.s.l. On each site, 4 sets of 30 clones (from grafting) were reciprocally planted; each set originating from adult trees selected in natural forest plots at nearly the same elevations (2,300, 2,000, 1,700 and 1,350 m). We considered that the absence of apical dominance in height growth is a consequence of the grafting effect, which prevents the use of plant height to quantify vertical growth; instead, we used the length of the terminal shoot. More details on the four elevation plots are in Escobar-Sandoval et al (2021). In each planting site, clones were initially (planting initiated in September 2013 and 2014) represented with ramets on 20 incomplete blocks of 30 trees, with 5 ramets per clone per block (600 trees in total in each elevation trial). We use the term ‘plots’ for the seed sources (in natural forest) instead of ‘population’, because the active gene flow along the elevational gradient could make possible to consider the continuous *Larix decidua* forest on the studied slope as a large single population, assuming that (likely) it were a single genetic neighborhood. We also use the term 'seedling' to refer to the young grafted trees being evaluated.

Due to clone mortality shortly after plantation, several ramets were replaced by surplus seedlings from the nursery. Seedling replacement ended in October 2020. On the present report, we conducted the analysis focusing only on seedlings from 2020 plantation.

Bud phenology was evaluated in 2024 with a 6 classes scoring-scale: “0” = leader bud in full dormancy, to “6” = leader bud fully elongated with needles well developed.

A model of elevational transfer distance response curve was fitted, using linear and quadratic regression and a statistical mixed model (Leites et al 2012a), with an analysis similar to the one conducted by Cruzado-Vargas et al (2021), using Procedure REG and MIXED, respectively, of SAS (SAS Institute 2014). The independent variable was the climatic transfer distance, defined as the test site climate, minus the seed source (where scions were collected) climate. Climate of the planting sites were derived from automatized micro-meteorological stations placed in each experimental site; data considered were for the trees growth period: 2014-2023 (ten years). Climate of the seed sources (where scions were collected) were derived from Meteo-France data, averaging 1964-2010 (47 years). Climate variables for this preliminary analysis are in Table 1 (precipitation variables were not included in this preliminary analysis).

Table 1. Climatic variables estimated for each provenance (average 1964-2010) and for each test site (average 2014-2023).

Code	Unit	Definition
MAT	°C	Mean annual temperature
MTCM	°C	Mean temperature in the coldest month
MMIN	°C	Mean minimum temperature in the coldest month
MTWM	°C	Mean temperature in the warmest month
MMAX	°C	Mean maximum temperature in the warmest month
DD5	°C	Degree-days > 5 °C

During the last field evaluation (4 to 6 June 2024), it was not possible to score the site at 2400 m elevation because it was still covered by snow. Then, we assumed that the seedlings in that site had not developed yet the bud leader, and a value of “0” of the bud development index was assumed for all seedlings in that site. For the same reason, in the statistical analysis we included, only for the site at 2400 m, the seedling length and survival scored in 2023 as the value of June 2024.

3. Results

Regressions (linear and quadratic) of plot population means per trial site against climatic transfer distance (climate of field planting minus climate of clone source) indicate that the best fit for the tree response variables examined (survival, seedling length and bud phenology) was for Mean Temperature of the Coldest Month (MTCM) transfer distance as independent variable. The quadratic regression model provided a better fit than the linear regression model.

Results indicate that survival rates are lower for the clones originating from elevations either below or above the planting site (R^2 = 0.37; P = 0.0483; Figure 1).

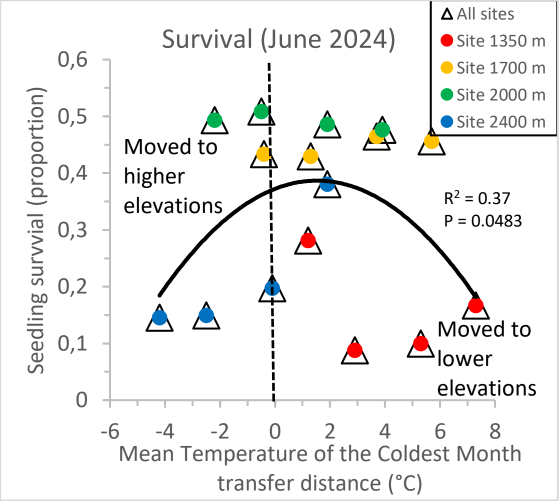

Figure 1. Mean per plot population per trial site of *Larix decidua* (clones) survival of seedlings, four years after plantation in an altitudinal reciprocal transplant field tests, near Briançon, French Alps. Symbol color indicate planting site elevation (not that of plot population). Vertical dotted line indicates a plot population of clones planted at a site with an elevation equal to that of their plot population of origin (zero transfer distance).

A quadratic regression of means per plot population per trial site of seedling length (as proxy of seedling height, because many seedlings were bended by snow pack, and also because the lack of leadership of the terminal bud, due to the cloning) against climatic transfer distance indicates that the clones grew more rapidly when they were planted at elevations lower than their original plot ($R^2 = 0.44$; $P = 0.0236$; Figure 2).

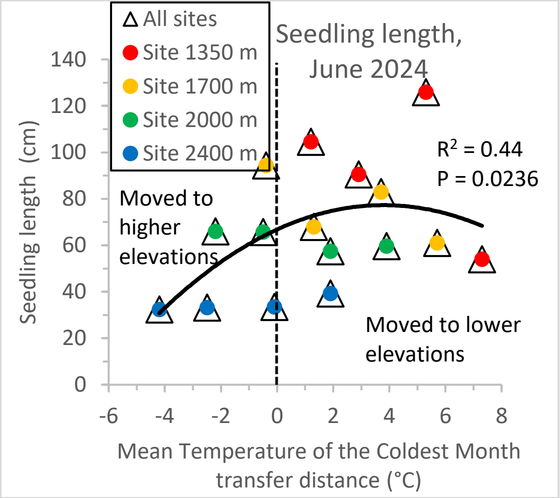

Figure 2. Mean per plot population per trial site of *Larix decidua* (clones) length of seedlings (proxy of seedling height), four years after plantation in the altitudinal reciprocal transplant field tests, near Briançon, French Alps. Symbol color indicate planting site elevation (not that of plot population). Vertical dotted line indicates a population of clones planted at a site with an elevation equal to that of their population of origin (zero climatic transfer distance).

Such differential growth is much more evident for bud phenology, where regressions of means of plot population per trial site indicate a general trend, where clones' buds are more developed (average index values above 3) when planted at elevation lower than their original plot ($R^2 = 0.63$; $P = 0.0014$; Figure 3).

When fitting a climatic transfer distance response function for MTCM with a Mixed model, both the linear and the quadratic terms of the climatic transfer distance were highly significant for tree length (height) ($P < 0.0001$). For bud phenology only, the linear term was significant ($P < 0.0001$). For survival the only significant term was the quadratic one ($P = 0.0024$); Table 2). For the three variables examined, there no significant ($P > 0.05$) differences among sites, plot populations or clones nested in plot populations (Table 2). In contrast, for seedling height and bud development, the block-nested-into-site effects were significant ($P < 0.043$; Table 2). For survival, block was not included in the model, since survival was estimated at clone level with one ramet per clone per block.

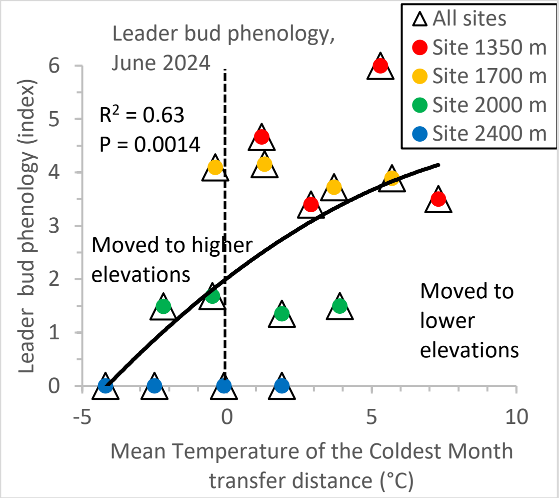

Figure 3. Mean per plot population per trial site of leader bud development (index; 0 = dormancy, to 6 = fully developed) *Larix decidua* (clones) seedlings, four years after planting in the altitudinal reciprocal transplant field tests, near Briançon, French Alps. Color of symbols indicates altitude of the planting site (not of the population origin). Vertical dotted line indicates a population of clones planted at a site with an elevation equal to that of their population of origin (zero transfer distance).

Table 2. Mixed model analysis for height, bud phenology (by 4 to 6 June 2024) and survival of seedlings originated from grafted trees. Akaike Information Criterion [AIC; Akaike (1973)], estimated parameters, contribution to total variance (only of random terms), and significance for each trait.

Parameter or source of variation	**Seedling height**		
Fixed effects	**Estimate**		**p**
Akaike Inf. Criterion (AIC)	6122.4		---
Intercept	65.8		0.0180
MTCM transfer distance	9.19		<0.0001
(MTCM transfer distance)^2	-1.20		<0.0001
Random Effects	**Variance**	**%**	**p**
Site	0	0.0	1
Population	304.2	26.2	0.1176

Block (Site)	79.7	6.9	0.0027
Clone (Population)	14.2	1.2	0.2595
Residual	764.9	65.8	

Table 2 continues ..

Parameter or source of variation	Bud phenology		
Fixed effects	**Estimate**		**p**
Akaike Inf. Criterion (AIC)	1953.7		
Intercept	2.10		0.0861
MTCM transfer distance	0.78		<0.0001
(MTCM transfer distance)^2	-0.013		0.2067
Random Effects	**Variance**	**%**	**p**
Site	0.30	10.47	0.1905
Population	1.39	48.52	0.1354
Block (Site)	0.04	1.55	0.0428
Clone (Population)	0.00	0.02	1
Residual	1.13	39.44	

Table 2 Continues …

Parameter or source of variation	Survival		
Fixed effects	**Estimate**		**p**
Akaike Inf. Criterion (AIC)	3943.3		
Intercept	36.93		0.0351
MTCM transfer distance	1.60		0.2559
(MTCM transfer distance)^2	-0.77		0.0024
Random Effects	**Variance**	**%**	**p**
Site	178.76	21.41	0.1236
Population	7.63	0.91	0.2848
Block (Site)	---		---
Clone (Population)	10.88	1.30	0.3487
Residual	637.63	76.37	

4. Discussion.

The general trend from our results shows that plot populations have lower survival rates when relocated to environmental extremes within the mountain range, either to colder sites at higher elevations or to warmer sites at lower elevations. Growth also decreases when they are moved to colder (higher elevation) sites, although in general it increases when they are moved to warmer (lower elevation) sites. Such growth pattern might be in part explained by the phenology of the leader bud elongation: by the end of spring, the leader buds have started already to elongate at lower elevations, meanwhile they are yet in full dormancy at the highest elevational site.

The fact that Mean Temperature of the Coldest Month transfer distance was the climatic variable that yielded the best climatic transfer distance response function, indicates that cold temperature is a strong limiting factor for growth and survival of the *Larix decidua* seedlings planted along the altitudinal gradient.

Although the quadratic regressions of each response variable against the Mean Temperature of the Coldest Month (MTCM) transfer distance was statistically significant, there is an important data dispersion around the means per plot population per trial site from the fitted curve (Figures 1 to 3). All these relatively weak trends suggest that it is necessary to:

(a) wait for more data at later ages: at minimum we should use measurements of height increment from the 2024 growing season for all plots, as soon as they are available. The lack of significant differences among clones within population, usually very high for these traits, indicates that the trials are still in a critical establishment phase.

(b) conduct the same analysis using precipitation variables

Even with the relative uncertainty of our early age evaluation, it seems that the genetic differentiation among plot populations is weak. A molecular genetic analysis obtained on the same seedling trees from the 4 plots showed a globally uniform genetic structure along the gradient, at least for neutral or nearly neutral to selection molecular markers (Nardin et al. 2015). This is also consistent with previous analysis of low genetic differentiation among populations (for quantitative traits) along altitudinal gradients of *Larix occidentalis* in the Rocky Mountains (Rehfeldt 1994).

The fact that the seed-source trees are represented by clones in the experiments is a strength of the experimental design, because it eliminates the variability usually provided by pollen-donating father trees on typical provenance trials composed of half-sib progenies. However, use of grafted seedlings raises the question of the unknown effect of the rootstock on seedlings behavior.

A risk for this project would be that at the young measurement age (four-year-old after the last replanting), the clones may not yet have fully expressed their growth potential and may still be in settlement phase. Additionally, an unexpected mortality due to non-climatic causes (e.g. gophers) necessitated the replanting of some ramets, which may have affected the statistical power of the experiment. On a later analysis, we will consider the use of clone age as a covariate to account for this ramets replanting effect.

5. Conclusion

Results indicate that: (a) Plot populations have lower survival rates when relocated to environmental extremes within the mountain range, whether to colder sites at higher elevations or to warmer sites at lower elevations. (b) Growth also decreases when they are moved to colder (higher elevation) sites, although in general it increases when they are moved to warmer (lower elevation) sites. (c) Such growth pattern might be in part explained by the phenology of the leader bud elongation: by the end of spring, leader buds have already started to elongate at lower elevations, meanwhile they are still in full dormancy at the highest elevational site.

6. Perspectives of future collaborations with the host laboratory

Since likely the *Larix decidua* clones have not expressed in full their growth potential at the planting sites, it is needed to continue the measurements some years more.

7. Articles published in the framework of the fellowship

There is in preparation a second paper in the framework of the collaboration between UMR-BioForA-INRAE and UMSNH, sponsored by Le Studium, for a different research project. The tentative title of the manuscript is: “Differential latewood density as response to climatic transfer distance among *Pseudotsuga menziesii* provenances planted in France.”

8. Funding

This project was funded by the following projects: Ministry of Ecology GICC GRAAL 10-MCGOT-GICC-8-CVS-139, France, European Union Horizon 2020 Framework programme MSCA-RISE-2014-645654-TOPWOOD, ECOS-Nord CONACYT bilateral France-Mexico M16A01, CONACYT Mexico, fellowship 439944 to previous PhD student Margarita Escobar-Sandoval**.** Foundation de France “Forêts Communes”. Funding was provided by Le Studium Loire Valley Institute for Advanced Studies, Orleans and Tours, France to CS-R.

9. Acknowledgements

The authors thank the INRAE experimental unit UE GBFOR and the INRAE plateau technique PHENOBOIS (formerly GENOBOIS), INRAE Val de Loire, Orléans (Frédéric Millier), France, for field and laboratory work along the larch altitudinal gradient, and the International Associated Laboratory (LIA) between INRAE, France, and INTA, Argentina FORESTIA. We also want to thank the municipalities of Villard-St-Pancrace, St-Martin-de-Queyrières and Puy-St-André, as well as the local forest service, ONF Hautes-Alpes, Briançon, France. The study experimental site “Gradient Altitudinal Mélèze” is part of the “Zone Atelier Alpes”. We want to thank Margarita Escobar-Sandoval, Ana Laura Cruzado-Vargas and other students and researchers who participated to early steps of field work in the study, and who provided help and advices for data analysis. Finally, we want to thank Lucie Lombard of the association Forêts Alpines.

10. References

Arnold, P. A., L. E. B. Kruuk and A. B. Nicotra. 2019. How to analyse plant phenotypic plasticity in response to a changing climate. *New Phytologist*, *222*, 1235–1241. https://doi.org/10.1111/nph.15656

Cruzado-Vargas, A.L. A. Blanco-García, R. Lindig-Cisneros, M. Gómez-Romero, L. Lopez-Toledo, E. de la Barrera, C. Sáenz-Romero. 2021. Reciprocal common garden altitudinal transplants reveal potential negative impacts of climate change on *Abies religiosa* populations in the Monarch

Butterfly Biosphere Reserve overwintering sites. *Forests*, 12(1):69-86. https://doi.org/10.3390/f12010069

Escobar-Sandoval M., L. Pâques, P. Fonti, A. Martinez-Meier, P. Rozenberg. 2021. Phenotypic plasticity of European larch radial growth and wood density along a-1,000 m elevational gradient. *Plant-Environment Interactions* 2:45–60. https://doi.org/10.1002/pei3.10040

Hammond, W.M., A.P. Williams, J.T. Abatzoglou, H.D. Adams, T. Klein, R. Rodríguez, C. Sáenz-Romero, H. Hartmann, D.D. Breshears and C.D. Allen, 2022. Global field observations of tree die-off reveal hotter-drought fingerprint for Earth's forests. *Nature Communications* 13, 176. https://doi.org/10.1038/s41467-022-29289-2

Hansen, J. E., M. Sato, L. Simons, L.S. Nazarenko, I. Sangha, P. Kharecha, J.C. Zachos, K. von Schuckmann, N.G. Loeb, M.B. Osman, Q. Jin, *et al.* 2023. Global warming in the pipeline. *Oxford Open Climate Change 3(1):*kgad008, , https://doi.org/10.1093/oxfclm/kgad008

Jump AS, Hunt JM, Peñuelas J. 2006 Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*. *Global Change Biology* 12:2163-2174.

Jump A.S., C. Mátyás, J. Peñuelas. 2009. The altitude-for latitude disparity in the range retractions of woody species. *Trends in Ecology and Evolution* 24(1):694-701.

Leites L.P., G.E. Rehfeldt, A.P. Robinson, N.L. Crookston, B.C. Jaquish. 2012a. Possibilities and limitations of using historic provenance tests to infer forest species growth responses to climate change. *Natural Resources Modeling*, 25, 409–433.

Leites L.P., A.P. Robinson, G.E. Rehfeldt, J.D. Marshall, N.L. Crookston. 2012b. Height growth response to climatic changes differs among populations of Douglas-fir: a novel analysis of historic data. *Ecological Applications*, 22, 154–165.

Lenoir, J. *et al. 2008.* A Significant Upward Shift in Plant Species Optimum Elevation During the 20th Century. *Science* 320, 1768-1771.DOI :10.1126/science.1156831.

Nardin, M., B. Musch, Y. Rousselle, V. Guerin, L. Sanchez, J.P. Rossi, S. Gerber, S. Marin, L.E. Pâques, P. Rozenberg. 2015. Genetic differentiation of European larch along an altitudinal gradient in the French Alps. *Ann. For. Sci.* 72: 517-527. DOI 10.1007/s13595-015-0483-8

Peñuelas J., R. Oyaga, M. Boada, A.S. Jump. 2007. Migration, invasion and decline: changes in recruitment and forest structure in a warming-linked shift of European beech forest in Catalonia (NE Spain). *Ecography* 30:830-838.

Rehfeldt, G. E., 1994. Evolutionary genetics, the biological species, and the ecology of the Interior cedar-hemlock forest. *In*: Proceedings of Interior cedar-hemlock-white-pine forest: ecology and management. Pullman, Washington State University, pp 91-100.

Sáenz-Romero, C. G. O'Neill, S.N. Aitken, R. Lindig-Cisneros. 2021. Assisted migration field tests in Canada and Mexico: Lessons, limitations, and challenges. *Forests*, 12, 9. https://dx.doi.org/10.3390/f12010009

SAS Institute (2014). *SAS/STAT (Version 9.4) Computer Software*. Cary, North Carolina, USA. SAS Institute Inc.