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Biologie intégrée pour la valorisation de la diversité des arbres et de la forêt

THÈSE présentée par :

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Trade-off of growth heterosis in Larch: Vigor at what cost?

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At the end:

सभी का दिल से धन्यवाद — इस यात्रा का हिस्सा बनने के लिए।

माताराणी के आशीर्वाद और मार्गदर्शन के लिए मैं सदा कृतज्ञ हूँ।

Conceptualization and structure of thesis:

Climate change has been having a marked effect on the way forests function for several decades now, with changes in growth, development, morphology, and phenology, and loss of diversity in all regions of the planet, leading to the more or less gradual death and loss of productivity of forests in both planted and natural production systems. The increasing frequencies and intensities of climate extremes, as well as new pest and disease outbreaks worldwide, have been reshaping the targets and dynamics of several traditional breeding strategies. This thesis is grounded on two main contextual drivers: the ongoing impacts of climate change and the shift in the paradigm of breeding programs from biomass-based to more adaptation-based assets. Breeding programs have been, until recently, embedded in the concept of higher production assurance to success; however, climate dynamics now question this fast growth and high yield forest reproductive material: Can these trees be used anymore for deployment, especially in this era of disturbance and shortage or depletion of resources, particularly of water?

There has been a marked mortality across all forested biomes worldwide, primarily due to the frequent "drought" episodes. A few decades ago, researchers studying tree responses to drought often focused on drought avoidance strategies, but with increased uncertainty regarding worldwide droughts, the drought tolerance strategy has become a preferred approach. These strategic shifts do not mean that growth is neglected; instead, they aim to sustain or enhance growth under stressful conditions. This raises important questions: Should strategies be tailored to specific site conditions? Should their inherent drought tolerance traits guide species selection? Breeding priorities need to be gradually adapted to align with the ongoing changes, *e.g., a* shift in target selection criteria, careful management of genetic diversity, new approaches to speed up recombination and evaluation processes, and overall breeding generations. Facing increasing uncertainty, breeding programs should keep a high level of flexibility, which depends on a greater insight and knowledge on how complex traits such as growth, wood formation, etc., are driven on one side, by more elementary functional traits and their inter-relationships, and on the other side, by pedo-climatic factors. More insight into these traits might inevitably highlight some unfavorable links between traits and trade-offs expressed differently in favorable vs stressful environmental conditions.

Our thesis finds its place in the frame of the INRAE *Larix* breeding program: this manages in parallel a recurrent selection program in European (*Larix decidua* Mill.) and Japanese (*Larix kaempferi* (Lamb.) Car.) larches and an interspecific hybridization program between the two. Interspecific hybridization has proved to be a powerful strategy through complementation of traits and generation of heterosis (or hybrid vigor), allowing rapid genetic gains. A pending question remains whether to keep both strategies running or to focus solely on interspecific hybridization and deployment of hybrids in the forest.

The interest in our thesis on interspecific hybridization and the dedicated mating designs established to study hybrid vigor lies in:

- Firstly, on the possibility of comparing the three larch taxa through connected pedigrees across contrasted environments. With this motivation, we formulated our core research questions around experimentally induced drought vs optimal watering, as a means to explore resource limitation and its effects on three larch taxa - European Larch, Japanese Larch, and their interspecific hybrid - focusing on contrasting responses regarding mortality, growth, and heterosis
- Secondly, on access to differential growth patterns among the three taxa, allowing evaluation of fast- vs slow-growing taxa (and genotypes), and detecting possible trade-offs between growth and 'adaptive' traits.

Therefore, this study aimed to explore and respond to core issues: (a) What is the differential response of pure larch species and their hybrid to mortality and growth across contrasted environments and over the years? (b) Is it possible to rely on NIRS as a high-throughput phenotyping technique to construct a large array of phenotypes for functional traits, with the aim of integrating them as 'adaptive' traits into breeding programs in relation to climate change? and (c) Is the fast growth of genotypes and taxa a factor of vulnerability to water constraints?

Since our main underlying environmental focus of the thesis is "water availability restriction" (called hereafter drought) and its effect on taxa-specific responses (like growth), the first Research chapter was laid on the foundation of response to drought episodes by examining mortality response and its relationship with climatic and soil water availability parameters between the three larch taxa, across two watering treatments and over a decade. Mortality was identified as a determinant trait to answer questions around drought response, as this reflects the utmost consequence of a tree's response to either tolerate drought or succumb to water stress. Radial growth - especially that of the year preceding mortality- was investigated to test its predictive capacity of mortality. In line with Chapter III, the link between growth and mortality was studied to verify if fast growth might be detrimental to survival.

To effectively address questions related to environmental challenges, like in Chapter I, especially taxon-specific responses- survival and growth, gives a global answer on trees' aptitude to cope with climate change. However, they result from complex interactions between physiological processes, and access to more functional traits is needed to disentangle the mechanisms driving the patterns. Unfortunately, phenotyping these traits is usually costly and labor-intensive, limiting the number of trees that can be analyzed, often well below the numbers needed to evaluate genotypes in genetic studies. It is therefore essential to have the possibility to screen a much larger number of trees to get more precision. This sets the stage for a methodological chapter, Chapter II, where High Throughput Phenotyping using NIRS is developed for functional traits essential for understanding drought resilience and growth performance in *Larix* species. We identified three groups of traits and proxies to evaluate them: water-use traits ($\Delta^{13}C$, P_{50}), which reflect how plants manage water under stress; photosynthetic capacity (C and N content, LDMC, SLA), indicating resource use and growth potential; and defense and structural support (lignin, H/G ratio, total phenolics).

Building on the environmental insights from Chapter I and the methodological advances from Chapter II, the last Research Chapter (Chapter III) focuses on i) the variability of expression of growth among the three *Larix* taxa, and thereby of heterosis, across contrasted environments – spatial, i.e., two watering treatments and, temporal (across ten years) and ii) possibly on how water-use efficiency, resistance to cavitation and drought resilience could drive growth performances. Finally, this chapter explores the links between growth and other 'adaptive' traits to answer whether fast growth could be detrimental to desirable traits related to water use efficiency, drought resilience, and overall resource allocation.

This thesis has been planned with first a General Introduction, which discussed the main underlining concept of the thesis: Climate change and the mechanism behind drought, challenges faced by breeders, interspecific hybridization, recurrent selection, and Larch as a tree model to answer the questions stated above. The next part presents the Materials and Methods, describing the experimentation and the material investigated, the methods of sampling and laboratory analyses to obtain the traits of interest, including growth, drought response, and functional characteristics across taxa, and the statistical procedures implemented. We have also included a dedicated section on Terminology, as this thesis involves several critical ecological terms that require clear definitions to ensure consistent understanding throughout the text.

The three main results chapters follow:

- Chapter I: "A Decade-long study on Mortality in European Larch (*Larix decidua* Mill.), Japanese Larch (*Larix kaempferi* (Lamb.) Car.) and their hybrid"
  Naincy Sagar, Luc E. Pâques, Stéphane Chantepie. (article published in Annals of Forest Science, doi: 10.1186/s13595-025-01294-7).

- Chapter II: "Prediction Models for *Larix* species via NIRS Spectroscopy as a High-Throughput Phenotyping Approach".
  Naincy Sagar, Nassim Belmokhtar, Anna Alves, Nathalie Boizot, David Chassagnaud, Régis Fichot, José Rodrigues, Luc E Pâques (article accepted with minor revision in Journal of Near Infrared Spectroscopy).

- Chapter III "Expression of heterosis for growth under contrasted soil watering treatments and over time in interspecific larch hybrids (*Larix x eurolepis*). Are fast-growing genotypes and taxa more vulnerable to water constraints?"
  Naincy Sagar, Luc E Pâques

Finally, a general Discussion addresses the overall findings of the study, integrates them with existing literature, and highlights their implications for breeding strategies and ecological adaptation. The Conclusion brings the concluding idea of the thesis and offers a path forward around these questions that we tried to formulate.

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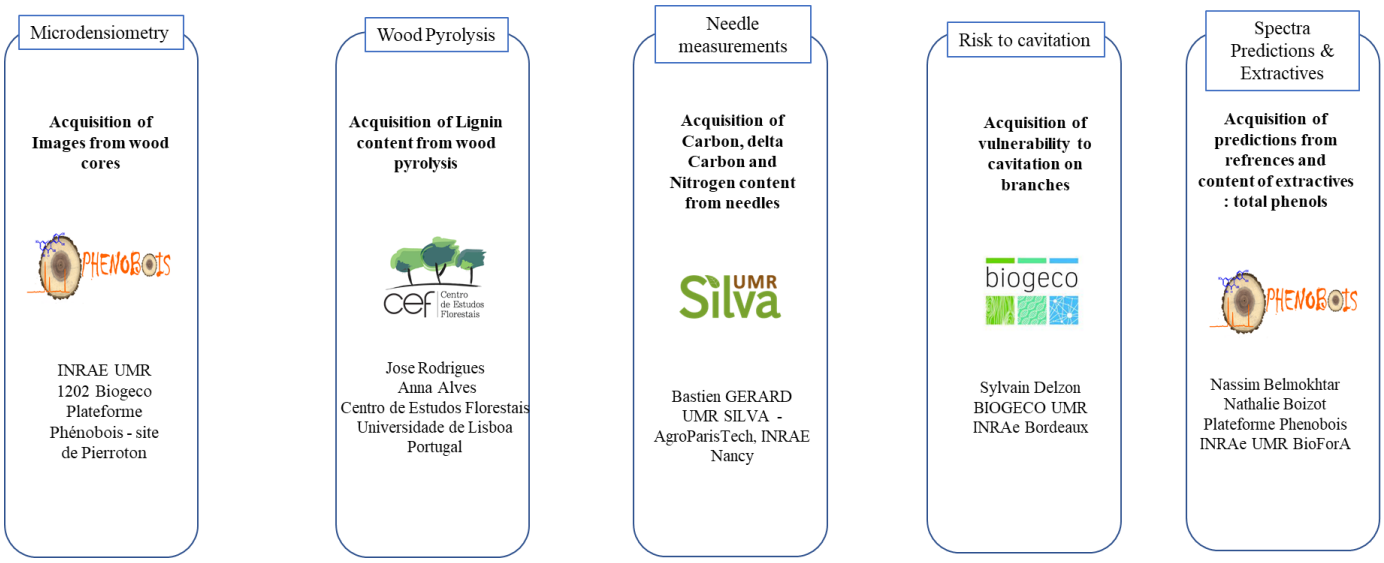

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Conceptualisation et structure de la thèse (en français) :

Le changement climatique affecte depuis plusieurs décennies le fonctionnement des forêts, entraînant des modifications de la croissance, du développement, de la morphologie et de la phénologie, ainsi qu'une perte de diversité dans toutes les régions du globe. Ces bouleversements se traduisent par un dépérissement progressif des forêts et une baisse de leur productivité, tant dans les systèmes de production naturels que dans les plantations. La fréquence et l'intensité croissantes des événements climatiques extrêmes, ainsi que l'émergence de nouveaux ravageurs et maladies à l'échelle mondiale, remodèlent profondément les objectifs et la dynamique des stratégies d'amélioration génétique traditionnelles. Cette thèse s'ancre dans deux grands facteurs contextuels : les effets persistants du changement climatique, et le changement de paradigme des programmes d'amélioration/sélection, qui passent de logiques de production de biomasse à des logiques davantage axées sur l'adaptation. En effet, jusqu'à récemment, les programmes d'amélioration visaient principalement à assurer des productions élevées avec des révolutions courtes. Or, dans un contexte de perturbations climatiques croissantes et de raréfaction des ressources – notamment de l'eau – cette stratégie soulève désormais des interrogations : ces arbres à croissance rapide sont-ils encore adaptés pour être déployés à grande échelle ? Peut-on encore les considérer comme du matériel forestier de reproduction pertinent ?

Les épisodes de mortalité en forêt, largement attribués aux sécheresses récurrentes et vagues de chaleur, se sont multipliés dans tous les biomes. Il y a quelques décennies, les recherches sur la réponse des arbres à la sécheresse portaient principalement sur les stratégies d'évitement. Cependant, face à l'imprévisibilité croissante des épisodes de sécheresse, les stratégies de tolérance à la sécheresse sont aujourd'hui privilégiées. Ce changement d'orientation ne signifie pas que la croissance soit négligée, mais plutôt que l'enjeu est de la maintenir, voire de l'améliorer, dans des conditions de stress. Cela soulève plusieurs questions essentielles : faut-il adapter les stratégies de sélection à chaque type de site ? Les traits fonctionnels liés à la tolérance à la sécheresse doivent-ils guider le choix des espèces ou des génotypes ? Les priorités des programmes de sélection doivent progressivement évoluer pour répondre à ces enjeux : révision des critères de sélection, meilleure gestion de la diversité génétique, nouvelles approches pour accélérer les cycles de sélection et d'évaluation, etc. Face à l'incertitude croissante, les programmes doivent aussi conserver une grande flexibilité. Celle-ci repose sur une meilleure compréhension des caractères complexes, tels que la croissance ou la formation du bois, qui dépendent à la fois de traits fonctionnels plus élémentaires et de leurs interrelations, et des conditions pédoclimatiques. Cette meilleure compréhension mettra très probablement en lumière certains compromis défavorables entre caractères, exprimés différemment selon que l'environnement soit favorable ou contraignant.

Notre thèse s'inscrit dans le cadre du programme de sélection *Larix* de l'INRAE, qui gère en parallèle un programme de sélection récurrente sur le mélèze d'Europe (*Larix decidua* Mill.) et le mélèze du Japon (*Larix kaempferi* (Lamb.) Car.), ainsi qu'un programme d'hybridation interspécifique entre les deux espèces. L'hybridation interspécifique s'est révélée être une stratégie puissante grâce à la complémentarité des traits et à la génération d'hétérosis (ou vigueur hybride), permettant des gains génétiques rapides. Une question demeure cependant : faut-il maintenir les

deux stratégies ou se concentrer exclusivement sur l'hybridation interspécifique et le déploiement des hybrides en forêt ?

L'intérêt de notre thèse pour l'hybridation interspécifique et les plans de croisements dédiés mis en place pour étudier la vigueur hybride repose sur plusieurs éléments :

- D'une part, sur la possibilité de comparer les trois taxa de mélèzes grâce à des pedigrees connectés, évalués dans des environnements contrastés. À partir de cette motivation, nous avons formulé nos principales questions de recherche autour d'un dispositif expérimental combinant stress hydrique (sécheresse induite) et condition hydrique 'optimale', afin d'explorer les effets de la limitation des ressources en eau sur trois taxa de mélèzes — le mélèze d'Europe, le mélèze du Japon et leur hybride interspécifique — en mettant l'accent sur leurs réponses contrastées en termes de mortalité, de croissance et d'hétérosis.
- D'autre part, sur la possibilité d'accéder 'par construction à des profils de croissance différenciés entre les trois taxa, permettant l'évaluation de taxa (et génotypes) à croissance rapide ou lente, et l'identification d'éventuels compromis entre croissance et traits dits « adaptatifs ».

Ainsi, cette étude vise à explorer et répondre à des enjeux majeurs : (a) Quelle est la réponse différentielle des espèces pures de mélèzes et de leur hybride en termes de mortalité et de croissance, à travers des environnements contrastés et au fil du temps ? (b) Est-il possible de s'appuyer sur la spectroscopie proche-infrarouge (NIRS) comme technique de phénotypage à haut débit pour construire un large éventail de phénotypes pour des caractères fonctionnels, dans l'objectif de les intégrer en tant que caractères 'adaptatifs' dans les programmes de sélection en lien avec le changement climatique ? (c) La croissance rapide des génotypes ou des taxa constitue-t-elle un facteur de vulnérabilité face aux contraintes hydriques ?

Puisque l'axe environnemental principal de cette thèse repose sur la 'restriction de la disponibilité en eau' du sol (ci-après appelée 'sécheresse') et à ses effets sur les réponses développementales des 3 taxa de mélèze, le premier chapitre de recherche analyse la mortalité en lien avec les épisodes de sécheresse. La mortalité a été considérée comme un caractère déterminant pour aborder la question de la tolérance à la sécheresse, car elle reflète l'issue ultime de la capacité ou non d'un arbre à résister au stress hydrique. Cette analyse a été menée en relation avec les paramètres climatiques et la disponibilité en eau du sol générée par deux traitements hydriques contrastés et sur une période de dix ans. La croissance radiale — en particulier celle de l'année précédant la mort — a été analysée afin de tester son pouvoir prédictif de la mortalité. Dans la continuité du chapitre III, le lien entre croissance et mortalité a été exploré pour évaluer si une croissance rapide pouvait s'avérer délétère pour la survie.

Pour répondre de manière pertinente aux enjeux environnementaux — comme dans le chapitre I — notamment aux réponses spécifiques des taxa en matière de survie et de croissance, il est nécessaire de disposer d'éléments plus précis sur les mécanismes sous-jacents. Ces réponses résultent en effet d'interactions complexes entre processus physiologiques : l'accès à davantage de caractères fonctionnels est essentiel pour comprendre les déterminants de ces trajectoires. Malheureusement, le phénotypage de ces caractères est souvent coûteux et demandeur en main-

d'œuvre, limitant ainsi le nombre d'arbres pouvant être analysés — souvent bien en deçà du seuil nécessaire à l'évaluation des génotypes dans les études génétiques. Il est donc essentiel de pouvoir cribler un plus grand nombre d'individus pour gagner en précision. Cela justifie l'introduction d'un chapitre méthodologique — le chapitre II — dédié au développement d'une approche de phénotypage à haut débit (HTP) par spectroscopie proche infrarouge (NIRS), appliquée à des caractères fonctionnels essentiels à la compréhension de la résilience à la sécheresse et de la performance de croissance chez les espèces du genre *Larix*. Nous avons identifié trois grands groupes de caractères et leurs proxys : (1) des caractères liés à l'hydraulique de l'arbre ($\Delta^{13}C$, P_{50}), qui reflètent la gestion hydrique sous stress, (2) des caractères liés à la capacité photosynthétique (teneurs en C et N, LDMC, SLA), indicateurs de l'utilisation des ressources et du potentiel de croissance, (3) des caractères de défense et de soutien structural (lignine, rapport H/G, composés phénoliques totaux).

En s'appuyant sur les enseignements du Chapitre I et les avancées méthodologiques du Chapitre II, le dernier chapitre de recherche (Chapitre III) se concentre sur : i) la variabilité de l'expression de la croissance parmi les trois taxa de *Larix* — et donc de l'hétérosis — à travers des environnements contrastés, à la fois dans l'espace (deux traitements hydriques) et dans le temps (sur une période de dix ans), ii) et potentiellement sur la manière dont l'efficience d'usage de l'eau, la résistance à la cavitation et la résilience à la sécheresse pourraient influencer les performances de croissance. Enfin, ce chapitre explore les liens entre croissance et autres caractères dits 'adaptatifs', afin de répondre à la question suivante : une croissance rapide peut-elle compromettre certains caractères favorables liés à l'utilisation de l'eau, à la résilience à la sécheresse et à l'allocation globale des ressources ?

Cette thèse a été structurée en commençant par une Introduction générale, qui présente les concepts fondamentaux sous-jacents : le changement climatique et les mécanismes associés à la sécheresse, les défis rencontrés par les sélectionneurs, les stratégies d'amélioration, et le choix du mélèze comme modèle pour répondre aux questions de recherche énoncées. La section suivante concerne les Matériels et Méthodes, où sont détaillés les dispositifs expérimentaux, le matériel végétal étudié, les méthodes d'échantillonnage et les analyses en laboratoire ayant permis d'obtenir les caractères d'intérêt, incluant la croissance, la réponse à la sécheresse et les caractéristiques fonctionnelles chez les différents taxa, ainsi que les méthodes statistiques utilisées. Nous avons également inclus une section dédiée à la Terminologie, étant donné que cette thèse mobilise plusieurs concepts écologiques clés nécessitant une définition claire afin d'assurer une compréhension cohérente tout au long du manuscrit.

Suivent les trois chapitres principaux de résultats :

- Chapter I: "A Decade-long study on Mortality in European Larch (*Larix decidua* Mill.), Japanese Larch (*Larix kaempferi* (Lamb.) Car.) and their hybrid"
  Naincy Sagar, Luc E. Pâques, Stéphane Chantepie. (Cet article a été publié dans Annals of Forest Science, doi: 10.1186/s13595-025-01294-7).

- Chapter II: "Prediction Models for *Larix* species via NIRS Spectroscopy as a High-Throughput Phenotyping Approach".

Naincy Sagar, Nassim Belmokhtar, Anna Alves, Nathalie Boizot, David Chassagnaud, Régis Fichot, José Rodrigues, Luc E Pâques (article accepté avec de légères révisions dans Journal of Near Infrared Spectroscopy).

- Chapter III "Expression of heterosis for growth under contrasted soil watering treatments and over time in interspecific larch hybrids (*Larix x eurolepis*). Are fast-growing genotypes and taxa more vulnerable to water constraints?"
  Naincy Sagar, Luc E Pâques

Enfin, une Discussion générale aborde l'ensemble des résultats de l'étude, les met en perspective avec la littérature existante et en souligne les implications pour les stratégies de sélection et l'adaptation écologique. La Conclusion présente l'idée finale de la thèse et propose une ouverture vers les pistes futures autour des questions que nous avons tenté de formuler.

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1. General Introduction

1.1. Evolving breeding paradigms

1.1.1. Climate change and shifting forest dynamics

Climate change is not a new concept and has been the subject of discussion among a diverse group of scientists, from climatologists and environmentalists to ecologists and breeders, all examining either the evidence of climate change or its potential impacts. Climate is the flow of heat between the geological, hydrological, and atmospheric components of Earth. As long as these particular components repeatedly cross their respective thresholds, climate change is bound to occur (Taylor 1999). Global temperatures are rising, and in Europe, the average has already increased by 1.1°C, impacting natural and human systems. Extreme events have become more frequent, with intensified temperature and precipitation anomalies (Intergovernmental Panel on Climate Change (IPCC) 2023). The extreme European heatwave of 2003 was one of the earliest clear demonstrations of climate change impacts (Schär and Jendritzky 2004). Climate change projections for Europe estimate a temperature increase of around 2 °C in Ireland and the UK, about 3 °C in Central Europe, and as much as 4–5 °C in Boreal regions and parts of the Mediterranean by 2100 (Christensen et al. 2007). In particular, temperatures in the southern and western parts of Europe are expected to rise by around 3 °C, accompanied by a projected 74% reduction in thermal comfort hours during summer (Intergovernmental Panel On Climate Change (IPCC) 2023).

These climatic changes have far-reaching consequences for both human systems and ecosystems. The livelihood of communities has been affected due to climate change perceptions and vulnerabilities that make communities highly marginalized with lack or depletion of basic resources (food and water), like the Chepang community in Nepal (Piya et al. 2019). Forests, as critical components of the Earth's biosphere, are increasingly affected by the accelerating pace of climate change. They are not only responsive to changing climatic conditions but also central to global carbon, water, and energy cycles. Across many regions, forest ecosystems are facing heightened uncertainty due to shifting climate regimes (Millar et al. 2007; Keenan 2015). Such uncertainties include increasingly spatially heterogeneous precipitation patterns, characterized by prolonged dry spells and more frequent episodes of heavy rainfall across the globe (Donat et al. 2013). The number of heatwave events has shown a sharp increase globally over recent decades—for example, the annual number of heatwave days has doubled in parts of Europe and Asia since the 1980s, reflecting a clear intensification of extreme heat under climate change (Zuo et al. 2015), and they are becoming more common (Dosio et al. 2018). Future droughts are likely to be more severe than those of the past due to anthropogenic climate change, even in regions where precipitation does not decrease, as warming enhances evaporative demand (Cook et al. 2018). With these increasing extremes, forest health is bound to decline due to heightened exposure to

pathogens, insect outbreaks, and diseases often triggered or intensified by climate stress. Their repeated occurrence can weaken tree defenses, reduce resilience, and lead to widespread dieback or mortality, especially in already vulnerable species or ecosystems (Sturrock et al. 2011).

Climate dynamics have also been reshaping forest productivity, with adverse effects on the growth and vitality of trees (Boisvenue and Running 2006). A stagnation or decline in basal area increment of *Fagus sylvatica* was found in southern France post - 1980s, largely due to increasing drought frequency and climate warming (Charru et al. 2010). *Populus tremuloides* had a 50% decline in basal area increment between 1976 and 1981, with additional growth reductions observed again during the 1990s, reflecting the compounded effects of drought and insect attacks (Hogg et al. 2005).

Along with these changes in forest productivity, migration or changes in species zones have also been reported. With increasing environmental stress, many species have begun to gradually shift toward more suitable habitats, revealing signs of recent migration lags. There is a reported shift in the Montseny mountains in NE Spain of Beech (*Fagus sylvatica*) forest by 70 m altitude and has been replaced by holm oak (*Quercus ilex*) forest at medium altitudes (800±1400 m) due to the warmer conditions and the land use changes (Peñuelas and Boada 2003). The most well-documented and visible impact of climate change on forests is the rise in tree mortality across all forested biomes worldwide (Hammond et al. 2022).

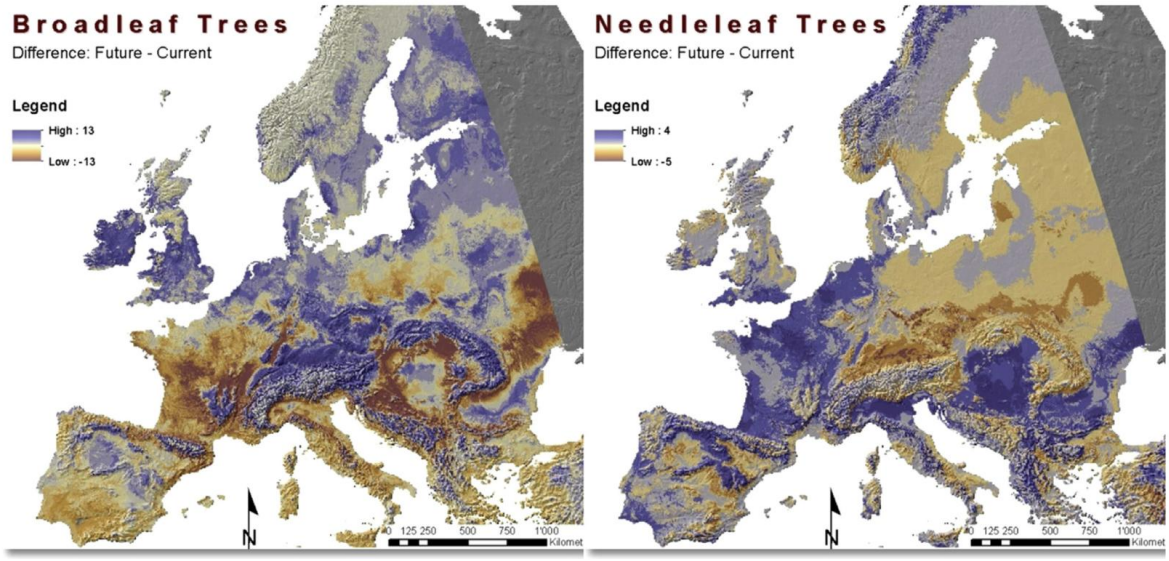


Fig. 1: Projected Impact of Climate Change on Tree Species Richness Across Europe by 2100 (Lindner et al. 2014). Maps show modeled changes in species richness for broadleaf and needleleaf trees between the historical period (1961–1990) and projected future climate (2071–2100) under the A1B scenario. Blue indicates areas of species gain; yellow to brown indicates areas of species loss.

Fig.1 shows the future projections that are likely to occur with considerable shifts of trees and biomass, likely as a consequence of climate change (Lindner et al. 2014). The left map shows changes for broadleaf trees (like oak or beech), and the right map shows changes for needleleaf trees (like pine or spruce). Areas in blue are expected to see an increase in the number of tree species, while regions in brown or yellow will likely see a decrease. For broadleaf trees, northern and central parts of Europe may become more suitable, leading to more species. At the same time, southern regions like Spain and Italy may lose diversity due to hotter and drier conditions. For needleleaf trees, the overall trend is more negative, with species losses expected across most of central and southern Europe.

These projected shifts in species distributions and diversity not only reflect the immediate ecological impacts of climate change but also signal the beginning of longer-term evolutionary responses. To fully understand forest futures, it is essential to move beyond short-term dynamics and consider how species and forest systems may evolve, adapt, or fail to keep pace with the rapidly changing climate

1.1.2. Shifting perspectives with forest evolution

Projections of future temperature, precipitation, forest cover, or species richness can point to significant losses in forest ecosystems in the future. However, these models offer no indication that the uncertainties surrounding future climate will resolve in the coming decades. Rather than waiting for certainty, these insights call for a fundamental shift in forest management toward proactive strategies that can accommodate and respond to an unpredictable future (Millar et al. 2007; Lindner et al. 2014; Malhi et al. 2020). In changing forest dynamics, integrating diverse management strategies is essential to maintain ecosystem resilience and productivity. Forest dynamics themselves are shaped by various interacting processes, from individual tree responses to resource limitations to broader shifts in species composition and structure. These responses, especially under climate-induced constraints like water or nutrient stress, can cascade from the tree level to influence stand and landscape-level trajectories. These mechanisms at the tree level response are explored further in Section 1.2, Response under Resource Limitation.

In light of these cascades, scientific perspectives are changing. We are in the era of the “toolbox” approach: a plan that can accommodate multiple stressors, such as high temperatures, reduced precipitation, or disease outbreaks that can either mitigate these damages or cope with them. This can only be achieved by implementing new practices over space and time (Millar et al. 2007).

Three main strategies for future forest management are discussed or proposed at the stand level: conservation of existing forest structures, active adaptation through silvicultural interventions, and passive adaptation via natural succession. Each strategy offers a different balance between intervention and flexibility and should be applied based on site-specific climate risks (Kolström et al. 2011). “Adaptation” is the process, action, or capacity of an individual or system to modify its inherent genetic or behavioral characteristics to better cope with change, often through adjustments (inspired by Zhai and Lee 2024). Adaptation strategies should incorporate an analysis of climate

vulnerability and an evaluation of current coping mechanisms under climate change. **Assisted migration** is one such active tool that involves intentionally relocating tree species to areas predicted to be more suitable under future climatic conditions. Bower et al. 2024 discussed that forestry-assisted migration, guided by a structured framework, including climate-based seed selection, risk assessment, and stakeholder engagement, can support climate-resilient reforestation. The assisted migration of Mediterranean oaks to Central Europe has already been established in southwest Germany (Koller et al. 2014). Among the different forms of assisted migration, **Assisted Species Migration** (ASM)—which involves moving entire species beyond their native ranges—is the most ambitious. While it carries greater ecological uncertainty, ASM can offer essential opportunities for conserving threatened species and sustaining ecosystem services in regions where native flora may no longer be viable (Bower et al. 2024).

Active adaptation also includes new **modifications in silvicultural methods** like thinning and tending that could alter the structure and composition of species and assist them in better adaptation (Gustafson et al. 2020). Thinning enables the tree to distribute risks through diversification, allowing the tree to recover after a drought period (Spiecker 2003). **Forest tree breeding** is an integral part of modern silviculture, aimed at enhancing genetic gains in growth through the selection and propagation of superior genotypes (White 1987; Ruotsalainen 2014). However, to remain effective under rapidly changing climatic conditions, traditional breeding approaches must evolve, emphasizing climate-driven strategies and integrating genomic tools, high-throughput phenotyping, and predictive modeling to develop climate-resilient forests at a pace that matches accelerating climate change (Cortés et al. 2020).

1.2. Tree Response under resource limitations

1.2.1. Shift in strategies

As breeding goals must evolve to incorporate resilience, it becomes essential to understand how trees allocate limited resources under stress. Under varying resource conditions, plants exhibit distinct shifts in their physiological processes. At moderate to high resource availability, plants tend to allocate resources towards increasing growth rates. However, under low resource conditions, there is a notable shift towards secondary metabolism. This phenomenon is encapsulated in the **growth-differentiation balance hypothesis** (GDB) proposed by Herms and Mattson 1992, which suggests that plants face a trade-off between growth and other mechanisms depending on resource availability. The GDB Hypothesis (**Fig.2**) suggests that these resource allocation patterns have significant implications for plant life history strategies. Plants in resource-rich environments tend to be fast-growing but poorly defended, relying on their ability to outcompete neighbors and recover quickly from damage. In contrast, plants in stressful environments grow more slowly but invest heavily in defensive traits, making them more resistant to herbivory and adverse conditions. Secondary metabolites, which are part of differentiation rather than growth, are particularly sensitive to shifts in resource allocation. When growth is suppressed

due to environmental constraints, plants may divert surplus carbon resources into producing these compounds, making them more resistant to herbivory and ecological stressors.

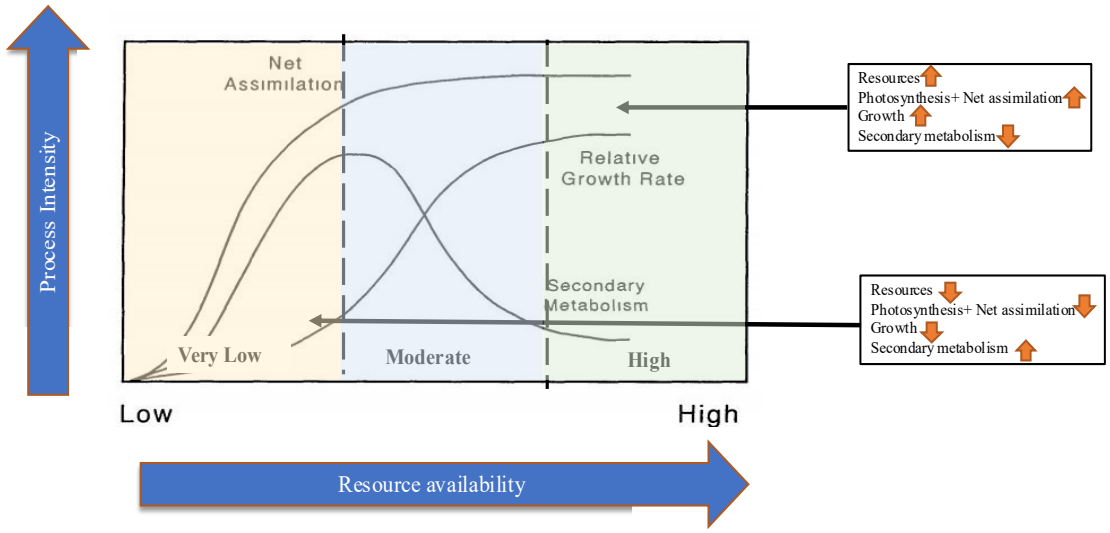


Fig 2: The Growth differentiation model as inspired by Herms and Mattson 1992

This shift implies that resource assimilation and transport mechanisms are adjusted, enabling plants to adopt strategies that enhance their survival under resource constraints. Such adjustments are not confined to a single pathway but involve modifications across the plant's entire life history, affecting various physiological and developmental processes (Lauder et al. 2019). Under such a strategic shift from growth to secondary metabolism, the plant faces a "fight or flight" decision: whether to continue investing in height and radial growth or to divert resources toward defense mechanisms and reproductive efforts - both aimed at ensuring survival under constrained conditions (Climent et al. 2008; Lauder et al. 2019). A depiction of the paths is shown in **Fig. 3**.

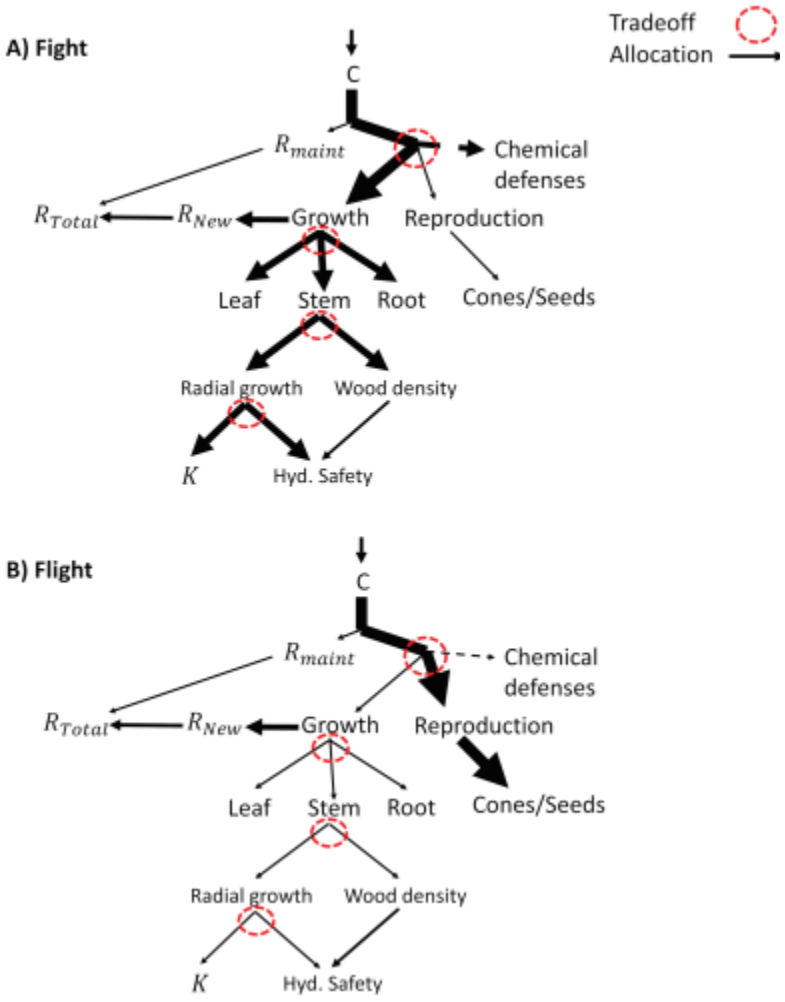


Fig 3: The two modes of path A) Fight to invest in growth or B) Flight to invest in reproduction (Lauder et al. 2019)

These pathways and models are easy to conceptualize in theory, but they are challenging to apply in real-world scenarios for specific species and environments due to the complex interactions among multiple traits (Stamp 2004). The Growth–Differentiation Balance Hypothesis has been critically reviewed by Stamp (2004), who questioned whether it can be rigorously tested, given the complexity of trait interactions. Traits like resin ducts, for instance, are often categorized under defense due to their role in deterring herbivory or pathogens. Yet, they also serve physiological functions such as storage and transport, as they are closely connected to the ray parenchyma cells. So, these models have to be integrated with multiple approaches of traits that coordinate and, as a whole, actually highlight this growth differentiation so that we do not underestimate the plant strategy behind it.

Specifically, under resource limitations such as drought, plants undergo significant changes in water relations and photosynthetic activity. Drought stress reduces water availability, causing stomatal closure to minimize water loss. This closure, however, restricts CO_2 intake, thereby

inhibiting photosynthesis. Consequently, plants experience reduced growth and productivity under such conditions.

1.2.2. Response under drought

"Drought" is one of the most pressing concerns in the climate change issue: it is one of the most discussed threats since the 1920s (Gorham and Kelly 2018). and research interest in drought has particularly increased in recent decades, according to worldwide forecasts (Alley et al. 2007). However, the first step toward raising awareness and understanding of drought is to define what drought means clearly. The definition of drought can either be conceptual or operational. Dictionary quoted definitions name drought as "a long period of no rain, especially during planting season," by the American Heritage Dictionary 1976. Drought, on a more practical or operational definition, would be one where daily precipitation values are compared to rates of evapotranspiration to determine the soil moisture content, and also visible signs of these depletions on plant conditions at various organ levels (Wilhite and Glantz 1985). Such definitions analyze drought frequency, severity, and duration for a given period. The World Meteorological Organization defines drought as a "period of abnormally dry weather sufficiently prolonged for the lack of precipitation to cause a serious hydrological imbalance". There are about four types of drought as identified by Subramanyam 1967 and reviewed by Wilhite and Glantz 1985:

1. Meteorological drought: This is the most common kind of drought, which is mostly "a sustained period without significant rainfall" (Linsley Jr et al. 1975).
2. Agricultural Drought: This drought is defined based on the effect of this lack of water on the characteristics of plants. Kulik 1976 defined 0.2 m of soil as critical for plant growth, and drying of this layer is an early indicator of drought intensity
3. Hydrological Drought: This drought is defined as "a period during which streamflows are inadequate to supply established uses under a given water management system" (Linsley Jr et al. 1975)
4. Socio-economic drought: Socio-economic drought is the effect of a drought period associated with the supply and demand for some economic good.

The concept of drought has been discussed, especially whether extensive studies have been done on this pressing concern. Ecological drought definitions have recently been proposed (e.g. "an episodic deficit in water availability that drives ecosystems beyond thresholds of vulnerability, impacts ecosystem services, and triggers feedbacks in natural and/or human systems" (Crausbay et al. 2017)). A literature survey was conducted on "drought" and "ecology" and included about 980 papers. It was found that most of the studies do not define what drought is in their particular experiments, which is a fundamental part of starting before talking about these results and impacts from drought (Slette et al. 2019).

This bar graph (**Fig. 4**: (Slette et al. 2019)) shows how scientific publications define "drought." The most common way is simply by using the term "dry" (over 30%), followed by descriptions like "differs from normal" or using a standardized drought index. Fewer studies define drought through

actual measurements like reduced rainfall, low water flow, plant water stress, or low soil moisture. The smallest category links drought to the dry season. The chart reveals that the majority of publications (about 70%) do not clearly define drought at all, while only around 30% do. This highlights a significant inconsistency in how drought is described in scientific research.

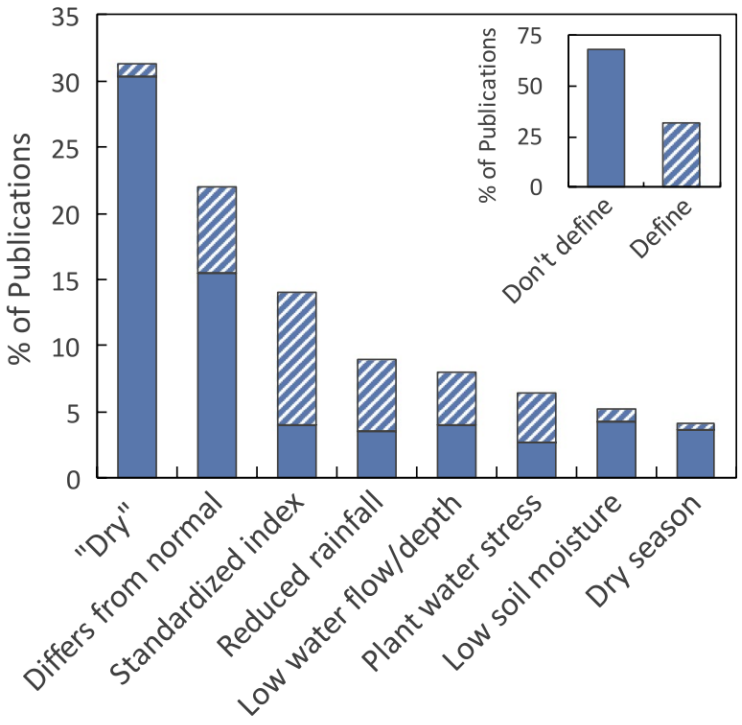


Fig 4: Definitions of "drought" used in scientific publications (Slette et al. 2019)

So, it has become necessary to develop a standardized indicator of drought that can assess whether drought is prevalent or not. However, understanding drought is not only about how it is defined, but also how plants and trees respond to it. While many tree mortality events have been linked to drought (Allen et al. 2010; Choat et al. 2018), the underlying physiological and structural responses that lead to such outcomes are still not fully understood. Are we truly aware of how trees react to water scarcity before reaching the point of mortality? Drought and heat stress significantly impact tree development, leading to gradual but profound functional modifications. One of the primary concerns in forestry and ecological studies is how trees respond to resource limitations, particularly water deficits. Drought affects tree growth by disrupting water uptake, reducing photosynthesis, and altering hormonal signaling, collectively influencing survival and productivity. Soil and atmospheric drought contribute to these challenges, with soil drought limiting water availability at the root level and air drought increasing evaporative demand (McDowell et al. 2008; Ryan 2011; Attia et al. 2015; Groover et al. 2025). As trees experience prolonged drought, they undergo structural and metabolic adjustments that may enhance resistance or resilience, depending on species-specific traits and environmental conditions **(Fig. 5)**.

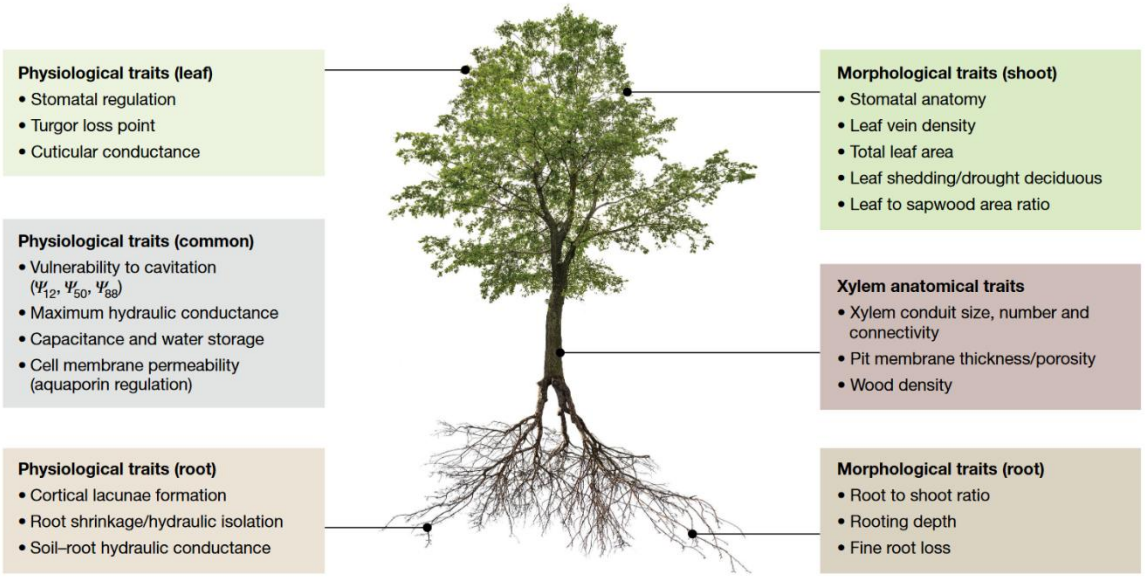


Fig 5: Overview of key traits influencing plant drought response across organs. Traits are categorized as physiological or morphological and are organized by plant part: leaf, shoot, root, or common to all tissues (Choat et al. 2018)

Morphologically, many species reduce leaf area through early leaf shedding or produce smaller leaves to limit transpiration water loss (McDowell et al. 2008). Droughted oak leaves exhibited visible symptoms such as marginal necrosis and chlorosis, alongside internal changes like >90% mesophyll starch depletion, epidermal collapse in up to 90% of cells, and increased plastoglobules (3–4 vs. 1–2), indicating severe physiological stress and cellular degradation (Vollenweider et al. 2016). Root architecture may also change, with increased allocation to deeper or finer roots to access residual soil moisture (Brunner et al. 2019). Such above- and below-ground structural adjustments are often accompanied by changes in wood anatomy.

Drought or stress often restricts growth processes before it significantly limits photosynthesis (Körner 2019). In conifers, drought can accelerate the transition to latewood, increasing tissue density and shrinkage risk. In several conifers including larch, stem cracks due to drought extended up to 3.78 m, reflecting mechanical failure associated with internal stress accumulation (Cameron et al. 2017, Pâques et al. 2022). Drought episodes can also lead to a significant reduction in cambial activity, as observed in *Quercus ilex*, where the number of xylem cells decreased by 70% and radial cell diameter was reduced by 30% compared to control years (Gil-Pelegrín et al. 2004). One symptom of this depletion in water availability is top dieback, observed at the canopy level and along the upper stem (Camarero at al. 2021). Dieback has significant ecological impacts, leading to localized declines in forest cover and productivity, disrupting carbon and water cycles, and driving shifts in vegetation composition, often favoring more drought-tolerant tree and shrub species or accelerating successional change.

Physiologically, one of the first responses is stomatal closure to reduce water loss, though this also limits CO_2 uptake and photosynthesis, affecting growth. Under drought, *Picea abies* showed a significant drop in predawn leaf water potential, decreasing from around –0.4 MPa (control) to –1.6 MPa after 22 days of water stress. This was accompanied by a sharp reduction in net photosynthetic rate, from 6.3 to 0.2 μmol CO_2 m^{-2} s^{-1}, and stomatal conductance, which declined from 210 to 10 mmol H_2O m^{-2} s^{-1}. Relative water content (RWC) also dropped by over 20%, indicating cellular dehydration. Despite this, trees maintained partial turgor through osmotic adjustment, suggesting a capacity for short-term drought tolerance (Ditmarová et al. 2010). Prolonged water stress can impair hydraulic conductivity, leading to xylem cavitation and embolism formation—major drivers of tree mortality during intense droughts (Choat et al. 2012; Anderegg et al. 2016). On a biochemical level, trees may accumulate osmo-protectants such as proline or soluble sugars and activate antioxidant defense systems to mitigate oxidative damage caused by drought-induced stress (Chaves et al. 2003; Rennenberg et al. 2006). Together, these responses reflect a coordinated effort to survive under water-limited conditions, but their effectiveness and reversibility vary widely across species and environmental contexts.

The impact of drought on tree function and survival is strongly influenced by its timing, duration, and frequency. Early-season droughts can interfere with budburst and early growth processes, while late-season events often affect resource accumulation and preparation for the next growth cycle (Bréda et al. 2006). Prolonged droughts increase the likelihood of cumulative hydraulic damage and carbon starvation (Choat et al. 2018). Moreover, repeated droughts may weaken defense capacity, resulting in reduced resilience and increased susceptibility to pests and pathogens (Guisset et al. 2024). Studies on Mediterranean and temperate species show that not only the intensity but also the recurrence interval of drought plays a decisive role in shaping long-term survival and productivity (Gazol et al. 2018; Lloret et al. 2011; Lloret et al. 2022).

To evaluate the impact of drought on trees across diverse environmental contexts, researchers have developed frameworks that quantify tree performance. Key indices include resistance (the ability to maintain growth during drought), recovery (the rate of return to pre-drought growth), and resilience (the overall capacity to absorb and rebound from stress), as proposed by Lloret et al. (2011). However, this framework has since been refined to address limitations in comparability and sensitivity to baseline growth levels. Schwarz et al. 2020 suggested that modifications to these indices are necessary, as the pre-drought growth period can be highly variable. They emphasized that resistance and resilience are sensitive to both the length and severity of drought, and highlighted the importance of carefully selecting consistent pre- and post-drought reference periods to avoid biased interpretations. Recent studies have expanded these assessments by incorporating functional traits, such as cavitation resistance (P_{50}) and stomatal sensitivity, which can predict hydraulic vulnerability and drought coping strategies. For instance, Bartlett et al. (2016) demonstrated that species with more negative P_{50} values, indicating greater resistance to embolism, tend to sustain hydraulic conductivity under severe drought, correlating strongly with survival outcomes (P_{50} explained 62% of variation in mortality). Similarly, Anderegg et al. 2016 found that stomatal regulation strategies interact with hydraulic traits to mediate drought-induced mortality risk across taxa, with species exhibiting tight stomatal control and low xylem vulnerability showing enhanced survival.

While these functional traits enhance drought survival, they do not come without cost. The ability to resist cavitation or maintain tight stomatal control often involves physiological or anatomical adjustments in their life history that limit carbon gain, growth potential, or resource use efficiency under favorable conditions.

1.2.3. Trade-offs as central to evolution

Life history theory provides a framework for understanding how organisms allocate their limited resources to growth, reproduction, and survival over their lifespan. Trade-offs, a central concept in life history evolution, are the actual costs organisms must pay when a beneficial trait is modified, especially under resource constraints. As Garland (2014) explains, a trade-off exists when an increase in one trait comes at the expense of another, primarily due to the limitation of resources. In trees, trade-offs exist across diverse traits, influencing their functional strategies and evolutionary pathways. Stearns (1989) highlights that trade-offs are shaped by genetic predisposition and environmental conditions, meaning that a tree's ability to balance growth, defense, and reproduction is influenced not only by inherited traits but also by external stressors like drought, soil fertility, and competition.

Demonstrating whether a trade-off truly exists remains difficult, as it requires disentangling complex interactions among traits, environmental conditions, and life stages, whether in plants or humans (Stearns 1989; Roff and Fairbairn 2007; Bolund 2020). Complex phenotypic correlations—those observed directly between traits in individuals—are often used to infer trade-offs, but they can be misleading. These correlations may arise not from underlying genetic constraints but from shared environmental influences, plasticity, or trait integration. In contrast, genotypic correlations, which reflect the genetic covariance between traits across genotypes, provide a more accurate picture of heritable trade-offs and are generally preferred in evolutionary and breeding studies (Laitinen and Nikoloski 2024). However, when only phenotypic data are available, they can still be informative if used cautiously.

The framework in **Fig 6** highlights four key axes for evaluating trade-offs (Laitinen and Nikoloski 2024). First, the type of measurement used (panel a) distinguishes between linear relationships (assessed via Pearson correlation) and nonlinear ones (captured by Spearman or Kendall methods), acknowledging that trade-offs may not always be proportionally expressed. Second, the environmental scope (panel b) separates absolute trade-offs, observable across all environments, from relative ones that appear only under specific conditions, such as drought. Third, the biological scale (panel c) asks whether the trade-off manifests at the individual level or reflects broader population-level patterns across generations.

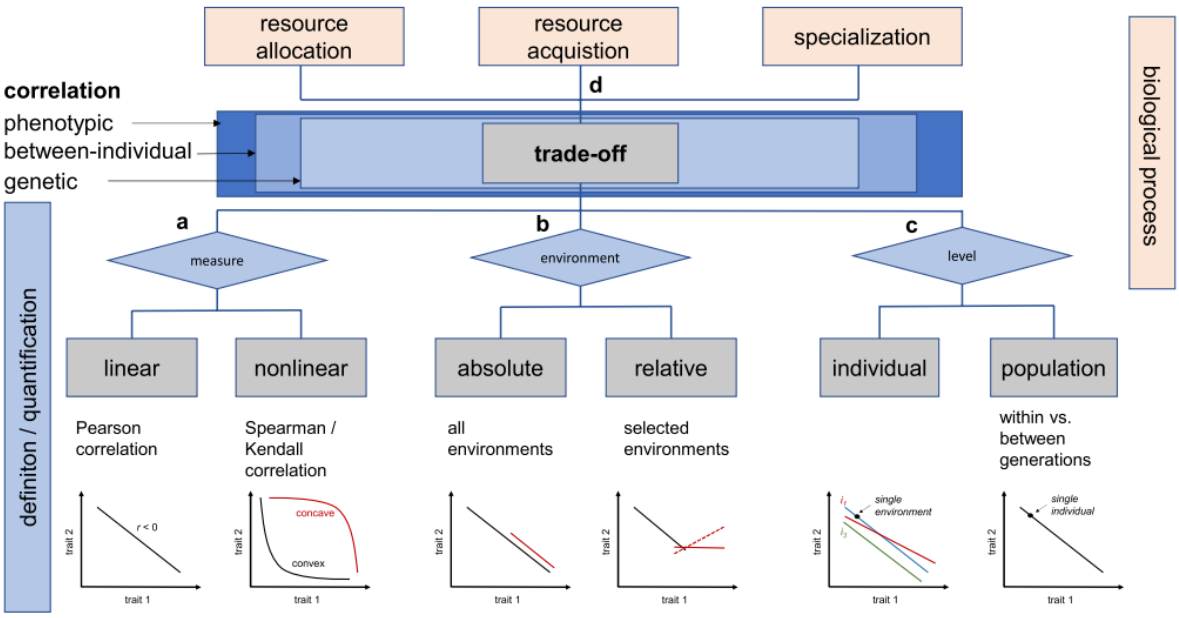


Fig 6: Studying trade-off is a multi-dimensional and context-dependent process (Laitinen and Nikoloski 2024) with (a) Measurements used to study trade-off, (b) Trade-off occurs differently in different environments, (c) Trade-off can occur at the individual to population level, (d) underlying biological process.

To improve reliability, phenotypic analyses should be conducted in controlled or common garden environments and complemented by replication across environments or years (Bolund 2020). Additionally, using mixed models, path analysis, or multivariate approaches (traits) can help isolate trait relationships and minimize the risk of spurious correlations (Lailvaux and Husak 2017). Thus, while genotypic correlations are ideal, carefully designed phenotypic analyses, when correctly interpreted, can still yield valuable insights into potential trade-offs.

One of the most evident trade-offs in trees is the balance between growth and reproduction, reflecting the allocation between somatic expansion and fecundity. Reproduction takes about 8-10% of carbon cost (Gower et al. 1995). There have been positive correlations between growth and reproduction in *Pinus halepensis* (Ayari and Khouja 2014) and *Abies* (Hisamoto and Goto 2017), but these are not compared between mast and non–mast years. However, in *Plantago coronopus* populations across Europe, growth had an adverse effect on the probability of reproduction under low soil fertility. In contrast, this relationship became positive or negligible under high-fertility conditions (Villellas and García 2018).

The trade-off between growth and survival is a central axis of life-history variation in tropical trees, and Wright et al. 2010 provided compelling quantitative evidence for this relationship based on a functional trait analysis of tree saplings on Barro Colorado Island, Panama. Across species, the 95th percentile of relative growth rate (RGR95) was negatively correlated with the mortality rate of the slowest-growing 25% of individuals (M25), with $r^2 = - 0.33$, $p<0.05$ for growth and $r^2 =$

$–0.31$, $p<0.05$ for mortality. Among the traits examined, wood density (WD) was the strongest predictor of reduced growth and increased survival, reflecting a classical trade-off where species

with dense wood grow slowly but are more resistant to mortality risks. Leaf mass per area (LMA) was also negatively correlated with both growth (r^2 =-0.24, $p < 0.05$ for RGR95) and mortality (r^2 = - 0.17, $p<0.05$ for M25), consistent with a resource-conservative strategy. Seed mass (SM) had weaker but significant effects (r^2 = –0.066 to –0.15, $p<0.05$), and maximum height (H_{max}) showed minimal explanatory power. Multiple regression models incorporating all traits explained up to 41% of the variation in mortality and 50% in growth, reinforcing that species' positions on the growth–mortality spectrum are trait-driven. These findings demonstrate that growth and survival trade-offs are deeply embedded in functional strategies, with wood density and leaf traits acting as key structural determinants of a species' ecological performance. In *Pinus edulis*, Redmond et al. (2019) found that growth and defense (measured via resin ducts) were negatively related at the individual level, while growth and reproduction showed no significant trade-off, suggesting flexibility in allocation depending on environmental context

As environmental pressures shift, further research into these trade-offs will be crucial for understanding forest dynamics, improving conservation efforts, and guiding sustainable forestry practices.

1.2.4. Role of growth: fast vs slow strategy

Tree growth strategies can be broadly classified into fast-growing and slow-growing species, each exhibiting distinct ecological adaptations that influence survival, resource allocation, and physiological resilience. Growth is a fundamental trait that shapes tree dynamics, influencing their ability to compete, survive environmental stress, and contribute to forest structure. Under constrained environments like drought, growth is affected before photosynthesis (Körner 2019). Growth and size can be an early indicator of future strategies in constrained environments (Dobbertin 2005).

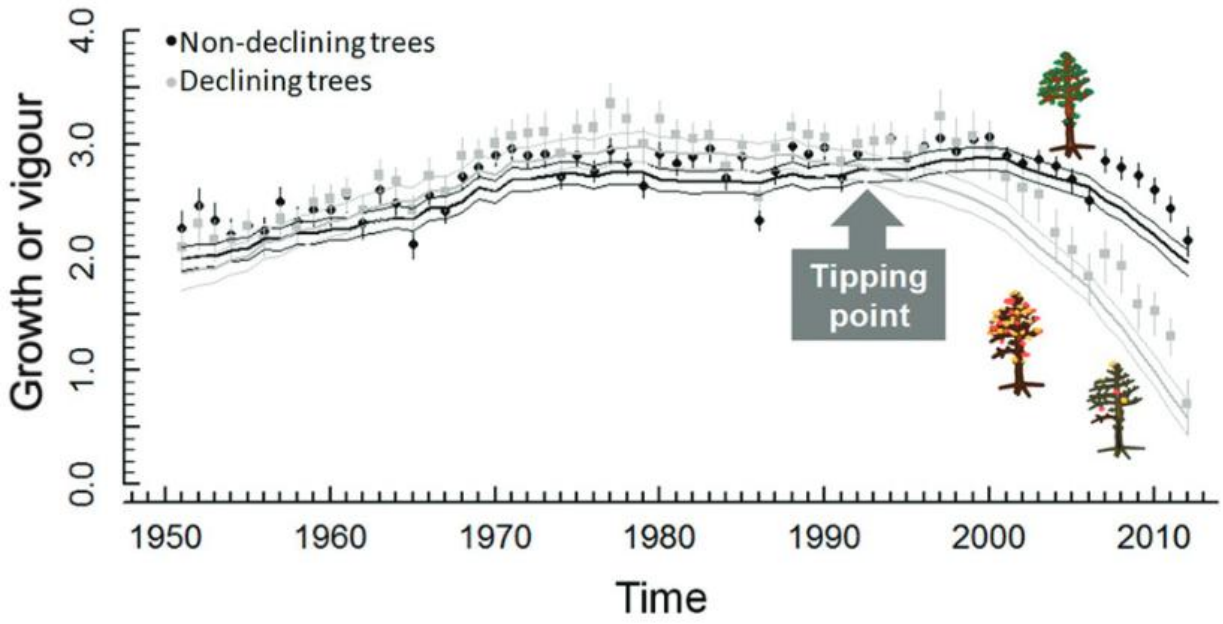


Fig 7: Plot showing the "tipping point" between declining and non-declining *Abies alba* trees over 20 years highlights how drought-triggered mortality is species-specific, revealing distinct responses (from Camarero 2021, which was modified from Camarero et al. 2015).

In **Fig. 7**, both groups initially show a steady increase in growth through the mid to late 20th century, with non-declining trees maintaining slightly higher growth levels. However, a clear tipping point occurs in the early 1990s, beyond which the growth trajectories sharply diverge. While non-declining trees sustain relatively stable growth, declining trees experience a marked reduction in vigor. This abrupt decline suggests that although some trees appear healthy for decades, they fall in growth after they may cross a physiological threshold. The figure underscores that tree decline may not be immediately detectable and often follows a long lag period after exposure to environmental stress, emphasizing the importance of early physiological warning signs before visual symptoms appear.

In recent years, there has been a growing trend of favoring slow-growing species ('Differentiation dominated plants') instead of fast-ones ('Growth dominated plants') due to their enhanced resilience and longevity, despite the widespread use of fast-growing species for timber, biomass, and carbon sequestration (Reich 2014). Fast-growing species, such *Larix* sp. prioritize rapid height and diameter expansion, allowing them to outcompete neighboring vegetation for light and space even exhausting their resources (Herms and Mattson 1992). These trees typically have lower resource use efficiency and storage reserves.

Plant trait	Growth dominated plants	Differentiation dominated plants
Relative growth rate	high	low
Resource acquisition rate	high	low
Resource use efficiency	low	high
Resource turnover rate	high	low
Allocation to leaf area	high	low
Storage reserves	low	high
Degree of sclerophylly	low	high
Secondary metabolism	low	high
Respiration rate	high	low
Proportion of respiratory energy supporting maintenance	low	high
Phenotypic plasticity	high	low
Constitutive resistance to herbivores	low	high
Induced resistance to herbivores	high	low
Competitive ability		
Resource-rich environments	high	low
Resource-limited environments	low	high

Sources: Grime, 1979; Chapin, 1980a; Coley et al., 1985; Coley, 1987; Huston and Smith, 1987; Chapin et al., 1990.

Fig 8: Comparison between growth dominated (fast) and differentiated dominated (slow) plants differential responses (from Herms and Mattson 1992)

In contrast, slow-growing species adopt a conservative strategy, allocating more resources toward defense, storage, and structural reinforcement rather than rapid biomass accumulation. These trees tend to develop denser wood, which enhances mechanical stability and resistance to pests and diseases (**Fig 8**). Eller et al. (2018) found that fast-growing tropical annuals exhibit a trade-off between high growth rates and hydraulic safety, as they tend to be more vulnerable to embolism in their xylem conduits with a sap flux density 12.9 times higher than that of slow-growing species. Signori-Müller et al. 2022 investigated non-structural carbohydrate (NSC) storage in Amazonian canopy trees and found that slow-growing species, such *as Eschweilera coriacea*, maintained significantly higher NSC reserves, particularly in their woody stems and roots. This increased carbohydrate storage allows these trees to buffer against periods of environmental stress, such as drought or nutrient scarcity, improving their survival compared to their fast-growing counterparts.

Kaczmarek et al. (2013) observed that some fast-growing *Populus clones* demonstrate improved survival under constrained environments. If the fast-growing trees can establish strong root systems

early in development, rooting success determines long-term survival in fast-growing species. This was also supported in a study on hybrid Larch, which was vigorous in above-ground biomass but also had finer roots than European and Japanese Larch, which suggests different allocation patterns (Pâques et al. 2022).

The debate between utilizing fast- and slow-growing species is particularly relevant in climate change and forestry management. Fast-growing trees are often favored for commercial purposes and afforestation projects due to their ability to sequester carbon and provide economic returns rapidly. However, their structural and hydraulic limitations can make them more vulnerable to environmental extremes. Conversely, slow-growing trees contribute to ecosystem stability and long-term carbon storage, making them valuable for conservation and restoration efforts. Tao et al. (2024) explored the relationship between growth rate and drought resilience in Northern Hemisphere forests. They found that slow-growing trees exhibited greater resistance to drought stress but required longer recovery times, highlighting the importance of balancing short-term productivity with long-term resilience.

Understanding the trade-offs between growth rate, survival, and resilience is essential for optimizing forest management strategies. While fast-growing species are valuable for rapid biomass production, integrating slow-growing trees into managed forests can enhance ecosystem stability, biodiversity, and long-term carbon storage. The decision to prioritize one strategy over the other depends on ecological goals, site conditions, and management objectives, necessitating a nuanced approach to forest conservation and silviculture. This calls for an integrated approach of an ecologist style and a breeding approach as a need in the era of climate change.

1.3. Bridging ecology and breeding as a need

1.3.1. Modern tree breeding: approaches and challenges

While some traditional tools still offer opportunities to accelerate breeding under climate change, tree breeders must develop innovative targeted strategies that deliver operational gains while minimizing potential losses due to climate change. At present, traditional breeding methods are not as rapid in responding to climate change, and that is why it is necessary to advocate for a modern, integrative approach. Crucially, it is essential to stress the importance of integrating genetic, ecological, and climatic data through modeling tools to predict tree performance under future conditions, enabling more informed breeding and deployment decisions (Luss et al. 2015; Gray et al. 2016; Chakhchar et al. 2017).

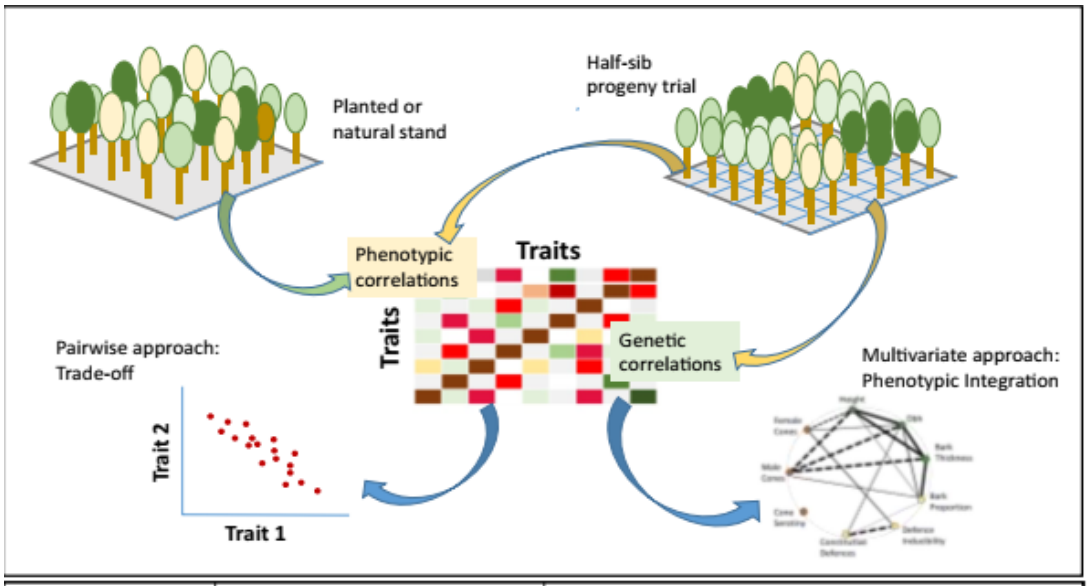
One of the key priorities for breeders is to enlarge the target traits beyond growth, stem form like stem straightness (Byram et al. 2005), and wood properties, like fiber length and stiffness (Ivković et al. 2009). Climent et al. (2024) discuss that overemphasizing rapid growth may lead to unintended trade-offs, such as reduced survival rates, lower reproductive success, and increased susceptibility to pathogens. Their study highlights the necessity of integrating additional traits, such as drought resistance, nutrient-use efficiency, and root system architecture, into breeding programs

to create more adaptable tree populations. The **multi-trait integration** is necessary to expand the target traits beyond growth, stem form, and wood properties, enabling the inclusion of functional characteristics such as root system architecture, water-use efficiency, nutrient acquisition, and stress tolerance - traits that are critical for enhancing adaptability and resilience under changing environmental conditions (York 2019).

Traditionally, breeders have relied on **site-specific performance** as a proxy for adaptability, usually including provenance and progeny testing (Mátyás 1996), but this method often overlooks the underlying genetic and physiological mechanisms of adaptation as well as the specific pedo-climatic factors driving trees responses.

To further refine breeding strategies, it is essential to decompose and study **complex traits such as growth or wood formation into more functional traits** and to evaluate their breeding potential. Many desirable characteristics, such as drought tolerance or pest resistance, are polygenic and influenced by multiple physiological and molecular factors. By breaking down these complex traits into functional components, breeders can more effectively identify genetic markers associated with resilience and adaptability. Since obtaining such a massive number of traits in a tree system is time-consuming and labor-intensive, **high-throughput techniques** assist in these methodologies. Developing high-throughput techniques to assess functional traits routinely is another primary requirement for advancing breeding efficiency. Advances in remote sensing, machine learning, and automated trait measurement systems now enable breeders to assess physiological and morphological traits at an unprecedented scale, especially under drought events (Kim et al. 2021). Studies have shown that high-throughput phenotyping can enhance selection in crop breeding by early detection of diseases (Shakoor et al. 2017).

Getting robust information on **levels of genetic variability,** heritabilities (h^2) and associations between traits (correlations, trade-offs) is critical for making informed breeding decisions. Some traits may exhibit high genetic variability, making them easier to improve through selection, while others may be tightly linked to undesirable trade-offs. Climent et al. (2024) discuss how breeding programs must carefully balance these **trade-offs** to avoid unintended consequences, such as sacrificing drought tolerance in favor of increased growth. Finally, **shortening breeding cycles** remains a key goal for accelerating genetic improvement. Traditional breeding cycles in forestry can take decades, but emerging technologies such as genomic selection, marker-assisted breeding, and controlled environment trials are helping to expedite the process (Grattapaglia et al. 2018).



		How we measure it	
Concept	What measures	Within populations	Among populations
Phenotypic correlation	Positive or negative relationship between two traits due to both genetic and environmental effects	Pearson's correlations among individual phenotypic values	Pearson's or Spearman's correlations among population means
Genetic correlation	Positive or negative relationship between two traits due to additive genetic effects	Quotient between the genetic covariance and the product of additive genetic variances in half-sib progeny tests	Pearson's correlations among population BLUPs
Trade-off	Balancing or antagonistic covariation between two traits	Pairwise regression of individual phenotypic values or genetic correlation	Pairwise regression among population means or BLUPs
Phenotypic integration	Level of relationship (positive or negative) among multiple traits of the phenotype	Phenotypic Integration indexes based on individual within-population values	Phenotypic Integration indexes based on population means or BLUPs

Fig 9: Conceptual framework illustrating approaches to study trait relationships in forest trees proposed by Climent et al. 2024.

Fig. 9 depicts a theoretical representation of how tree breeders could study the relationships between tree traits to understand better growth, adaptation, and trade-offs (Climent et al. 2024). Traits can be observed in natural or planted stands or measured in controlled breeding trials like half-sib/full-sib progeny tests. Using a pairwise approach, researchers could examine how two traits interact, such as whether fast growth reduces defense, revealing possible trade-offs. With a multivariate approach, they could look at how multiple traits are connected at once, known as phenotypic integration. Both phenotypic (based on observed values) and genetic correlations (based on inherited genetic effects) help scientists understand how traits co-vary within and across populations.

Even though there are such innovative strategies under the influence of climate change, breeding for resilience is still not much explored in plants (Xiong et al. 2022) and trees, as breeders face challenges due to some drawbacks. Forest trees have **long lifespans**, making breeding inherently slow. Even with genomic tools, generation turnover remains a limiting factor.

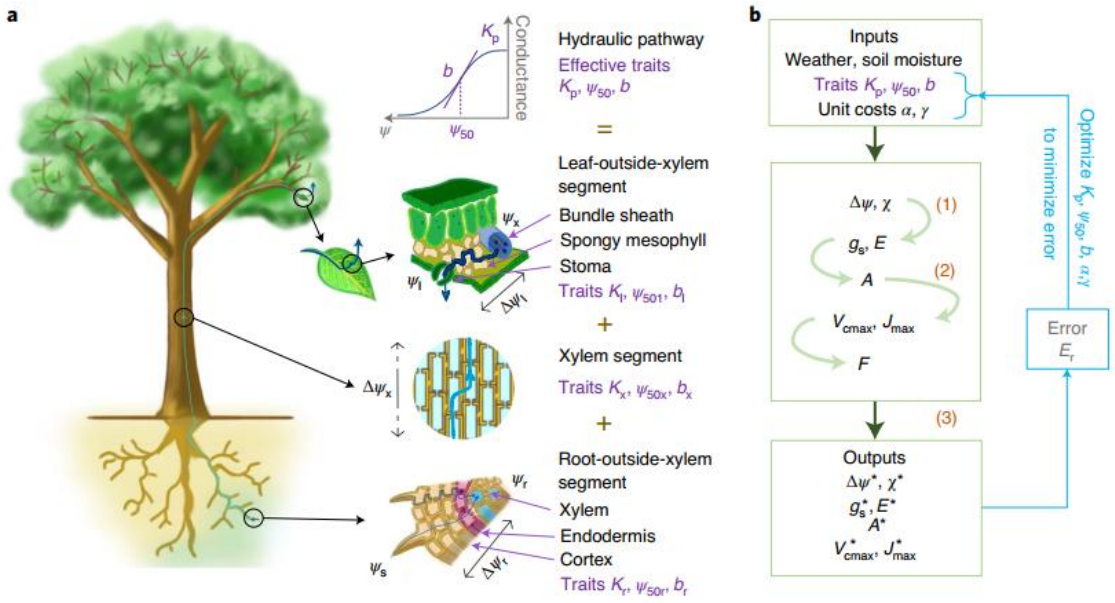


Fig 10: Schematic representation of the unified approach of tree traits integration towards water–transport pathway by Joshi et al. 2022 where (a) Purple labels are the hydraulic traits with segments: Ψ_s in soil, Ψ_r in roots, Ψ_x at the end of the xylem, and Ψ_l in leaves representing the traits. Each segment has its own set of traits, and the overall performance results from the combined effect of multiple traits. (b) traits like conductance (k), vulnerability to cavitation (Ψ_{50}), and sensitivity must be balanced (depicted by the proposed models)

Combining genomic, ecological, and climatic data requires interdisciplinary expertise and advanced analytical frameworks, which are still developing in forestry. In addition, the multi-trait complexity, where the traits are integrated across different organs (**Fig. 10**), and how selection of one trait might come with costs in other characteristics, makes tree breeding more challenging (Joshi et al. 2022). Each level of the tree's functioning comprises its own traits: at root level, traits related to cortex, endodermis, and root xylem, at stem xylem level, with traits related to hydraulic conductivity and cavitation in the stem, and at leaf level, traits associated with bundle sheath, mesophyll, and stomata control (Joshi et al. 2022).

These proposed strategies and challenges recommend a path forward in modern tree breeding, but also with caution. To integrate such tools into breeding programs, it is essential to assess tree responses under resource-limited conditions and to identify specific desired traits that can be reliably passed on to the next generation.

1.3.2. Selection of trees with desired traits

Previous sections outlined key ecological concepts such as the trade-offs between traits and the implications of fast growth. Breeding, focusing more on the production goal, should now better consider environmental conditions supporting survival and growth together with functional elementary traits driving trees adaptation. Bridging ecology and breeding emphasizes using ecological principles to inform breeding programs, ensuring that selected species are not only productive but also resilient to environmental changes (Laughlin et al. 2017). Trees are long-lived organisms that must withstand changing environments over decades. Species that dominated historical ecosystems did so because they possessed traits that conferred high survival under the prevailing environmental conditions (Shipley 2010). By selecting for specific traits, such as disease resistance, fire tolerance, efficient water use, or strong structural properties, breeders and ecologists should ensure that the trees planted today will survive, grow, and reproduce under current and future conditions (Carson and Carson 1989). If we could understand which traits would confer fitness, we could more effectively guide species selection, breeding, and restoration efforts.

Functional traits have recently been increasingly used as tools to understand, predict, and manage plants' responses to environmental changes and the species assembly process. Traits such as specific leaf area (SLA), wood density, bark thickness, and xylem vulnerability (P_{50}) are now central in ecological studies because they link directly to key physiological functions like photosynthesis, hydraulic safety, fire resistance, and growth strategies. Wright et al. (2004) introduced the concept of the *Leaf Economics Spectrum*, showing that traits like SLA and leaf nitrogen concentration form global axes of plant function. Chave et al. (2009) demonstrated how wood density varies across climates and predicts species survival and mechanical strength. Fulé et al. (2004) emphasized bark thickness as a key trait in fire-prone ecosystems. Choat et al. (2012) revealed a global convergence in tree species' vulnerability to drought using P_{50} as an indicator of hydraulic failure. Some of such functional traits have been discussed in **Table 1.**

These functional traits are now incorporated into restoration models (Laughlin et al. 2017) and climate adaptation strategies (Brodribb et al. 2020) to select species or genotypes that balance productivity with resilience. This shift marks a move from taxonomic-based management to a trait-based approach that is both mechanistic and predictive. There have also been discussions to make future models that could incorporate multivariate trait filters—reflecting both historical disturbance regimes and future climate projections—to predict which species or functional groups are most likely to persist and restore ecosystem resilience. The one by Laughlin et al. 2017 uses plant traits and environmental filters, such as fire, drought, and soil conditions, to choose species that can restore ecosystems and survive future changes.

In the context of breeding, most gains in acquiring such desirable traits across generations have been achieved through two powerful tools: interspecific hybridization and recurrent selection.

Table 1: Some functional traits relevant for tree breeding and ecological resilience, including their importance, example species, and supporting literature.

Trait	Species	Why is it important?	Key Literature
Thick bark	*Pinus ponderosa*	Protects the cambium from fire damage; fire-prone ecosystems	Fulé et al. 2004
High wood density	*Quercus sp, Eucalyptus*	Drought resistance, mechanical strength	Chave et al. 2009
Moderate Leaf nitrogen	Deciduous and evergreen species	Balances photosynthetic efficiency and resource conservation	Wright et al. 2004
Low P_{50}	conifers	Reflects drought tolerance	Delzon et al. 2010; Choat et al. 2012
Phenotypic plasticity	*Populus, Pinus*	Flexible response to environmental variability, hints about survival in a changing climate	Valladares et al. 2006; Nicotra et al. 2010
Early reproduction	*Betula sp, Populus*	Persistence or short life span	Grubb 1998; Moles and Westoby 2004
Deep-rooting system and fine roots	*Quercus, Larch*	Enhances access to soil moisture during drought	Padilla and Pugnaire 2007; Pâques et al. 2022
Effective stomatal control	Temperate conifers to Tropical wet	Maintains water use efficiency under fluctuating temperatures	Bartlett et al. 2012
Canopy architecture	*Fagus sp*	Affects light capture, transpiration rates and competitive ability	Reich et al. 2003
Resprouting ability	*Quercus sp*	Rapid recovery after disturbance (drought or fire)	Clarke et al. 2013
Leaf shedding	*Acacia*	Reduces water loss under extreme drought conditions	Wolfe et al. 2016

1.3.3. Interspecific Hybridization vs Recurrent Selection

The historical evidence of hybridization comes from written records by Theophrastus (c. 371 – 287 B.C.) in *Historia plantarum* and *De causis plantarum* on the function of plant flowers and their relation to fruits (Negbi 1995). Hybridization between plants of two distinct species has long been employed in plant breeding (Mason and Batley 2015) to serve multiple purposes: (1) combining the complete genomes of both parental species to broaden the gene pool of one or both species, (2) merging genomes to create amphiploids that integrate desirable traits from each parent, and (3) introducing specific beneficial traits-often from a wild species—into elite cultivars of another species through introgression (Prohens et al. 2017). Hybridization is thus becoming of interest, especially in transferring genetic material, which can be assisted by transferring desired traits from generation to generation.

Establishment of trees takes time in the context of climate change, especially for forest trees with long generation cycles and less ample variation in population and possibility of migration (Kremer et al. 2012). Thus, interspecific hybridization by creating hybrids is essential (Janes and Hamilton 2017) for accelerating adaptation in forest trees, as it enables the combination of complementary traits. The term "hybrid" can be the offspring of an initial cross between parental lineages (F1) or subsequent ones (backcrosses, F2) within populations. Some species that have been planted as hybrids are *Acacia, Araucaria, Eucalyptus, Larix, Picea, Pinus, and Populus* (Carron et al. 1992). Interspecific hybridization can assist in adaptation by enhancing environmental tolerance, as seen in *Eucalyptus* and *Araucaria* hybrids (Carron et al. 1992); hybrids can be well adapted outside their natural parental zones, like in *Pinus* hybrids (Dungey 2001), or inherit disease resistance, like in *Larix* (Sylvestre-Guinot et al. 1999). Nevertheless, there are also several barriers to interspecific hybridization, such as difficulties in hybrid seed production, especially in larch species, where asynchronous flowering among parent species limits cross-compatibility. Moreover, breeding programs for hybrids are generally more complex and costly than those for pure species, and large-scale deployment poses additional logistical challenges.

These limitations have been recently highlighted in a study on conifers by Klápště et al. (2022a), demonstrating that species hybridization is not a universal solution for developing resilient genotypes suited to future environments. In their study on spruce (*Picea glauca* × *P. engelmannii*), the authors found that while hybrids can exhibit advantageous trait combinations, outcomes were highly dependent on parental origin and elevation against the weevil *Pissodes strobi* (Peck). For instance, progenies from resistant-by-resistant (RxR) crosses showed strong negative genetic correlations between growth and sensitivity traits ($r = -0.74$ between height at 5 years and weevil attack severity), suggesting that fast-growing individuals in these crosses were less attacked. In contrast, susceptible-by-susceptible (SxS) families showed positive genetic correlations (up to $r = 0.34$), indicating greater susceptibility in faster-growing individuals. Moreover, the RxS hybrids had low and inconsistent correlations across traits, underscoring that hybrid vigor and resilience cannot be assumed a priori. The study also reported that the elevation of parental origin significantly influenced weevil resistance (an increase in elevation of parental origin decreased the likelihood of no egg puncture occurrence), revealing that reproductive or phenological mismatches might buffer against pest attacks in certain hybrids (Klápště et al. 2022a). These findings

collectively caution that hybridization outcomes must be evaluated case by case, and not all interspecific hybrids will necessarily offer robust solutions for climate resilience in forestry.

Recurrent selection is also another important tool in breeding. It is mainly used to improve traits by increasing, generation after generation, the frequency of favorable alleles, while maintaining genetic variability for selection. Recurrent selection has been well documented on Tropical trees like oil palm (*Elaeis guineensis*), cocoa (*Theobroma cacao*), coffee (*Coffea arabica*), rubber tree (*Hevea brasiliensis*), and *Eucalyptus* by Baudouin et al. (1997), but also on most temperate and boreal conifers (*Pinus sp., Picea sp., Pseudotsuga*). In recurrent selection, a base population with a broad genetic diversity—often including wild types- is first established. This population is then evaluated through multi-site trials, after which the best-performing individuals are selected. These selected individuals are intercrossed to form the next generation, and the cycle is repeated to improve desired traits progressively. The comparison between Interspecific hybridization and Recurrent selection is depicted in **Table 2**.

Taken together, both interspecific hybridization and recurrent selection offer complementary strategies in forest tree breeding. While hybridization enables the rapid introduction of novel trait combinations and of heterosis -often observed in F_1 hybrids—it comes with limitations related to reproductive barriers, trait instability, and deployment complexity. Recurrent selection, in contrast, supports the long-term improvement of polygenic traits within a managed breeding population, allowing for gradual genetic gain and conservation of diversity. In some breeding programs, hybrids have been integrated into recurrent schemes, blurring the boundary between these two approaches.

In this context, larch (*Larix* spp.) represents a particularly insightful model. With hybrids such as *Larix* × *eurolepis* (a cross between European larch and Japanese larch), and variation in growth and drought adaptation of hybrids and their parents, larch offers a unique opportunity to explore how hybridization and selection interact under real-world constraints. The present study builds upon this background by using a long-term nursery experimentation combining European larch (EL), Japanese larch (JL), and their interspecific hybrid (HL) to evaluate growth performance, plasticity, and resilience under contrasting soil water availability. This model system enables a comparative analysis of breeding potential and ecological adaptability, particularly under increasing climate stress.

Table 2: Comparison between Recurrent selection and Interspecific hybridization

Aspect	Recurrent selection	Interspecific Hybridization	Literature
Definition	Cyclical selection and recombination within a species to accumulate favorable alleles	Crossing of two different species to combine complementary genomes and traits	(Mason and Batley 2015)
Genetic base	Intraspecific (within one species)	Interspecific (between different related species)	(Klápště et al. 2022)
Goal	Gradual improvement of complex traits (e.g., yield, resilience, quality) while preserving diversity	Introduce novel traits (e.g., heterosis, disease resistance, adaptability)	(Grattapaglia and Kirst 2008; Potts and Dungey 2004)
Timeframe	Long-term; each cycle may take 7–15 years in trees	Often one or few generations; fertility and performance may drop in later generations	(Namkoong 1979)
Challenges	Slow genetic gain per cycle; requires long-term trials and synchronization of flowering	Cross-compatibility issues, asynchronous flowering, low fertility, unstable performance	(Pâques 1989a; Dungey 2001)

1.4. **Larch, a tree model for the study**

1.4.1. European and Japanese Larch

Larch is among the most widely found species in the northern hemisphere, distributed over North America, Europe, and Asia (Pâques et al. 2013). European Larch (*Larix decidua* Mill.) is mostly found in the Alps, the Sudeten Mountains, and the Western Carpathians, with some presence in Central Poland and Romania. Native European Larch can grow from less than 1000 to 2900 m elevations. European Larch is one of the few conifers with deciduous leaves, a trait that enhances its tolerance to harsh conditions and allows it to thrive in alpine climates (Wagner et al. 2015). *Larix decidua* is highly prized for its good mechanical wood properties, but even more so for its abundant and highly naturally durable heartwood (Pâques 2001). It is particularly appreciated for structural uses. Outside its native range, its extension across Europe -mainly with Sudetan and Central Poland populations- is mostly in continental climates.

Japanese Larch is native to the Hondo islands in Japan and is found in about 25 native populations. *Larix leptolepis* Gord., also known as *Larix kaempferi* or Japanese larch, derives its name from the Greek words *leptos* (thin) and *lepis* (scale), referring to the tree's slender cone scales. This species is the focus of a genetic improvement program in Japan aimed at hybrid breeding, enhancing wood quality, and increasing resistance to pests and diseases (Kurinobu 2005). It is widely utilized in the production of furniture and paper. In Europe, it has been appreciated for its fast growth, better resistance to canker and tolerance to shade than European larch. Due to its sensitivity to summer drought, its use is mostly restricted to oceanic climates.

The distribution of the species of *Larix sp* has been depicted in **Fig 11**.

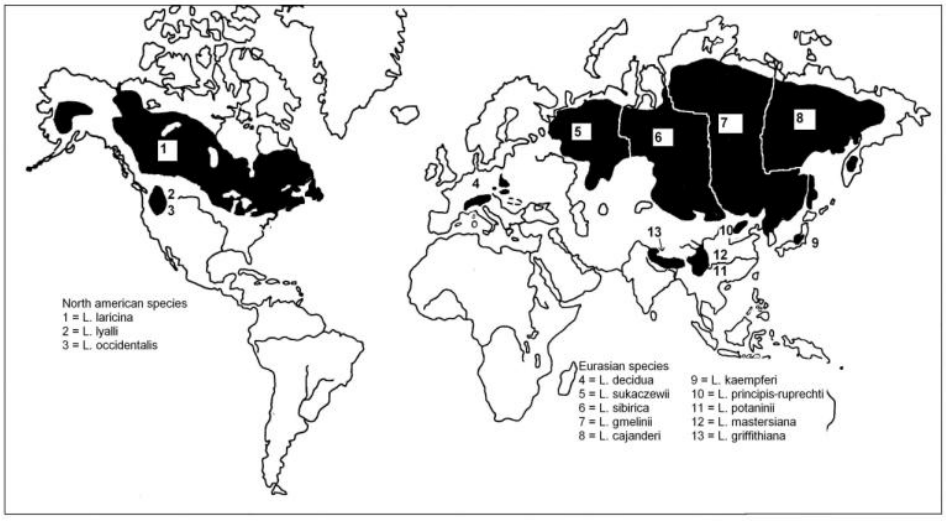


Fig 11: The worldwide distribution of the *Larix* genus (map from Karlman 2010).

Although its interest for reforestation, larch remains a minor group of species among European conifers: the native range of European larch across Europe represents around 600 000 ha and the cultivation range of the 3 taxa (European, Japanese and hybrid) outside European larch native range exceeds 900 000 ha. With a total area of around 1.5 million ha, larches represent around 4% of European coniferous forests (Pâques et al. 2013). In France, no detailed statistics per taxa are available, excepted for European larch which covers around 126 000 ha (source: IFN https://inventaire-forestier.ign.fr/). Erratic reproduction and difficulties in mass-propagation of larch by seed remain main drawbacks in larch deployment.

1.4.2. Hybrid Larch and breeding

Larix × *eurolepis* A. Henry**,** i.e., Hybrid Larch, which is the interspecific hybrid between European Larch and Japanese Larch, was first described in the 1900s, but the first crossing was made by the Georgian breeder Salomon Kurdiani in Poland in the early 20th century and since then the parents and hybrids have been tested in several parts of Europe (Oleksyn and Fritts 1991). Interspecific hybridization is one of the most effective strategies for genetic improvement in larch, capitalizing on trait complementation between parent species, hybrid vigor (heterosis), and hybrid homeostasis (Pâques et al. 2013) (see also **Table 3**). Since then, hybrid larches have attracted growing interest in countries such as Denmark, Germany, Belgium, France, and the Netherlands—primarily for their growth advantages.

Since its launch in the 80s, the INRAE interspecific hybridization program, supported by breeding populations of over 250 'wild' clones for each parental species, has created by control crosses over one thousand full- or half-sib 1st-generation hybrid combinations, currently tested across France. More recently (mid-90s), advanced hybridization or composite breeding is evaluating the interest of 2nd-generation hybrids. The success of hybrids highly depends on the genetic background of their parents, particularly on the origins of their European parents (Alps, Sudetan and central Poland), which is currently evaluated in dedicated trials.

To better understand the genetic basis of hybrid superiority, a full 18x18 diallel mating design was implemented in the 90s and planted across four ecologically contrasted sites in France in 1997 as a complement to previous factorial designs. It allowed to properly evaluate heterosis, its genetic basis and differential phenotypic plasticity among hybrids and European and Japanese larches for growth over a soil water gradient (Pâques 2002; Marchal et al. 2017, 2018; Escobar 2020). A subset of this diallel design was planted in 2013 on two sites (Orléans and Beaumont) and three contrasted water regimes (dry-Orléans, intermediate-Beaumont and irrigated-Orléans) to approach more finely conditions of hybrid superiority expression for growth and its link with morphological, structural, phenological, and physiological traits. The trial planted at INRAE-Orléans supports this thesis.

It follows two previous studies conducted with young seedlings of the three larch taxa in the nursery (Pâques 2009) and in a greenhouse with a controlled drought stress (Sassani et al. 2021). The first one showed that growth rhythm more than phenology (length of growing season) better explains heterosis and, illustrated over one growing season through weekly assessments, different hybrid combination-dependent strategies to reach height heterosis. The second one highlighted clear

difference in water use and growth traits among the three *Larix* taxa under control (C) and controlled drought stress (S) conditions (**Fig. 12**). Hybrid larch (HL) generally showed intermediate to high values across traits like transpiration (a), needle surface (d), and biomass accumulation (g–h), suggesting both vigor and moderate drought resilience. In contrast, European larch (EL) tended to maintain higher relative water content (c), whereas Japanese larch (JL) showed larger stem diameter and needle area but a sharper decline under stress. These differences provided a preliminary functional basis for interpreting growth–water use and evaluating adaptive responses in hybrid vs. parental species (Sassani et al. 2021).

As discussed previously, interspecific hybridization in larch and its mating plan provides a sound framework:

- To explore resource limitation vs optimal conditions and their effects on survival and growth of different taxa, as it offers the possibility to compare the three larch taxa - European Larch, Japanese Larch, and their interspecific hybrid - through connected pedigrees across contrasted environments,
- To allow the evaluation of fast- vs slow-growing taxa (and genotypes), and detection of possible trade-offs between growth and 'adaptive' traits, thanks to a 'direct' access by construction to differential growth patterns among the three tested taxa.

Table 3. Comparison of European Larch, Japanese Larch, and Hybrid Larch with respect to traits of interest. These comparisons are not all based on direct experimental tests but synthesize patterns reported in the literature.

Trait	What Was Measured / How	EL	JL	HL	Environmental context /Provenance	Literature
Growth (Height / Diameter)	Height / Diameter	Moderate	Good	High	Multi-site trials in France	(Pâques 2009; Greenwood et al. 2015; Marchal et al. 2017)
Budburst Phenology	No of days	Late	Early	Intermediate	Nursery experiment with 2-year-olds	(Pâques 2009)
Phenotypic plasticity	Ring area	Less	Intermediate	High	Reaction norms to REW	(Marchal et al. 2019)
Transpiration efficiency	Biomass increase / cumulative transpiration (g/kg)	Lowest (≈4.0 g/kg)	Highest (≈6.1 g/kg)	Intermediate (≈5.9 g/kg)	Greenhouse drought vs. control; saplings from seed orchards	(Sassani et al. 2021)
δ13C (WUE proxy)	Isotope ratio mass spectrometry on apical needles	low WUE	high WUE	Intermediate; largest increase under drought	Greenhouse drought vs. control; saplings from seed orchards	(Sassani et al. 2021)
Needle Surface	Estimated from needle dry mass using allometric equations and scanned area	Low, declined under drought	Very high in control, reduced under drought	Maintained similar under both conditions	Greenhouse drought vs. control; saplings from seed orchards	(Sassani et al. 2021)
Relative Water Content (RWC)	Stem fresh, saturated, and dry weights from 5 cm segment above root collar	Maintained high even under drought	Slight decrease	Significant decrease under drought	Greenhouse drought vs. control; saplings from seed orchards	(Sassani et al. 2021)
Relative Biomass Increase (%)	% change from initial biomass	Low, reduced under drought	High in control, significantly reduced under drought	Very high in control, reduced under drought	Greenhouse drought vs. control; saplings from seed orchards	(Sassani et al. 2021)
Heartwood proportion	Increment cores at breast height, virtual assessment of color	71% at 34 yrs	80% at 36	60-70% at 15	Two provenance trials (EL and JL) and 3 HL trials	(Pâques 2001)

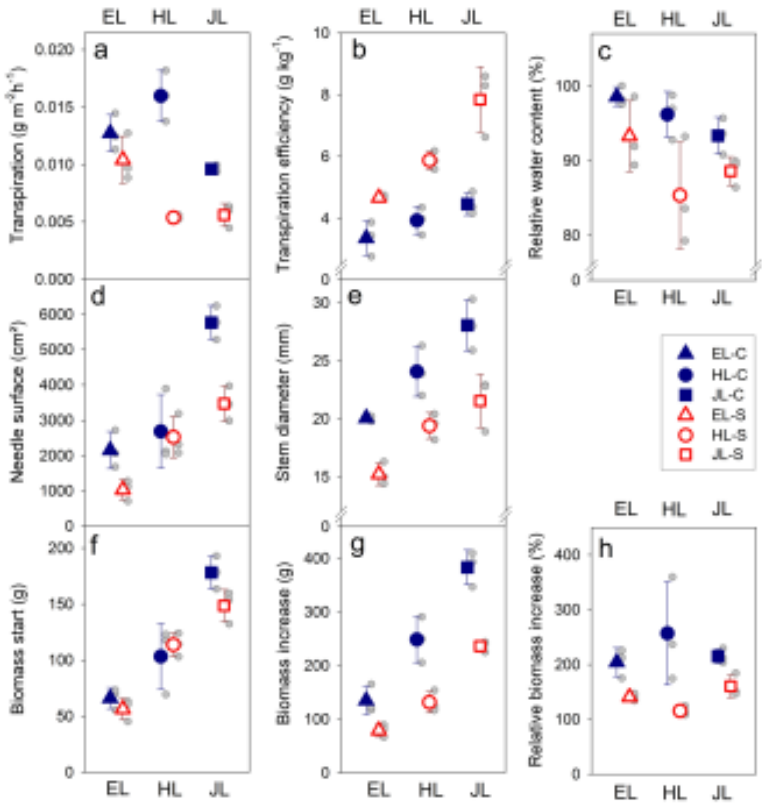


Fig 12: Trait comparisons among European larch (EL), Hybrid larch (HL), and Japanese larch (JL) under control (C) and stress (S) conditions. Panels show (a) transpiration, (b) transpiration efficiency, (c) relative water content, (d) needle surface, (e) stem diameter, (f) initial biomass, (g) biomass increase, and (h) relative biomass increase. Symbols represent treatments: solid for control (C) and open for stress (S). Error bars denote standard deviations (Sassani et al. 2021).

1.5. **Approach / Questions for Chapters of study**

Building on this foundation, where hybrid vigor is seen as a breeding opportunity, the thesis is structured around three complementary chapters that examine mortality patterns, high-throughput trait prediction, and growth–resilience interactions under environmental stress. A central aim unifies these chapters: understanding how the parents and the hybrids interact with environmental constraints (differential soil water regimes) to shape tree performance and adaptability. The conceptual framework of each chapter is presented in detail at the beginning of the respective chapters. In this section, we try to introduce the main questions in each chapter.

1.5.1. Chapter I: A Decade-long Study on Mortality in European Larch (*Larix decidua* Mill.), Japanese Larch (*Larix kaempferi* (Lamb.) Car.) and their hybrid

This chapter investigates 'long'-term tree mortality trends and their relationship with growth history and climatic stress. Using a decade of observational data from irrigated and non-irrigated plots, we address the following questions:

- Do the three larch taxa exhibit different susceptibilities to mortality?
- Which climatic parameter(s) and time window best predict mortality variation over time, and are they similar for the three taxa?
- Does high vigor affect the vulnerability of trees to mortality when subjected to water constraints?

1.5.2. Chapter II: Prediction Models for *Larix* species via NIRS Spectroscopy as a High-Throughput Phenotyping Approach

This chapter explores how Near-Infrared Spectroscopy (NIRS) can be used as a rapid, non-destructive tool to predict some key adaptive and functional traits in *Larix* taxa. It evaluates the feasibility of building robust models for traits linked to:

- Tree water economy: proxy to water use efficiency ($\Delta^{13}C$) and vulnerability to cavitation(P_{50})
- Photosynthetic capacity (C and N content, LDMC, SLA)
- Defense and structural support (lignin, H/G ratio, phenolics)

1.5.3. Chapter III: Expression of heterosis for growth under contrasted soil watering Treatments and over time in interspecific larch hybrids (*Larix x eurolepis*): Are fast-growing genotypes and taxa more vulnerable to water constraints?

This chapter analyzes growth patterns, plasticity, and resilience traits across hybrid and parental *Larix* taxa, focusing on heterosis expression and growth–adaptation trade-offs under drought. The key research questions are:

- How important is heterosis for growth: how does it vary with soil water availability, and how does it develop over time?
- Do EL, JL, and HL taxa differ in their growth responses to drought and, more globally, to soil water availability, as quantified by resilience parameters and phenotypic plasticity?
- Is there a trade-off between growth and adaptive traits in larch taxa: does fast growth come at a cost?

2. Materials and Methods

2.1. Experimental Site and Setup

The experimental site was established in INRAe Nursery close to Orléans (Lat. 47.83 N, Long. 1.91 E, alt. 108m) on a former oak-hornbeam coppice. It is a flat area on a former river bed of the Loire River, characterized by poor sandy gravel soil with a low water retention capacity (around 35 mm). The site is characterized by an oceanic climate with a mean annual temperature of 11.9°C (mean growing season temperature: 15.9 °C) and a mean precipitation of 742 mm (growing season precipitation: 461.9 mm). This location was chosen on purpose, as it is close to the institute to facilitate observations and measurements and, above all, to get access to contrasted soil water availability (**Table 4** for details).

The species model selected for the experiment was European Larch (EL, *Larix decidua*), Japanese Larch (JL, *Larix kaempferi*), and their hybrids (HL). The plant material is a subset of full-sib progenies obtained from an inter- and intra-specific diallel mating design involving 9 European Larches (Sudetan origin) and 9 Japanese Larches (various origins from Hondo Island). The complete diallel design has been planted on four sites across France in 1997 to study hybrid superiority and, more precisely, heterosis "*sensu stricto*," i.e., to compare the hybrid performance to its parent's performances. Based on growth performances in one of these four trials (St-Saud, 24), a subset of genotypes was chosen based on hybrid heterotic performances – high, medium, and low levels of heterosis for growth.

Table 4: Characteristics of the site utilized for the study

Site Name	Orléans, France
Latitude	47.83 N
Longitude	1.91 E
Altitude	108 m
Soil	Poor sandy gravel soil
Climate type	Oceanic
Average annual Temperature (°C)- 2012 to 2023	11.857
Growing season Temperature (°C) - 2012 to 2023	15.85
Average annual Precipitation (mm) - 2012 to 2023	742.35
Growing season Precipitation (mm) - 2012 to 2023	461.85

The subset consisted of 12 full-sib (FS) families of European larch (EL x EL), 12 FS families of Japanese Larch (JL x JL), and 12 FS hybrid families (EL x JL) sharing common EL and JL parents (**Fig 13(a).**). Each family consisted of 12 individuals, which were cloned by grafting in the spring of 2010 on a common rootstock (progeny from "Lavercantière" hybridization seed orchard). The 9 EL and 9 JL parental clones were also vegetatively propagated by grafting and added to the trial. The number of ramets per individual varies around six. Subset design is shown in **Fig. 13(a).** Plants were kept in the nursery for two years, and in the winter of 2013,

they were planted in the experimental site. A split-plot design was implemented. In order to minimize competitive interactions among the three taxa, each taxon was separately planted in bands and replicated three times, with four incomplete randomized blocks within each band. **(Fig. 13(b))**. Clones were fully randomized within the blocks to reduce experimental bias. The trees were spaced at a distance of 2.5 meters between rows and 3 meters within rows.

To understand the different responses of Larch under water stress, we established differential water treatments to evaluate the expression of heterosis in 'optimal' and stressful soil water availability conditions. A third site with the same genotypes was established in Limousin (Massif Central) at Beaumont-du-Lac, a region recommended for larch plantations. This site is out of the scope of this study.

The INRAe-Orléans experimental site was divided into two distinct plots, referred to as Treatments: irrigated (IRR) and non-irrigated (NIRR). The irrigated plot has been equipped with drip irrigation since 2015 (one line on both sides of trees, at 50 cm from trees and 20 cm depth), ensuring steady daily irrigation directly to the root zone throughout the growing season, *i.e.*, April-May to September. The IRR plot is considered a plot where the tree experiences minimal water stress, whereas the NIRR plot is under recurrent 'drought' stress: by drought, we refer to periods of low water availability ascertained by **Fig. 14**. At the onset of the experiment, there were 1049 trees in the irrigated and 851 in the non-irrigated part of the experiment, sharing the same genetic material.

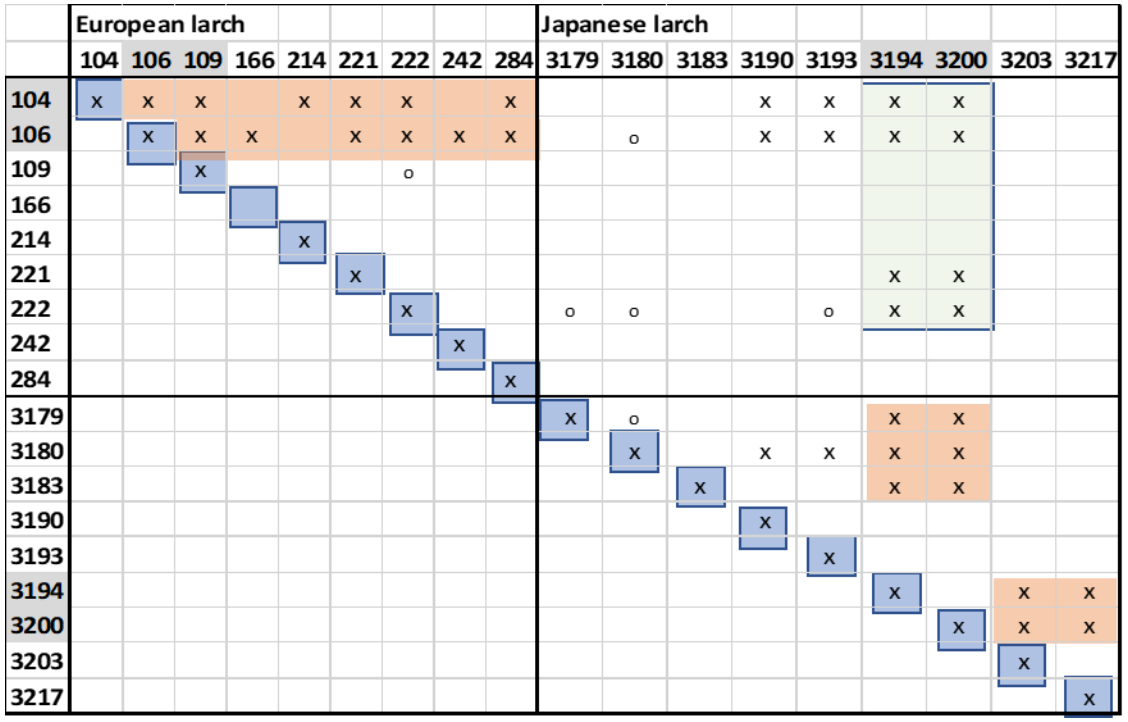


Fig 13(a): Subset of the (9 European × 9 Japanese larches) diallel mating design: x = the 12 full-sib families per taxon plus parental clones (underlined in blue) planted in the trial. (Note: in green, 8 hybrid combinations and their related parental combinations in red on which a special focus is made in some studies; o = extra material, including individuals that were mislabeled, as revealed by post-plantation molecular marker analysis.)

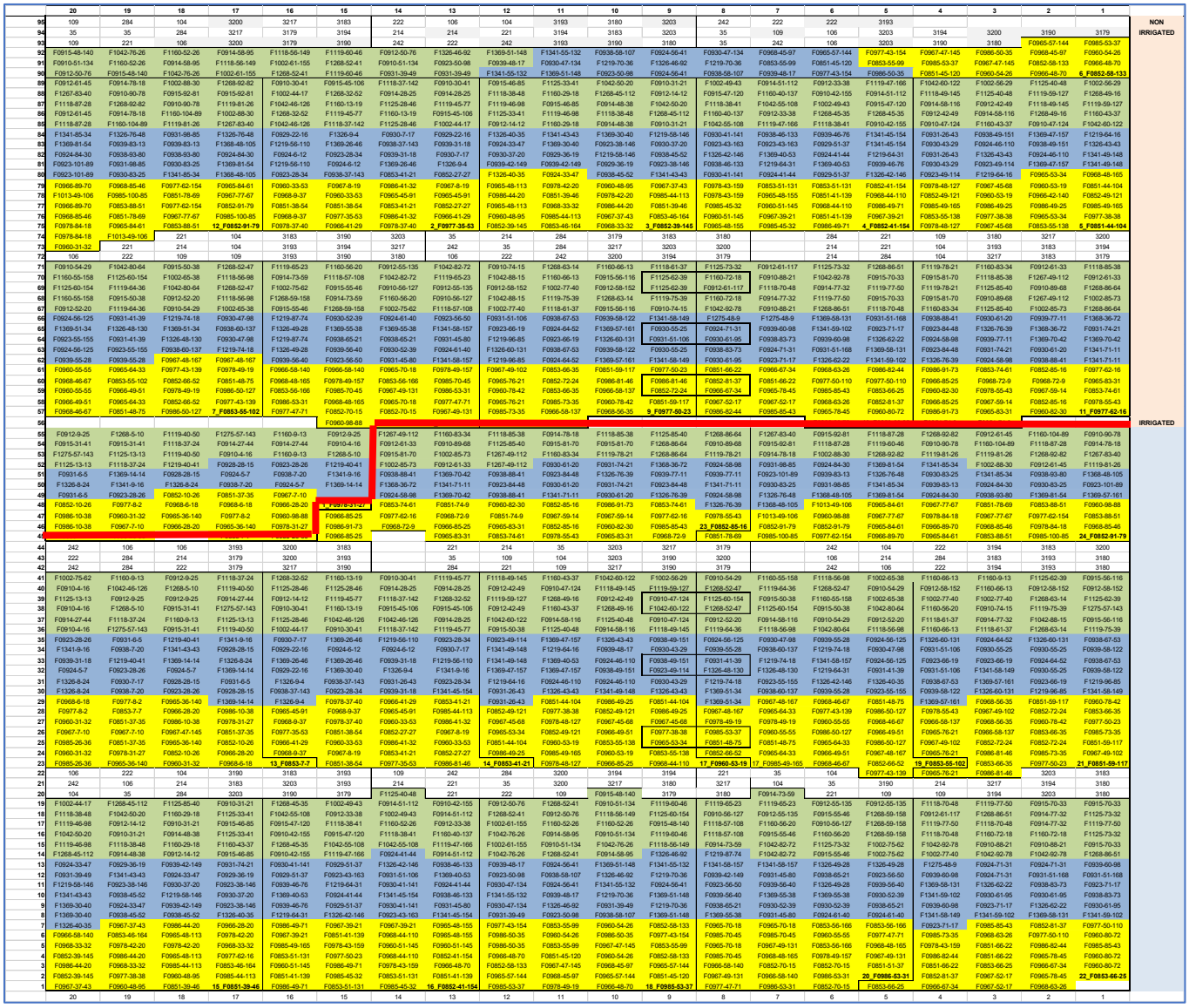


Fig 13(b): Map of the experimental trial at INRAe-Orléans nursery with the two treatments (non-irrigated and irrigated); taxa are planted across Blocks in bands: yellow = hybrid larch; blue = Japanese larch; green = European larch; white = clonal parents. Each boxed cell represents the Identification of the respective tree (clone).

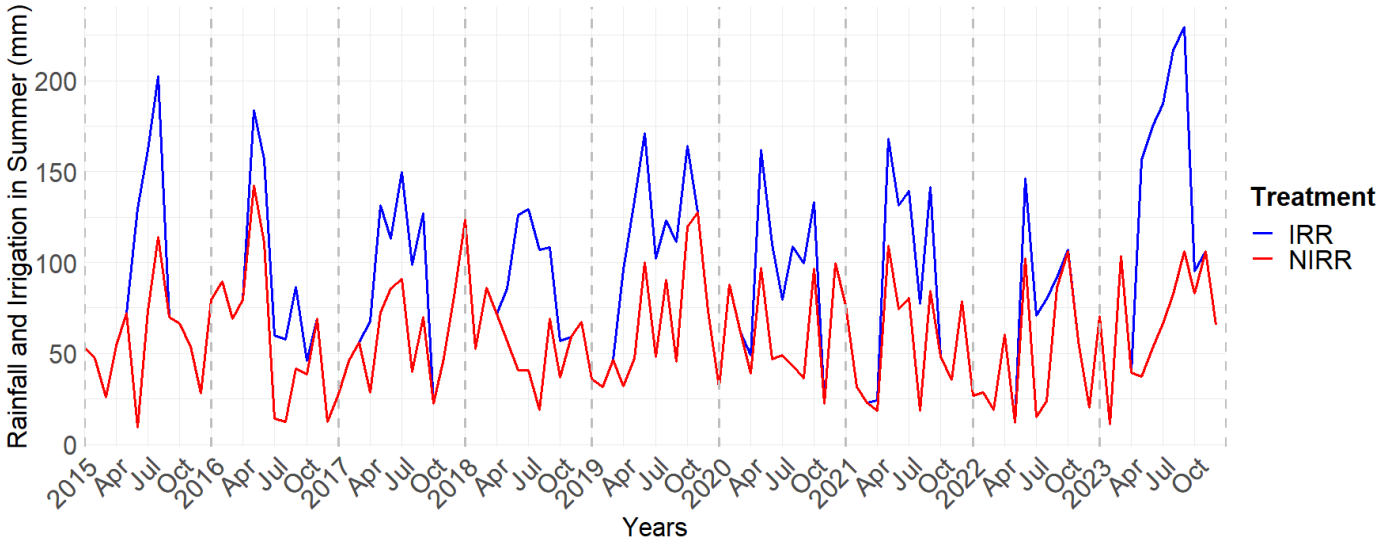


Fig 14: The temporal water supply by irrigation and/or rain in the two plots of the experimental site: Irrigated(blue) and non-irrigated(red) over the years 2015 to 2023.

Minimal cultivation practices were employed, with regular weeding being the primary method to manage grass competition and maintain optimal growth conditions for the larch trees. Pedigrees of all clones were controlled with microsatellites, and a few errors were detected. Rouging of erroneous trees followed. A first thinning was implemented in the IRR plot in 2020 to reduce competition.

2.2. Data collection

The site's close proximity to the INRAE facility facilitated regular data collection. Some traits were measured for all years of the experiment from 2013 to 2023, while others were assessed in specific years. These traits were obtained through a combination of direct field measurements, computational estimations, and laboratory analysis. Most of the work was done on the 1900 trees to represent the families and species accurately. **Table 5** presents a summary of the traits used in this study

2.2.1. On-site measurements and computations

Mortality (S) was recorded every year ("0" for the alive and "1" for dead trees). The trees that experienced a reduction in height due to drought were recorded as trees with Top dieback (1 for trees with top dieback and 0 without) **(Fig 15).**

Fig 15: Top dieback illustration

Total height (HT) and BH diameter (D) were measured on all trees from 2013 to 2023. Height measurements were done twice, once at the end of the growing season, which is November to December, and the second at the onset of spring for a particular year. Height measurements were done with a pole until trees were less than 10 m and later with a Vertex. The diameter of the trees was recorded at breast height (1.3 m above ground) with a diameter tape done at the end of the growing season.

To monitor growth over time, the current annual height increment was calculated from height measurements as the difference between height in year *n* and height in year *n-1*. In the case of top-dieback, frequent in the NIRR plot, height was re-measured during the following early spring to consider height at which height growth resumed: in this case, annual height increments of year *n* were calculated as the difference between height measured in early spring of year *n+1* and height measured in year *n-1* to consider only the living part of the increment contributing to the final height. If top-dieback involved several annual increments, these increments were set at 0 until a new shoot (future main stem) bypassed the final height previously recorded (**Fig 16** for details).

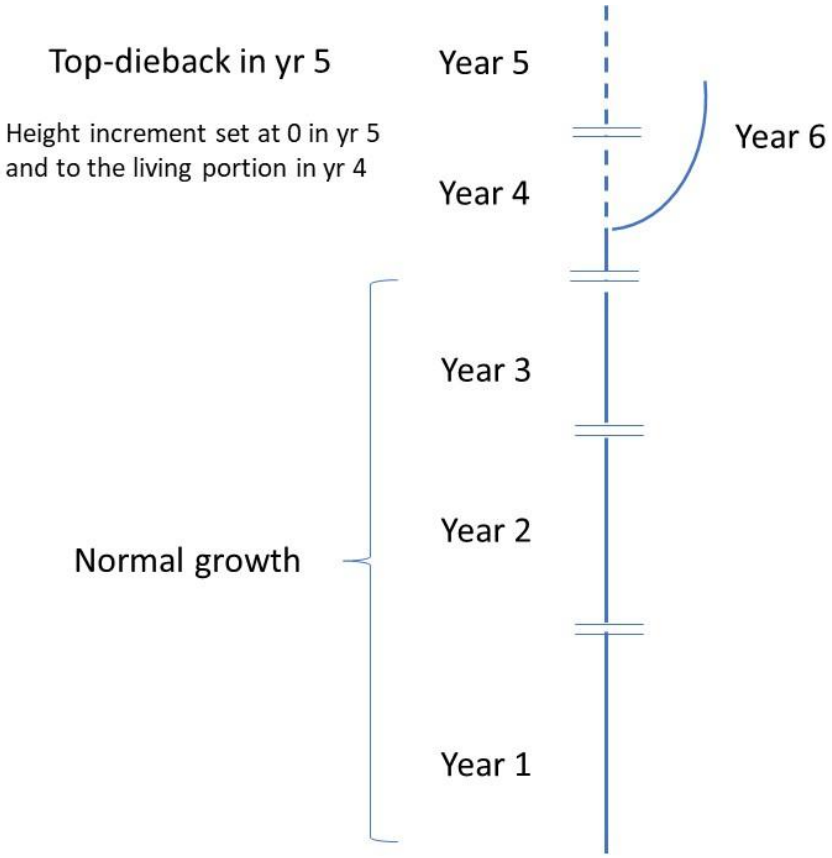


Fig 16: Schematic representation of tree height measurement considering top-dieback over multiple years. During normal growth (Years 1-3), the tree increases in height annually. When top dieback occurs (Year 5), the upper portion of the tree dies including part of increment of yr 4. The annual height increment of yr 4 was restricted to length of its living part whereas that of yr 5 was set to 0. Dashed lines indicate dead portions of increments, while solid lines represent the living portion contributing to the final height measurement.

For the diameter increment over the years, the change in diameter across consecutive measurements was evaluated. To estimate overall productivity, stem volume was calculated using the formula developed for Larch by Pauwels and Rondeux (1999) which typically follows a relationship involving diameter at breast height (D) and tree height (HT).

To characterize the three taxa's response to drought stress, resilience parameters were calculated following Lloret et al. (2011), based on changes in annual height and diameter increments before, during, and after an identified drought period (see CIII for details on their determination). To minimize the impact of ontogenetic effects, the data were standardized according to the method described by Helama (2015).

2.2.2. Increment core sampling and measurements

Increment core sampling was done to ensure a good representation across the two plots and the three taxa. Increment core collection was conducted from January to March 2022. Cores were extracted diametrically at 1 m from the ground with a 5 mm-diameter increment core borer. Whenever a core showed defects (a lot of missing initial rings, major cracks, compressed wood, or resin duct), a second collection was made for the particular tree. These extracted wood cores were carefully kept in plastic boxes, properly labeled with their true identity. Following sample

collection, further processing was conducted at the INRAe-Phénobois platform in Pierroton. This included drying in a kiln at 35°C, sawing into 2 mm-thick boards, and x-raying the samples. Finally, the X-ray films were scanned to obtain high-resolution radiographic images for further analysis. The X-ray images were analyzed under WinDENDRO^{TM} (Regent Instruments Inc., 2008) at INRAe BioForA to get microdensiometry profiles. Additional work was to control for breaks along profiles and ring limits carefully and to ascertain the chronology to avoid misidentifications, thanks to a script in R developed at INRAe BioForA (R Core Team, 2024). A peak in the microdensitometric profile was considered a year, and a low peak was either a year of stress or a false ring. The growth rings from 2014 to 2021 were mostly visible in all the profiles and were kept for further analysis. Additionally, a second round of collection was conducted in March 2022 to replace certain damaged cores. An example of a microdensitometric profile is shown in **Fig. 17**, where annual density fluctuations between 2015 and 2021 are clearly visible. The high-density peaks correspond to latewood, while the lower-density troughs represent earlywood zones. These profiles were used to identify ring boundaries and subdivide each annual ring into earlywood, transition wood, and latewood. In some years, especially under drought conditions, atypical patterns such as shallow peaks or irregular density transitions were observed, potentially indicating stress events or false rings.

The main parameters obtained from the dated ring series included annual ring width (in µm) and ring area (in μm^2), the latter calculated to assess annual radial increments in terms of cross-sectional area. In most cores collected, especially from the non-irrigated (NIRR) treatment, the pith was either missing or damaged - likely due to difficulties encountered while extracting the borer, or because the wood was too dry or resinous, which can lead to fragmentation or loss of the innermost rings. To compensate for this, the total stem diameter was measured and converted to radius, from which the bark thickness was subtracted to approximate the wood radius. Subtracting the bark thickness from the total radius enabled reconstruction of the inner wood radius, allowing reliable ring width calculation even without a visible pith.

The annual ring area was estimated using ring width data and geometric reconstruction. Specifically, each annual ring was treated as a concentric circular band, and its area was calculated using the difference in squared radii of successive rings, multiplied by π. Along with the total ring width, the widths of earlywood, transition wood, and latewood were also identified when anatomically distinguishable. These subdivisions allowed for a more precise estimation of ring area by summing the areas of each component zone. The area of each segment was determined by subtracting the squared radius of its inner boundary from that of the outer boundary and multiplying the result by π. As trees increase in size, a given radial increment corresponds to a significantly larger cross-sectional area, making ring area a better measure of actual wood production. Additionally, the ring area more accurately reflects biomass accumulation and avoids biases associated with older trees, which may have small radial increments but still produce substantial amounts of wood.

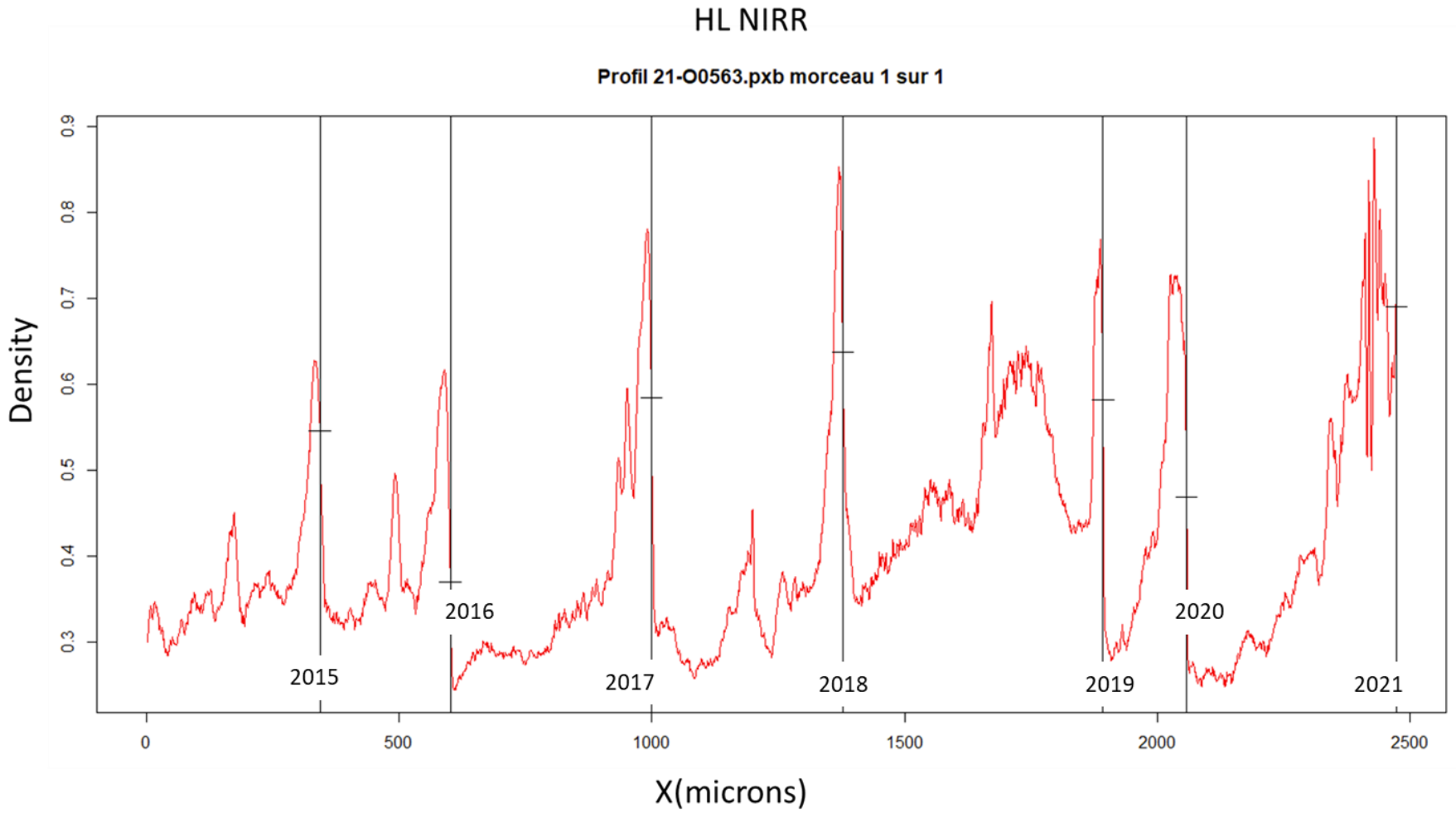


Fig. 17: Microdensitometric profile showing annual wood density variations from 2015 to 2021 in a NIRR part of the experiment for a hybrid larch sample. Peaks represent latewood zones, while troughs indicate earlywood.

Table 5: The description of the traits employed for the overall study of larch across the experimental site

S. no	Trait	Unit	Years measured	Sample size (at planting)	Sample size (latest)	Role/Proxy	Major Functional Role
1	Height (HT)	cm	2013-2023	1814	1442	Apical growth	
2	Height increment (GU)	cm	2013-2023	1820	1442	Apical growth units	
3	Current height increment (P)	cm	2013-2023	1814	1438	Potential Apical growth	
4	Diameter (D)	mm	2014-2023	1699	1444	Stem width	GROWTH
5	Current diameter increment (accrD)	mm	2015-2023	1610	1442	Stem width growth rate	
6	Stem Volume (V)	dm^3	2018-2023	1699	1437	Tree Growth and productivity / C storage	
7	Ring area (RA)	μm^2	2014-2021		1213	Tree radial Growth	
8	Specific Leaf Area (SLA)	cm^2g^{-1}	2023		194	Leaf resource use efficiency	
9	Leaf dry matter content (LDM)		2023		194	Photosynthetic capacity	
10	Needle nitrogen content (N)	mg g^{-1}	2021-2023	120	200	Nutrient content	LEAF ECONOMICS
11	Needle carbon content (C)	mg g^{-1}	2021-2023	120	200	Photosynthetic capacity Nutrient content	
12	Δ^{13}C-Water Use Efficiency ($\Delta^{13}C$)	‰	2021-2023	120	200	water use in relation to carbon assimilation	
13	Resistance to cavitation (P_{50})	MPa	2024		137	Drought tolerance	WATER HYDRAUILICS
14	Mortality(S)	0/1	2013-2023	1900	1845	Water availability on trees survival	
15	Trees with drought damage (Drought)	0/1	2015-2022	1892	1422	Water availability on trees vitality	DEFENSE (Abiotic)
16	Top dieback in length (desc)	cm	2015-2022	1892	1422	Water availability on trees vitality	
17	Lignin and H/G ratio (L, HG)	%	2022		191	Structural input	
18	Total Phenols (Phen)	mg eq. Tax/g DW	2023		198	Defense	STRUCTURE/CHEMICAL DEFENSE

19	Recovery after drought (no abbreviation used)	Based on height increment and ring area	2015-2021	1719	1445	Tree recovery	
20	Resistance to drought (no abbreviation used)	Based on height increment and ring area	2015-2021	1719	1445	Resistance to drought	ADAPTIVE RESPONSE
21	Resilience during drought (no abbreviation used)	Based on height increment and ring area	2015-2021	1719	1445	Resilience	
22	Average Growth Reduction (AGR)	Based on height increment and ring area	2015-2021	1719	1445	Growth reduction during the drought period	
22	Phenotypic plasticity (no abbreviation used)	based on diameter	2013-2023	1814	1442	Response to drought	

2.2.3. Laboratory measurements

Needle Trait Analysis

Needles were collected to evaluate key physiological and biochemical traits, including Specific Leaf Area (SLA) and Leaf Dry Matter (LDM), total phenol content, and carbon (C), nitrogen (N), and $\Delta^{13}C$. Needle samples were collected over three years to cover different stages of maturation: in September 2021, July 2022, and late August 2023 from 120, 200, and 200 trees, respectively. For each set of samples, trees were chosen in both irrigated and non-irrigated plots among the three taxa and different progenies so to catch the maximum a priori variability (environmental and genetic). Over the years, the trees in the samples were of different identities. All collections were taken from the mid-canopy, focusing on fresh, green current-year shoots at the sunny part of the crown with about five brachyblasts per tree. Immediately after collection, samples were stored in bags to minimize moisture loss.

Needles were collected in 2021 and 2022, oven-dried at 60 °C for at least 24 hours, and then finely milled using a high-throughput ball mill to obtain a particle size of 500–1000 µm. In 2023, needles were frozen at –80 °C and lyophilized (Freeze Dryer Christ Alpha 1-4) to preserve chemical integrity. Dried needle powders (approx. 1 g) were stored in glass vials at 20 °C and 45% humidity for NIRS and elemental analyses. Carbon (C), nitrogen (N), and carbon isotope discrimination ($\triangle^{13}C$) were measured from 1 mg subsamples, combusted at 1025 °C in an elemental analyzer coupled to an isotope ratio mass spectrometer (EA IsoLink CN IRMS System, Thermo Fisher Scientific, Bremen, Germany). Analyses were conducted at the INRAE SILVATECH platform (doi: 10.15454/1.5572400113627854E12).

Carbon and nitrogen concentrations were analyzed to determine nutrient allocation and growth potential. C and N contents were expressed in % of dry matter, while $\delta^{13}C$ was expressed in ‰ relative to the Vienna Pee Dee Belemnite (VPDB) standard (Craig 1957):

$$\delta^{13} = \left\{\frac{R_{sample} - R_{standard}}{R_{standard}}\right\} \times 1000$$

where, R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and the standard, respectively.

To estimate carbon isotope discrimination (ΔC^{13}) as a proxy for water-use efficiency, $\delta^{13}C$ values were converted (Farquhar and Richards 1984):

$$\Delta^{13}C = \frac{\delta_{air} - \delta_{plant}}{1 + \left(\frac{\delta_{plant}}{1000}\right)}‰$$

where, δ_{air} was assumed to be -8 ‰

Carbon and nitrogen content were converted to mg g^{-1} of dry weight.

In 2023, leaf traits—Specific Leaf Area (SLA) and Leaf Dry Matter (LDM)—were measured on selected trees. Fresh needles from five brachyblasts per tree were separated from the small twig, weighed to get the fresh weight, and flattened in Petri dishes. To ensure accurate area measurement, the needles were repositioned to avoid overlap and scanned using an EPSON V750 Pro scanner. The scanned images were archived and later analyzed using ImageJ to

determine needle count and total needle area. After scanning, the samples were oven-dried in a kiln at 40°C until a constant weight was reached, and then re-weighed to get the dry weight.

Needles were collected for chemical analysis in 2021, 2022, and 2023, with samples oven-dried in 2021 and 2022, while in 2023, they were lyophilized at approximately -70°C to preserve biochemical integrity. Total phenol content was measured in 2023 at the Phénobois platform in Orléans using high-performance liquid chromatography (HPLC). For this analysis, needle samples were ground into a fine powder, and phenolic compounds were extracted using an extraction method before filtration. The extracts were analyzed using an HPLC system equipped with a C18 column and a UV-visible detector, and phenol content was quantified using calibration curves.

Analysis of lignin content

After completing the ring-width analysis, the same wood cores used for microdensitometric profiles were further processed for chemical analysis. The wood samples were dried and finely ground into a homogeneous powder (1 gm) with ball milling at INRAe Phénobois Orléans platform. Lignin content and H/G ratio were determined using the pyrolysis method. Lignin is a complex aromatic polymer that provides structural rigidity and hydrophobicity to cell walls, contributing to wood strength and resistance to decay. The H/G ratio represents the relative abundance of p-hydroxyphenyl (H) and guaiacyl (G) lignin units, indicating the chemical composition and degree of lignin condensation. Approximately 1 gram of wood powder was used to analyze lignin content and composition for wood samples. Analytical pyrolysis was performed to assess the relative proportions of lignin monomers. This analysis followed the methods described in Alves et al. (2006). The lignin analyses were conducted at the Centro de Estudos Florestais, Universidade de Lisboa in Portugal.

Cavitation measurements

Branches were collected exclusively from trees in the irrigated (IRR) plot, as individuals in the non-irrigated (NIRR) plot had already experienced prolonged water stress. In total, samples were taken from approximately 39 European larch (EL), 77 hybrid larch (HL), and 21 Japanese larch (JL) trees. Sampling was conducted to ensure adequate representation of the 8 priority hybrid families and related parental progenies within the sample set. Each branch was labeled correctly, wrapped in water-soaked tissue, and sealed in plastic bags to minimize moisture loss during transport to the laboratory in Bordeaux.

Upon arrival at the laboratory, the branches were debarked and immediately analyzed using a Cavitron system equipped with a 15 cm rotor. This technique measures hydraulic conductivity loss by spinning stem segments at increasing centrifugal speeds to simulate negative xylem pressures (i.e., increasing tension), which progressively induces cavitation. The resulting vulnerability curve reflects the loss of conductivity as a function of applied xylem pressure, providing a quantitative assessment of drought-induced embolism resistance (Delzon et al. 2010). The collected data were used to generate vulnerability curves, and the vulnerability curve to cavitation at 50% loss was calculated using the "fitplc" function in R (see **Fig 18** for example on a sample).

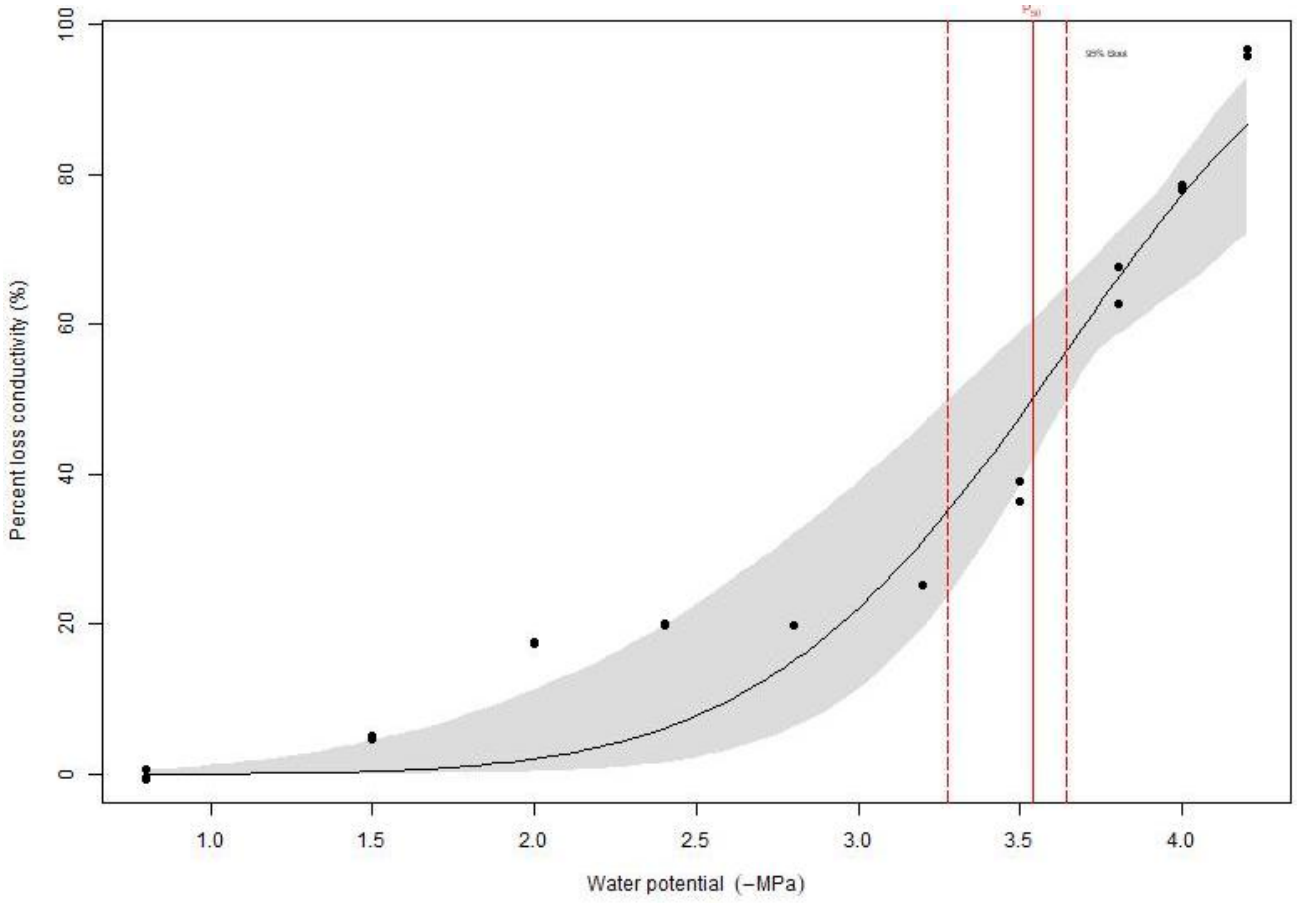


Fig 18: Vulnerability curve illustrating the relationship between percent loss of conductivity (PLC) and water potential (−MPa) for Sample 1. The black dots represent measured data points. The shaded gray region indicates the 95% confidence interval from bootstrapping. The red vertical solid line represents P_{50}, the water potential at which 50% loss of conductivity occurs, a key indicator of cavitation resistance.

NIRS spectral Analysis

Near-infrared spectroscopy (NIRS) was used as a high-throughput, non-destructive method to predict functional and chemical traits of various tree tissues based on their spectral properties. The goal was to develop predictive models using a reference set (training population) with known trait values, and then apply these models to the entire sample set (prediction population) to estimate traits of interest. Samples included:

- Needles collected in 2021, 2022, and 2023 for predicting chemical traits (carbon, nitrogen, and $\Delta^{13}C$)
- Wood cores collected in 2023 for lignin content estimation
- Branches collected in 2024 for predicting P_{50} (a functional trait related to xylem vulnerability)

All samples were oven-dried (or lyophilized in the case of 2023 needles), finely ground using a ball mill to ensure homogeneity, and stored in clean, dry glass vials at room temperature (20 °C, 45% RH) to preserve their chemical and structural integrity until spectral scanning.

Spectral analysis was performed using a Frontier spectrometer (Perkin Elmer, USA), which operated within a wavelength range of 10,000 cm^{-1} to 4,000 cm^{-1}. Each sample underwent 64 scans with a spectral resolution of 8 cm^{-1}, performed at intervals of 2 cm^{-1}, following the

methodology of Gebreselassie et al. (2017). The resulting spectral data were analyzed to detect specific biochemical signatures associated with key plant compounds, such as lignin, cellulose, and phenolic compounds, providing insights into tree physiology and stress responses. This standardized approach allowed for the precise characterization of chemical traits relevant to plant growth, resilience, and adaptation to environmental stressors.

2.2.4. Environmental data

The environmental dataset used in this study consisted of climate variables recorded by a meteorological station near the experimental trial at the INRAE Orléans nursery. Data were accessed through the INRAE CLIMATIK portal. Daily climate data included average (T_avg), maximum (T_max), and minimum (T_min) temperatures (°C), relative humidity (%), rainfall (mm), and global radiation (G_rad, W/cm²). Vapor Pressure Deficit (VPD, kPa) was calculated using T_avg and relative humidity, following Brice and Hall (2020). In 2015, six soil moisture sensors (TDR CS616 and WM 257L) were installed at 24 cm and 60 cm depths in both IRR and NIRR plots to monitor soil water content and potential. TDR sensors measure soil volumetric water content, whereas WM sensors measure soil matric potential, i.e., the suction or energy required by plants to extract water from the soil. Soil matric potential typically ranges from 0 kPa in saturated soil to about –1000 kPa in very dry soil, with progressively negative values indicating increasingly difficult water availability for plants.

To quantify site water availability, the BILJOU water balance model (Granier et al., 1999) was employed. This model integrates precipitation, evapotranspiration, soil moisture, and soil characteristics to estimate Relative Extractable Water (REW). For computation, the model requires inputs such as stand type, Leaf Area Index (LAI), soil composition, etc. Due to difficulties in monitoring LAI annually, especially in plots with canopy gaps observed in NIRR, a fixed arbitrary LAI value of 5 was used across years to consider a closed canopy.

Rather than interpreting REW directly as a drought stress indicator, it was used as a climatic index to characterize years based on water availability, similar to the Standardized Precipitation-Evapotranspiration Index (SPEI) but incorporating soil and vegetation characteristics. While REW values below 0.4 indicate potential water limitations, in this study, REW primarily served to classify climatic conditions rather than assess drought stress on trees, which depends on species-specific physiological responses and site-specific factors.

With this information, from 2012 to 2023, interannual variation in climate at the NIRR site is characterized by increasing mean annual maximum temperatures and elevated vapor pressure deficits (VPD) in recent years, notably 2019, 2020, and 2022. These years also coincide with lower total annual precipitation, as indicated by the color gradient. Earlier years, such as 2012–2014, were cooler and wetter, whereas the post-2018 period reflects a shift toward warmer and drier conditions (**Fig 19**).

Fig. 20 displays the timing and distribution of peak drought conditions (expressed as day of year) under irrigated (IRR) and non-irrigated (NIRR) treatments. The years are arranged from left to right based on the increasing number of drought-affected days within each treatment. The IRR plot shows narrower distributions with generally delayed drought events, while NIRR plots reflect broader and earlier drought occurrences, highlighting more substantial interannual variability in the absence of irrigation.

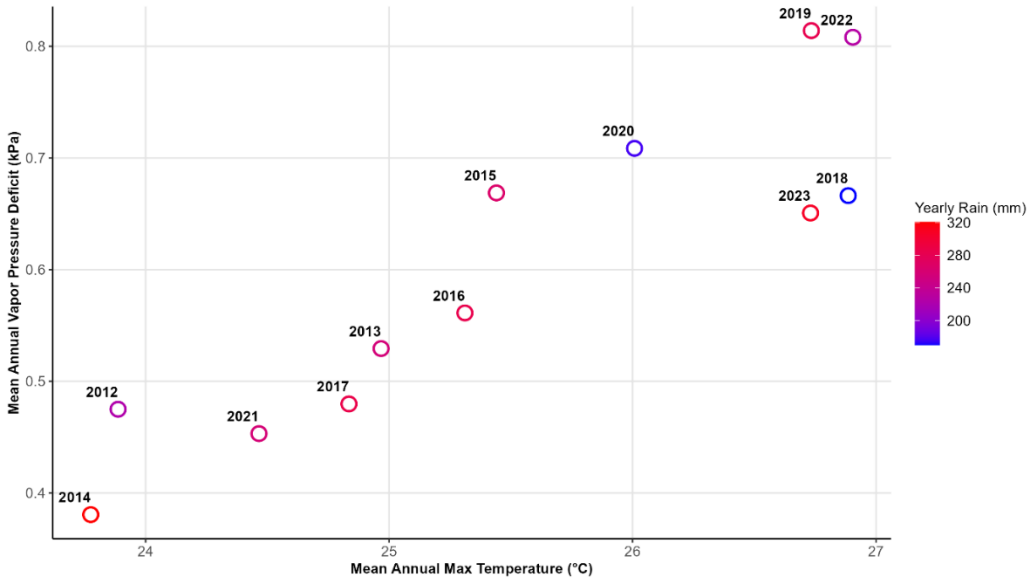


Fig 19: Mean annual maximum temperature and mean yearly vapor pressure deficit (VPD) from 2012 to 2023 in the non-irrigated part of the experiment. Each point represents a single year, labeled accordingly. The color gradient indicates the total annual precipitation, with warmer colors representing drier years.

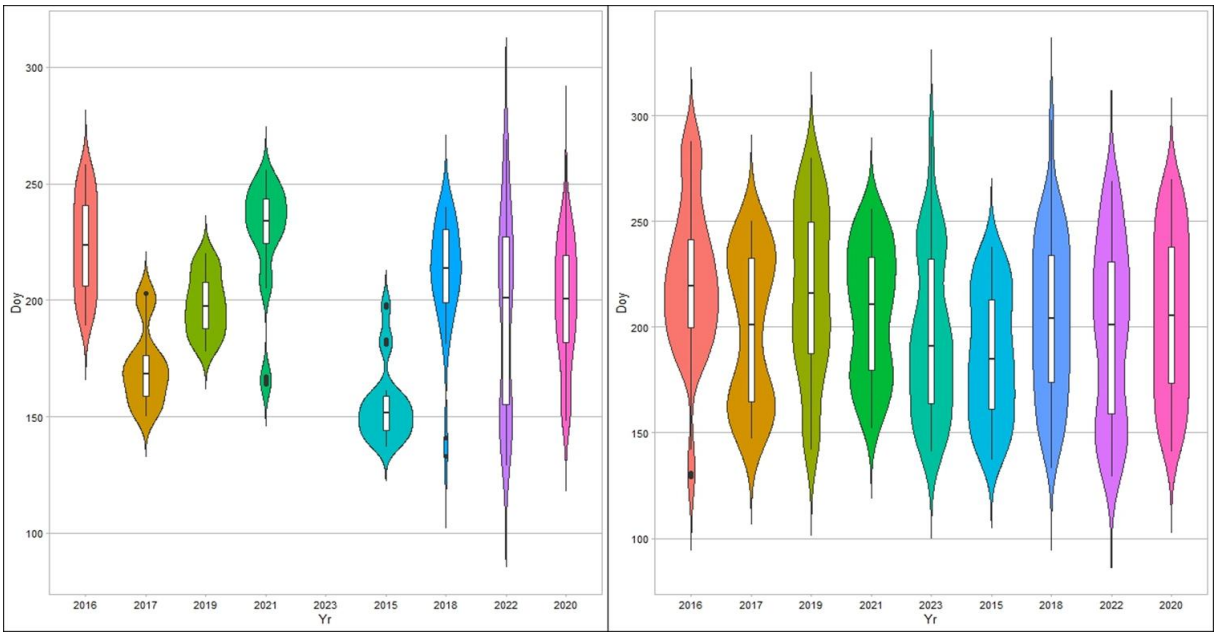


Fig 20: Violin plots showing the distribution of the day of year (DOY) for peak drought conditions across study years—left panel: IRR treatment; Right panel: NIRR treatment. Years are ordered by increasing number of drought days within each treatment. Drought days were identified based on **Relative Extractable Water (REW)** values simulated by the **BILJOU model**, where days with **REW < 0.4** were considered **dry**. This threshold corresponds to the soil-water level at which **stomatal closure typically begins**, marking the onset of physiological water stress in trees.

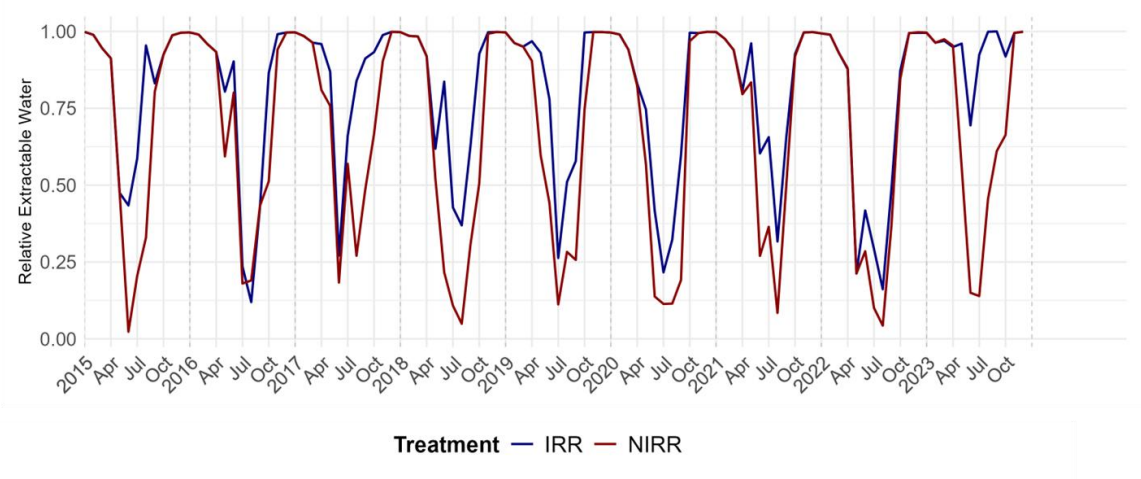


Fig 21. Monthly mean Relative Extractable Water (REW) at the NIRR site from 2015 to 2023. The red line represents temporal variation in REW in NIRR part of the experiment and blue lines the IRR parts simultaneously.

Monthly REW data from 2015 to 2023 show clear seasonal fluctuations, with low values during summer and recovery in winter (**Fig 21**) in the non-irrigated part of the site. Several years, especially 2017, 2019, 2020, and 2022, experienced extended periods below the REW threshold of 0.4, indicating repeated summer drought stress.

This information will be used in further analysis to detect 'normal' and stressful years, evaluate resilience parameters, identify key climatic variables related to mortality, and evaluate phenotypic plasticity of growth.

2.3. Models/Statistical analysis:

This section outlines the primary statistical methods and modeling approaches used to analyze the experimental data and address the research objectives across different chapters.

2.3.1. Linear Regression and Bayesian Analysis

For Chapter I, which focused on long-term mortality dynamics, we applied linear regression models and Bayesian logistic regression to explore the relationship between mortality probability and environmental and growth factors (growth trends, temperature, VPD, REW).

Linear Regression was employed to model the relationship between dependent and independent variables, where the dependent variable represents the phenotypic trait and mortality (binary trait), and the independent variables represent environmental factors such as temperature, soil moisture, and radiation. Linear regression assumes a linear relationship, allowing for the prediction of trait values based on these environmental variables. For this, a package was used in R "climwin," which explores each climate window to get the critical time period when there is the strongest correlation. In logistic regression, so that the coefficients do not tend to estimate toward infinity(separation), we fitted the regression using the penalized likelihood (Kosmidis 2022) to provide well-balanced likelihood estimates (Firth 1993).

Also, to get a trivariate model with the years, mortality, and diameter growth among the three taxa of Larch, a Bayesian model was utilized (linear regressions), and the variances and covariances matrix among the traits at the clonal level was used to predict the merit. We analyzed our data using trivariate mixed models in a Bayesian framework (Hadfield 2010). This approach allowed us to assign different statistical distributions to each trait in our multivariate model. We modeled diameter growth using a Gaussian (normal) distribution, while the live/dead (mortality) trait was modeled using a binomial distribution. We applied a weakly informative Gelman prior for the mortality trait's fixed effects. A Gelman prior is a weakly informative prior used to regularize fixed effects in Bayesian models, particularly in logistic regressions. It combines the inherent variance of the logistic distribution ($\pi^2/3$) with an extra variance term from random effects (σ^2), which constrains parameter estimates and improves model stability, especially when data are limited (Chapter I for detail).

2.3.2. PLS Regression

In Chapter II, we aimed to develop predictive models based on near-infrared spectroscopy (NIRS) to estimate key functional and chemical traits from powdered plant tissues. To achieve this, we applied multivariate statistical techniques that link spectral data to reference measurements from a training population.

One of the best approaches relies on Projection to Latent Structures (PLS) regression (Wold et al. 2001), a supervised method modeling the relationship between the predictive matrix (X) and the responses matrix (Y). It is very effective on high-dimensional data such as near-infrared spectra, which are composed of highly correlated wavelengths. The predictive data are projected in a new and reduced dimensional space based on data variability, generating new variables called Latent variables. The models are obtained by regressing these latent variables and the Y variable (Agelet and Hurburgh 2010). For the multivariate analysis after the acquisition of spectral information, PLS was used to get predictive models and the computation of Predictive R^2 and RMSECV. These models were used in Chapter II to get predictive models for traits of Water use economics, Carbon, Nitrogen, Leaf economics, total phenols, and Lignin.

2.3.3. ANOVA, Models, Stability Analysis, and Random Regression for Reaction Norms

To evaluate growth performance, heterosis expression, and genotype-by-environment (G×E) interactions in Chapter III, several statistical approaches were applied:

- ANOVA was used to test the effects of taxon (European larch, Japanese larch, hybrid larch), treatment (irrigated vs. non-irrigated), and their interaction on traits such as height, diameter, and volume. Post-hoc comparisons were conducted using Tukey's HSD test to determine statistically significant pairwise differences among taxa and treatments. This was done at taxa level and family level
- Mixed-effects models were implemented using the "breedR" package with "spatial" for the environmental correction.
- Stability and adaptability of genotypes across years and treatments were assessed using both parametric and non-parametric methods: Wricke's Ecovalence was calculated to quantify each genotype's contribution to the overall G×E interaction. Hühn's stability statistics (S(1), S(2)) were applied to evaluate the consistency of genotype rankings and variance across environments, particularly under drought stress.

To assess phenotypic plasticity in annual height and diameter increments across an environmental gradient of water availability, we implemented linear mixed-effects modeling using the lmer function from the lme4 package in R. Analyses were conducted separately for each taxon, allowing us to quantify taxon-specific responses to environmental variation.

The response variables were standardized annual increments in height and diameter at breast height (DBH), derived by z-transforming values within species to ensure comparability and to control for differences in scale. The main fixed effect was water availability and the yearly variation, expressed as a continuous environmental gradient. To capture potential nonlinear responses, we modeled the environmental gradient as a quadratic polynomial, using mean-centered values to reduce multicollinearity between the linear and quadratic terms.

Random intercepts were included for both genotype and site to account for repeated measures within genotypes and potential spatial structure across the trial. This hierarchical structure allowed us to disentangle fixed environmental effects from genotype- and site-level variability. Several candidate models were tested at the genotype (combination) level within each taxon (European larch, Japanese larch, and hybrid larch) to evaluate the best way to capture growth response under varying irrigation treatments. Model selection was based on the Akaike Information Criterion (AIC) to assess goodness of fit and parsimony (Valladares et al. 2006). To obtain robust estimates of model uncertainty, we computed 95% confidence intervals for the fixed effects using parametric bootstrapping with 1,000 simulations, implemented via the bootMer function in the lme4 package. This approach accounts for fixed and random effects structure during simulation and is particularly suitable for complex mixed-effects models (Lovell and McKay 2015). In addition, Pearson and Spearman correlation analyses were conducted to explore the relationships between growth traits and other traits, helping to identify potential trade-offs across taxa and treatments. Please see Chapter III for details

3. Chapter I: A Decade-long study on Mortality in European Larch (*Larix decidua* Mill.), Japanese Larch (*Larix kaempferi* (Lamb.) Car.) and their hybrid

3.4. Conceptualization

The central purpose of Chapter I was to assess the impact of drought episodes over the decade on hybrid larch and its parental species. For this, we identified mortality as the most extreme symptom, both complex and multifactorial, but it has rarely been investigated in long-term studies and is often overlooked compared to growth performance-related traits. (The growth-related traits will be studied later in Chapter III.)

There have been several studies on Larch species development focusing on growth, physiological, and phenological traits. They are central in making decisions for the selection of appropriate individuals and thus contributing to effective breeding populations. However, these traits only explain a part of the whole development. Ultimately, a tree's contribution to a forest stand depends on its capacity to survive to abiotic (climate, competition) as well as to biotic stresses. It is in this context that the study presented in this chapter was designed and carried out.

3.4.1. Drought in our Experimental Context

Studying the response of trees under drought has been an effort for decades, with studies conducted either in natural conditions by comparing favorable and stressful climatic years or soil types, or in experimentation with either deprivation of water or alternatively supply of water. The latter approach was used in our study, especially designed to induce intensive drought stresses through the choice of a high-drainage soil in a site with a naturally low level of precipitation, and to compare it to an 'optimal' water availability situation obtained by continuous irrigation. Following ClimEssences (https://climessences.fr/) modelling tool based on three major climatic indices (water deficit, heat-sum and minimum temperatures), the non-irrigated plot climatic conditions at INRAe-Orléans would correspond to those predicted for West Massif Central conditions – a recommended place for larch plantation- in 2050 following model RCP 4.5 (optimistic).

Many types of droughts have been identified (meteorological, agricultural, hydrological, socioeconomic, etc.). We conceptualized our framework of drought based on a recognized risk assessment model. So, this framework consists of (**Fig 22**):

1. Hazard: This represents the climate event or stressor itself – in our case, the prolonged period of low soil water availability in the non–irrigated part of the experiment compared to the irrigated one. This can be represented in our experiment through soil matric potential over the years.
2. Exposure: Exposure defines the degree to which the system (here, clones or taxa) is subjected to the hazard. Trees planted in the non-irrigated site of the experiment are consistently and heavily exposed to this drought hazard.
3. Vulnerability: Vulnerability reflects the inherent sensitivity or adaptive capacity of the system. Here, that includes all factors/traits that make trees more sensitive to the stress: e.g. genotype, size and growth rate of trees, age, soil characteristics. The modeling of

mortality as a function of growth, genetics (taxa and clones), and environmental conditions comes directly into this vulnerability concept.

Drought experiments need to define and explain 'drought' before ascertaining results based on it. The definition of drought can be conceptual or operational. Conceptual definitions are mostly dictionary-quoted definitions (American Heritage Dictionary), which define drought as "a long period with no rain, especially during a planting season," whereas a practical one defines the onset, severity, and time. There are different types of droughts, as reviewed by Wilhite and Glantz (1985):

(a) Meteorological drought: sustained period of time without significant rainfall" or "deficit of water below a given reference value, with both deficit duration and deficit magnitude taken into account"
(b) Agricultural drought: This is a more practical one with "drought intensity as the difference between plant water demand and available soil water."
(c) Hydrological drought: "period during which streamflows are inadequate to supply established uses under a given water management system"
(d) Socioeconomic drought: "shortage of water harmful to man 's agricultural activities. It occurs as an interaction between agricultural activity (i.e., demand) and natural events (i.e., supply), which results in a water volume or quality inadequate for plant and/or animal needs."

In this study, we did not have access to direct physiological measurements of drought stress in larch trees, such as predawn water potential or $\delta^{13}C$ measured annually at the needle level, which usually assist in actually determining drought in individual trees. Instead, we characterized drought intensity and timing using site-specific data on soil water availability and climate-derived drought indices. The daily temperature and the amount of rainfall received also helped identify the actual years that affected the trees in the experiment.

Two soil water measuring probes (WM and TDR, at 24 and 60 cm depth) continuously monitored soil moisture. Various parameters describing the site and tree stand assisted in measuring the soil's Relative Extractable Water (REW) (Granier et al. 1999), which aided in discriminating harsh (dry) years. Finally, a Vapor Pressure deficit (VPD) was also computed to evaluate air dryness.

Periods of high temperatures (T_max), VPD, and soil matric potential values coincided with observable drought-related damage in the field (especially mortality). In particular, symptoms such as top dieback, longitudinal stem cracks (Pâques et al. 2023), abrupt growth reductions in ring width and height increment, and tree mortality were frequently recorded in the non-irrigated plot during the severe drought years (e.g., 2015 and 2019).

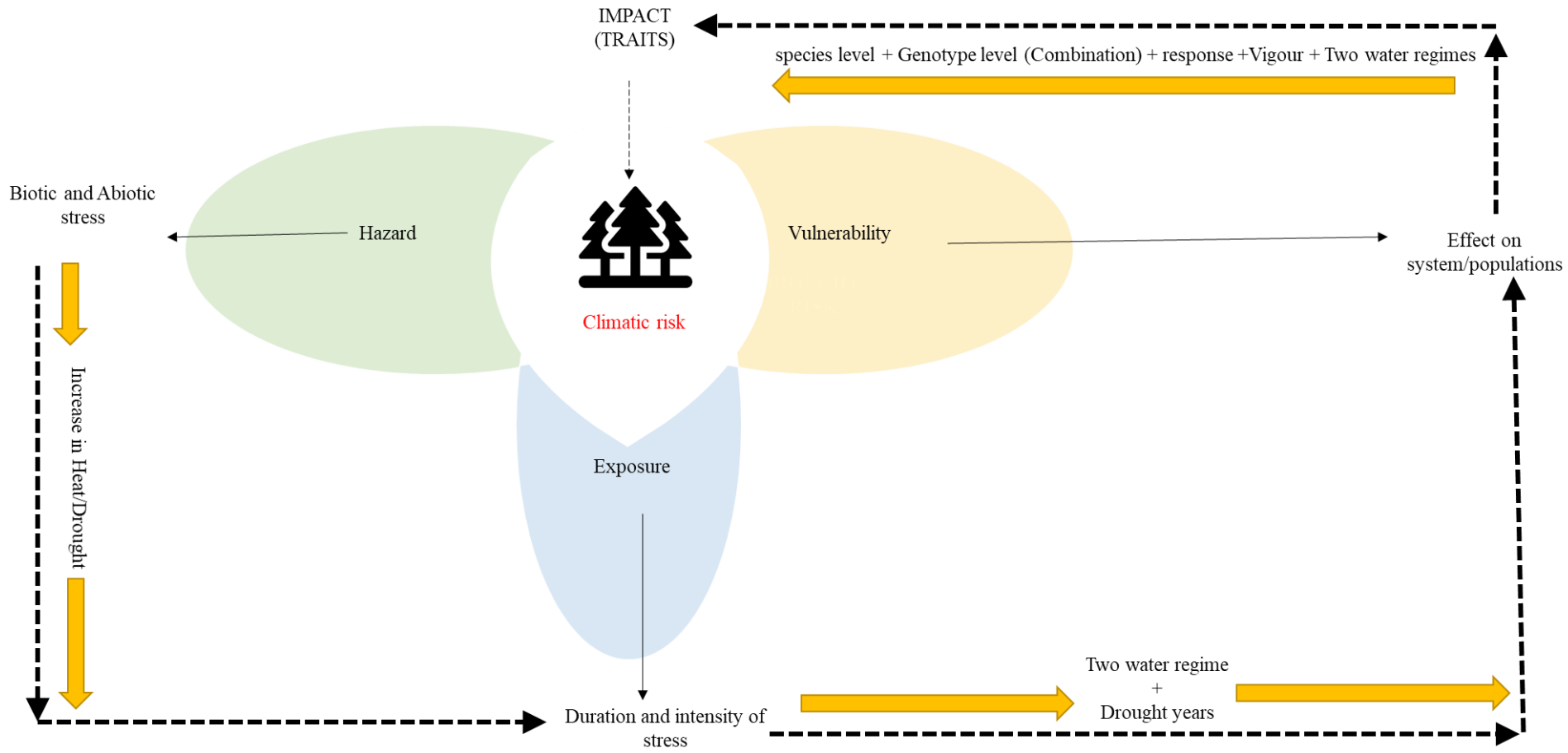


Fig 22: The schematic diagram inspired by Sergent (2011) (thesis) to quantify drought risk, through the hazard, exposure, and vulnerability, with its response on impact traits (here, mortality and growth)

Our operational definition of drought was, therefore, ecological and comparative.

3.4.2. Methodological Considerations

While annual mortality rates could be directly assessed, our objective was to model the probability of mortality events, integrating various sources of variation (climatic factors, site, taxa, genetic components, and growth). Characterizing the susceptibility of genotypes (taxa, families, clones) to drought and identifying the key climatic drivers of mortality are necessary to i) predict how well suited they will be in a context of more frequent and long drought episodes, ii) adjust deployment to suitable climate and soil, and iii) orientate breeding and selection goals. In this context, there are some considerations:

1. The experimental design

The study of mortality in our experiment, which involved an organized planting design and identical genotypes (clones) exposed to both irrigated and non-irrigated treatments, offers a controlled framework to assess drought response, unlike studies conducted under 'natural' forest conditions. Our design differs from natural forest conditions, where environmental variation is spatially heterogeneous and competition dynamics are more complex. Nonetheless, the experimental approach provided clear contrasts in water availability and reduced confounding factors (competition), enabling more robust attribution of mortality patterns to drought stress. Although the artificial setting may limit direct extrapolation to natural forest ecosystems, it offers a valuable insight to test the drought response of a wide array of genotypes under replicated (identical clones in IRR and NIRR conditions) and monitored conditions (soil water). Moreover, including pure species (European and Japanese larch) and their hybrid (*Larix* × *eurolepis*) allows for comparative analysis of species-specific and hybrid-specific mortality risks under controlled drought scenarios.

2. Mortality as a binary trait

Mortality was assessed on an individual basis as a binary trait – alive (0) or dead (1)- making generalized linear models (GLMs) with a binomial distribution and logit link a suitable choice for modelling. Thanks to the setup of the experimentation, mortality is suspected to be attributed quite exclusively to drought episodes: indeed, the same genotypes (clones) tested in irrigated and non-irrigated plots were submitted to common environmental factors (soil, climate, competition, age) except for the level of water supply.

This enabled the prediction of mortality with other explanatory variables such as watering treatments, years, taxa, and their interactions comparable. However, applying GLMs to mortality data was a bit complex, which came from the structure itself: mortality events were relatively rare, and unevenly distributed across years and taxa. This introduced issues such as data separation and convergence instability, which is why the penalized likelihood method was adopted to stabilize estimation and model comparison. This was also one of the primary reasons why we restricted this modelling to the non-irrigated part of the experiment where more mortality events occurred, after having confirmed the primary role of absence of irrigation on mortality.

Environmental window analysis and Bayesian network

In modeling the relationship between climatic variables and tree mortality, a major challenge lies in the temporal alignment of the predictor (here, environment) and response (here, mortality) variables. Often, we focus on seasonal or monthly time periods, which are arbitrary and fixed. In biological systems, where the response could be delayed, successive, cumulative, and vary across years, such fixed time windows might not be a suitable method. To address this, it is necessary to systematically test multiple time windows across a defined range, to enable identification of periods that are most correlated with the key interest, here, mortality (under a series of windows tested under the climwin package in R – **Fig 23**).

We thus used a sliding window approach across a range of time intervals (start and end date) within a defined period. This approach treats the timing of environmental influence as an estimable parameter, allowing the data to inform both the temporal window and the effect size of each covariate. By scanning overlapping windows of varying lengths, the method identifies those intervals that maximize model fit, typically measured via changes in AICc relative to a null model. This process increases the likelihood of capturing lagged or cumulative effects that might otherwise be obscured.

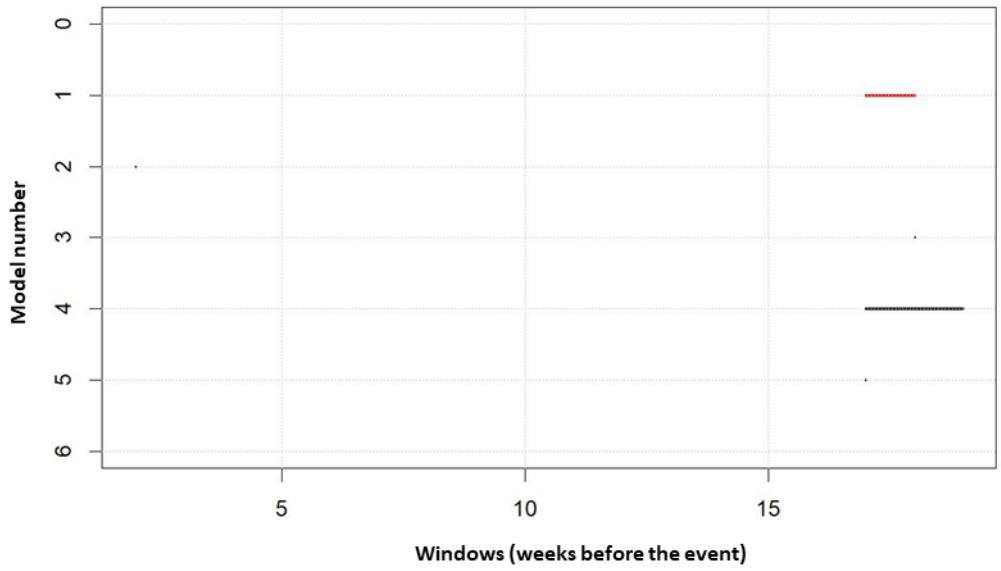


Fig 23. Most explanatory climatic window periods for tree mortality in hybrid larch (HL). Each horizontal line represents the time window (in weeks before the reference date) during which a climate variable was averaged and tested in a model. The length of each line corresponds to the duration of the window. The red line highlights the best-performing model based on the lowest ΔAICc value, indicating the window that most strongly explains variation in mortality across years. Black lines correspond to alternative candidate models.

Beyond climate, growth (size) can also be attributed as a predictor of mortality. In the context of our study, it was necessary to determine whether faster-growing individuals were more or less vulnerable to mortality. This refocuses the classical insights into growth-survival trade-

offs, especially under stress. To test for such effects, we modeled the relationship between prior growth and subsequent mortality events across years and genotypes. Our objective was to determine whether growth traits (e.g., annual diameter increment) act as risk factors, protective factors, or show no consistent association with survival.

Frequentist inferences rely on sampling distributions and treat model parameters as fixed, while Bayesian inferences treat parameters as random with probability distributions, allowing uncertainty to be modelled. Our study aimed to quantify the relationship between growth and mortality over years and across clones, which required a flexible multivariate modelling capable of handling traits (binary for mortality and continuous for growth). The Bayesian approach enabled us to achieve this by modeling the traits jointly while also considering covariation at the clonal level.

Abstract in French : Etude sur une décennie de la mortalité chez le mélèze d'Europe (*Larix decidua* Mill.), le mélèze du Japon (*Larix kaempferi* (Lamb.) Car.) et leur hybride

Résume :

Message clé - Cette étude, basée sur une expérimentation clonale de mélèze avec des conditions contrastées de disponibilité en eau du sol, a montré que le mélèze du Japon présentait la mortalité induite par la sécheresse la plus élevée, suivi de l'hybride et du mélèze d'Europe. La mortalité était liée à une faible disponibilité en eau du sol, à des températures élevées et à un déficit de pression de vapeur fin de printemps, et était négativement corrélée à la croissance, indiquant un potentiel de sélection de génotypes combinant forte croissance et meilleure survie.

Contexte - La mortalité induite par les extrêmes climatiques, en particulier par la sécheresse, suscite une inquiétude croissante en raison de l'augmentation de la fréquence, de l'intensité et de la durée des épisodes de sécheresse. La prédiction de la mortalité des arbres est une problématique complexe et multifactorielle, impliquant à la fois des caractères spécifiques aux arbres et des facteurs environnementaux, ce qui en complique l'analyse.

Objectif - Notre objectif était de modéliser la mortalité spécifique aux trois taxa de mélèze en interaction avec la sécheresse du sol et d'autres facteurs environnementaux, ainsi que la relation entre mortalité et performances de croissance.

Méthode - Nous avons estimé la variation de la probabilité de mortalité de *Larix decidua*, *Larix kaempferi* et de leur hybride sur une période de dix ans dans deux placettes présentant des disponibilités en eau du sol contrastées. À l'aide de régressions logistiques, nous avons identifié les variables environnementales et les fenêtres temporelles expliquant le mieux cette variation selon les deux traitements (irrigué ou pas). Enfin, nous avons testé l'association entre la croissance radiale et la mortalité.

Résultats - La mortalité était négligeable pour les trois taxa dans la parcelle irriguée. Dans la parcelle non irriguée, le mélèze du Japon présentait la mortalité la plus élevée, le mélèze d'Europe la plus faible, et l'hybride un niveau intermédiaire. La température maximale et le déficit de pression de vapeur à la fin du printemps expliquaient la dynamique temporelle de la mortalité. La mortalité était associée à une réduction de la croissance radiale l'année précédant celle-ci et corrélée négativement à la vigueur des clones.

Conclusion - La réponse différentielle des deux espèces et de leur hybride à la sécheresse en termes de mortalité apporte des pistes intéressantes pour les programmes de sélection. La sélection de génotypes combinant forte croissance et meilleure survie semble envisageable.

Mots-clés : Changement climatique, Mortalité, Croissance, Sécheresse, *Larix*

3.5. *Article:* A Decade-long study on Mortality in European Larch (*Larix decidua* Mill.), Japanese Larch (*Larix kaempferi* (Lamb.) Car.) and their hybrid

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A decade-long study on mortality in European larch (*Larix decidua* Mill.), Japanese larch (*Larix kaempferi* (Lamb.) Car.) and their hybrid

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Abstract

Key Message This study, based on a clonal larch experiment under contrasting soil water conditions, showed that Japanese larch exhibited the highest drought-induced mortality, followed by the hybrid and European larch. Mortality was driven by low soil water availability, high temperature, and vapor pressure deficit in late spring, and was negatively correlated with growth, indicating potential for selecting genotypes that combine high growth with greater survival.

Context Mortality induced by climate extremes, particularly by drought, has been of growing concern due to the increasing frequency, intensity, and duration of drought episodes. Predicting tree mortality is a complex and multifaceted issue that involves not only tree-specific traits but also the environment, making it more challenging.

Aim We aimed to identify species-specific mortality induced by the interaction between drought and other environmental factors, as well as the relationship between mortality and growth performance.

Method We estimated the variation in the probability of mortality of *Larix decidua* Mill., *Larix kaempfer* (Lamb.) Car. and their hybrid over ten years in two plots with contrasting soil water availability. Using logistic regression, we identified the environmental variables and time windows that best explained this variation under two watering treatments. Finally, we tested the association between radial growth and mortality.

Results Mortality was negligible for all three taxa in the irrigated plot. In the non-irrigated plot, Japanese larch exhibited the highest mortality, European larch the lowest, and the hybrid an intermediate level. Maximum temperature and vapor pressure deficit in late spring explained the temporal dynamics of mortality. Mortality was associated with reduced radial growth and was negatively correlated with mean long-term growth performance.

Conclusion The differential mortality response of the two species and their hybrid to drought can provide insights for breeding programs. Selection for combined growth and enhanced survival seems possible.

Keywords Climate change, Mortality, Growth, Drought, *Larix*

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1 Introduction

A growing body of scientific literature highlights the large negative impact of climate change across all forested biomes, particularly due to more severe and recurrent drought episodes (Solomon et al. 2007; Cook et al.

2018), irregular precipitation (Donat et al. 2013), fire hazards (Halofsky et al. 2020), and increased impacts of pests and diseases (Sturrock et al. 2011). Tree mortality due to drought events has been measured worldwide (Hammond et al. 2022), and the effect of drought on mortality is often amplified by an increase in air temperature (Van Mantgem et al. 2009; Peng et al. 2011; Wolf and Paul-Limoges 2023).

Predicting tree mortality is a complex and multifaceted issue. The reasons behind mortality risk are multiple (abiotic, biotic, human) and often interact with each other (Will et al. 2013). This is particularly true for drought-induced mortality. As highlighted in a review by Teskey et al. (2015), the impact of drought on mortality is highly dependent on the characteristics of the event itself (timing, duration, intensity, frequency). In addition, intrinsic tree factors such as size and age, along with the local environment (e.g. soil, stand density), can affect the vulnerability of trees and stands (Dobbertin 2005; Trugman et al. 2021). Moreover, drought often occurs with other stressors, creating interplay effects that can exacerbate tree mortality. High temperatures, for example, can increase drought severity by enhancing evapotranspiration rates, further stressing water resources, and causing physiological stress, leading to increased mortality (Allen et al. 2010; Hartmann et al. 2018; Matusick et al. 2018). This complexity means that a better understanding of the processes driving tree mortality, as well as the impact of drought intensity and duration, is required in order to improve predictions of tree responses to climate change.

Under drought conditions, trees often undergo changes in the foliage (discoloration, leaf shedding) (Vollenweider et al. 2016; Rosner et al. 2016) and experience stem structure alterations, often accompanied by top dieback and cracks (Cameron et al. 2017). These modifications, combined with a marked reduction in both apical and radial growth, with a decrease in the size and number of meristematic cells, are commonly associated with mortality induction or can cause death directly (Orwig and Abrams 1997; Gil-Pelegrín et al. 2004).

The timing, duration, and frequency of drought have an effect on tree responses. An early-season drought episode decreases effective apical and radial expansion that usually occurs during the spring-early summer period, while a late drought depletes carbohydrate reserve, which is necessary for winter survival (McDowell et al. 2008). Drought can lead to immediate tree death within the year (Wolf and Paul-Limoges 2023), but can also cause damage that extends to the following or subsequent years (Bigler et al. 2007). With prolonged or consecutive droughts, radial growth in Douglas fir exhibited more severe decreases in resistance and recovery than during single episodes of a similar intensity (Guisset et al. 2024).

The mortality risk caused by drought also depends on tree size (Mueller et al. 2005) and growth rate (Dobbertin 2005), which means that it is critical to understand the differential response of individual trees. The pace of growth can serve as an early indicator of drought susceptibility, as reduced growth is commonly observed closer to mortality events (Reich 2014). Variation in growth rate is influenced by how trees distribute their carbon resources and how resilient they are to environmental stress and thus to mortality (Wang et al. 2012). Whilst faster-growing trees have a lower resistance to drought stress, they usually exhibit higher recovery (Tao et al. 2024). Overall, faster-growing clones (and taller trees) can achieve higher survival, as shown by some authors (Giovannelli et al. 2007; Kaczmarek et al. 2013). However, the ability to allocate carbon resources is species- and genotype-specific, indicating that while fast-growing trees often show higher survival (McLaughlin et al. 2003; Bennett et al. 2015), their resilience to drought may depend not only on growth rate but also on their hydraulic traits (Trugman et al. 2021).

Different tree species exhibit varying levels of susceptibility to climate-induced stress and, thereby, to mortality. Overall, angiosperm trees seem less affected by drought-related mortality than conifers (Klein 2019; Lloret et al. 2022) due to their higher stomatal sensitivity, leading to early closure of stomata to conserve water. Nevertheless, this stomatal strategy can lead to carbon deprivation when extreme conditions persist (McDowell et al. 2008).

Like a few other gymnosperms, the larch genus (*Larix*) uniquely combines the needle-like leaves of conifers with the seasonal leaf shedding of deciduous trees. Larches are highly valued by foresters and industry for their rapid juvenile growth and the exceptional quality of their wood. In Europe, three main taxa are cultivated (Pâques 2013): European larch (*Larix decidua* Mill.), Japanese larch (*Larix kaempferi* (Lamb.) Car.), and their hybrids. Compared to some evergreen conifers, European larch seems poorly adapted to dry conditions (Eilmann and Rigling 2012; Lévesque et al. 2013; Oberhuber et al. 2014, 2015). Japanese larch, native to the high-elevation volcanoes of Hondo Island, with their very humid environments even during summer, is highly sensitive to summer drought (Masson 2005), and its planting is restricted to oceanic climates. Compared to its parent species, hybrid larch exhibits greater growth (known as heterosis) and plasticity to water availability (Marchal et al. 2019) and more effectively buffers environmental conditions overall (Pâques 2013). Although the physiological, anatomical, and growth responses to drought of the three larch taxa have been compared in studies with seedlings grown in nurseries or greenhouses (Haasemann 1986; Sasani et al. 2021), drought-induced mortality and growth have

not been studied in plantations. Growth rate (and size) can be a source of vulnerability (Dobbertin 2005), so the faster growth of the hybrid compared to European and Japanese larches (Marchal et al. 2017), which is favored by breeding, may be inappropriate at water resource-limited sites.

Our study takes advantage of a unique experiment, involving the three larch taxa planted under two contrasting watering treatments, to address the pressing need to examine the responses of the different taxa, the impact of climate factors, and time variations on tree mortality. Based on logistic regressions, we estimated the probability of mortality across the watering treatments and years for the three taxa and answered the following questions: (1) Do the three larch taxa exhibit different susceptibilities to mortality? (2) Which climatic parameter(s) and time window best predict mortality variation over time, and are they similar for the three taxa? (3) Does high vigor affect the vulnerability of trees to mortality when subjected to water constraints?

2 Materials and methods

2.1 Experimental site and genetic material

The material monitored in this study is a subset of an inter-/intra-specific diallel mating design involving nine European larches (EL) and nine Japanese larches (JL) (Marchal et al. 2017). The European larches were from the Sudetan Mountains population (*sudetica*) and the Japanese larches were from different populations on Hondo Island. This subset includes 12 full-sib families of EL, 12 of JL, and 12 full-sib hybrid larch (HL) families, all sharing the same EL and JL parents. Each family includes 12 individuals (clones), each represented by a variable number of ramets produced by grafting in the spring of 2011. In order to reduce a possible impact of the rootstock on tree development, seedlings from a unique seed source (FH201-Lavercantière-PF hybridization seed orchard) were used. Progenies from this orchard recombining one EL clone as mother and around 30 full-sib clones of JL as father are characterized by a narrow genetic basis (Philippe and Pâques 2003). A total of 1900 trees were included in the analyses.

Grafted plants were established when they were 2 years old at the nursery of INRAe-Orléans (latitude: 47.83 N, longitude: 1.91 E, elevation: about 108 m) during winter 2013. The experimental site is on a flat area characterized by a poor sandy soil with heavy gravel loads and a poor water retention capacity (around 35 mm). Combined with a low annual rainfall (less than 700 mm), conditions are far from ideal for reforestation with larch but highly suitable for testing its susceptibility to soil water deficit. To this end, the trial was divided into two plots to study the behavior of the three taxa of larch (EL, JL, HL) with two different irrigation treatments (hereafter "Treatments"): irrigated (IRR) and non-irrigated (NIRR). The irrigated plot has been drip irrigated daily (since 2015) during the whole growing season (April to September), and the other (NIRR) has not received any irrigation except during the first 2 years following planting. Monthly differences in water supply (rain + irrigation) can be seen in Fig. 1 together with the soil water potential. In the context of our experiment, drought is characterized by lower soil water potential in summer compared to other seasons and is amplified in the NIRR plot. Within each plot, a split-plot design is used with taxa planted in bands replicated three times and four incomplete randomized blocks within bands with complete randomization of clones. This design was selected to reduce competition between taxa. Trees were planted at a spacing of 2.5 × 3 m. Regular mowing is the only cultivation treatment used to reduce grass competition.

2.2 Meteorological, edaphic variables, and mortality assessment

The meteorological data from 2013 to 2023 originate from a Meteo-France meteorological station located within the nursery of INRAe-Orléans (Lat: 47.83 N, Long: 1.91 E). The daily data included temperatures (average, maximum, and minimum) in degrees Celsius, relative humidity (%), rain (mm), and global radiation (W/cm^{-2}). Based on these data, the Vapor Pressure Deficit (VPD, KPa) was calculated following Brice and Hall (2020), using relative humidity and average air temperatures. In 2015, the site was equipped with six soil humidity probes (TDR model CS616 and WM model 257L) installed at depths of around 24 cm (TDR24; WM24) and 60 cm (TDR60, WM60) in both IRR and NIRR plots to record the soil water content and potential. In addition, BILJOU, a water balance model (Granier et al. 1999; https://appgeodb.nancy.inra.fr/biljou/fr/), was used to estimate the soil relative extractable soil–water (REW). Due to the difficulty in monitoring Leaf Area Index (LAI, a major parameter in the model) across years and with canopy gaps, we fixed it at a stable value of 5. REW was thus used as an index to compare years and to characterize the variation in soil water availability within and over the years.

Data on tree mortality were collected yearly from 2013 to 2023 (Pâques 2025a). Each tree observed during the following spring with persistent height elongation was considered alive, and a tree with a dried or dead stem was considered dead. A year after the tree was considered dead, it was excluded from the analysis. Absence or presence of stem top dieback was also included in the study and recorded annually. Data can be found at https://doi.org/10.57745/RRUYLW.

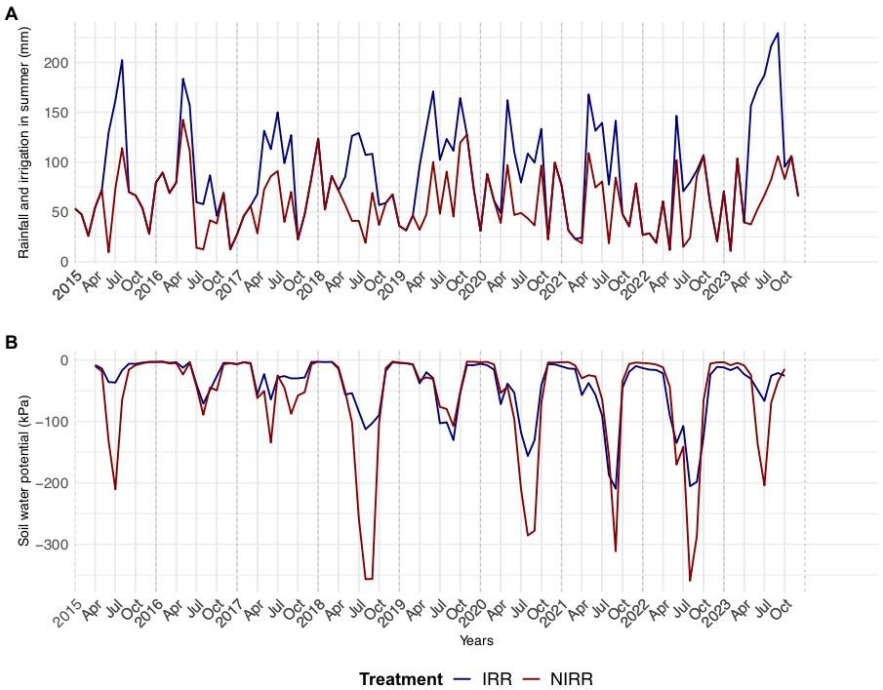

Fig. 1 **A** Available water, i.e., monthly cumulated water supply (rain and irrigation) for trees in the two treatments over the years 2015 to 2023, and **B** soil water potential (kPa) over the years (at 24 cm soil depth) measured by means of watermark soil probes at the experimental site. The blue line refers to the irrigated treatment (IRR), and the red line corresponds to the non-irrigated treatment (NIRR)

2.3 Statistical analysis

2.3.1 Effect of treatment, year, and taxa on mortality

The effect of soil water availability, year, and taxa on the probability of mortality of trees was assessed by modeling the live/dead outcomes with logistic regressions (i.e., a generalized linear model with a binomial distribution for outcome and logit link function). All possible combinations of the factors (year, taxa, and treatment) and their interactions were tested to comprehensively evaluate their effects on tree mortality. The models were evaluated using the Akaike Information Criterion corrected for small sample size (AICc, Hurvich and Tsai 1995), with the smallest AICc identifying the model that best fits the data, and where a difference of at least 2 units from other candidate models is taken as substantial support (Burnham and Anderson 2004).

The analysis used the "glm" function from the R programming language (R Core Team, 2024). In logistic regression, when the predictor variables perfectly predict the outcome variable, their coefficient estimates tend toward infinity (called separation) and may prevent the model from converging. To address this issue, we fitted the logistic regressions using penalized likelihood methods implemented in the "brglm2" package (Kosmidis 2022), providing finite and well-behaved likelihood estimates even in complete or quasi-complete separation cases (Firth 1993).

2.3.2 Best environmental predictor of mortality and relevant window periods

Before testing for the best environmental predictor of mortality, we selected uncorrelated meteorological and edaphic variables.

We applied logistic regression to determine the meteorological and edaphic variables, along with their most relevant window periods, that best predict tree mortality. We focused exclusively on the non-irrigated area of the experimental site, as almost no mortality was observed in the irrigated (IRR) area. The analysis was conducted separately for the three larch taxa. To explore

different window periods, we utilized the package "climwin" which systematically tests a range of possible windows to determine those that best explain the variation in mortality between years (Van De Pol et al. 2016). For each meteorological and edaphic variable, weekly means were tested over the period from mid-April to October 30 th. We determined the meteorological and edaphic variables that provided the most explanation of the data, along with the optimal climate window, by comparing the ΔAICc of each model with a model without weather data included (i.e., a constant model). Two strategies were used to ensure the environmental signal's robustness. First, to decrease the risk of overfitting, the mean ΔAICc of each model was estimated using repeated *K*-fold cross-validation with $k = 5$ (Van De Pol et al. 2016), repeated 200 times, resulting in 1000 evaluations for each mean. Then, we used the mean ΔAICc to find the models with the best support ($\Delta AICc_{best}$). The *K*-fold implementation from the "climwin" package was slightly modified to measure the predictive accuracy of logistic regressions using a logarithmic loss function. Second, to ensure that the environmental signal identified by the best candidate models did not result from chance, the "environmental" variables were randomized, and a new search for window periods was initiated. This procedure was repeated 1000 times, and the best candidate model from each randomized dataset was used to build a distribution of $\Delta AICc_{rand}$ (Van De Pol et al. 2016). The probability of observing $\Delta AICc_{best}$ by chance (termed pΔAICc) was estimated as the percentile of $\Delta AICc_{rand}$ that exceeded the value of $\Delta AICc_{best}$ (Bailey et al. 2016). The amount of deviance explained by the best "environmental" variables on mortality and the significance of the relationship were assessed through an analysis of deviance (ANODEV, Skalski 1996).

2.3.3 Link between tree growth and probability of mortality at individual and clonal level

We first investigated the relationship between individual live/dead status and the annual diameter increments, measured the year preceding mortality. The analysis was conducted only in the non-irrigated plot, in which the highest loss of individual trees was detected. We fitted a logistic regression with the taxa effect interacting with both the annual diameter increment (hereafter diameter growth) and year as categorical fixed effects to account for potential year-to-year variation. To facilitate comparability across taxa, the diameter growth was centered-reduced before analysis. As no diameter growth was estimated for 2014, the period included in the analyses ranged from 2015 to 2023. The study used the "glm" function with a penalized likelihood (see above for details) from the R programming language (R Core Team, 2024). The significance of the interaction between taxa and diameter growth was assessed using the Wald test implemented in the 'lmtest' R package (Zeileis and Hothorn 2002). The probability of mortality was predicted using the 'marginal effect' R package (Arel-Bundock et al. 2024).

The covariation between the diameter growth increment and the probability of mortality was explored at the clonal level using trivariate mixed linear regressions. The models were built to test for the covariation between the mortality of clones in the non-irrigated plot (trait 1), the diameter growth measured on identical clones located in the non-irrigated plot (trait 2), and the diameter growth of their respective clones in the irrigated plot (trait 3). We accounted for the average yearly effect (confounded with age) on diameter growth (IRR and NIRR) and mortality by fitting years as a fixed effect for all three traits. The variances and covariances between both diameter growth and probability of mortality among clones were modeled as a multivariate clone random effect. This allowed us to estimate a variance–covariance matrix among traits at the clone level and predict the merit of each clone for each trait. The correlations between the three traits (COR_{trait}) were estimated by dividing the covariance estimates by the product of the squared variances of the traits.

Trivariate mixed models were fitted under a Bayesian framework with the MCMCglmm package (Hadfield 2010), which allows setting different distributions for each trait of a multivariate model (here gaussian for both diameter growth and binomial for live/dead trait). We used a weakly informative Gelman prior for fixed effects of the mortality trait where $D = (\pi^2/3) + \sigma^2$ (with σ^2, the total variance due to the random effect). For the random effect, we used a parameter expanded prior with a scale of 10e4 (Hadfield 2010). As the residual variance is not identifiable in the likelihood of the binary trait, the residual variance for the mortality trait was set to one (Hadfield 2014). Models were run on 11e6 iterations with a burning phase of 1e6 iterations and a thinning of 5e3. Then, the posterior distribution of each parameter consists of 2e3 posterior samples. Convergence was checked through visual inspection and measure of autocorrelation of the posterior samples (we ensured that autocorrelation was lower than 0.05). To estimate the statistical differences in COR_{trait} between taxa, we first calculated the difference in posterior samples between taxa for each COR_{trait}. We then estimated the 95% credible interval on these differences and considered the difference statistically significant at a *p*-value of 0.05 if the credible interval did not include 0.

3 Results

Tree mortality and reduced growth were observed across all taxa in the non-irrigated (NIRR) compared to the irrigated (IRR) treatment (Figs. 2, 3, and 4). Under almost all conditions, hybrid larch exhibited the highest growth performance, either matching or exceeding that of European and Japanese larch species (Fig. 2). Japanese larch appeared to be the most vulnerable, exhibiting the highest loss in the number of trees in both treatments (Fig. 4), with more than 45% of dead trees in 2023 in the NIRR plot. European larch (EL) followed, with a mortality rate of around 35%, while hybrid larch (HL) showed slightly lower mortality, reaching just over 30% in the NIRR treatment, approximately 15% less than Japanese larch. In parallel, the top dieback symptoms were nearly absent in the irrigated plot but were more frequently severe in the non-irrigated plot. Japanese larch was the most affected taxon, with almost 50% of trees with symptoms, European larch the least (25%), and hybrid larch intermediate (around 35%) in 2023. The incidence of top dieback in 2015 was highest in the case of Japanese larch (almost 50%), but for 2018, the highest increase in top dieback was for European larch (15% in 2017 to around 25% in 2018), while

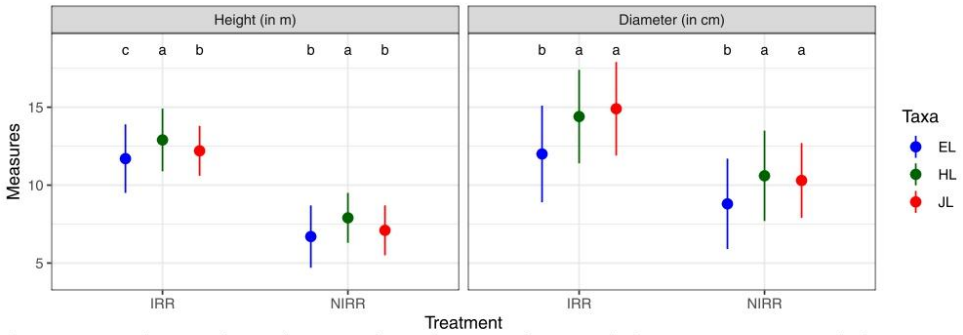

Fig. 2 Average growth in irrigated (IRR) and non-irrigated (NIRR) plots at age 10 for European larch (IRR: 317; NIRR: 180), Japanese larch (IRR: 320; NIRR: 140), and hybrid larch (IRR: 308; NIRR: 178). Bars correspond to the standard deviation. Significant differences in means between taxa were tested using Tukey's test for each treatment and each trait separately. Different letters indicate significant differences

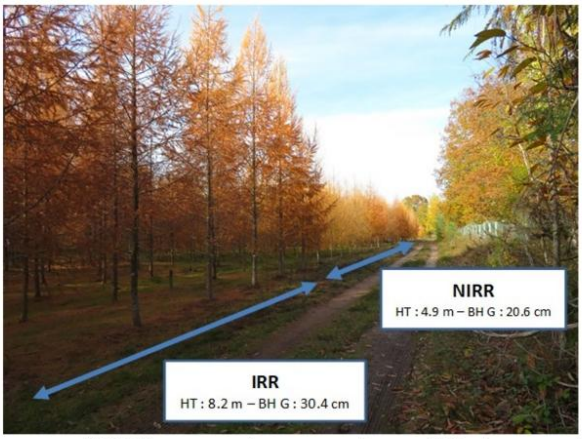

Fig. 3 General view of the experiment at INRAE Orléans nursery in Autumn 2019, with irrigated (IRR) and non-irrigated (NIRR) plots. Average height (HT) and breast height girth (BH G) in Fall 2019 are presented

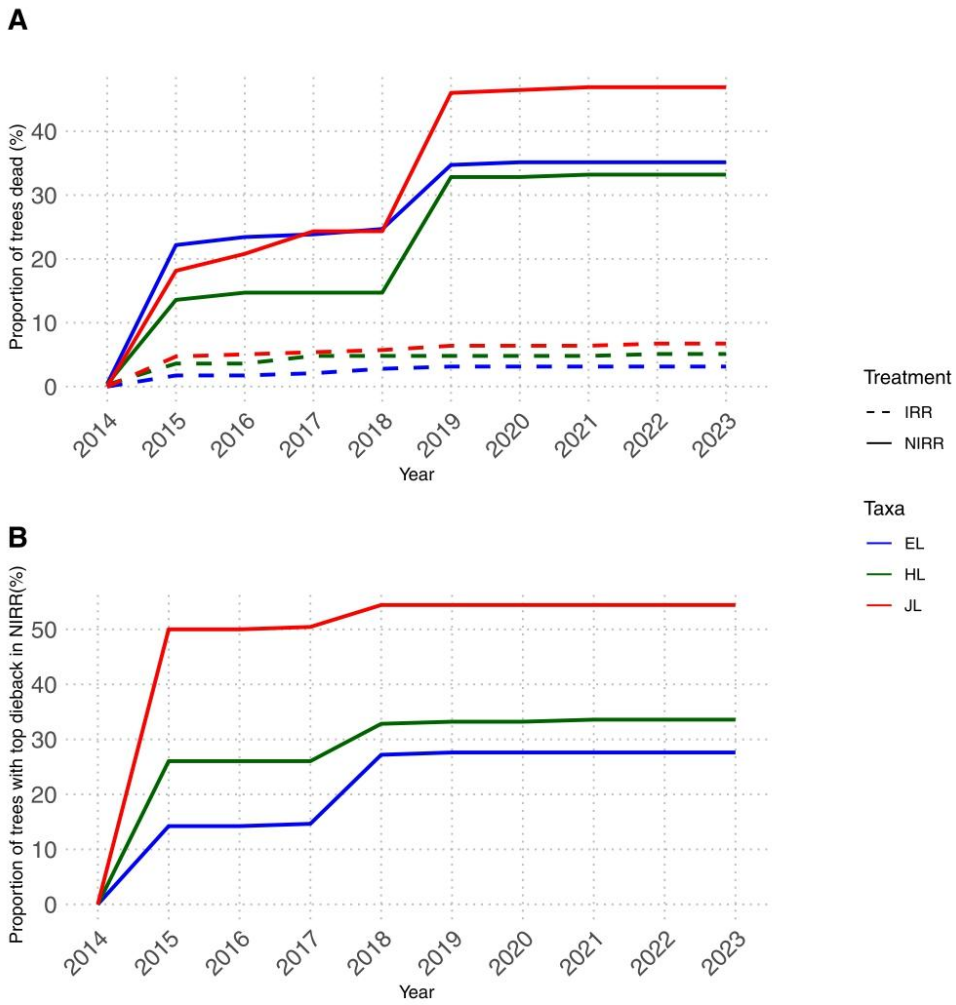

Fig. 4 **A** Cumulative percentage of trees lost from 2014 to 2023 for the two treatments (IRR and NIRR) and the three taxa, EL (blue), JL (red), and HL (dark green). **B** Cumulative percentage of top dieback in the non-irrigated (NIRR) plot

hybrid larch showed a gradual increase, reaching 35% in the same year.

3.1 Effect of treatment, year, and taxa on mortality

Among all logistic regressions that tested the impact of treatment, year, and taxa on mortality, the AICc comparison provided substantial statistical support for the model with an additive effect of taxa and interaction between treatment and year (ΔAICc = 7.951 when compared with the second-best model Treatment × Year) (Table 1).

According to this best model, Japanese larch mortality was higher than that of hybrid and European larch. The probability of mortality increased in the non-irrigated plot compared to the irrigated plot. A significant variation in the probability of mortality was estimated among years, with a higher probability of mortality in 2015 and

Table 1 Result of the model selection based on AICc (corrected Akaike information criterion). Models are ranked from lowest to highest AICc. Taxa refers to the European, Japanese, and Hybrid larches. Treatment corresponds to the two water treatments: irrigated and non-irrigated and the years are from 2014 to 2023. ΔAICc is estimated relative to the most parametrized model (Taxa × Treatment × Year)

Code	Model	AICc	ΔAICc
1	Taxa + Treatment × Year	2099.752	− 78.649
2	Treatment × Year	2107.703	− 70.698
3	Taxa + Treatment + Year	2128.803	− 49.598
4	Taxa × Treatment + Year	2130.673	− 47.728
5	Treatment + Year	2136.552	− 41.849
6	Taxa × Treatment × Year	2178.401	0
7	Taxa + Year	2421.401	243
8	Year	2426.181	247.8
9	Taxa × Year	2437.666	259.3
10	Taxa + Treatment	2781.751	603.4
11	Taxa × Treatment	2783.617	605.2
12	Treatment	2791.124	612.7
13	Taxa	3091.297	912.9
14	Constant	3096.847	918.4

2019 for the non-irrigated plot. In the irrigated plot, the greatest probability of mortality was detected in 2015, but it was below 0.3 (Fig. 5).

3.2 Predictor of mortality with the explicative environmental variable, climate window, and influence of years

3.2.1 Environmental variable selection and extreme years

Strong correlations were found between several of the climatic variables (Table 2). We removed T_avg and T_min from the climatic analyses to avoid redundancy, as they correlated with T_max (0.99 for T_avg, 0.93 for T_min). VPD and R_hum were negatively correlated (− 0.89); R_hum was excluded from further analysis, and VPD, which combines temperature and humidity, was a preferred variable because it provides a more comprehensive measure of plant physiological stress linked to air drought. TDR24 and TDR60 provide a direct measure of volumetric water content at 24 cm and 60 cm depth and are highly correlated with REW (0.91 between REW and TDR24 and 0.89 between REW and TDR60). Soil water mark, which measures the pressure needed for the roots to draw up water (WM24 and WM60), was also found to be correlated with REW (−0.73, WM24 and −0.704,

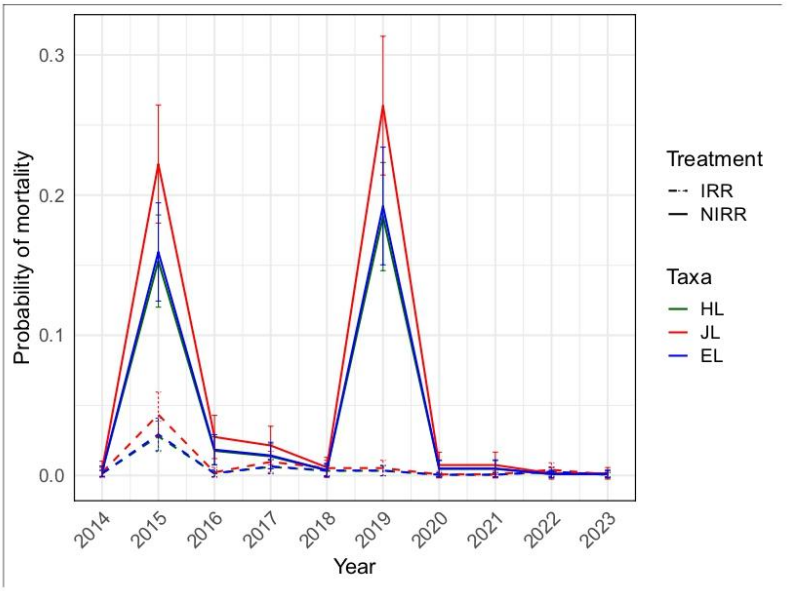

Fig. 5 Estimates of mortality probabilities from 2014 to 2023 for the two treatments among the three taxa of Larch according to the best mortality model (model 1, Table 2). EL, JL, and HL are European, Japanese, and Hybrid larches, respectively; IRR and NIRR are irrigated and non-irrigated plots. Bars around estimates represent the 95% confidence interval

Table 2 Pearson correlations between environmental variables recorded at INRAe Orléans meteorological station. T_avg is the average temperature, T_min is minimum temperature, T_max is maximum temperature, R_hum is relative humidity, G_rad is global radiation. REW is relative extractable water, VPD is vapor pressure deficit, TDR is Time domain reflectometry at 24 and 60 cm soil depth, and WM is Watermark soil probe measurement (soil water potential) at 24 and 60 cm depth. The values are bold when significant ($p < 0.05$)

	WM24	WM60	TDR24	TDR60	Rain	T_avg	R_hum	G_rad	T_min	T_max	VPD	REW
WM24	1	0.97	− 0.81	− 0.80	− 0.11	0.64	− 0.49	0.49	0.61	0.66	0.65	− 0.73
WM60		1	− 0.77	− 0.78	− 0.11	0.63	− 0.45	0.44	0.61	0.64	0.62	− 0.70
TDR24			1	0.95	0.18	− 0.84	0.67	− 0.76	− 0.77	− 0.87	− 0.81	0.91
TDR60				1	0.08	− 0.84	0.58	− 0.70	− 0.79	− 0.85	− 0.76	0.89
Rain					1	0.11	0.18	− 0.05	0.22	0.03	− 0.08	0.10
T_avg						1	− 0.71	0.85	0.97	0.99	0.89	− 0.91
R_hum							1	− 0.89	− 0.55	− 0.76	− 0.89	0.69
G_rad								1	0.73	0.88	0.89	− 0.80
T_min									1	0.93	0.80	− 0.86
T_max										1	0.90	− 0.90
VPD											1	− 0.88
REW												1

WM60). REW was preferred because it is a more integrative variable to reflect soil water availability. Thus, the climate variables selected for further analysis were maximum temperature (T_max), vapor pressure deficit (VPD), rain (Rain), relative extractable water (REW), and global radiation (G_rad).

3.2.2 Explicative environmental parameter and window for each taxon

For European Larch, T_max was the most explicative climate variable (based on AICc, Table 3), and the climate windows with the strongest correlations to mortality were concentrated in June and July. Specifically, the most

Table 3 Output from the "climwin" analysis for each European (EL), Japanese (JL), and hybrid (HL) larches. Models are ranked based on the ΔAICc (constant model − climatic model). For each taxon, only the first six models are presented. Model with substantial support are displayed in bold along with the result of the analysis of deviance (*F* statistics, *p*-value significance level, R^2). R^2 corresponds to the annual variation in mortality explained by the variation in the climatic variable

	Variable	ΔAICc	Window period	pΔAICc	*F* statistics	*p*-value	R^2 (%)
European larch	T_max	− 106.547	June 21 to July 8	< 0.001	47.29	< 0.001	85.53%
	T_max	− 101.55	June 21 to July 4	< 0.001			
	T_max	− 99.82	June 21 to July 6	< 0.001			
	T_max	− 98.782	September 3 to September 9	< 0.001			
	T_max	− 98.07	June 25 to July 1	< 0.001			
	T_max	− 98.07	September 3 to October 31	< 0.001			
Japanese larch	VPD	− 75.27	June 14 to July 4	< 0.001	20.46	0.002	71.89%
	VPD	− 74.56	June 21 to July 4	< 0.001	20.29	0.0019	71.72%
	T_max	− 73.83	June 21 to July 4	< 0.001	21.84	0.002	73.19%
	T_max	− 71.77	June 14 to July 8	< 0.001			
	T_max	− 71.12	June 21 to June 28	< 0.001			
	VPD	− 70.49	July 4 to July 6	< 0.001			
Hybrid larch	VPD	− 128.97	June 21 to July 4	< 0.001	6.958	0.029	46.51%
	T_max	− 127.07	June 21 to June 27	< 0.001	7.31	0.026	47.75%
	T_max	− 119.74	June 21 to July 4	< 0.001			
	VPD	− 116.47	June 14 to July 4	< 0.001			
	VPD	− 114.51	June 21 to July 1	< 0.001			
	T_max	− 110.73	June 21 to July 8	< 0.001			

significant climate window opened and closed between June 21 and July 8. The model with T_max explained more than 85% of the annual variation in probability of mortality ($p < 0.05$) (Table 3). As the mean weekly T_max increased, the probability of European Larch mortality increased sharply, particularly at temperatures above 28 °C. This trend was driven by extreme years like 2015 and 2019, which had the highest probabilities of mortality (Fig. 6A).

In hybrid Larch, VPD and T_max explained the annual variation in mortality with the same support (ΔAICc between best VPD and best T_max models equal − 1.90, Table 3). The climate window for VPD that best explained mortality spanned June and July (June 21 to July 4). The mortality was positively correlated with VPD in this window, with a steep increase in mortality observed for values over 1–1.25 kPa. VPD explained 47% of the annual variation in the probability of mortality ($p < 0.05$) (Table 3) (Fig. 6C). The climate window for T_max that best explained mortality was from June 21 to June 27. An increase in T_max during this window was related to an increase in mortality and explained 48% of the yearly variation in mortality.

For Japanese Larch, VPD and T_max explained the most annual variation in mortality (Table 3). Although two models showed similar and close AICc values for VPD, the best-fitting model was selected. For VPD, the climate windows ranged from June 14 to July 4, and the model explained 71.9% of the total variance. The probability of mortality increased with an increase in VPD: 2019 had the highest probability of mortality for VPD above 0.8 kPa (Fig. 6E). T_max had the same statistical support as VPD and explained 73.2% of variation in mortality with the best climate window from June 21 to July 4.

3.3 Tree growth and probability of mortality at the individual annual diameter growth and clonal level

Analyzing the link between the probability of mortality and diameter growth (Fig. 7) at the individual ring level, considering the ring growth in the preceding year, revealed a significant relationship between reduced diameter growth and increased probability of mortality among the three larch taxa in the non-irrigated plot. All three taxa had increased mortality when there was lower diameter growth (0% to approx. 20%), with a significant difference between species (Wald test on interaction term: $p = 0.026$). European Larch exhibited higher mortality trends, with mortality probabilities rapidly decreasing as diameter growth increased.

At the clonal level, we also found a significant negative correlation between the probability of mortality and diameter growth in the non-irrigated plot for all three taxa (Table 4). While the correlation was higher for Japanese larch, no significant differences could be detected between the species (Table 5). For hybrid larch, the variance in probability of mortality was small compared to the other taxa, and the correlation with diameter growth in NIRR was only marginally significant when considering the 95% Credible Interval (CI) (Table 4, Fig. 8). The probability of mortality of clones in the non-irrigated plot was not correlated with the diameter growth of their respective copies (ramets) in the irrigated plot (Table 4, Fig. 8) for any of the taxa, even though the diameter growth at clonal level was significantly correlated between the non-irrigated and the irrigated plots (Table 4).

4 Discussion

Predicting tree mortality induced by the combined effects of drought and heat is highly challenging, whether using physiological approaches or analyzing experimental setups on a large spatial scale. Indeed, although some studies have attempted to predict tree mortality based on physiological characteristics, such as water potential and hydraulic safety limits, these methods have proven relatively ineffective (Anderegg et al. 2016; Li et al. 2022). In large-scale mortality studies conducted across multiple geographic sites in natural forests or plantations, the exact causes of mortality are often unknown and most probably multifactorial. In addition, the high variability in factors such as soil, stand structure, age, and genetic origin affecting susceptibility and vulnerability to mortality makes it difficult to disentangle how these diverse influences interact across different temporal and spatial scales (Anderegg et al. 2016; Trugman et al. 2021). In our study, we were able to make use of observations on the same cloned trees from the three *Larix* taxa, established

(See figure on next page.)

Fig. 6 The probability of mortality in European (EL), hybrid (HL), and Japanese (JL) larch taxa as related to the key climatic variables. **A** European larch: probability of mortality related to mean weekly maximum temperature. **B** Hybrid larch: probability of mortality based on mean weekly maximum temperature. **C** Hybrid larch: probability of mortality based on mean weekly vapor pressure deficit. **D** Japanese larch: probability of mortality based on mean weekly maximum temperature. **E** Japanese larch: probability of mortality based on mean weekly vapor pressure deficit. The sites illustrate the relationship between mortality and climatic factors (climate windows are displayed at the top), with shaded areas representing 95% confidence intervals. The red points represent the percentage of mortality in a particular year

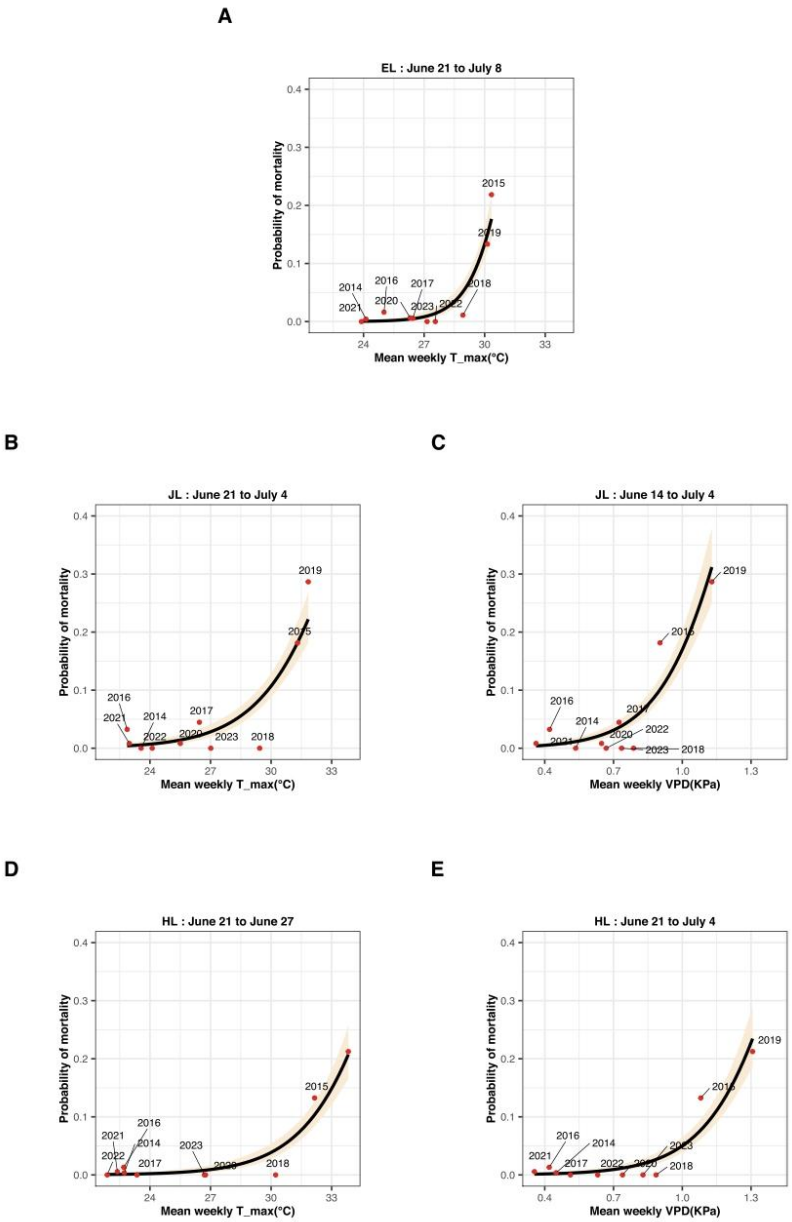

Fig. 6 (See legend on previous page.)

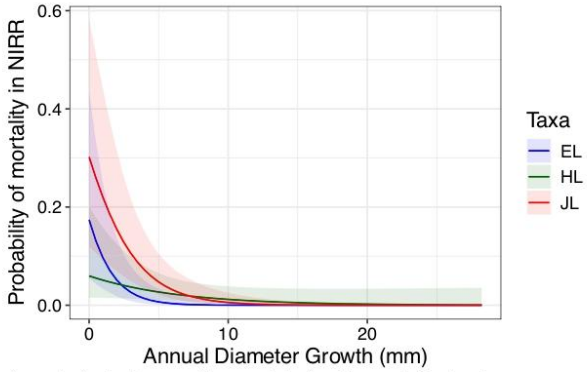

Fig. 7 Predicted probability of mortality for the three taxa (European, hybrid and Japanese) of larch in the non-irrigated plot (NIRR) as a function of annual diameter growth increment measured in the preceding year. The shaded areas represent the 95% confidence intervals

Table 4 Variance estimates of probability of mortality and of diameter growth in irrigated and non-irrigated plots at clone level and correlations between these traits. Estimates correspond to the posterior mode and brackets to the 95% credible interval. NIRR and IRR refer, respectively, to the non-irrigated and irrigated plots of the experiment. Significant correlations are in bold

	European larch	Hybrid larch	Japanese larch
Variance mortality	2.02 [0.47:6.24]	0.55 [0.00:1.99]	1.67 [0.77:4.34]
Variance growth IRR	0.11 [0.08:0.15]	0.13 [0.09:0.17]	0.11 [0.08:0.15]
Variance growth NIRR	0.19 [0.13:0.28]	0.17 [0.12:0.24]	0.23 [0.15:0.33]
Correlation (mortality, growth IRR)	− 0.09 [− 0.32:0.21]	0.12 [− 0.21:0.52]	− 0.17 [− 0.43:0.09]
Correlation (mortality, growth NIRR)	− 0.37 [− 0.78: − 0.03]	− 0.38 [− 0.83: − 0.05]	− 0.76 [− 0.95: − 0.49]
Correlation (growth IRR, growth NIRR)	0.24 [0.02:0.45]	0.28 [0.05:0.41]	0.22 [0.03:0.48]

Table 5 Difference in variance and correlation estimates between taxa for mortality probability and diameter growth in irrigated and non-irrigated plots. Variance and correlation values for each taxon are provided in Table 3. Estimates correspond to the posterior mode and brackets to the 95% credible interval. NIRR and IRR refer, respectively, to the non-irrigated and irrigated plots of the experiment. Significant correlations are in bold

	Difference HL-EL	Difference HL-JL	Difference EL-JL
Correlation (mortality, growth IRR)	0.18 [− 0.26:0.71]	0.29 [− 0.18:0.77]	0.10 [− 0.25:0.48]
Correlation (mortality, growth NIRR)	− 0.07 [− 0.57:0.54]	0.26 [− 0.20:0.79]	0.44 [− 0.09:0.80]
Correlation (growth IRR, growth NIRR)	0.03 [− 0.27:0.33]	0.04 [− 0.31:0.30]	0.03 [− 0.34:0.29]

on the same site under two contrasting watering treatments, in even-age plots with the same stand density and cultivation management. The grafting strategy used in our study was implemented by ensuring that the source of hybrid larch rootstocks was as genetically homogeneous as possible, in order to limit variable impacts of rootstock on mortality. While vegetative propagation by cutting allows the trees to develop on their own roots, this technique was not possible in this case because of the loss of juvenility of the ortets (age 17 at time of collection). Recently, a heteroplastic grafting experiment on hybrid and Japanese larches rootstocks showed that the scion had a significant effect on mortality but not the rootstock species (Pâques 2025b), suggesting a low or limited impact of grafting on our results.

Vegetative propagation by cutting of the individuals used in this study could have been a better alternative than grafting (i.e., trees development on their own

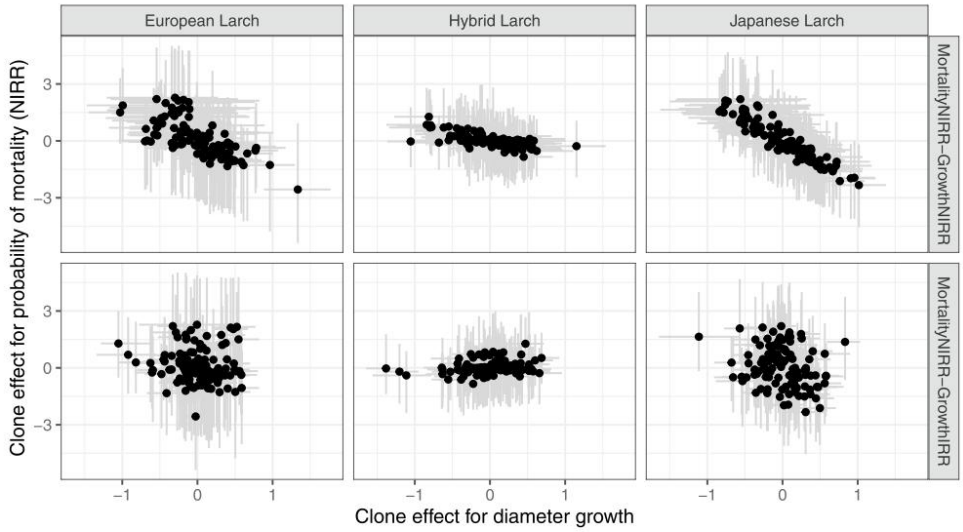

Fig. 8 Clonal effect on probability of mortality and diameter growth for European, hybrid, and Japanese larch. Gray bars represent the 95% credible intervals. The first row represents the covariation between the clonal effect on probability of mortality and diameter growth for trees in the experiment's non-irrigated plot (NIRR). The second row represents the covariation between the clonal effect on probability of mortality for trees in the non-irrigated plot and the diameter growth for trees in the irrigated plot (IRR)

roots) but it was technically not possible because of the loss of juvenility of the ortets (age 17 at time of collection). Grafting was the only possibility left, and we took care to use the most genetically homogeneous source of hybrid larch rootstocks available. Recently, we showed, thanks to a heteroplastic grafting experimentation on either hybrid or Japanese larches, that the scion had a significant effect on mortality but not the rootstock species (Pâques 2025b). This comforts our results.

The significant reduction in diameter and height in the non-irrigated plot compared to the irrigated plot indicates that the environmental conditions at the experimental site, in terms of soil water capacity and annual precipitation, were suboptimal for all larch taxa. In contrast, irrigation allowed growth to be at levels comparable to those of optimal plantation sites in France (corresponding to the best yield class according to Hamilton and Christie 1971; Pauwels and Marenne 1999). Apart from the additional water supply in the irrigated plot, all other environmental factors (climatic variables, soil characteristics, cultivation management (e.g., soil preparation, weeding)) were similar in the two plots. In addition, our experimental approach, which utilized vegetative propagation of the same genotypes, eliminated bias from uncontrolled genetic variability. Notable variations in mortality between the two plots (IRR and NIRR) suggest, therefore, in the absence of any other known causes of mortality, that soil water availability was the crucial factor in determining the survival of trees, whatever the taxa.

During the experiment, almost all dead trees were located in the non-irrigated plot (45% in NIRR compared to 5% in IRR). Most of the mortality occurred between 2015 and 2019, with variable levels in the three taxa. The highest mortality was linked to high maximum temperatures and vapor pressure deficits, particularly at the beginning of the summer, when growth is at its maximum. During these exceptional years and periods, mean maximum temperatures and VPD exceeded 30 °C and 0.9 kPa, respectively, meaning that, at times, maximum daily temperatures could reach over 38 °C (2015) and 42 °C (2019), and VPD 1.9 and 2.4 kPa, respectively. It was also observed in the experiment that these two sources of stress were highly detrimental to trees, with leaf discoloration observed during summer, a high frequency of stems with top dieback in 2015 and 2018, and, finally, mortality. For the non-irrigated trees, neither rain nor REW (Relative Extractable Water) were significant factors, as evidenced by the models tested; however, it should be highlighted that the effects of the watering treatments did significantly affect mortality. As additional water supply was the only difference between irrigated and

non-irrigated plots, water limitation in NIRR should be considered a major cause of mortality. During this period, trees in the irrigated plot were exposed to the same extreme temperature and VPD conditions, but they remained vital with fast growth, green foliage, little top dieback, and less mortality. This highlights the importance of the compensatory effect of water supply in the irrigated plot and of the soil water stress exacerbated by maximum temperature and VPD in the non-irrigated plot.

While in 2015 there was a clear association between top dieback and mortality of young trees, this direct link was not observed in 2018 on larger trees. Instead, mortality peaked in 2019, suggesting that trees exposed to stress in 2018, as indicated by top dieback, did not die immediately but succumbed the following year. Top dieback seriously weakens the tree with a reduced green crown and a compromised vascular system (Klesse et al. 2021), making it more susceptible to further damage. This pattern indicates that prior stress events, reflected here by top dieback, can predispose trees to increased mortality in subsequent years, especially under continued or exacerbated adverse conditions (Guisset et al. 2024).

Cavitation and carbon starvation are considered the primary drivers of dieback and tree death under drought conditions (McDowell et al. 2008). Although no formal test is possible based on the 2015 and 2019 years, the high frequency of stems affected by top dieback observed in the same year or the year preceding that with the highest mortality rate suggests that, among the two major factors of mortality due to drought, cavitation can be implicated. Under adverse conditions, elevated transpiration rates lead to the formation of air bubbles in the xylem, causing cavitation, which impairs water transport (Anderegg et al. 2016). Portions of trees not supplied with water may become so dehydrated that they die. Prolonged and severe drought conditions, as experienced by trees in nearly all years, but particularly in 2015 and 2019, could also lead to carbon starvation. However, contrary to common belief, Hajek et al. (2022) found no evidence that carbon starvation—marked by reduced starch and increased soluble sugars—was the leading cause of mortality in temperate species with canopy dieback. Instead, changes in non-structural carbohydrates (NSCs) could be a reaction to stress rather than a direct cause of mortality.

Among the three larch taxa tested, Japanese Larch was found to be the most vulnerable to water limitation. Native to high elevation mountains of Hondo Island in Japan, Japanese larch is well adapted to environments with high rainfall and high air humidity. Our experiment confirms its high sensitivity to high VPD (in addition to T_max). Japanese larch is known by foresters to suffer from summer drought when transferred to a continental climate; there, European larch is preferred for plantations (Masson 2005). For European larch, our results identified T_max (and not VPD) as the main climatic variable affecting its survival. European larch, described in previous studies as an anisohydric taxon (Anfodillo et al. 1998; Swidrak et al. 2013; Klein 2014; Leo et al. 2014) was also shown, at the sapling stage, to exhibit slower growth and a hydraulically safer wood (higher wall/lumen ratio and smaller pit cavity area better equipped to tolerate water stress) than the two other taxa (Sasani et al. 2021): this would predispose it to be more tolerant to drought. These characteristics would, indeed, suggest that it would be less prone to cavitation during severe drought episodes. This is confirmed by the overall reduced proportion of trees with top dieback over the ten years of observation (Fig. 4B), particularly in 2015 but not in 2018. The extreme heat recorded in 2018, a shift in wood hydraulic characteristics with tree aging, or any underlying unknown factor might explain this difference. Together with Japanese larch, hybrid larch was described as isohydric by Sasani et al. (2021). With a higher phenotypic plasticity of growth in relation to soil water availability than its parents, hybrid larch proved to have a high reactivity to soil water deficit with a substantial decline of growth (Marchal et al. 2019). Hybrid larch also proved susceptible to air humidity (VPD) and T_max, like Japanese larch, but to a lower degree and exhibited an overall lower mortality than Japanese and European larches. The well-known fast growth of the hybrid compared to its parent taxa (Greenwood et al. 2015; Marchal et al. 2017) does not seem to adversely affect its survival during drought. Our study relying on progeny sharing common parental clones thus allowed a sound comparison of the three taxa. However, the limited number of genotypes tested for each taxon potentially restricts the generalization of results at the taxon level.

The use of slow-growing species for afforestation purposes is increasingly advocated in the context of global changes. Because of their better resource-saving, more efficient carbon storage (Atkinson et al. 2012; Signori-Müller et al. 2022) and safer hydraulic wood properties (Eller et al. 2018), they would resist climatic stresses better and exhibit better survival (Zhu et al. 2018). However, species-specific behavior in terms of aspects of resilience (resistance and recovery) to climate stresses and possible trade-offs (Tao et al. 2024) complicates the generalization. The link between tree growth and mortality is another important part of predicting tree mortality. We considered it at two levels: at the individual diameter level, approximated by annual diameter increment (ring width), and at the clonal level.

The analysis of the probability of mortality and diameter growth at the individual diameter level showed a

significant relationship, where reduced growth one year was linked to increased mortality the following year across all three larch taxa. This partially aligns with findings summarized by Cailleret et al. (2017), who observed a consistent trend across various sites and species, showing that a general decline in radial growth often precedes mortality.

At the clonal level, mortality and growth in non-irrigated conditions also had a negative correlation for all taxa: the faster-growing clones had a better survival rate than the slow-growing ones. Similar conclusions were reached, for example, for Poplar (Kaczmarek et al. 2013). Giovannelli et al. (2007) observed that the fast-growing clones such as *P. deltoides* Marsh. "Dvina" exhibited lower mortality under drought conditions, suggesting more efficient water use and resource allocation than slower-growing counterparts. Similarly, a strong positive correlation ($r = 0.49$) between growth and survival was found in poplar clones at age 10, as recalculated from Kaczmarek et al. (2013), highlighting the advantage of selecting vigorous clones for areas with water stress. Nevertheless, these results contrast with those of Monclus et al. (2006), who observed that most productive poplar (*Populus* × *euramericana*) genotypes displayed low drought tolerance (meaning they are more prone to mortality). More globally, Bennett et al. (2015) concluded that larger trees suffer more (are more at risk) during water stress events than slow-growing trees. Fast growth appears advantageous in high-resource environments, but excess costs make it disadvantageous in low-resource ones (Grime 1977, 1979 in Reich 2014). Our results, together with the ones cited above on poplar, challenge this view. Faster-growing trees might be associated with rapid root establishment and deeper rooting, which could enhance survival (Kaczmarek et al. 2013). This is supported at the taxon level by a previous study on larch (Pâques et al. 2022): hybrid larch, the most vigorous taxa, not only had greater above-ground biomass but also greater coarse and fine root biomasses than both European and Japanese parents, potentially improving resource acquisition and seedling establishment (Hamberg et al. 2018). The significant positive correlation between survival and growth exhibited at the clonal level suggests that selecting vigorous clones may enhance afforestation in drought-prone areas. For hybrid larch, where this correlation is only marginally significant, selecting vigorous clones is unlikely to negatively affect survival.

These results bring some preliminary answers to breeders' concerns about selecting for fast growth and its consequences on tolerance to stress and, finally, on survival. From this perspective, fast growth does not seem detrimental and supports the use of hybrids in place of parent species and the selection of clones, combining fast growth and low susceptibility to mortality. However, the absence of a link between the growth of clones in the non-limiting water site and their survival in water stress conditions indicates the need to test genotypes and select those that perform best in stressful conditions.

A primary limitation of this study, as in many studies of this type, was our inability to partition the climate variable effects, as could be done in a factorial design testing the separate factors and their interactions at different levels. Another drawback of such a study is the underestimation of ontogenic effects and the progressive genetic erosion of the material over the years through mortality from stress events (once dead, a genotype can no longer be tested at an older age or under other climatic conditions). Temporally disconnected trials could solve this problem by isolating yearly and ontogenic effects.

5 Conclusion

In this study, we were able to establish differential water availability with contrasting water constraints across sites, resulting in distinct responses between the three larch taxa. This experimental approach with a control of potential sources of bias (e.g., soil, age, stand management) allowed us to answer our questions about taxa-specific susceptibility to mortality, key climatic predictors of mortality, and the effect of high vigor under soil water deficit. Under general drought conditions, European larch emerged as the least sensitive taxon and Japanese larch as the most susceptible, with maximum temperature and vapor pressure deficit during the early summer being the most explicative factors influencing mortality.

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Code availability
This work is a part of the thesis that is yet to be defended, which is why we would still use the data to answer other questions. The code developed for this study's analysis will be available upon reasonable request from the corresponding authors.

Authors' contributions
Naincy Sagar: methodology, formal analysis; investigation; visualization; writing—original draft. Luc E. Pâques: conceptualization; writing—review and editing; visualization; supervision; project administration; funding acquisition. Stéphane Chantepie: conceptualization; methodology; investigation; data analysis; writing—review and editing; supervision. The authors read and approved the final manuscript.

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Data availability
The dataset has been deposited at https://doi.org/10.57745/RRUYLW. It will be fully accessible by September 30, 2025. Meanwhile, the data and materials used in this study are available upon reasonable request from the corresponding authors.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors consent to the publication of this manuscript.

Competing interests
The authors declare that they have no competing interests.

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References

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH, Ted Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim J-H, Allard G, Running SW, Semerci A, Cobb N (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manag 259:660–684. https://doi.org/10.1016/j.foreco.2009.09.001

Anderegg WRL, Klein T, Bartlett M, Sack L, Pellegrini AFA, Choat B, Jansen S (2016) Meta-analysis reveals that hydraulic traits explain cross-species patterns of drought-induced tree mortality across the globe. Proc Natl Acad Sci 113:5024–5029. https://doi.org/10.1073/pnas.1525678113

Anfodillo T, Rento S, Carraro V, Furlanetto L, Urbinati C, Carrer M (1998) Tree water relations and climatic variations at the alpine timberline: seasonal changes of sap flux and xylem water potential in *Larix decidua* Miller, *Picea abies* (L.) Karst. and *Pinus cembra* L. Ann Sci for 55:159–172. https://doi.org/10.1051/forest:19980110

Arel-Bundock V, Greifer N, Heiss A (2024) How to Interpret Statistical Models Using marginal effects for *R* and *Python*. J Stat Softw 111. https://doi.org/10.18637/jss.v111.i09

Atkinson RRL, Burrell MM, Osborne CP, Rose KE, Rees M (2012) A non-targeted metabolomics approach to quantifying differences in root storage between fast- and slow-growing plants. New Phytol 196:200–211. https://doi.org/10.1111/j.1469-8137.2012.04274.x

Bailey LD, Van de Pol M (2016) climwin: an R toolbox for climate window analysis. PLoS ONE 11(12):e0167980. https://doi.org/10.1371/journal.pone.0167980

Bennett AC, McDowell NG, Allen CD, Anderson-Teixeira KJ (2015) Larger trees suffer most during drought in forests worldwide. Nat Plants 1:1–5. https://doi.org/10.1038/nplants.2015.139

Bigler C, Gavin DG, Gunning C, Veblen TT (2007) Drought induces lagged tree mortality in a subalpine forest in the Rocky Mountains. Oikos 116:1983–1994. https://doi.org/10.1111/j.2007.0030-1299.16034.x

Brice T, Hall T (2020) US Dept of Commerce National Oceanic and Atmospheric Administration National Weather Service [WWW Document]. Vapor Pressure Calculator. URL: https://www.weather.gov/epz/wxcalc_vaporpressure

Burnham KP, Anderson DR (eds) (2004) Model Selection and Multimodel Inference. Springer, New York, NY

Cailleret M, Jansen S, Robert EMR, Desoto L, Aakala T, Antos JA, Beikircher B, Bigler C, Bugmann H, Caccianiga M, Čada V, Camarero JJ, Cherubini P, Cochard H, Coyea MR, Čufar K, Das AJ, Davi H, Delzon S, Dorman M, Gea-Izquierdo G, Gillner S, Haavik LJ, Hartmann H, Hereş A-M, Hultine KR, Janda P, Kane JM, Kharuk VI, Kitzberger T, Klein T, Kramer K, Lens F, Levanic T, Linares Calderon JC, Lloret F, Lobo-Do-Vale R, Lombardi F, López Rodríguez R, Mäkinen H, Mayr S, Mészáros I, Metsaranta JM, Minunno F, Oberhuber W, Papadopoulos A, Peltoniemi M, Petritan AM, Rohner B, Sangüesa-Barreda G, Sarris D, Smith JM, Stan AB, Sterck F, Stojanović DB, Suarez ML, Svoboda M, Tognetti R, Torres-Ruiz JM, Trotsiuk V, Villalba R, Vodde F, Westwood AR, Wyckoff PH, Zafirov N, Martínez-Vilalta J (2017) A synthesis of radial growth patterns preceding tree mortality. Glob Change Biol 23:1675–1690. https://doi.org/10.1111/gcb.13535

Cameron A, Orr D, Clark J (2017) Variation in the incidence and severity of drought crack in three conifer species in North East Scotland. Scand J for Res 32:658–662. https://doi.org/10.1080/02827581.2017.1360936

Cook BI, Mankin JS, Anchukaitis KJ (2018) Climate change and drought: from past to future. Curr Clim Change Rep 4:164–179. https://doi.org/10.1007/s40641-018-0093-2

Dobbertin M (2005) Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. Eur J for Res 124:319–333. https://doi.org/10.1007/s10342-005-0085-3

Donat MG, Alexander LV, Yang H, Durre I, Vose R, Dunn RJH, Willett KM, Aguilar E, Brunet M, Caesar J, Hewitson B, Jack C, Klein Tank AMG, Kruger AC, Marengo J, Peterson TC, Renom M, Oria Rojas C, Rusticucci M, Salinger J, Elrayah AS, Sekele SS, Srivastava AK, Trewin B, Villarroel C, Vincent LA, Zhai P, Zhang X, Kitching S (2013) Updated analyses of temperature and precipitation extreme indices since the beginning of the twentieth century: The HadEX2 dataset. J Geophys Res Atmospheres 118:2098–2118. https://doi.org/10.1002/jgrd.50150

Eilmann B, Rigling A (2012) Tree-growth analyses to estimate tree species' drought tolerance. Tree Physiol 32:178–187. https://doi.org/10.1093/treephys/tps004

Eller C, De V, Barros F, R.L. Bittencourt P, Rowland L, Mencuccini M, S. Oliveira R, (2018) Xylem hydraulic safety and construction costs determine tropical tree growth: tree growth vs hydraulic safety trade-off. Plant Cell Environ 41:548–562. https://doi.org/10.1111/pce.13106

Firth D (1993) Bias reduction of maximum likelihood estimates. Biometrika 80:27–38. https://doi.org/10.1093/biomet/80.1.27

Gil-Pelegrín E, Corcuera L, Camarero JJ (2004) Effects of a severe drought on *Quercus ilex* radial growth and xylem anatomy. Trees - Struct Funct 18:83–92. https://doi.org/10.1007/s00468-003-0284-9

Giovannelli A, Deslauriers A, Fragnelli G, Scaletti L, Castro G, Rossi S, Crivellaro A (2007) Evaluation of drought response of two poplar clones (*Populus*×*canadensis* Mönch 'I-214' and *P. deltoides* Marsh. 'Dvina') through high resolution analysis of stem growth. J Exp Bot 58:2673–2683. https://doi.org/10.1093/jxb/erm117

Granier A, Bréda N, Biron P, Villette S (1999) A lumped water balance model to evaluate duration and intensity of drought constraints in forest stands. Ecol Model 116:269–283. https://doi.org/10.1016/S0304-3800(98)00205-1

Greenwood MS, Roth BE, Maass D, Irland LC (2015) Near rotation-length performance of selected hybrid larch in Central Maine, U.S.A. Silvae Genet 64:73–80. https://doi.org/10.1515/sg-2015-0006

Guisset C, Dendoncker M, Vincke C, Ponette Q (2024) Drought timing, intensity, and consecutiveness have more influence on Douglas fir growth response than site conditions and stand density in European temperate climate. For Ecol Manag 569:122177. https://doi.org/10.1016/j.foreco.2024.122177

Haasemann W (1986) Untersuchungen zur Ökologie der Europäer lärche, Japaner lärche und ihrer Hybriden im Nass-Trockenfeld. Beitr Forstwirschaft 20:184–188

Hadfield JD (2010) MCMC Methods for multi-response generalized linear mixed models: the MCMCglmm R Package. Journal of Statistical Software, 33:1–22. https://doi.org/10.18637/jss.v033.i02

Hadfield JD (2014) MCMCglmm course notes. Retrieved from: https://cran.rproject.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf

Hajek P, Link RM, Nock CA, Bauhus J, Gebauer T, Gessler A, Kovach K, Messier C, Paquette A, Saurer M, Scherer-Lorenzen M, Rose L, Schuldt B (2022) Mutually inclusive mechanisms of drought-induced tree mortality. Glob Change Biol 28:3365–3378. https://doi.org/10.1111/gcb.16146

Halofsky JE, Peterson DL, Harvey BJ (2020) Changing wildfire, changing forests: the effects of climate change on fire regimes and vegetation in the Pacific Northwest, USA. Fire Ecol 16:4. https://doi.org/10.1186/s42408-019-0062-8

Hamberg L, Velmala SM, Sievänen R, Kalliokoski T, Pennanen T (2018) Early root growth and architecture of fast- and slow-growing Norway spruce (*Picea abies*) families differ—potential for functional adaptation. Tree Physiol 38:853–864. https://doi.org/10.1093/treephys/tpx159
Hamilton GJ, Christie JM (1971) Forest Management Tables (metric). Forestry Commission, London
Hammond WM, Williams AP, Abatzoglou JT, Adams HD, Klein T, López R, Sáenz-Romero C, Hartmann H, Breshears DD, Allen CD (2022) Global field observations of tree die-off reveal hotter-drought fingerprint for Earth's forests. Nat Commun 13:1761. https://doi.org/10.1038/s41467-022-29289-2
Hartmann H, Moura CF, Anderegg WRL, Ruehr NK, Salmon Y, Allen CD, Arndt SK, Breshears DD, Davi H, Galbraith D, Ruthrof KX, Wunder J, Adams HD, Bloemen J, Cailleret M, Cobb R, Gessler A, Grams TEE, Jansen S, Kautz M, Lloret F, O'Brien M (2018) Research frontiers for improving our understanding of drought-induced tree and forest mortality. New Phytol 218:15–28. https://doi.org/10.1111/nph.15048
Hurvich CM, Tsai CL (1995) Model selection for extended quasi-likelihood models in small samples. Biometrics. 51:1077–1084
Kaczmarek DJ, Coyle DR, Coleman MD (2013) Survival and growth of a range of *Populus* clones in central South Carolina USA through age ten: do early assessments reflect longer-term survival and growth trends? Biomass Bioenergy 49:260–272. https://doi.org/10.1016/j.biombioe.2012.12.005
Klein T (2014) The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. Funct Ecol 28:1313–1320. https://doi.org/10.1111/1365-2435.12289
Klein T (2019) A nation-wide analysis of tree mortality under climate change: Forest loss and its causes in Israel 1948–2017. For Ecol Manag 432: 840-849. https://doi.org/10.1016/j.foreco.2018.10.020
Klesse S, Von Arx G, Gossner MM, Hug C, Rigling A, Queloz V (2021) Amplifying feedback loop between growth and wood anatomical characteristics of *Fraxinus excelsior* explains size-related susceptibility to ash dieback. Tree Physiol 41:683–696. https://doi.org/10.1093/treephys/tpaa091
Kosmidis I (2022) Mean and median bias reduction: a concise review and application to adjacent-categories logit models. arXiv :2112.02621 https://doi.org/10.48550/arXiv.2112.02621
Leo M, Oberhuber W, Schuster R, Grams TEE, Matyssek R, Wieser G (2014) Evaluating the effect of plant water availability on inner alpine coniferous trees based on sap flow measurements. Eur J for Res 133:691–698. https://doi.org/10.1007/s10342-013-0697-y
Lévesque M, Saurer M, Siegwolf R, Eilmann B, Brang P, Bugmann H, Rigling A (2013) Drought response of five conifer species under contrasting water availability suggests high vulnerability of Norway spruce and European larch. Glob Change Biol 19. https://doi.org/10.1111/gcb.12268
Li X, Xi B, Wu X, Choat B, Feng J, Jiang M, Tissue D (2022) Unlocking drought-induced tree mortality: physiological mechanisms to modeling. Front Plant Sci 13. https://doi.org/10.3389/fpls.2022.835921
Lloret F, Jaime LA, Margalef-Marrase J, Pérez-Navarro MA, Batllori E (2022) Short-term forest resilience after drought-induced die-off in Southwestern European forests. Sci Total Environ 806:150940. https://doi.org/10.1016/j.scitotenv.2021.150940
Marchal A, Muñoz F, Millier F, Sánchez L, Pâques LE (2017) Hybrid larch heterosis: for which traits and under which genetic control? Tree Genet Genomes 13:92. https://doi.org/10.1007/s11295-017-1177-1
Marchal A, Schlichting CD, Gobin R, Balandier P, Millier F, Muñoz F, Pâques LE, Sánchez L (2019) Deciphering Hybrid Larch Reaction Norms Using Random Regression. G3 Genes Genomes Genetics, 9:21–32. https://doi.org/10.1534/g3.118.200697
Masson G (2005) Autoécologie des essences forestières. Lavoisier, Paris
Matusick G, Ruthrof KX, Kala J, Brouwers NC, Breshears DD, Hardy GESJ (2018) Chronic historical drought legacy exacerbates tree mortality and crown dieback during acute heatwave-compounded drought. Environ Res Lett 13:095002. https://doi.org/10.1088/1748-9326/aad8cb
McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–739. https://doi.org/10.1111/j.1469-8137.2008.02436.x
McLaughlin SB, Wullschleger SD, Nosal M (2003) Diurnal and seasonal changes in stem increment and water use by yellow poplar trees in response to environmental stress. Tree Physiol 23:1125–1136. https://doi.org/10.1093/treephys/23.16.1125
Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit J, Barbaroux C, Le Thiec D, Bréchet C, Brignolas F (2006) Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* × *Populus nigra*. New Phytol 169:765–777. https://doi.org/10.1111/j.1469-8137.2005.01630.x
Mueller RC, Scudder CM, Porter ME, Talbot Trotter Iii R, Gehring CA, Whitham TG (2005) Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. J Ecol 93:1085–1093. https://doi.org/10.1111/j.1365-2745.2005.01042.x
Oberhuber W, Gruber A, Kofler W, Swidrak I (2014) Radial stem growth in response to microclimate and soil moisture in a drought-prone mixed coniferous forest at an inner Alpine site. Eur J for Res 133:467–479. https://doi.org/10.1007/s10342-013-0777-z
Oberhuber W, Kofler W, Schuster R, Wieser G (2015) Environmental effects on stem water deficit in co-occurring conifers exposed to soil dryness. Int J Biometeorol 59:417–426. https://doi.org/10.1007/s00484-014-0853-1
Orwig DA, Abrams MD (1997) Variation in radial growth responses to drought among species, site, and canopy strata. Trees 11:474. https://doi.org/10.1007/s004680050110
Pâques LE (2013) Forest tree breeding in Europe: current state-of-the-art and perspectives. Springer, Dordrecht
Pâques LE (2025a) Mortality dataset Larch sub-sample diallel 18x18 INRAE Orléans, Recherche Data Gouv, *version provisoire*, https://doi.org/10.57745/RRUYLW
Pâques LE (2025b) Rootstock effects on growth, reproduction and wood properties in hybrid larch (*Larix x eurolepis* Henry) and in some other *Larix* sp grafting associations. Ann For Sci 82:9. https://doi.org/10.1186/s13595-025-01281-y
Pâques LE, Lejeune V, Veisse D (2022) Do biomass partitioning and growth efficiency contribute to growth heterosis in inter-specific hybrid larch *Larix* x *eurolepis*? Forestry 95:466–476. https://doi.org/10.1093/forestry/cpac004
Pauwels D, Marenne S (1999). Comment estimer la productivité des peuplements de mélèzes en région wallonne? Gembloux, FUSAGx, Unité de Gestion et Economie forestières, note 12
Peng C, Ma Z, Lei X, Zhu Q, Chen H, Wang W, Liu S, Li W, Fang X, Zhou X (2011) A drought-induced pervasive increase in tree mortality across Canada's boreal forests. Nat Clim Change 1:467–471. https://doi.org/10.1038/nclimate1293
Philippe G, Pâques LE (2003) Dossier de demande d'admission d'un matériel de base destiné à la production, par voie générative, de matériels forestiers de reproduction qualifiés : 'FH201-Lavercantière-PF'. Ministère de l'Agriculture, Comité technique permanent de la sélection des plantes cultivées, section Arbres forestiers. Domaine des Barres Nogent sur Vernisson
Reich PB (2014) The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. J Ecol 102:275–301. https://doi.org/10.1111/1365-2745.12211
Rosner S, Světlík J, Andreassen K, Børja I, Dalsgaard L, Evans R, Luss S, Tveito OE, Solberg S (2016) Novel Hydraulic Vulnerability Proxies for a Boreal Conifer Species Reveal That Opportunists May Have Lower Survival Prospects under Extreme Climatic Events. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.00831
Sasani N, Pâques LE, Boulanger G, Singh AP, Gierlinger N, Rosner S, Brendel O (2021) Physiological and anatomical responses to drought stress differ between two larch species and their hybrid. Trees 35:1467–1484. https://doi.org/10.1007/s00468-021-02129-4
Signori-Müller C, Oliveira RS, Valentim Tavares J, Carvalho Diniz F, Gilpin M, de V. Barros F, Marca Zevallos MJ, Salas Yupayccana CA, Nina A, Brum M, Baker TR, Cosio EG, Malhi Y, Monteagudo Mendoza A, Phillips OL, Rowland L, Salinas N, Vasquez R, Mencuccini M, Galbraith D, (2022) Variation of non-structural carbohydrates across the fast–slow continuum in Amazon Forest canopy trees. Funct Ecol 36:341–355. https://doi.org/10.1111/1365-2435.13971
Skalski JR (1996) Regression of abundance estimates from mark recapture surveys against environmental covariates. Can J Fish Aquat Sci 53:196–204. https://doi.org/10.1139/f95-169
Solomon S, Intergovernmental panel on climate change, intergovernmental panel on climate change, (eds) (2007) Climate change 2007: the physical science basis: contribution of working group i to the fourth assessment

report of the intergovernmental panel on climate change. Cambridge University Press, New York
Sturrock RN, Frankel SJ, Brown AV, Hennon PE, Kliejunas JT, Lewis KJ, Worrall JJ, Woods AJ (2011) Climate change and forest diseases. Plant Pathol 60:133–149. https://doi.org/10.1111/j.1365-3059.2010.02406.x
Swidrak I, Schuster R, Oberhuber W (2013) Comparing growth phenology of co-occurring deciduous and evergreen conifers exposed to drought. Flora - Morphol Distrib Funct Ecol Plants 208:609–617. https://doi.org/10.1016/j.flora.2013.09.004
Tao W, He J, Smith NG, Yang H, Liu J, Chen L, Tao J, Luo W (2024) Tree growth rate-mediated trade-off between drought resistance and recovery in the Northern Hemisphere. Proc R Soc B Biol Sci 291:20241427. https://doi.org/10.1098/rspb.2024.1427
Teskey R, Wertin T, Bauweraerts I, Ameye M, Mcguire MA, Steppe K (2015) Responses of tree species to heat waves and extreme heat events. Plant Cell Environ 38:1699–1712. https://doi.org/10.1111/pce.12417
Trugman AT, Anderegg LDL, Anderegg WRL, Das AJ, Stephenson NL (2021) Why is Tree Drought Mortality so Hard to Predict? Trends Ecol Evol 36:520–532. https://doi.org/10.1016/j.tree.2021.02.001
Van De Pol M, Bailey LD, McLean N, Rijsdijk L, Lawson CR, Brouwer L (2016) Identifying the best climatic predictors in ecology and evolution. Methods Ecol Evol 7:1246–1257. https://doi.org/10.1111/2041-210X.12590
Van Mantgem PJ, Stephenson NL, Byrne JC, Daniels LD, Franklin JF, Fulé PZ, Harmon ME, Larson AJ, Smith JM, Taylor AH, Veblen TT (2009) Widespread Increase of Tree Mortality Rates in the Western United States. Science 323:521–524. https://doi.org/10.1126/science.1165000
Vollenweider P, Menard T, Arend M, Kuster TM, Günthardt-Goerg MS (2016) Structural changes associated with drought stress symptoms in foliage of Central European oaks. Trees 30:883–900. https://doi.org/10.1007/s00468-015-1329-6
Wang W, Peng C, Kneeshaw DD, Larocque GR, Luo Z (2012) Drought-induced tree mortality: ecological consequences, causes, and modeling. Environ Rev 20:109–121. https://doi.org/10.1139/a2012-004
Will RE, Wilson SM, Zou CB, Hennessey TC (2013) Increased vapor pressure deficit due to higher temperature leads to greater transpiration and faster mortality during drought for tree seedlings common to the forest–grassland ecotone. New Phytol 200:366–374. https://doi.org/10.1111/nph.12321
Wolf S, Paul-Limoges E (2023) Drought and heat reduce forest carbon uptake. Nat Commun 14:6217. https://doi.org/10.1038/s41467-023-41854-x
Zeileis A, Hothorn T (2002) Diagnostic checking in regression relationships. R News 2(3):7–10 (https://CRAN.R-project.org/doc/Rnews/)
Zhu Y, Queenborough SA, Condit R, Hubbell SP, Ma KP, Comita LS (2018) Density-dependent survival varies with species life-history strategy in a tropical forest. Ecol Lett 21:506–515. https://doi.org/10.1111/ele.12915

Publisher's Note

Correction note

-Table 2: Since the bold formatting indicating significance in Table 2 is not visible in the paper, the variables that **did not** show a significant correlation are listed here and the rest remain significant. Correlations of Rain with: WM24, WM60, TDR24, TDR60, T_avg, R_hum, G_rad, T_max, VPD, and REW.

- The published paper retains the term: *soil water potential*, whereas in the thesis the more precise term *soil matric potential* is used throughout.

3.6. Insights from Chapter I

This chapter addressed the dynamics of drought-induced mortality in three larch taxa—European larch (EL), Japanese larch (JL), and their hybrid (HL)—across a decade-long period under controlled variation in soil water availability. Mortality was selected as the starting point of this thesis because it represents the most severe and irreversible outcome of environmental stress, and thus provides a clear lens through which to assess vulnerability and adaptation. Understanding *which taxa and under what conditions* mortality occurs is essential to defining limits, setting the foundation for further inquiry into performance under stress, and the traits that underlie survival.

By integrating classical logistic models with time-sensitive sliding window analyses and multivariate trait-based frameworks, we disentangled the factors of soil water limitation, climatic extremes, and growth performance to tree death. Taxon-specific trends emerged clearly: European larch showed the lowest sensitivity to drought-induced mortality, Japanese larch the highest, while the hybrid held an intermediate position. High early-summer temperatures and vapor pressure deficit (VPD), particularly in conjunction with limited soil moisture, were the strongest environmental predictors of mortality. The occurrence of top dieback in the preceding year to mortality suggests that xylem cavitation or tree water economics might be a proximate cause of death in many trees. Mortality was closely associated with growth at individual and clonal levels, but in different ways. Annual radial growth in the year preceding death was consistently lower in trees that eventually died. Importantly, we showed that clonal vigor was, whatever the taxa, positively correlated with survival.

The chapter highlights the necessity of trait-based approaches grounded in long-term field data to evaluate adaptive capacity under climate stress. However, mortality is only one endpoint in a broader continuum of drought responses. In subsequent chapters, we shift the focus from mortality *per se* to pre-mortality indicators, including growth, growth plasticity, resilience components (resistance, recovery, and resilience), and physiological responses such as carbon isotope discrimination (Chapter III). These traits, evaluated over time and across irrigation regimes, provide deeper insights into how hybrid larch and its parental species cope with chronic and acute water limitations.

However, this broad spectrum of traits requires rigorous measurements and labor-intensive work. A larger array of traits is needed to effectively answer this breakdown of mortality and growth components. At this stage, high-throughput phenotyping methodologies come into play. In particular, Near-Infrared Spectroscopy (NIRS), coupled with predictive calibration models, offers a powerful tool for rapid, non-destructive estimation of complex traits related to physiology, stress response, and wood chemistry. This represents the next logical step in advancing our approach.

Ultimately, the thesis's main aim is that this progression - from mortality as an outcome to growth and physiological traits as predictors – could support a more integrated breakdown of drought response and adaptation.

4. Chapter II: Prediction Models for *Larix* species via NIRS Spectroscopy as a High-Throughput Phenotyping Approach

4.1. Conceptualization

Building on the findings from Chapter I, where mortality was identified as a critical trait influenced by environmental factors, we recognized the need to understand better the underlying mechanism that leads to tree mortality and reduction of growth under drought conditions. While mortality emerges as a final outcome of stress that explains the tree's ability to withstand stress or succumb to such conditions, we would still need to break down the processes leading to or not to it. To bridge this, we selected traits based on roles of water hydraulics, photosynthetic capacity, and structural characteristics. Among soil fertility and climatic factors affecting the growth and survival of larches, water limitation (soil and air) appears as most critical. Indeed, the sensitivity to water availability often justifies excluding larch plantations from certain zones or restricting them to only the most tolerant species. As a result, priority was given to two key traits: water use efficiency (WUE) and vulnerability to cavitation.

Water use efficiency, often estimated through carbon isotope discrimination ($\Delta^{13}C$), reflects the balance between carbon gain and water loss and thus indicates a genotype's ability to maintain growth under limited water availability. Vulnerability to cavitation (P_{50}) represents the sensitivity of xylem conduits to embolism formation under water stress. Together, these traits provide critical insight into a tree's capacity to cope with water and water stress while maintaining physiological function.

A second set of traits directly related to growth potential is linked to photosynthetic capacity. As previously shown by Matyssek and Schulze (1988), maximum CO_2 assimilation (Amax, non limiting climatic factors, ambient CO_2 concentration) did not vary significantly among larch taxa. However, the major difference in photosynthetic output was associated with the amount of foliage biomass (Matyssek and Schulze 1987), highlighting the role of crown structure and resource allocation. To capture this variation, we included specific leaf area (SLA)-the ratio of needle area to dry weight—which influences light capture and growth rate. We also included foliar nitrogen content (N%), as following Pérez-Harguindeguy et al. (2016), N concentration tends to be closely correlated with mass-based maximum photosynthetic rate.

Finally, foliar carbon (C) content and leaf dry matter content (LDMC), and lignin concentration and H/G ratio in woodwere considered to provide insights into structural investment. Lignin concentration and phenols were also included in the study of defense under stress. These chemical and structural traits are associated with defense and resource conservation.

However, direct measurements of such traits across a large experiment (with more than 1000 trees) are time-consuming, labor-intensive, and expensive, and in some cases, the sampling can be invasive. What remained clear, however, was that an extensive array of measurements is required to answer these questions regarding the mechanisms behind the differential responses of taxa and genotypes over the years across the two different watering regimes.

This prompted the use of a high-throughput phenotyping methodology. Near-infrared spectroscopy (NIRS) has already proven powerful in predicting chemical and physical properties in wood (Nantongo et al. 2021).

In this chapter, we focused on the development and calibration of predictive models for the traits mentioned above, which, besides lignin, have been little studied through this approach in trees. For some traits, we had the possibility to test for several years samples collections.

If robust, these models, based on sampling a limited number of trees that have been phenotyped for a given trait, these 'reference data' represent the 'training set', would allow the prediction of the phenotypes of individuals from the whole trial (the 'testing set').

4.1.1. Near–infrared Spectra and calibration of models

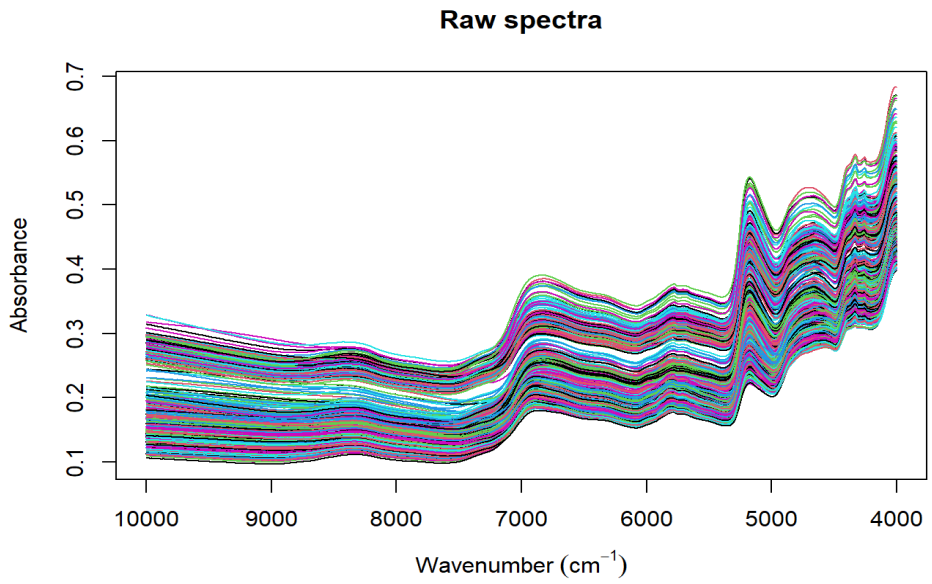

Fig 24: The distribution of raw spectra with overtones and combinations from our spectra acquisition on needles.

Before diving into the calibrations and type of methodology, it is necessary to understand the fundamental concept of light energy. The energy of light is indirectly proportional to wavelength, and the varying energy levels from light can be arranged along the electromagnetic spectrum. The near-infrared region is one of the regions at the center of this electromagnetic spectrum, with three regions together (far-infrared: 10 µm; mid-infrared: 10 to 2.5 µm, and near-infrared: 2,500 to 750 nm). Near infrared (it is used as it has higher energy than other wavelengths) was discovered by Herschel in 1800 while working on an experiment that measured heat by filtering out sunlight based on colors, yet NIRS was not worked on for more than 150 years. When light is passed to a sample, fractions are reflected, absorbed, and transmitted; we worked with absorption.

Data analysis and chemometrics start with a first step, pretreatment or pre-processing of the raw spectra: in this study, we considered eight pretreatments. Pre-treatments help reduce noise and increase the signal from chemical information. Standard Normal Variate (SNV) treats each

spectrum individually by centering and scaling it. In contrast, Multiplicative Scatter Correction (MSC) involves averaging the spectra first, then regressing each spectrum against the average to correct for scatter effects. The choice of spectral pre-treatment is mainly empirical and often based on trial and error, depending on which method yields the most informative results for a given trait. Our study tested different pre-treatments for each trait to determine which approach provided the most accurate and meaningful predictions.

Outliers were then identified and removed based on Principal Component Analysis (PCA) score plots, where samples falling far from the main cluster in the principal component space were considered abnormal. This step was performed prior to model validation to prevent distortion of the calibration process and ensure more robust and reliable model performance (**Fig. 25).**

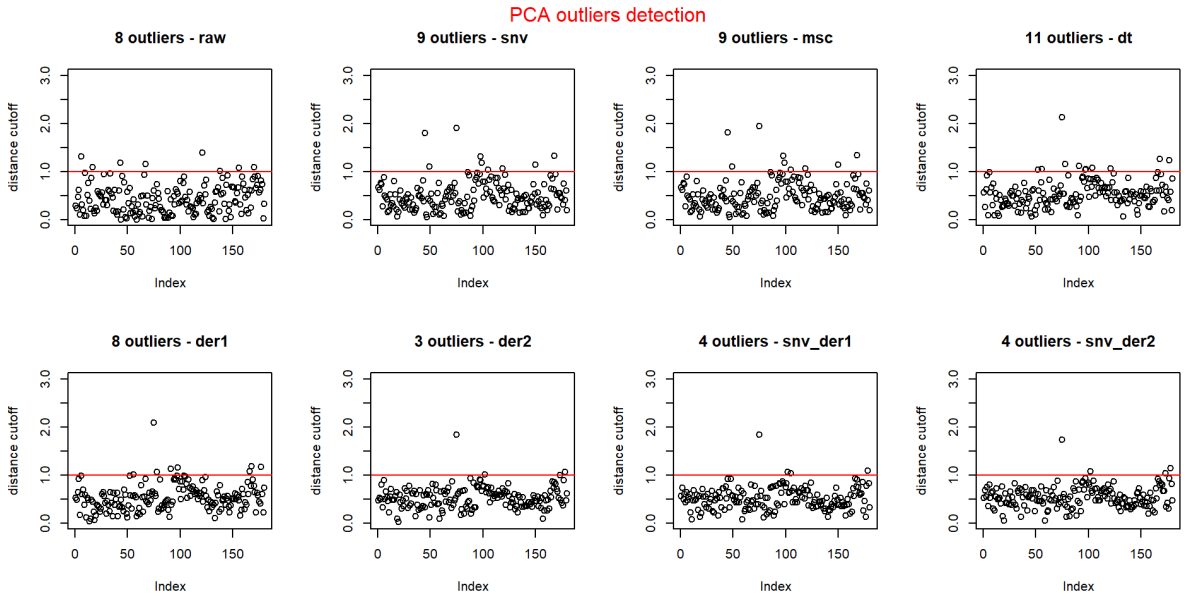

Fig 25: PCA-based outlier detection across different spectral pretreatments. The plots show the Mahalanobis distance of samples from the PCA center for each pretreatment method. Samples above the red threshold line are considered outliers. The number of detected outliers varies by method: raw (8), SNV (9), MSC (9), detrend (11), first derivative (8), second derivative (3), SNV + first derivative (4), and SNV + second derivative (4).

For calibration methods, the best known are Multiple Linear Regression (MLR), Principal Component regression (PCR), and Partial Least Squares (PLS). We chose PLS for our analysis as it is more supervised and considers wavelength multicollinearity, which is mostly the case. Validation of the calibration should ideally be done with a distributed sample that was not previously used. The basic idea of this method is to leave out one sample (in full cross-validation) or a group of samples (in k-fold cross-validation), and build a model using the rest of the data. We employed 5-fold cross-validation. In 5-fold cross-validation, the data is divided into five parts. The model is trained on four parts and tested on one part that was left out. This is done 5 times so that each part is used once as the test set. this whole process is repeated 100 times with different random data splits. So, every sample in the dataset gets tested many times, but never on the same data it was trained on.

4.1.2. Pre-work on traits from the training sample (reference phenotypes)

Analysis of variance was conducted on reference data to test for significant differences among taxa and watering treatments. (**Figs 26, 27,28 and 29**). The following ANOVA analyses were conducted separately for the two watering treatment sites (IRR and NIRR), with *taxa* as a factor, before proceeding to their predictive models.

Distinct differences were observed for these traits. For P_{50}, which is a proxy for vulnerability to drought-induced embolism, HL was significantly different than JL in being less vulnerable, and EL was the least vulnerable to cavitation (**Fig. 26**). Our results align with findings from Delzon et al. (2010), who reported significant interspecific variability in P_{50} among conifers, including *Larix decidua*, which exhibited relatively less vulnerability to cavitation than other species.

Lignin production in wood was higher in the non-irrigated plot compared to the irrigated one. HL had significantly more lignin than EL, but not comparable to JL, in the irrigated site of the experiment. However, in the non-irrigated part, there was no significant difference between the three taxa. H/G had no significant difference in either the irrigated or the non-irrigated site (**Fig. 27**). For phenolics as for lignin, content increased when water was limiting (NIRR plot); there was no significant difference between the three taxa in the irrigated part, but in the non-irrigated part, JL had a significantly higher content than HL. In an experiment of 9 years on *Quercus sp*, drought-treated plants show a substantial increase in phenolics compared to controls, estimated at around 40–50% higher (Nogués et al. 2014).

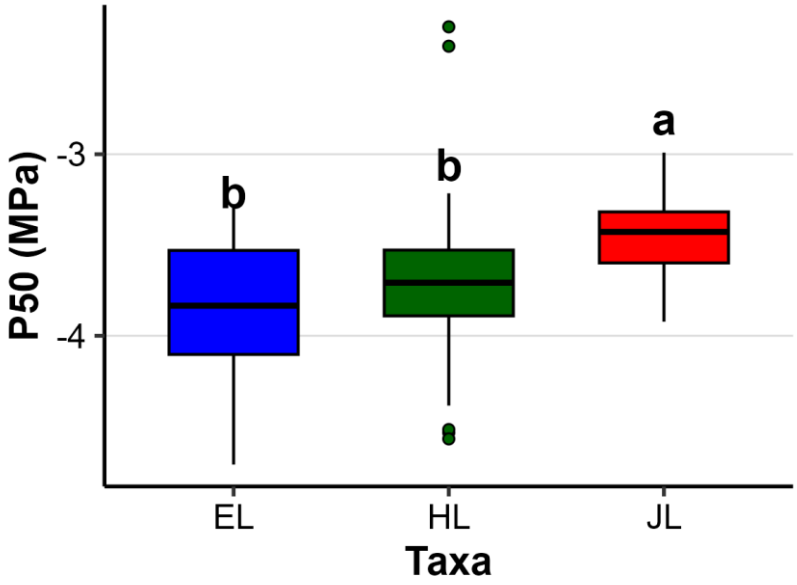

Fig 26: Boxplot of P_{50} (MPa) measured in 2024 (trees collected in the irrigated plot) across the three taxa (EL = European larch, HL = hybrid larch, JL= Japanese larch). The letters represent significant differences ($p<0.05$) from the ANOVA test and Tukey post hoc test.

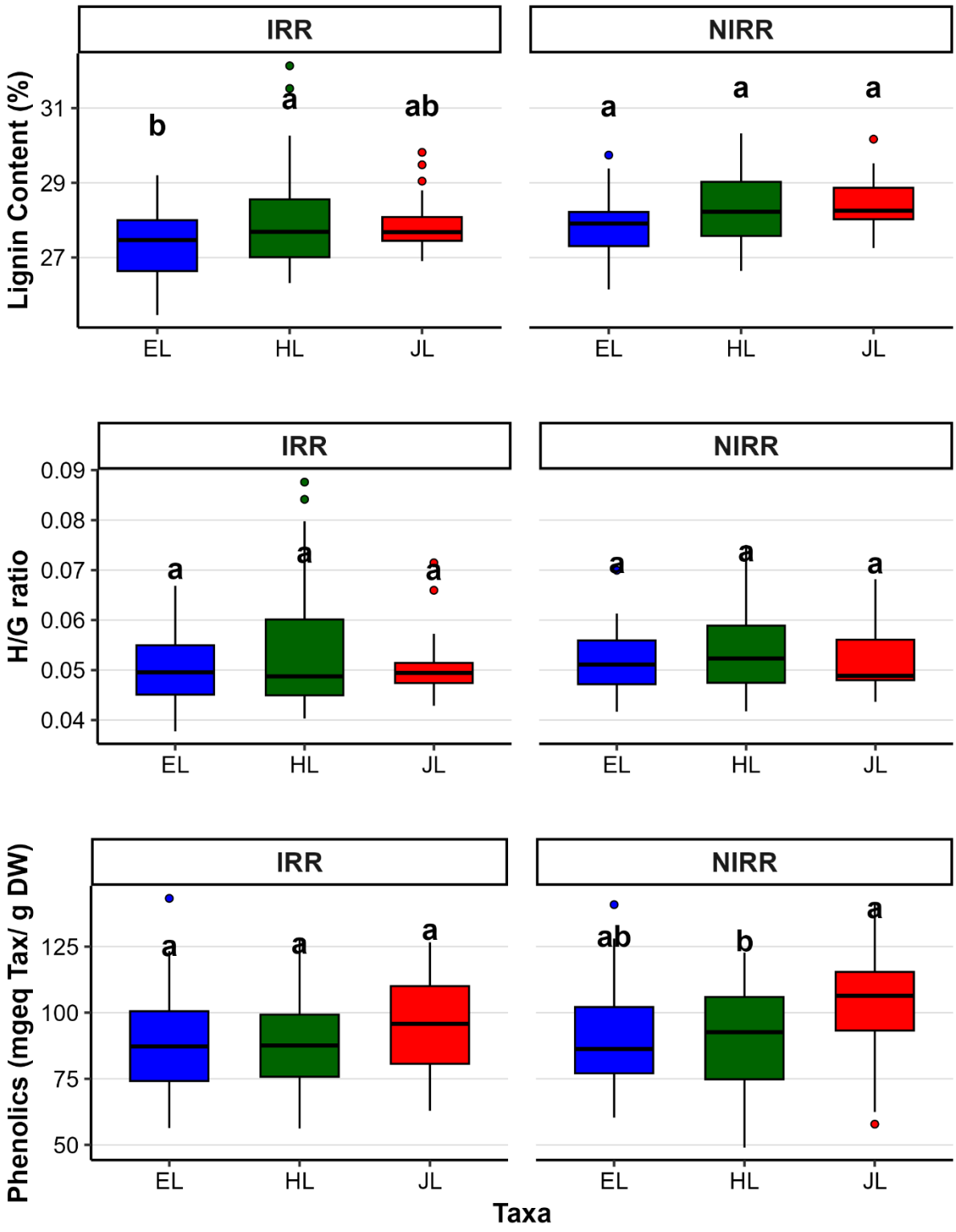

Fig 27: Boxplots of Lignin content (%) -2022, H/G ratio -2022, and Phenolics -2023 across three species (EL, HL, JL) under irrigated (IRR) and non-irrigated (NIRR) treatments. The letters represent significant differences ($p<0.05$).

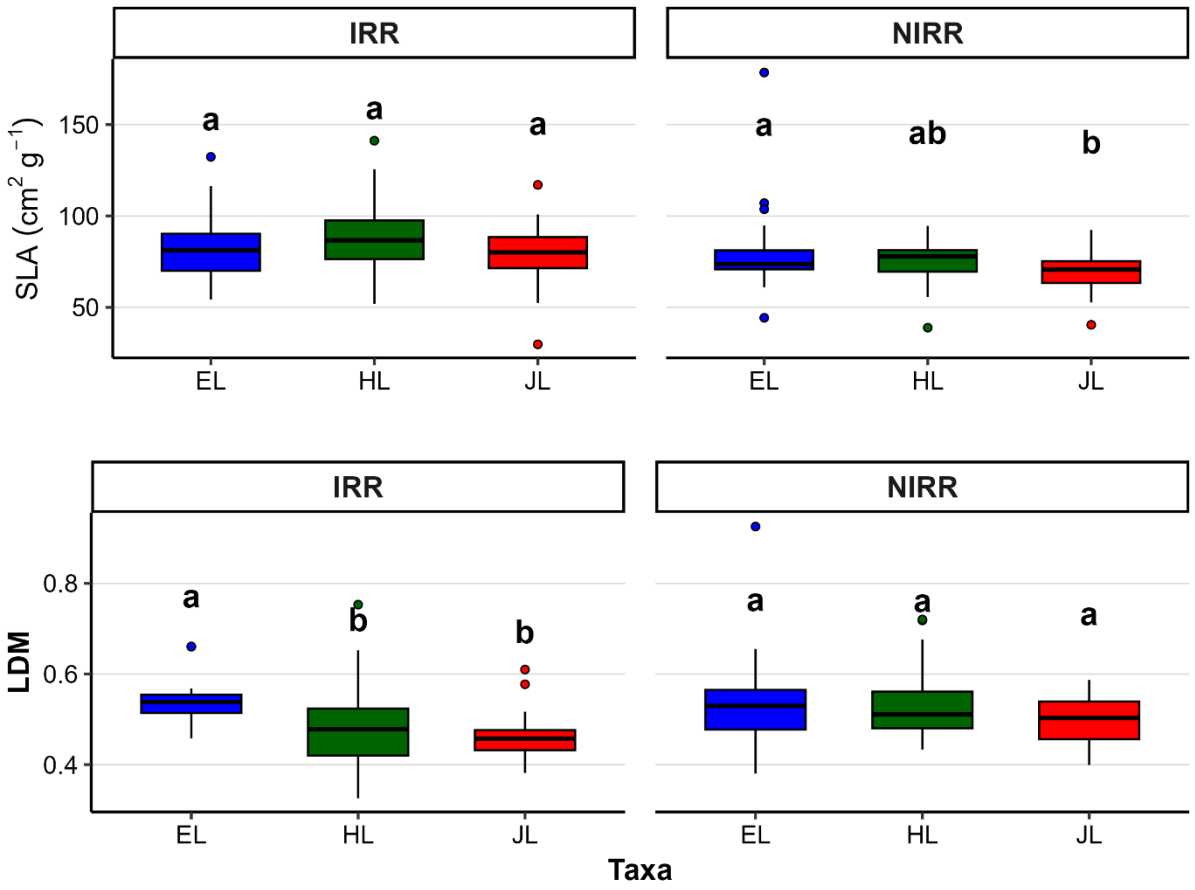

Fig 28: Boxplots of Specific Leaf Area (SLA, cm^2 g^{-1}) and Leaf Dry Matter content (LDM) in 2023 across three species (EL, HL, JL) under irrigated (IRR) and non-irrigated (NIRR) treatments. The letters represent significant differences ($p<0.05$).

Under irrigation (IRR), HL displayed high specific leaf area (SLA) and low leaf dry matter content (LDM), along with elevated $\Delta^{13}C$ values and intermediate nitrogen (N) concentrations. Under drought (NIRR) (**Figs 28 and 29**), HL showed a moderate reduction in SLA and $\Delta^{13}C$, while maintaining stable LDM, N, and carbon (C) levels. JL exhibited consistently low SLA and LDM across treatments. EL had the highest N content and lowest $\Delta^{13}C$ under IRR, suggesting a lower water-use efficiency; under NIRR, EL experienced declines in SLA and N, and an increase in $\Delta^{13}C$. Sassani et al. (2021) found that Carbon isotope composition ($\delta^{13}C$) values increased in all 2-year-old *Larix* taxa under drought, indicating improved water-use efficiency through reduced stomatal conductance. Both Japanese larch (JL) and the hybrid (HL) had higher $\delta^{13}C$ than European larch (EL).

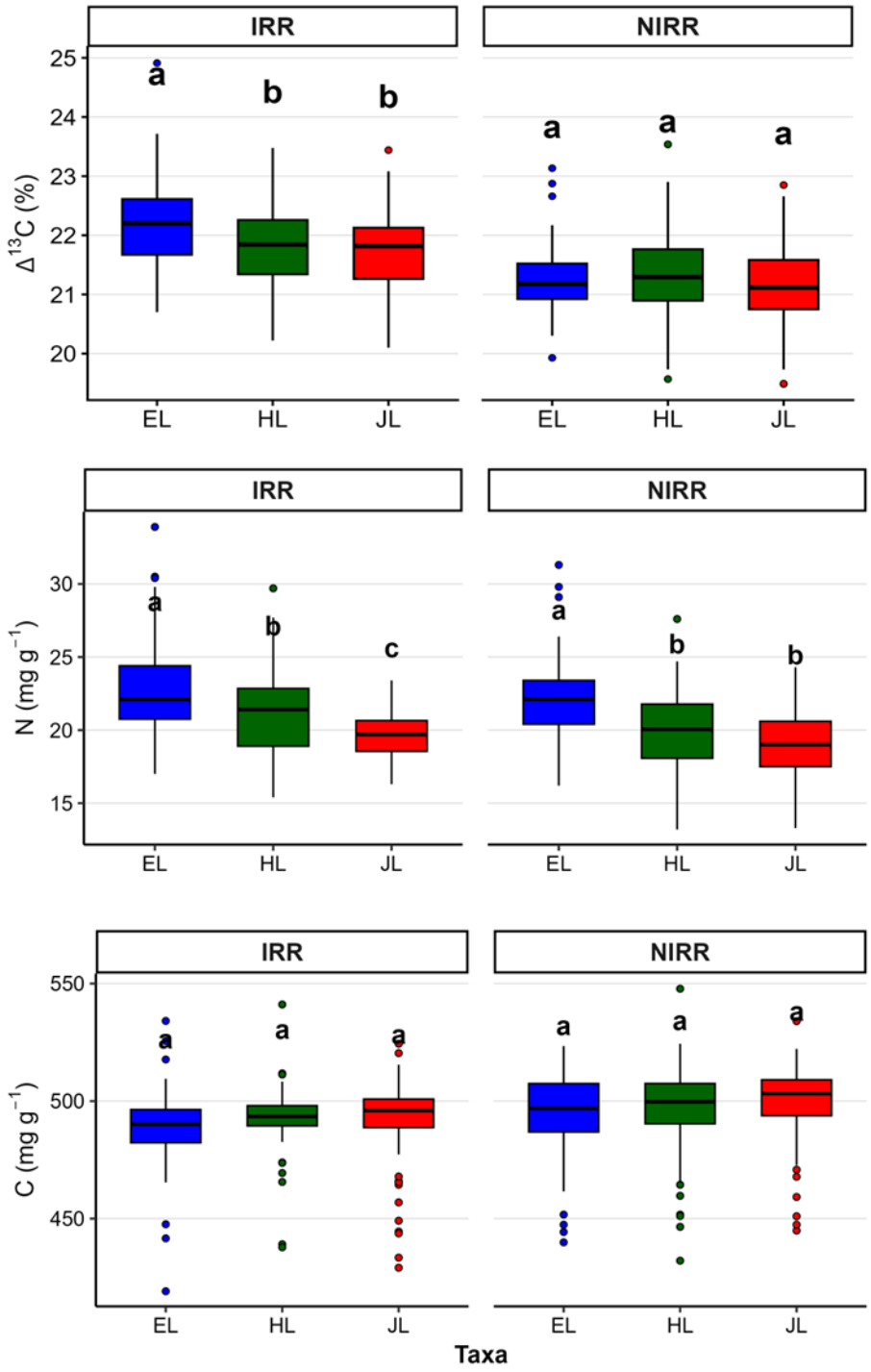

Fig 29: Boxplots of N, C and $\Delta^{13}C$ in 2023 across three species (EL, HL, JL) under irrigated (IRR) and non-irrigated (NIRR) treatments. The letters represent significant differences ($p<0.05$)

Model performance was evaluated using R^2 and RMSEP, indicating reliable predictive capacity across key traits. Year-wise and combined-year models were tested to identify the best-performing models per trait and time point, particularly for carbon (C), nitrogen (N), and carbon isotope discrimination ($\Delta^{13}C$). Successful models were obtained for $\Delta^{13}C$, N, C, lignin, and the H/G lignin ratio, showing moderate to good R^2 values (detailed in Chapter II), but not for SLA and P_{50}. Model validity was further supported by ANOVA tests within treatments, comparing

results from observed (training set) and predicted (testing set) values, which confirmed the robustness of the approach for trait prediction under the two watering treatments.

Abstract in French : Modèles prédictifs par spectrométrie proche-infrarouge (SPIR), une approche de phénotypage à haut-débit pour le *Larix*

Résumé

Le phénotypage de larges populations reste une contrainte majeure dans les programmes de sélection des arbres, en raison notamment du caractère chronophage et coûteux des méthodes conventionnelles. La spectroscopie dans le proche infrarouge (SPIR), en tant que méthode de phénotypage à haut débit, constitue une alternative offrant une approche rapide et économique pour évaluer des caractères physiologiques liés à la croissance sur un grand nombre d'arbres. Parmi celles-ci, nous nous sommes intéressés aux teneurs en $\triangle^{13}C$ (efficience d'utilisation de l'eau), C, N, SLA, LDM et composés phénoliques sur aiguilles ; P_{50} (vulnérabilité à la cavitation) sur rameaux ainsi que lignine et rapport H/G sur carottes de bois. Nous avons cherché à évaluer le potentiel de modèles basés sur la SPIR pour prédire ces caractères chez le mélèze à partir de données issues d'une expérimentation sur différentes espèces de *Larix*, et en utilisant la modélisation multivariée, notamment la régression par moindres carrés partiels (PLS). Des modèles fiables ont été obtenus pour la teneur en azote ($R^2_{training}$= 0.95, $R^2_{testing}$= 0.94), la lignine ($R^2_{training}$= 0.95, $R^2_{testing}$= 0.94), et le rapport H/G ($R^2_{training}$= 0.88, $R^2_{testing}$= 0.89), tandis que des performances prédictives modérées ont été observées pour la teneur en carbone ($R^2_{training}$= 0.79, $R^2_{testing}$= 0.79) et $\triangle$13C ($R^2_{training}$= 0.76, $R^2_{testing}$= 0.69). Cette approche méthodologique et ses résultats encouragent le passage des méthodes de laboratoire traditionnelles vers des techniques d'évaluation des caractères plus efficaces et à grande échelle en foresterie.

Mots-clés : Individus atypiques en ACP, Mélèze, Lignine, Phénols totaux, Efficience de l'utilisation de l'eau, $\triangle^{13}$CD13C, Carbone, Azote, Amélioration génétique, Spectroscopie proche infrarouge

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4.2. **Prediction Models for *Larix* species via NIRS Spectroscopy as a High-Throughput Phenotyping Approach**

Abstract:

Phenotyping extensive populations remains a major constraint in tree breeding programmes, particularly due to the time-consuming and labor-intensive nature of conventional methods. Near-infrared Spectroscopy (NIRS), which is a high-throughput phenotyping method, offers an alternative solution, providing a rapid and cost-effective approach for assessing growth- and function-based traits on large numbers of trees. We aimed to evaluate the potential of NIRS-based models for predicting such traits in Larch. In this study, we attempted to predict $\Delta^{13}C$, C, N, SLA, LDM, and phenolics on needles; P_{50} on branches, and Lignin and H/G ratio on wood cores from an experimental study on *Larix* species using multivariate modelling, i.e., through partial least squares. Reliable models were obtained for N content ($R^2_{training}$= 0.95, $R^2_{testing}$= 0.94), lignin ($R^2_{training}$= 0.95, $R^2_{testing}$= 0.94), and H/G ratio ($R^2_{training}$= 0.88, $R^2_{testing}$= 0.89), while moderate predictive performance was observed for C content ($R^2_{training}$= 0.79, $R^2_{testing}$= 0.79) and $\Delta^{13}C$ ($R^2_{training}$= 0.76, $R^2_{testing}$= 0.69). This methodological approach and its results encourage the transition from traditional laboratory methods to efficient, large-scale-based trait evaluation techniques in forestry.

Keywords: PCA outliers, Larch, Lignin, Total phenolics WUE, D13C Carbon, Nitrogen, Tree breeding, Near-infrared spectroscopy

1. Introduction

In the pursuit of global climate stability, forest tree breeding has traditionally emphasized improving growth characteristics, together with some external traits (e.g., stem straightness)[1], and internal wood properties (e.g., fiber length and stiffness)[2] while also aiming to enhance species-specific resistance to various biotic and abiotic stresses. Historically, the primary objective was to select and deploy tree materials well-adapted to specific sites, with traditional approaches largely relying on analysing site effects - encompassing[3] soil, climate[4], and topography[5] - on performance. However, the advent of climate change has reshaped the forest management and tree breeding perspectives with increasing uncertainties due to drought and heat waves[6,7], new pests and diseases[8], and fire hazards[9]. Tree breeding programmes now require more flexibility and reactivity to face these new challenges.

Progress in high-throughput phenotyping and genotyping is speeding up genetic evaluation and selection. Modern tree breeding requires addressing more traits supporting yield and adaptation to biotic and abiotic threats. To tackle these, complex traits, such as survival or growth, can be dissected into more functional ones, analysed for their interactions, and the most critical traits—i.e., those that are both beneficial to the trees and genetically variable, heritable, and not unfavourably correlated with other important traits—should be identified, as these are the ones most responsive to selection.

However, assessing these traits – often already proxies of more complex traits - typically involves labour-intensive, sometimes invasive or destructive sampling methods and costly laboratory analyses, which are incompatible with the evaluation of hundreds to thousands of trees as expected in genetic studies. Measuring chemical wood properties (e.g., total lignin and monomers content) requires wet chemistry approaches like the analytical Klason method[10] (requires larger samples) and py-lignin[11], and physiological traits such as water-use efficiency or vulnerability to drought-induced embolism traditionally involve heavy field sampling, expensive and/or time-consuming measurements in the lab[12,13]. The cost and time needed thereby seriously limit the number of samples and traits that can be evaluated in breeding programmes.

High-throughput phenotyping offers a transformative solution that should enable large-scale, low-cost screening of plants for various traits[14]. Several non-destructive high-throughput methodologies have already been developed to assess physical (e.g., Pilodyn, Resistograph, SilviScan), mechanical (e.g., Rigidimeter, acoustic tools), chemical (e.g., NIR) or structural (e.g., Computed Tomography scanning) properties of wood and needle[15]. These methods have proved to be efficient, cost-effective, and are widely used in tree breeding, forest inventory, and wood product evaluation[16]. Pyrolysis method takes less time than the wet chemical method to extract lignin content from wood but is still demanding when large measurements have to be undertaken[11].

Among these methodologies, NIR spectroscopy has emerged as a cost-effective and efficient technique for rapidly analysing large numbers of (wood and needle) samples[15]. This powerful vibrational technique is based on studying the molecular vibration of functional groups of organic matter in the range of 12,000 and 4000 cm^{-1} or 780 and 2500 nm. NIR spectra contain information related to the chemical composition and molecular structure of the analysed materials[17]. This indirect approach utilizes multivariate statistical analysis to correlate NIR absorbance spectra with reference measurements of target attributes through a representative sample set. NIRS calibration models have shown promising results for estimating various traits in several forest tree species (e.g. *Pinus pinaster*, *Pinus radiata*, *Eucalyptus grandis* × *E. urophylla* hybrids and *Populus nigra (L.)*[18–21].), highlighting its potential as a reliable methodology in forestry[22,23].

Regarding *Larix* species, qualitative and quantitative NIRS models have been reported to efficiently discriminate *Larix* species and predict wood density, natural durability, and some mechanical properties[24–27]. While these efforts have advanced the use of NIRS in wood-related traits, they have primarily focused on biochemical and mechanical traits. In contrast, little is known about the potential of NIRS to predict functional traits related to resource use, such as water-use efficiency, structural allocation, and hydraulic capacity - all of which are critical under increasing climate variability[28].

Our study builds on this foundation by targeting these underrepresented but ecologically and physiologically relevant traits to support breeding and selection of more performant and resilient trees. Because of several favourable properties (fast growth, abundant heartwood, natural durability, and mechanical strength), Larch is receiving growing interest in plantations and is the object of intensive breeding. However, the question about the relative place in planting programmes of the three main larch taxa in Europe (European Larch (EL), Japanese Larch (JL), and their hybrids (HL)) is raised with climatic pending issues, mostly drought and

extreme temperatures. Whereas these species are known to exhibit diverse growth strategies and responses to climate[29–32], it is becoming crucial to dig into functional traits supporting these complex traits and to better understand key traits that differentiate the three species and in particular support the faster growth of hybrids, known as heterosis. Among these, we identified three groups of traits: a first set is related to tree water economy (water-use efficiency ($\Delta^{13}C$) and vulnerability to cavitation (P_{50}))[33,34], which are of interest to understand how plants manage water under stress; a second set linked to photosynthetic capacity (C and N content, LDMC, SLA)[35,36], indicating resource use and growth potential; and a third one related to defense and structural support (lignin, H/G ratio, phenolics)[11,37]. We aimed to develop NIRS predictive models for each of them to assist in selecting genotypes suited to future climate conditions. By applying NIRS to this expanded set of traits, our work bridges the gap between traditional trait measurement methods and cost-effective and less time-consuming models of prediction, marking a novel application of spectroscopic methods in tree breeding.

2. Materials and Methods:

2.1.Study Site and Material

The traits for this study were measured from samples collected in a *Larix* species clonal-progeny trial at the INRAE Orleans nursery (Latitude:47.83°N and Longitude:1.91°E) planted in the winter of 2013. It is part of a diallel mating design combining intra- and inter-specific crossings between 9 European larch (EL, *Larix decidua Mill.*) and 9 Japanese larch (JL, *Larix kaempferi (Lamb.) Car.*) parents[29]. The three taxa: EL, JL, and their hybrid (*Larix* × *eurolepis*) were represented by 12 full-sib families, each represented by 12 individuals (clones); copies of these clones were obtained by grafting. The experimental site was established on a flat area characterized by a sandy-heavy gravel soil with poor water retention (approximately 35 mm) and limited annual rainfall (less than 700 mm). The trial area was divided into two plots: irrigated (IRR) and non-irrigated (NIRR); the irrigated plot has been supplied yearly since 2015 with water (100 to 200 mm - rainfall + water supply) during the growing period, i.e., April to October (drip-irrigated) to maintain suitable conditions for growth of Larch species whereas the non-irrigated plot corresponded to a particularly dry site. This created two contrasted sites with the same genetic material clonally propagated from the three species.

2.2. Sampling and trait measurements

In order to develop NIRS calibration models, trees to be sampled were selected in order to capture most of the genetic diversity existing across taxa and sites: each taxon was represented by individuals from different genotypes (progenies and clones) collected in both irrigated and non-irrigated sites, except for resistance to drought-induced embolism (see below). A full description of the number of samples collected for each trait is given in **Table 1**. The sampling was done on needles, branches, or wood cores in various years according to the trait of interest.

Table 1: Description of the sampling by traits, years, species, and irrigation treatment. EL: European larch, HL: Hybrid larch, JL: Japanese larch, IRR: irrigated, NIRR: not-irrigated

Group	*Trait*	*Year*	*Sampling*	*Species*	*No of trees in IRR*	*No of trees in NIRR*
Water Hydraulics	Resistance to cavitation (P_{50})	2024	Branch	EL	39	x
				HL	77	x
				JL	21	x
Photosynthetic capacity and Water hydraulics	Carbon, Nitrogen and $\Delta^{13}C$	all years	Needle	EL	83	83
				HL	98	98
				JL	79	79
		2021		EL	20	20
				HL	20	20
				JL	20	20
		2022		EL	31	31
				HL	40	40
				JL	29	29
		2023		EL	32	32
				HL	38	38
				JL	30	30
	Leaf dry matter Content (LDM)	2023		EL	30	31
				HL	37	38
				JL	29	29
	Specific Leaf Area (SLA)	2023		EL	30	31
				HL	37	38
				JL	29	29
Mechanical support and development	Lignin and H/G ratio	2022	Wood core	EL	31	28
				HL	38	35
				JL	32	27
	Phenolics	2023		EL	31	31
				HL	39	39
				JL	29	30

2.2.1. Needle C, N and $\triangle^{13}C$

Needle samples were collected over three years to cover different stages of maturation: in September 2021, July 2022, and late August 2023 from 120, 200, and 200 trees, respectively. Over the years, the trees in the samples were of different identities. All collections were taken from the mid-canopy, focusing on fresh, green current-year shoots at the sunny part of the crown with about 15 brachyblasts per tree.

We used two drying methods. In 2021 and 2022, the needles were placed into labelled paper bags and oven-dried (60° C) for at least 24 hours. Then, these needles (about 1 g selected) were taken to be finely milled using a high throughput ball milling facility to achieve a particle size between 500 and 1000 µm. In 2023, the needles after the collection were stored in a freezer (about -80°C) and then lyophilized (Freeze dryer Christ Alpha 1-4) to maintain their chemical integrity before being milled as described above. These dried samples were used for carbon (C), nitrogen (N), and stable carbon isotope discrimination ($\triangle^{13}C$) analyses. Approximately 1 g of powdered needles was stored in hermetically sealed glass vials in an air-conditioned room (20 °C and 45% humidity) awaiting the NIRS measurements (see below).

All measurements were performed within three months of sampling and preparation. C and N contents and δ^{13} analyses were performed from 1 mg subsamples encapsulated in tin capsules and combusted at 1025°C in excess of oxygen by an elemental analyser coupled via a gas box interface to a continuous flow isotope ratio mass spectrometer (EA IsoLink CN IRMS System: Flash IRMS Elemental Analyzer - Conflo IV Universal Interface - Delta V Advantage IRMS, ThermoFischer Sc, Breme, Germany). Analyses were performed at SILVATECH (Silvatech, INRAE, 2018. Structural and functional analysis of tree and wood Facility, doi: 10.15454/1.5572400113627854E12). C and N contents were expressed in % of dry matter while $\delta^{13}C$ was expressed in ‰ relative to the Vienna Pee Dee Belemnite (VPDB) standard[38] .

$$\delta^{13} = \left\{\frac{R_{sample} - R_{standard}}{R_{standard}}\right\} \times 1000$$

where, R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and the standard, respectively.

To estimate carbon isotope discrimination (ΔC^{13}) as a proxy for water-use efficiency, $\delta^{13}C$ values were converted[39]:

$$\Delta^{13}C = \frac{\delta_{air} - \delta_{plant}}{1 + \left(\frac{\delta_{plant}}{1000}\right)}‰$$

where, δ_{air} was assumed to be -8 ‰

Carbon and nitrogen content were converted to mg g^{-1} of dry weight.

2.2.2. Specific leaf area (SLA) and leaf dry matter (LDM) content

Measurements of SLA and LDM content were performed on 194 samples collected in late August 2023 (when needles were fully developed), following the same collection protocol as above. Fresh needles were collected from five brachyblasts per tree and stored into plastic bags. They were then separated from the small twigs in the laboratory and immediately weighed to

determine their fresh mass. They were then carefully placed in glass Petri dishes, with the lids used to flatten them to ensure uniform positioning. Before scanning, the needles were carefully repositioned to avoid overlap. Images of the five sets of needles were obtained using a scanner (EPSON V750 pro), and these images were archived for further analysis. The archived images were later analysed using ImageJ software[40], to determine the number of needles and their individual areas. Following imaging, the needle samples were oven-dried in a kiln at 40°C until their weight stabilized and re-weighed to determine dry mass. SLA was calculated as the ratio of total leaf area to dry mass (cm^2g^{-1}). At the same time, LDM content was derived from the ratio of dry mass to fresh mass, reflecting the average density of needle tissues[41].

2.2.3. Needle Phenolics concentration

The phenolics concentration in the 2023 needle samples was analysed at the INRAE Phenobois platform in Orleans (PHENOBOIS, INRAE, 2018. Platform for Phenotyping Hydraulic and Physicochemical Properties of Wood from Woody Genetic Resources, https://doi.org/10.15454/1.5572410490640864E12) using an adapted Folin-Ciocalteu method[42,43]. Phenols were released from the needle samples using an acetone/water extraction process flowed by sonication to separate the soluble fraction. The extracted solution was distributed in 96-well plates to measure its absorbance at 735 nm.

2.2.4. Vulnerability to drought-induced embolism

Vulnerability to drought-induced embolism sets the functional limit to water transport under water deficit and is typically measured as a proxy for drought tolerance[44]. Measurements were performed on branches from trees sampled only in the irrigated plot (IRR) to reduce the bias associated with possible native embolism. Branch collection was conducted from January to February 2024 from 137 trees. Lateral branches were collected from the upper canopy using pruning shears, with two samples taken from each tree and carefully labelled. The priority was on apical branches, straight, approximately 60 cm long, and about 10 mm thick at the thicker end. All branches were wrapped in tissue soaked with water and placed into plastic bags while transported and later analysed at the respective laboratory.

Samples were analysed between January and February 2024 at the INRAE Phenobois platform in Bordeaux using the Cavitron technique[45]. The Cavitron system employs a rotor that applies centrifugal force to perfused branch segments, simulating increasing levels of water stress. Spinning the segments at progressively higher speeds creates pressure within the xylem, inducing cavitation events. The branch samples were perfused continuously with a solution containing potassium chloride (KCl) and calcium chloride ($CaCl_2$), and the diameter of the branch was measured before every analysis. Hydraulic conductance and the percent loss of hydraulic conductance (PLC) were measured at increasing pressures, with data collected to generate vulnerability curves. The critical pressure at which 50% loss of conductivity (P_{50}) occurred was determined as a key indicator of xylem vulnerability to embolism. The data analysis was conducted using the “fitplc” R package (version 1.2-3). After measurements, the branches were shredded using a RETSCH SM 2000 cutter and milled into powder with ball milling. One gram of powder with a particle size ranging from 500 to 1000 µm was stored in glass vials in an air-conditioned room (20 °C and 45% humidity) until NIRS measurements.

2.2.5. Wood lignin content and composition

Analyses were performed on wood increment cores sampled between January and March 2022 from the main trunk (at 1 m above ground) from 191 trees in total. One core per tree was collected to provide enough material for chemical and spectral analyses. Wood cores were cut into finer pieces with a cutter and grinded by ball milling to get 1 g of powder dedicated to lignin and phenolics analysis and NIRS measurements. Analytical pyrolysis was performed to assess the relative proportions of lignin monomers[11,46]. The lignin analyses were conducted at the Centro de Estudos Florestais, Universidade de Lisboa in Portugal to determine total lignin content and p-hydroxyophenyl/guaiacyl unit (H/G) ratios.

2.2.6. Needle, branch, and wood core sampling for NIRS prediction

In parallel, approximately 1200 samples were collected from nearly all trees in the experiment, using similar methods to ensure consistent quality and synchronized timing for reliable NIRS predictions.

2.3.NIR Spectra acquisition

NIR Spectra were collected on powdered samples using an FT-NIR spectrometer (Frontier Perkin Elmer, Waltham, MA, USA). Measurements were performed on freshly milled samples of needles, wood, and branches after they had been kept for at least 24 hours in an air-conditioned room (20 °C and 45% RH). The samples were scanned between 10,000 and 4000 cm^{-1} in a diffuse reflectance mode using the Perkin Elmer NIRA accessory. Each spectrum consisted of 64 averaged scans with a spectral resolution of 8 cm^{-1}, an interval of 2, and a zero-filling factor of 4[20].

2.4.Statistical analysis

All analyses were performed using R Statistical Software[47].

2.4.1. Variability in data

Descriptive statistics were performed on the traits analysed in the laboratory (called 'reference' traits) to summarize the dataset and provide an overview of the variability across different conditions. In addition, an Analysis of Variance (ANOVA) was conducted to assess the significance of the effects of treatments (irrigated, non-irrigated), taxa (European Larch, Japanese Larch, and their hybrids), and years (2021, 2022, 2023) on the measured traits.

2.4.2. Reference data analysis

For collections made across several years, we used samples and data from all possible combinations of the collection years). To select an accurate reference sample, outliers were removed using boxplots. This method is based on the interquartile range (IQR)[48]:

$$IQR = Q3 - Q1$$

Where Q1 and Q3 are the first and third quartiles, respectively.

The outlier's boundaries are calculated as follows:

Lower limit $Q1 - 1.5 \times IQR$

Upper limit $Q2 + 1.5 \times IQR$

The constant 1.5 is a coefficient specifying how far the outlier should be from the edge of their box.

2.5.Chemometric approach

2.5.1. Spectra pre-treatment

Many undesirable factors can hamper the quality of data obtained from spectral information. To address this, pre-treatment algorithms were used before any further multivariate analysis. The selected pre-treatments included normalization, detrending, and applying first and second derivatives on raw or pre-processed spectra[49]. In all, seven-year combinations (wherever possible) have been applied to the NIRS data in order to identify a more effective one for each trait of interest. Standard Normal Variate (SNV) and polynomial de-trend transformations have been applied to the raw spectra. A smoothing by derivation (1st and 2nd order) was also applied on raw and normalized spectra using the Savitzky-Golay filter implemented in the rchemo package[50]. A multiplicative scatter correction method was also applied to the raw spectra to remove physical light scatter by accounting for additive and multiplicative effects[51].

The spectral data used for the predictions ranged from 7500 to 4500 cm^{-1}, with the exception of lignin, H/G ratio, and P_{50}, which ranged from 8500 to 4500 cm^{-1}.

2.5.2. Unsupervised NIR spectra analysis

A Principal Component Analysis (PCA) was performed on the pre-processed spectral data to reduce dimensionality and explore variance patterns that can negatively impact the training of the models[52]. Then, we removed the outlier spectra despite the application of pre-treatments. This was carried out using the 'PCA-scores' method, which calculates the Mahalanobis score distance (SD) of the projection of an observation on the score plan to the centre of this plan. It relies on the use of a distance cut-off, which is calculated using a moment estimation of the parameters of the chi-squared distribution for SD^2 [53]. For this purpose, we used the *scordis* function scores calculated by the function "*pcasvd"* implemented in the "rchemo" R package[51].

2.5.3. Supervised NIR spectra analysis

First, the data sets were divided into training (75%) and testing (25%) subsets using the Kennard and Stone algorithm to select representative samples for the calibration and testing steps[54]. The development of predictive models was carried out using Projection to Latent Structures (PLS) regression[55]. The models were obtained by carrying out a regression between these latent variables and Y variables[56]

The model tuning and evaluation relied on five-fold repeated cross-validation with 100 repetitions on the training sets, which allowed in the identification of the more effective spectra pre-treatment and the suitable numbers of latent variables (*LVs*) of the PLS, based on the minimal value of Root Mean Square Error (*RSME*) and optimal R squared (R^2). The selected models were then applied to the testing sets for their evaluation.

The computation of this method was performed using the *sampks*, *gridvclc,* and *plskern* function of the rchemo package[50].Finally, the best models were used to predict traits from NIRS spectra collected on the whole experiment.

3. Results and discussion

3.1. Description of the reference samples data

The descriptive statistics of the reference data of the traits selected for the study are shown in **Table 2**. The range of values found in this study aligns with previous findings on several traits in the literature. $\Delta^{13}C$, an indirect proxy for water use efficiency, varied in our study from 19.49 to 23.54 ‰, which is in the range (18.78 to 22.2 ‰) found in the literature for multiple *Larix* species, including *L. decidua, L. kaempferi, L. eurolepis, L. occidentalis, L. lyallii, L. laricina, L. sibirica,* and *L. gmelinii* [32,57,58]. P_{50} values for Larch species in this study reached on average -3.7 MPa (-4.71 to -2.30 MPa), which is consistent with reported values for different larch species (-4.5 to -3.0 MPa)[59–62]. For Nitrogen content, values ranged from 13.2 to 33.9 mg g^{-1} in line with published data for *Larix* (13.5 – 29.7 mgg^{-1}) [57,62,63]. Finally, SLA reached on average 79 mg cm^{-2}, which is in the lower range of values found by several authors for larch (64 – 144 mgg^{-1})[57,62,64–66].

$\Delta^{13}C$ exhibited one of the lowest observed variabilities (CV = 3.54 %) among traits, but with significant treatment and taxa effects ($p<0.001$). Resistance to cavitation (P_{50}) showed a higher variability (CV = 10.19%) but with only a significant taxa effect (<0.001). Traits associated with photosynthetic capacities, such as carbon (CV = 3.31%) and nitrogen (CV = 13.89%), revealed significant treatment and taxon effects, and only for C, year effects ($p<0.001$), indicating genetic variability that can be leveraged in model calibration. Leaf dry matter content and particularly SLA were characterized by the highest variability (CV > 12 %) and were both significantly (or close to being) influenced by taxa ($p<0.01$), but treatments had a significant effect only for SLA. Secondary metabolite traits like total phenol content in needles displayed one of the highest variability levels (CV = 21.3%), with a significant taxon effect but no treatment effect, whereas lignin content showed both a significant taxon and treatment effect. As reflected by mean values and CVs in **Table 2**, the variability across traits underscores their utility in predictive models by capturing species-specific and treatment (watering) differences.

Table 2: Descriptive statistics and the significant effect of Treatment (irrigated and non-irrigated plot), species (European Larch, Japanese Larch, and Hybrid Larch) and years in $\Delta^{13}C$, Resistance to cavitation, Carbon, Nitrogen, Leaf dry matter, Specific leaf area and Leaf dry matter, Lignin, H/G ratio and phenolics.

Trait	Unit	Year of collection	No	Min	Max	Mean	SD	CV (%)	ANOVA		
									Factors	F value	Pr(>F)
$\Delta^{13}C$	‰	2021,2022,2023	520	19.49	23.54	21.59	0.76	3.54	Taxa	3.547	0.0295*
									Treatment	124.6	<2e-16***
									Year	30.36	3.43e-13 ***
Resistance to cavitation(P_{50})	MPa	2024	137	-4.71	-2.30	-3.715	0.38	10.19	Taxa	8.77	0.000263***
Carbon	mg g^{-1}	2021,2022,2023	520	419.1	547.8	494.08	16.37	3.31	Taxa	3.012	0.05008ns
									Treatment	20.005	9.54e-06***
									Year	17.149	9.54e-06***
Nitrogen	mg g^{-1}	2021,2022,2023	520	13.2	33.9	20.76	2.88	13.89	Taxa	63.667	<2e-16***
									Treatment	12.982	0.000345***
									Year	0.016	0.89995ns
Leaf Dry matter		2023	194	0.32	0.92	0.506	0.07	14.68	Taxa	9.338	0.000136***
									Treatment	6.575	0.011122*
Specific leaf Area	cm^2g^{-1}	2023	194	29.78	178.47	78.98	16.84	21.32	Taxa	3.290	0.0394*
									Treatment	16.320	7.79e-05***
Lignin	%	2022	191	25.462	32.13	27.99	1.025	3.66	Taxa	6.912	0.00127**
									Treatment	8.956	0.00314**
H/G ratio		2022	191	0.038	0.088	0.052	0.0085	16.20	Taxa	2.630	0.0748.
									Treatment	0.498	0.4813ns
Phenolics	mg eq. Tax/g DW	2023	198	48.973	143.22	92.66	19.738	21.30	Taxa	4.220	0.0161*
									Treatment	1.969	0.1622ns

3.2.Calibration of NIRS models

To assess the potential of NIR spectroscopy for predicting structural and functional traits in larch needles and wood samples, we compared the R^2 and RMSE obtained from all training and testing procedures (**Table 3**).

3.2.1. $\Delta^{13}C$

The predictive performance for $\Delta^{13}C$ was inconsistent across the years **(Fig. 1a)**. Model 2023 outperformed the others with a training R^2 of 0.76 and a test R^2 of 0.69, accompanied by the lowest RMSE testing of 0.40 for this trait. The model obtained from the years 2021 and 2023 also highlighted close results, particularly during the testing ($R^2_{testing}$ = 0.60 and $RMSE_{testing}$ = 0.45). The model 2022 showed the poorest predictive ability ($R^2_{training}$ = 0.47 and $R^2_{testing}$ = 0.25), possibly due to a lack of variability within this dataset as revealed by the strong and significant positive Pearson correlation between the standard deviation of $\Delta^{13}C$ and NIRS models training (R^2 = 0.83) and testing (R^2 = 0.98) R^2 values **(Fig.2a)**. These findings suggest that a greater variability in the data may provide spectral signal for the prediction. Such variability among samples could be explained by needles physiology affected by environmental changes across the years. The year 2022 was characterized by one of the harshest climatic years (maximum temperature and drought) during the experimentation, compared to 2021, one of the most favourable ones. A similar range of R^2 values to our best models was also found in *Picea rubens* and *Abies balsamea* (R^2 = 0.76)[39].

Table 3: Result of NIRS calibration models and validation statistic for $\Delta^{13}C$, resistance to cavitation for water use efficiency, Carbon, Nitrogen, Leaf dry matter, Specific Leaf area for photosynthetic capacity, Lignin, H/G ratio, and total phenolic content for mechanical support and defense. Reliable models are in bold

Trait	Part	Year of Collection	Total	No of outliers	nobs	PT	LVs	$R^2_{training}$	RMSE training	nobs	$R^2_{testing}$	RMSE testing
$\Delta^{13}C$	Needle	2021,2022,2023	506	22	363	dt	15	0.62	0.43	121	0.48	0.53
		2021,2022	316	23	219	dt	13	0.61	0.37	74	0.40	0.46
		2022,2023	387	12	281	dt	10	0.61	0.44	94	0.57	0.49
		2021,2023	304	11	219	der2	9	0.63	0.46	74	0.60	0.45
		2021	116	7	81	der2	7	0.50	0.41	28	0.50	0.47
		2022	198	13	138	dt	10	0.47	0.33	47	0.25	0.49
		2023	**188**	**3**	**138**	**snv_der2**	**9**	**0.76**	**0.41**	**46**	**0.69**	**0.40**
Cavitation P_{50}	branches	2024	134	29	78	dt	04	0.22	0.27	27	0.01	0.34
Carbon	Needle	2021,2022,2023	469	25	333	snv_der1	9	0.52	0.75	111	0.52	0.75
		2021,2022	294	10	213	der2	3	0.18	1.00	71	0.18	1.20
		2022,2023	361	10	263	dt	9	0.45	0.98	88	0.44	0.87
		2021,2023	**307**	**2**	**228**	**snv_der1**	**12**	**0.79**	**0.44**	**77**	**0.79**	**0.37**
		2021	119	8	83	dt	10	0.76	0.36	28	0.64	0.47
		2022	188	20	126	snv	1	0.01	1.60	42	0.03	1.70
		2023	187	5	136	snv_der1	9	0.86	1.37	46	0.79	0.43
Nitrogen	Needle	2021,2022,2023	497	7	367	der1	13	0.91	0.08	123	0.83	0.09
		2021,2022	299	11	224	snv_der1	12	0.93	0.07	75	0.90	0.07
		2022,2023	384	9	281	snv_der1	12	0.89	0.80	94	0.84	0.09
		2021,2023	299	10	222	snv_der1	10	0.88	0.08	75	0.84	0.08
		2021	**116**	**4**	**84**	**snv_der1**	**8**	**0.95**	**0.06**	**28**	**0.94**	**0.07**
		2022	200	13	140	dt	11	0.91	0.06	47	0.93	0.06
		2023	187	5	136	snv_der1	9	0.84	0.08	46	0.79	0.08
Leaf dry matter content	Needle	2023	184	4	138	snv_der2	7	0.49	0.044	46	0.02	0.068
Specific Leaf Area	Needle	2023	184	10	128	msc	10	0.36	8.9	43	0.01	12.00
Lignin	**Wood core**	**2022**	**189**	**29**	**120**	**raw**	**14**	**0.95**	**0.2**	**40**	**0.94**	**0.26**
H/G	**Wood core**	**2022**	**184**	**28**	**117**	**raw**	**14**	**0.88**	**0.0025**	**39**	**0.89**	**0.0025**
Phenolics	**Needle**	**2023**	**187**	**6**	**135**	**dt**	**11**	**0.75**	**9.7**	**46**	**0.75**	**9.5**

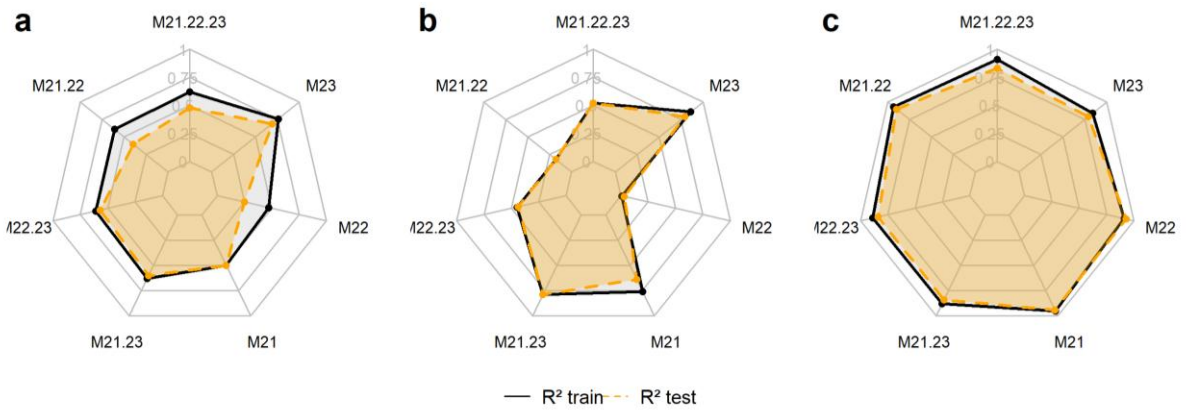


Fig 1: Performance of Near-Infrared Spectroscopy (NIRS) Models over the years and their respective combinations (2021, 2022, and 2023) for predicting: (a) $\Delta^{13}C$, (b) Total Carbon, and (c) Total Nitrogen. The black lines represent the R^2 values for training, while the orange lines indicate the R^2 values for testing.

The selected pre-processing methods highlight variations in spectral noise across the various pre-treatments. The combination of SNV normalization and second derivative preprocessing yields the highest R^2 and lowest RMSE for both training and testing, likely due to its dual effect on noise reduction and signal enhancement[49]. Conversely, the number of selected latent variables, ranging from 7 to 15, appears to correlate with sample size. Regarding spectral outliers detected by the PCA-scores approach, their proportions relative to sample size vary between 2% and 10%. A Pearson correlation analysis between these proportions and the R^2 values of NIRS models in training and testing indicates a significant negative correlation (Supplementary **Table 1**), consistent with findings in the literature[67]. The 2023 model has emerged as the most suitable for application to over one thousand larch needle spectra from the same plot.

3.2.2. Carbon content

The comparison of the models' performances revealed a strong contrast across the years **(Fig. 1b)**. The M2023 and M2021.2023 models exhibited the highest training performances, with R^2 values of 0.86 and 0.79, respectively, while both achieved a test R^2 of 0.79. The 2021 model demonstrated moderate performance (R^2 train = 0.76, R^2test = 0.64), whereas the other models (M2022, M2022.2023, M2021.2022, and M2021.2022.2023) showed weaker metrics, with R^2 test values below 0.52. For Mid-Infrared spectra, R^2 values were 0.62 for poplar and 0.92 for *Picea* and *Abies* [68,69], while NIR showed R^2_{CV} >0.94 but was considered weak [68,69]. In contrast to $\Delta^{13}C$, the carbon content variability across the seven datasets of year combinations appears to be negatively correlated with R^2 values, especially with test-R^2 values **(Fig. 2b).** This trait dispersion within these datasets seems to introduce more noise, negatively impacting NIRS calibration. The combination of SNV and first-derivative pre-treatment appears to be more suitable for total carbon calibration, confirming its effectiveness in correcting the multiplicative noise effect in spectral data. Furthermore, the number of selected latent variables, ranging from 1 to 12, seems to be strongly and positively correlated with the R^2 values of the model's training and testing phases (Supplementary **Table 2**). In contrast, the proportion of PCA-scores outliers

does not correlate significantly with the models' performance. Based on our findings, we have thus selected a model based on 2023 needle spectra for the prediction of this trait in the whole collection of samples.

3.2.3. Nitrogen content

The nitrogen models exhibit consistently high performance across the seven-year combination modalities. The testing R^2 values ranged between 0.79 and 0.94 **(Fig. 1c).** This falls in line with published results on total nitrogen (range, R^2= 0.81 to 0.98)[68]. Total nitrogen dispersion across these datasets was limited, with standard deviations ranging from 0.22 to 0.34. This limited variability explains the lack of correlation between total nitrogen dispersion and model performance **(Fig. 2c).**

Overall, the pre-treatment methods used resulted in similar performance across the nitrogen models. The combination of SNV and first-derivative pre-treatment slightly outperformed the others, confirming its effectiveness. The number of latent variables is strongly and positively correlated with dataset size, indicating the need for additional data decomposition when handling complex and large sample sets (data not shown).

While nitrogen was well predicted by NIRS, the best nitrogen model didn't always yield the best results for $\Delta^{13}C$. Since $\Delta^{13}C$ represents the ratio of carbon gain to water loss, it is influenced not only by photosynthesis (which is linked to nitrogen[70]) but also by other factors. Therefore, variation in $\Delta^{13}C$ model performance across years likely reflects changes in photosynthetic capacity as well as some additional processes affecting carbon–water balance.

3.2.4. Specific leaf area and Leaf dry matter content

The models for specific leaf area (SLA) and leaf dry matter content (LDMC) showed minimal to no predictive power. SLA yielded calibration results in $R^2_{training}$ of 0.29 and $R^2_{testing}$ of 0.01, while LDM content had calibration results of $R^2_{training}$ = 0.38 and $R^2_{testing}$ = 0.02. These poor results could be explained if one considers that NIRS spectra were obtained from wood powder destroying leaf structural traits such as density and thickness on which depends for example SLA. However, these results are not in agreement with recently published work on the prediction of these traits where high R^2 and low RMSE could be found[71,72], suggesting that our experimental approach may be inadequate for this purpose.

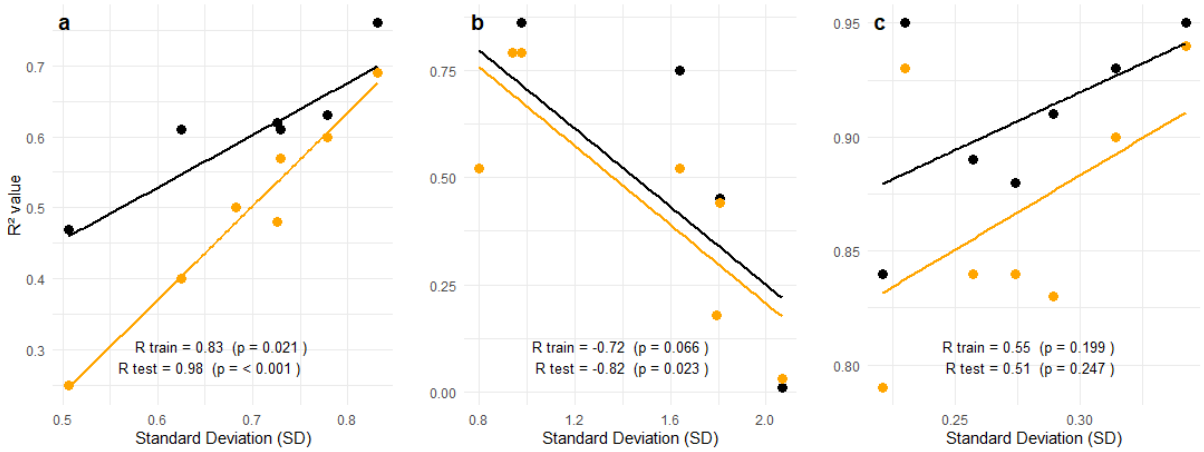


Fig 2: The correlation between standard deviation (SD) and model performance (R^2) for models predicting - (a) $\Delta^{13}C$, (b) Total Carbon, and (c) Total Nitrogen datasets. Black lines and dots represent training models; Orange lines and dots represent testing models.

3.2.5. Lignin and H/G ratio

For lignin content, the dataset consisted of 191 spectra from increment core wood samples, with 29 identified as outliers using PCA-scores, leaving 120 samples for training and 40 for testing. The PLS model, utilizing raw spectra, achieved the highest coefficients of determination, with 0.95 in the training set and 0.94 in the test set. The RMSEs were also low and closely aligned between training (0.2) and testing (0.26), indicating consistent model performance across both datasets.

For the H/G ratio, the total sample size was 184 spectra, with 28 outliers resulting in 117 training samples and 39 test samples. As for lignin, raw spectra were informative enough to achieve performant model metrics ($R^2_{training}$ = 0.88, $R^2_{testing}$ = 0.89). The identical RMSE of 0.0034 in training and testing datasets suggests minimal loss of accuracy when applied on independent data.

We confirm the high potential of near-infrared spectroscopy (NIRS) for accurately predicting lignin content and the H/G ratio, consistent with findings in the literature[16]. A high coefficient of determination (R^2) ranging from 90% to 92% was observed for the H/G ratio in *Pinus pinaster*[24]. Additionally, extractives such as arabinogalactan content, a key extractive in larch, were successfully predicted using Fourier-transform near-infrared spectroscopy (FT-NIR). This was particularly evident in the heartwood of *Larix decidua* and *Larix sibirica*, where predictive power ranged from 0.8 to 0.9 [73].

Our approach suggests that the raw spectral dataset was sufficiently information-rich, and the PLS model effectively compensated for any baseline shifts or scattering without additional manipulation. This could be due to the homogeneity of the ground wood core samples or the robustness of PLS in handling collinear and noisy data by projecting it into a lower-dimensional space.

3.2.6. Needle Phenolics

The predictive model for phenolic content, a secondary metabolite, demonstrated relatively good performance ($R^2_{training}$ = 0.75, $R^2_{testing}$ = 0.75) and reliability. The RMSE values were also relatively low (9.7 for training and 9.5 for testing) compared to the mean phenolic content (92.66). The optimal model was developed using detrended spectra and 11 latent variables. The number of spectral outliers was moderate, suggesting good spectral quality. This model was successfully generalized to a larger dataset.

3.2.7. Cavitation

The models developed for P_{50} exhibited inconsistent performance ($R^2_{training}$ = 0.22, $R^2_{testing}$ = 0.01; **Table 3**), far below the results obtained in *E. globulus* and *E. viminalis* (R^2_cal > 7.50, on branch samples)[28] and Norway spruce sapwood (R^2_cv = 0.65, solid sapwood cubes)[74]. P_{50}, the xylem pressure inducing 50% loss of hydraulic conductivity, is a key trait for assessing drought-induced cavitation in conifers. It is generally measured under controlled, artificial conditions using techniques like the Cavitron, which simulate increasing water tension by centrifugation. Cavitation resistance is known to depend on structural properties such as tracheid wall thickness and pit membrane characteristics[75,76]. In our study, however, NIRS spectra were acquired from finely milled wood powder, which disrupts structural integrity and likely impairs prediction accuracy for such a complex anatomical trait. This highlights a key limitation: while NIRS can effectively predict biochemical traits (like Lignin), its application to hydraulic properties like P_{50} remains challenging due to the importance of structural features destroyed during sample preparation.

3.3.Validation of the methodology

Among the traits investigated, we identified $\Delta^{13}C$, carbon and nitrogen contents, total phenolics, lignin content, and H/G ratio as traits which could be successfully assessed by NIRS-based prediction. To validate the NIRS models, we aimed to confirm that the biological trends observed in measured reference data were preserved in the predictions. This included checking whether the predicted trait values across taxa and treatments followed the same patterns as the measured ones, in both direction and significance. As shown in the boxplots (**Figs. 3 and 4**), we compared ANOVA results and pairwise differences to assess whether the ranking and separation among larch taxa were consistent.

Carbon isotope discrimination ($\Delta^{13}C$) showed significant variation (p = 0.018) among the three larch taxa. Hybrids exhibited the highest median $\Delta^{13}C$ value, followed by Japanese and European larch with lower values (Fig. 3A). The NIRS-predicted $\Delta^{13}C$ displayed a similar pattern, with a comparable p-value (0.016) and significant pairwise differences, mirroring the measured results (**Fig. 3D**). In addition to the similarities between the measured and predicted $\Delta^{13}C$ pattern, there was also significant agreement in statistical significance of the means, indicating robust accuracy of the model. The NIRS model does take out a little more variability (e.g., in JL), and it produced a few outliers (e.g., in HL and JL), suggesting that the model dampened some of the natural variability. This NIRS model confirms the ability of this method to accurately predict $\Delta^{13}C$ on tree needles as published before.

The carbon contents that were measured and predicted were also significantly different (p = 0.00041) among larch taxa, at p=0.00041 and 4.4e-10, respectively **(Fig. 3B, E)**. The lower p-

value on predicted carbon content could be attributed to the larger sample size. Thus, we investigated the effect of sample size on it, and a Welch-anova was calculated and yielded similar results (data not shown). Additionally, we observed a significant difference in predicted data between European larch and hybrid larch, in contrast to the measured data. The NIRS model for carbon is accurate enough to pick up clear differences between the three larch taxa, supporting the reliability of NIRS for carbon prediction[77,78]. Still, it tends to smooth out some of the carbon variability within each species, which hints that it might overlook certain biological nuances. This could be important when we are trying to understand how the trees handle extreme environmental stress.

The nitrogen content in larch needles that was measured highlighted significant differences ($p = 6.2e^{-10}$) among larch taxa, with European larch exhibiting the highest nitrogen content (**Fig. 3C**). The nitrogen content predicted by NIRS was nearly equivalent to the measured nitrogen values, having an even lower p-value ($< 2.2e^{-16}$) for taxa effects and supporting the same trend (**Fig. 3F**). However, the data also showed outliers, particularly for Japanese larch; indicating the potential sensitivity of the model to spectral noise in new samples. These results highlight the accuracy of NIRS for predicting nitrogen, consistent with extensive literature[79,80].

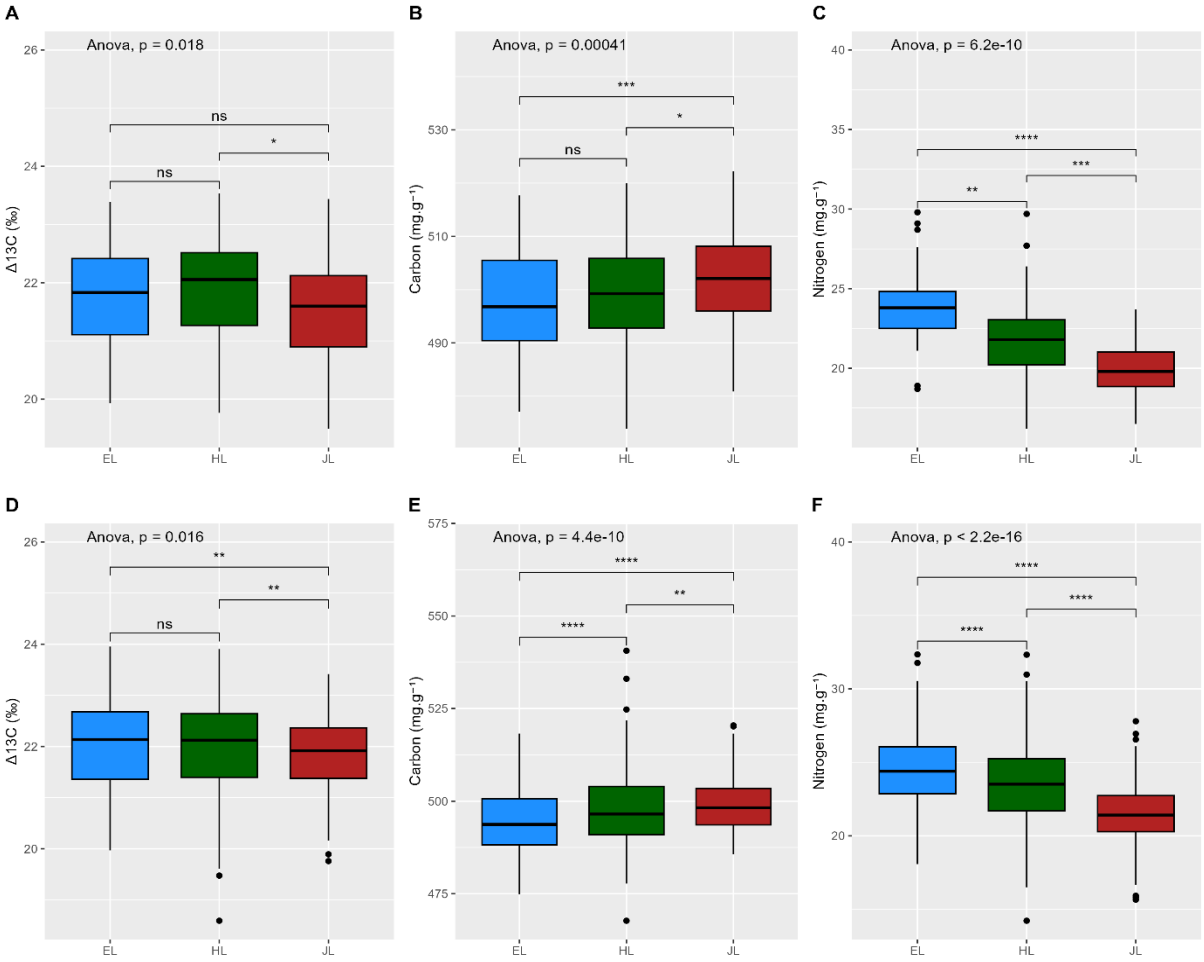


Fig 3: Boxplots showing the measured (reference) and predicted traits. Panels (A–C) represent the measured traits, including $\Delta^{13}C$ (A), carbon content (B), and nitrogen content (C), while panels (D–F) represent the predicted traits for $\Delta^{13}C$ (D), carbon content (E), and nitrogen content (F). The taxa include European Larch (EL), Hybrid Larch (HL), and Japanese Larch (JL). Statistical significance was assessed using ANOVA, with p-values indicated for each trait: ns (not significant), $p < 0.05$, $p < 0.01$, *$p < 0.001$, ****$p < 0.0001$.

Regarding the measured total phenolics in needles, we observed significant ($p = 0.016$) differences between the three taxa, with a higher content for JL (**Fig. 4A**). The predicted results highlighted the same pattern with a stronger significance ($p < 2.2e\text{-}16$) **(Fig. 4D)**. However, the spread of predicted values is slightly increased as well as the number of outliers. This suggests that the model may overestimate the range of variability.

The comparison of measured total lignin content highlighted significant differences between EL and the other taxa, but not between HL and JL. These findings have been confirmed using the predicted values. The reduced variability in predicted values (Fig. 4E) compared to measured ones (Fig. 4B) suggests that the model may miss some biological variability, potentially limiting its ability to detect extreme responses to environmental conditions. The H/G ratio appears to be not impacted by larch taxa as indicated by measured values (**Fig. 4C**) while the predicted ones indicate a significant difference between HL and JL (**Fig. 4F**). For both traits, we observe an increased number of outliers that can be explained by the presence of noisy

spectra within the predicted dataset. As a solution for this issue, we can suggest cleaning the spectra using NIR spectra outliers' removal based on PCA or PLS.

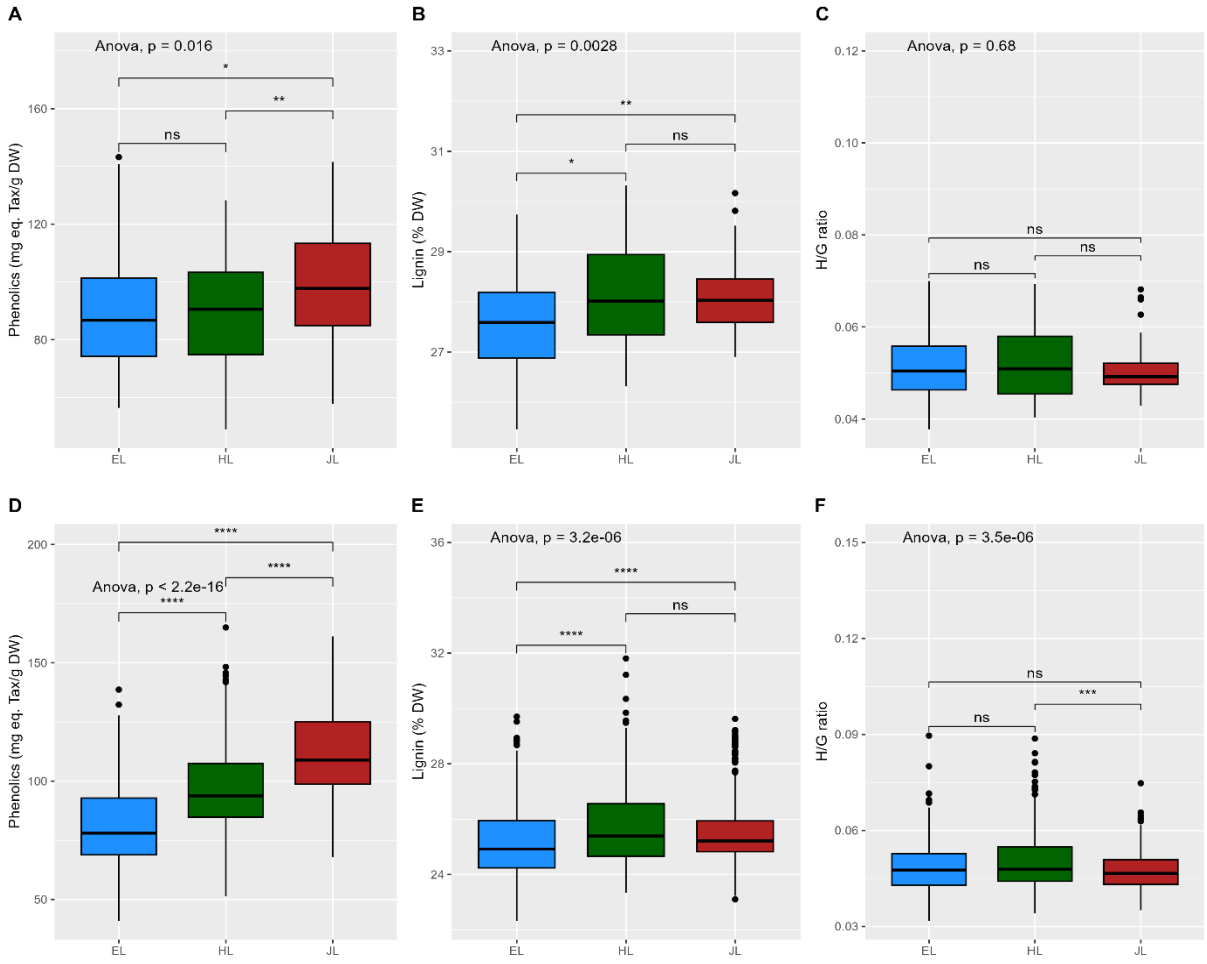


Fig 4: Boxplots showing the measured (reference) and predicted traits. Panels (A–C) represent the measured traits, including Phenolics (A), Lignin (B), and H/G ratio (C), while panels (D–F) represent the predicted traits for Phenolics (D), Lignin (E), and H/G ratio (F). The taxa include European Larch (EL), Hybrid Larch (HL), and Japanese Larch (JL). Statistical significance was assessed using ANOVA, with p-values indicated for each trait: ns (not significant), $p < 0.05$, $p < 0.01$, *$p < 0.001$, ****$p < 0.0001$.

The NIRS models generally perform well, as they replicate the measured patterns and significance for all traits. This supports the use of NIRS as a rapid, non-destructive method for predicting plant traits, as noted in the literature. However, the models show limitations: reduced variability for $\Delta^{13}C$ and Carbon, and increased outliers for Nitrogen. These issues are common in NIRS applications and can be mitigated by improving calibration datasets, using larger sample sizes during the prediction, or applying advanced chemometric techniques. Considering these results, we can conclude that NIRS models will accurately enough predict a range of traits related to water-use efficiency ($\Delta^{13}C$), carbon and nitrogen contents, as well as phenolics in

needles and lignin, and H/G ratios. The possibility to screen these traits through NIRS prediction in hundreds of trees (more than one thousand in this study) is a significant improvement for genotype evaluation in breeding programs. Indeed, genetic gains are heavily dependent on selection intensity, that is, on the number of genotypes tested.

4. Conclusion

NIRS modelling is still a new concept in forestry which is restricted to a few species (particularly few for conifers) and mostly to wood traits. This study is a primary mark to encourage the use of this high-throughput phenotyping method: it could significantly reduce the extensive measurements and time needed to assess complex traits like WUE or wood and needles chemical contents. Breeding needs now to address these traits, directly or indirectly linked to key structural and functional traits. This approach will assist the selection of efficient and more resilient trees, especially for breeding/deployment/afforestation programs.

While NIRS with multivariate analysis provides a solution for reducing the time frame for measurements, the next step would be to assist in further reducing the time-consuming sample collection and preparation, and somehow to reduce the invasiveness of sampling. Collecting samples (e.g., core collection) might indeed damage trees. A better way would be to get portable spectral equipment that measures the sample without damaging the tree.

5. References

1. Byram TD, Myszewski JH, Gwaze DP, et al. Improving wood quality in the western gulf forest tree improvement program: the problem of multiple breeding objectives. *Tree Genet Genomes* 2005; 1: 85–92.

2. Ivković M, Gapare WJ, Abarquez A, et al. Prediction of wood stiffness, strength, and shrinkage in juvenile wood of radiata pine. *Wood Sci Technol* 2009; 43: 237–257.

3. Manzanedo RD, Schanz FR, Fischer M, et al. *Fagus sylvatica* seedlings show provenance differentiation rather than adaptation to soil in a transplant experiment. *BMC Ecol* 2018; 18: 42.

4. Chakraborty D, Jandl R, Kapeller S, et al. Disentangling the role of climate and soil on tree growth and its interaction with seed origin. *Sci Total Environ* 2019; 654: 393–401.

5. Schwartz NB, Feng X, Muscarella R, et al. Topography and traits modulate tree performance and drought response in a tropical forest. *Front For Glob Change* 2020; 3: 596256.

6. Cook BI, Mankin JS, Anchukaitis KJ. Climate Change and Drought: From Past to Future. *Curr Clim Change Rep* 2018; 4: 164–179.

7. AR4 Climate Change 2007: The Physical Science Basis — IPCC, https://www.ipcc.ch/report/ar4/wg1/ (accessed 8 April 2025).

8. Sturrock RN, Frankel SJ, Brown AV, et al. Climate change and forest diseases. *Plant Pathol* 2011; 60: 133–149.

9. Halofsky JE, Peterson DL, Harvey BJ. Changing wildfire, changing forests: the effects of climate change on fire regimes and vegetation in the Pacific Northwest, USA. *Fire Ecol* 2020; 16: 4.

10. Dence CW. The determination of lignin. In: *Methods lignin chem*. Springer, 1992, pp. 33–61.

11. Alves A, Schwanninger M, Pereira H, et al. Analytical pyrolysis as a direct method to determine the lignin content in wood - Part 1: Comparison of pyrolysis lignin with Klason lignin. *J Anal Appl Pyrolosis* 2006; 76: 209–213.

12. Cochard H, Badel E, Herbette S, et al. Methods for measuring plant vulnerability to cavitation: a critical review. *J Exp Bot* 2013; 64: 4779–4791.

13. Seibt U, Rajabi A, Griffiths H, et al. Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia* 2008; 155: 441–454.

14. Mazis A, Choudhury SD, Morgan PB, et al. Application of high-throughput plant phenotyping for assessing biophysical traits and drought response in two oak species under controlled environment. *For Ecol Manag* 2020; 465: 118101.

15. Wang Y, Xiang J, Tang Y, et al. A review of the application of near-infrared spectroscopy (NIRS) in forestry. *Appl Spectrosc Rev* 2022; 57: 300–317.

16. Schimleck L, Dahlen J, Apiolaza LA, et al. Non-Destructive Evaluation Techniques and What They Tell Us about Wood Property Variation. *Forests* 2019; 10: 728.

17. Ozaki Y, Morita S, Morisawa Y. Spectral Analysis in the NIR Spectroscopy. In: Ozaki Y, Huck C, Tsuchikawa S, et al. (eds) *Near-Infrared Spectroscopy: Theory, Spectral Analysis, Instrumentation, and Applications*. Singapore: Springer Singapore,2020; 63–82.

18. da Silva Perez D, Guillemain A, Alazard P, et al. Improvement of *Pinus pinaster* Ait elite trees selection by combining near infrared spectroscopy and genetic tools. 2007; 61: 611–622.

19. Gebreselassie MN, Ader K, Boizot N, et al. Near-infrared spectroscopy enables the genetic analysis of chemical properties in a large set of wood samples from *Populus nigra* (L.) natural populations. *Ind Crops Prod* 2017; 107: 159–171.

20. Hein PRG, Chaix G. NIR Spectral Heritability: A Promising Tool for Wood Breeders? *J Infrared Spectrosc* 2014; 22: 141–147.

21. Nantongo JS, Potts B, Rodemann T, et al. Developing near infrared spectroscopy models for predicting chemistry and responses to stress in *Pinus radiata* (D. Don). *J Infrared Spectrosc* 2021; 29: 245–256.

22. Tsuchikawa S, Kobori H. A review of recent application of near infrared spectroscopy to wood science and technology. *J Wood Sci* 2015; 61: 213–220.

23. Tsuchikawa S, Inagaki T, Ma T. Application of Near-Infrared Spectroscopy to Forest and Wood Products. *Curr For Rep* 2023; 9: 401–412.

24. Alves A, Santos A, Rozenberg P, et al. A common near infrared—based partial least squares regression model for the prediction of wood density of *Pinus pinaster* and *Larix × eurolepis*. *Wood Sci Technol* 2012; 46: 157–175.

25. Fujimoto T, Yamamoto H, Tsuchikawa S. Estimation of Wood Stiffness and Strength Properties of Hybrid Larch by Near-Infrared Spectroscopy. *Appl Spectrosc* 2007; 61: 882–888.

26. Gierlinger N, Schwanninger M, Wimmer R. Characteristics and Classification of Fourier-Transform near Infrared Spectra of the Heartwood of Different Larch Species (*Larix* sp.). *J Infrared Spectrosc* 2004; 12: 113–119.

27. Sykacek E, Gierlinger N, Wimmer R, et al. Prediction of natural durability of commercial available European and Siberian larch by near-infrared spectroscopy. 2006; 60: 643–647.

28. Barotto AJ, Martínez-Meier A, Segura V, et al. Use of near-infrared spectroscopy to estimate physical, anatomical and hydraulic properties of *Eucalyptus* wood. *Tree Physiol* 2022; 43: 501–514.

29. Marchal A, Muñoz F, Millier F, et al. Hybrid larch heterosis: for which traits and under which genetic control? *Tree Genet Genomes* 2017; 13: 92.

30. Greenwood M, Roth B, Maass D, et al. Near rotation-length performance of selected hybrid larch in Central Maine, USA. *Silvae Genet* 2015; 64: 73–80.

31. Paques LE. Growth rhythm parameters as components of hybrid vigour in young seedlings of hybrid larch (*Larix decidua x L. kaempferi*). *Silvae Genet* 2009; 58: 42–53.

32. Sasani N, Pâques LE, Boulanger G, et al. Physiological and anatomical responses to drought stress differ between two larch species and their hybrid. *Trees* 2021; 35: 1467–1484.

33. Churakova OV, Lehmann MM, Siegwolf RT, et al. Compound-specific carbon isotope patterns in needles of conifer tree species from the Swiss National Park under recent climate change. *Plant Physiol Biochem* 2019; 139: 264–272.

34. Cochard H. Cavitation in trees. *Comptes Rendus Phys* 2006; 7: 1018–1026.

35. Niinemets Ü. Distribution patterns of foliar carbon and nitrogen as affected by tree dimensions and relative light conditions in the canopy of *Picea abies*. *Trees* 1997; 11: 144–154.

36. Wilson PJ, Thompson K, Hodgson JG. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytol* 1999; 143: 155–162.

37. Witzell J, Martín JA. Phenolic metabolites in the resistance of northern forest trees to pathogens—past experiences and future prospects. *Can J For Res* 2008; 38: 2711–2727.

38. Craig H. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochim Cosmochim Acta* 1957; 12: 133–149.

39. Farquhar G, Richards R. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Funct Plant Biol* 1984; 11: 539–552.

40. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 2012; 9: 671–675.

41. Vile D, Garnier É, Shipley B, et al. Specific Leaf Area and Dry Matter Content Estimate Thickness in Laminar Leaves. *Ann Bot* 2005; 96: 1129–1136.

42. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 1965; 16: 144–158.

43. Pâques LE, García-Casas M del C, Charpentier J-P. Distribution of heartwood extractives in hybrid larches and in their related European and Japanese larch parents: relationship with wood colour parameters. *Eur J For Res* 2013; 132: 61–69.

44. Blackman CJ, Brodribb TJ, Jordan GJ. Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytol* 2010; 188: 1113–1123.

45. Delzon S, Douthe C, Sala A, et al. Mechanism of water-stress induced cavitation in conifers: bordered pit structure and function support the hypothesis of seal capillary-seeding. *Plant Cell Environ* 2010; 33: 2101–2111.

46. Alves A, Schwanninger M, Pereira H, et al. Calibration of NIR to assess lignin composition (H/G ratio) in maritime pine wood using analytical pyrolysis as the reference method. 2006; 60: 29–31.

47. R Core Team. R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria., https://www.R-project.org/ (2024).

48. Tukey JW. Exploratory data analysis. Reading, MA: Addison-wesley. 1977; Vol. 2,131-160.

49. Rinnan Å, Berg F van den, Engelsen SB. Review of the most common pre-processing techniques for near-infrared spectra. *TrAC Trends Anal Chem* 2009; 28: 1201–1222.

50. Brandolini-Bunlon M, Jallais B, Roger JM, et al. *R package rchemo: Dimension Reduction, Regression and Discrimination for Chemometrics*. 2023.

51. Stevens A, Ramirez-Lopez L, Stevens MA, et al. Package 'prospectr'. In: *Package 'prospectr'*. 2015.

52. Chessel D, Dufour AB, Thioulouse J. The ade4 package-I-One-table methods. *R News* 2004; 4: 5–10.

53. Pomerantsev AL. Acceptance areas for multivariate classification derived by projection methods. *J Chemom* 2008; 22: 601–609.

54. Kennard RW, Stone LA. Computer aided design of experiments. *Technometrics* 1969; 11: 137–148.

55. Wold S, Sjöström M, Eriksson L. PLS-regression: a basic tool of chemometrics. *Chemom Intell Lab Syst* 2001; 58: 109–130.

56. Agelet LE, Hurburgh CR. A Tutorial on Near Infrared Spectroscopy and Its Calibration. *Crit Rev Anal Chem* 2010; 40: 246–260.

57. Kloeppel BD, Gower ST, Treichel IW, et al. Foliar carbon isotope discrimination in *Larix* species and sympatric evergreen conifers: a global comparison. *Oecologia* 1998; 114: 153–159.

58. Piñol J, Sala A. Ecological implications of xylem cavitation for several Pinaceae in the Pacific Northern USA. *Funct Ecol* 2000; 14: 538–545.

59. Mayr S, Hacke U, Schmid P, et al. Frost Drought in conifers at the alpine timberline : xylem dysfunction and adaptations. *Ecology* 2006; 87: 3175–3185.

60. Charra-Vaskou K, Charrier G, Wortemann R, et al. Drought and frost resistance of trees: a comparison of four species at different sites and altitudes. *Ann For Sci* 2012; 69: 325–333.

61. Dulamsuren C, Abilova SB, Bektayeva M, et al. Hydraulic architecture and vulnerability to drought-induced embolism in southern boreal tree species of Inner Asia. *Tree Physiol* 2019; 39: 463–473.

62. Matyssek R, Schulze E-D. Heterosis in hybrid larch (*Larix decidua x leptolepis*). *Trees* 1987; 1: 219–224.

63. Walters MB, Gerlach JP. Intraspecific growth and functional leaf trait responses to natural soil resource gradients for conifer species with contrasting leaf habit. *Tree Physiol* 2013; 33: 297–310.

64. Fellner H, Dirnberger GF, Sterba H. Specific leaf area of European Larch (*Larix decidua* Mill.). *Trees* 2016; 30: 1237–1244.

65. Higgins SS, Black RA, Radamaker GK, et al. Gas exchange characteristics and water relations of *Larix occidentalis*. *Can J For Res* 1987; 17: 1364–1370.

66. Williams G, Nelson A. Spatial variation in specific leaf area and horizontal distribution of leaf area in juvenile western larch (*Larix occidentalis* Nutt.). *Trees* 2018; 32: 1621–1631.

67. Cozzolino D. Use of Infrared Spectroscopy for In-Field Measurement and Phenotyping of Plant Properties: Instrumentation, Data Analysis, and Examples. *Appl Spectrosc Rev* 2014; 49: 564–584.

68. Guet J. *Expression d'une variabilité génétique pour la phénologie de croissance, l'efficience d'utilisation de l'eau et la résistance à la cavitation au sein de populations naturelles de peuplier noir (Populus nigra L.)*. Université d'Orléans.2015

69. Richardson AD, III JBR. Quantitative reflectance spectroscopy as an alternative to traditional wet lab analysis of foliar chemistry: near-infrared and mid-infrared calibrations compared. *Can J For Res* 2005; 35: 1122–1130.

70. Farquhar GD, O'Leary MH, Berry JA. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Funct Plant Biol* 1982; 9: 121–137.

71. Ge Y, Atefi A, Zhang H, et al. High-throughput analysis of leaf physiological and chemical traits with VIS–NIR–SWIR spectroscopy: a case study with a maize diversity panel. *Plant Methods* 2019; 15: 66.

72. Kothari S, Beauchamp-Rioux R, Blanchard F, et al. Predicting leaf traits across functional groups using reflectance spectroscopy. *New Phytol* 2023; 238: 549–566.

73. Forsthuber B, Ecker M, Truskaller M, et al. Rapid prediction of surface characteristics of European and Siberian larch wood by FT-NIRS. *Eur J Wood Wood Prod* 2017; 75: 569–580.

74. Luss S, Schwanninger M, Rosner S. Hydraulic traits of Norway spruce sapwood estimated by Fourier transform near-infrared spectroscopy (FT-NIR). *Can J For Res* 2015; 45: 625–631.

75. Jansen S, Choat B, Pletsers A. Morphological variation of intervessel pit membranes and implications to xylem function in angiosperms. *Am J Bot* 2009; 96: 409–419.

76. Tixier A, Herbette S, Jansen S, et al. Modelling the mechanical behaviour of pit membranes in bordered pits with respect to cavitation resistance in angiosperms. *Ann Bot* 2014; 114: 325–334.

77. Kleinebecker T, Schmidt SR, Fritz C, et al. Prediction of δ13C and δ15N in plant tissues with near-infrared reflectance spectroscopy. *New Phytol* 2009; 184: 732–739.

78. Petisco C, García-Criado B, Mediavilla S, et al. Near-infrared reflectance spectroscopy as a fast and non-destructive tool to predict foliar organic constituents of several woody species. *Anal Bioanal Chem* 2006; 386: 1823–1833.

79. Ecarnot M, Compan F, Roumet P. Assessing leaf nitrogen content and leaf mass per unit area of wheat in the field throughout plant cycle with a portable spectrometer. *Field Crops Res* 2013; 140: 44–50.

80. Foley WJ, McIlwee A, Lawler I, et al. Ecological applications of near infrared reflectance spectroscopy – a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. *Oecologia* 1998; 116: 293–305.

Supplementary:

Table S1: Number of Outliers in Boxplots of Trait in Reference Data used for predictions

Trait	numbers of outliers
$\Delta^{13}C$	1
Carbon	1
Nitrogen	4
Total phenolics	0
Lignin	2
H/G ratio	7

Table S2: Summary of missing samples and correlation between latent variables (LV) and R^2 values for NIRS model predictions.

Trait	NIRS models	Sampled	Total	difference	Missing spectra	boxplot outliers	spectra outliers	Training size	proportion of PCA outliers	Pre treatment	no of LVs	R2 train	RMSE train	testing size	R2 test	RMSE test
	model_2021.2022.2023	520	506	14	12	2	22	363	6.06%	dt	15	0.62	0.430	121	0.48	0.530
	model_2021.2022	320	316	4	0	4	23	219	10.50%	dt	13	0.62	0.370	74	0.40	0.460
	model_2022.2023	400	387	13	12	1	12	281	4.27%	dt	10	0.61	0.440	94	0.57	0.490
$\Delta^{13}C$	model_2021.2023	320	304	16	12	4	11	219	5.02%	der2	9	0.63	0.460	74	0.60	0.450
	model_2021	120	116	4	0	4	7	81	8.64%	der2	7	0.50	0.410	28	0.50	0.470
	model_2022	200	200	0	0	2	13	138	9.42%	dt	10	0.47	0.330	47	0.25	0.490
	model_2023	200	187	13	12	1	3	138	2.17%	snv_der2	9	0.76	0.440	46	0.69	0.420
Correlation numbers of LVs and R^2 of prediction				0								0.17			-0.29	
Correlation spectra outlier vs R^2 of prediction				0								-0.74			-0.86	
	model_2021.2022.2023	520	469	51	12	39	25	333	7.51%	snv_der1	9	0.52	0.750	111	0.52	0.750
	model_2021.2022	320	294	26	0	26	13	213	6.10%	snv_der1	3	0.18	1.000	71	0.18	1.200
	model_2022.2023	400	361	39	12	27	10	263	3.80%	dt	9	0.45	0.980	88	0.44	0.870
C	model_2021.2023	320	307	13	12	1	2	228	0.88%	snv_der1	12	0.79	4.400	77	0.79	3.700
	model_2021	120	119	1	0	1	8	83	9.64%	dt	10	0.76	0.360	28	0.64	0.470
	model_2022	200	188	12	0	12	20	126	15.87%	snv	1	0.01	1.600	42	0.04	1.700
	model_2023	200	187	13	12	1	5	136	3.68%	snv_der1	9	0.86	0.370	46	0.79	0.430
Correlation numbers of LVs and R^2 of prediction				0								0.91			0.93	
Correlation spectra outlier vs R^2 of prediction				0								-0.65			-0.70	
	model_2021.2022.2023	520	497	23	12	11	7	367	1.91%	der1	13	0.91	0.080	123	0.83	0.099
	model_2021.2022	320	310	10	0	10	11	224	4.91%	snv_der1	12	0.93	0.070	75	0.90	0.079
	model_2022.2023	400	384	16	12	4	9	281	3.20%	snv_der1	12	0.89	0.084	94	0.84	0.095
N	model_2021.2023	320	299	21	12	9	2	222	0.90%	snv_der1	10	0.88	0.084	75	0.84	0.087
	model_2021	120	116	4	0	4	4	84	4.76%	snv_der1	8	0.95	0.630	28	0.94	0.710
	model_2022	200	200	0	0	0	13	140	9.29%	dt	11	0.91	0.069	47	0.93	0.067
	model_2023	200	187	13	12	1	5	136	3.68%	snv_der1	9	0.84	0.087	46	0.79	0.088
Correlation numbers of LVs and R^2 of prediction												0.09			-0.17	
Correlation spectra outlier vs R^2 of prediction												0.32			0.68	

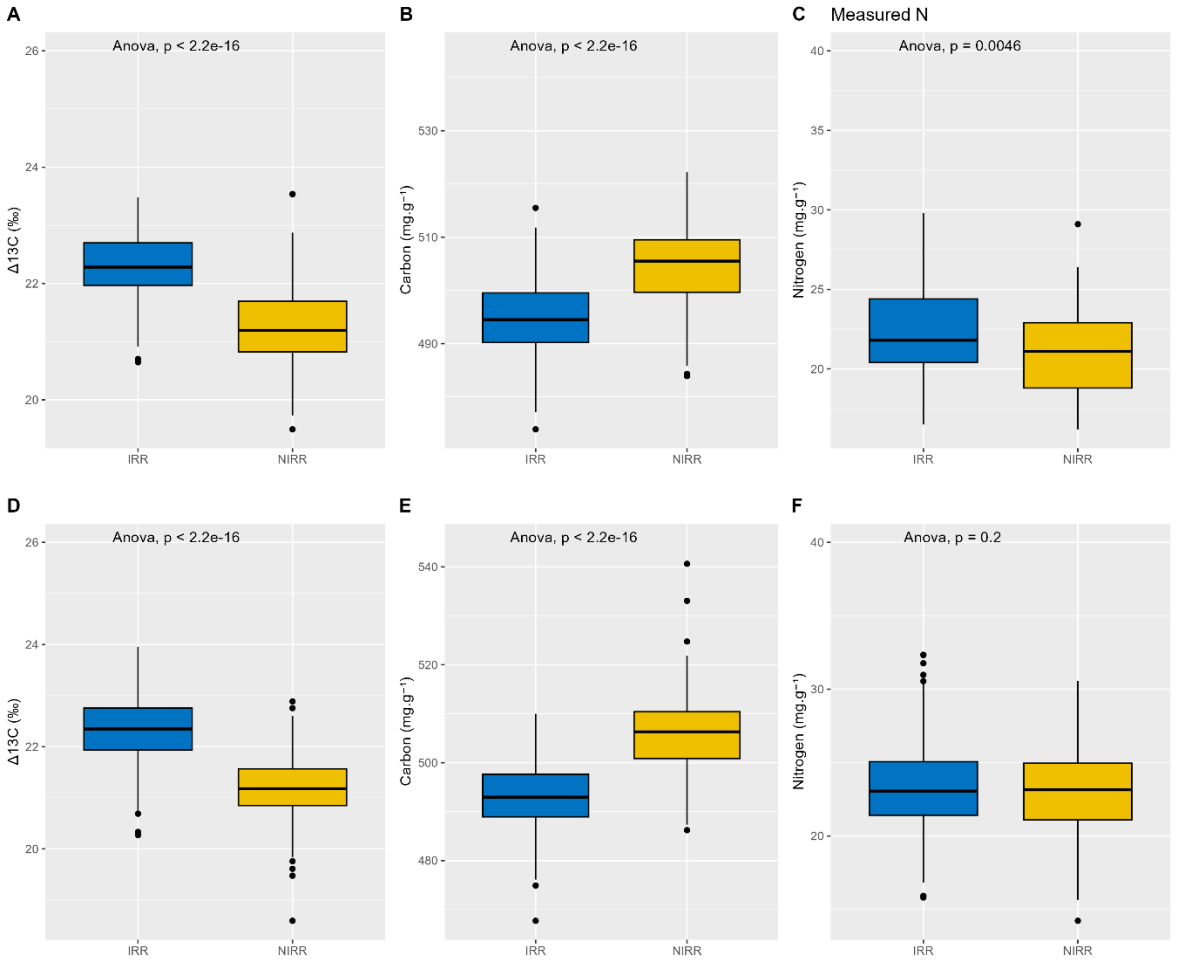


Fig S1: Boxplot showing Traits in two watering treatments with ANOVA test ($p < 0.05$), (A–C) Measured traits: $\Delta^{13}C$ (A), Carbon (B), and Nitrogen (C). (D–F) Predicted traits: $\Delta^{13}C$ (D), Carbon (E), and Nitrogen (F). IRR: irrigated; NIRR: not irrigated

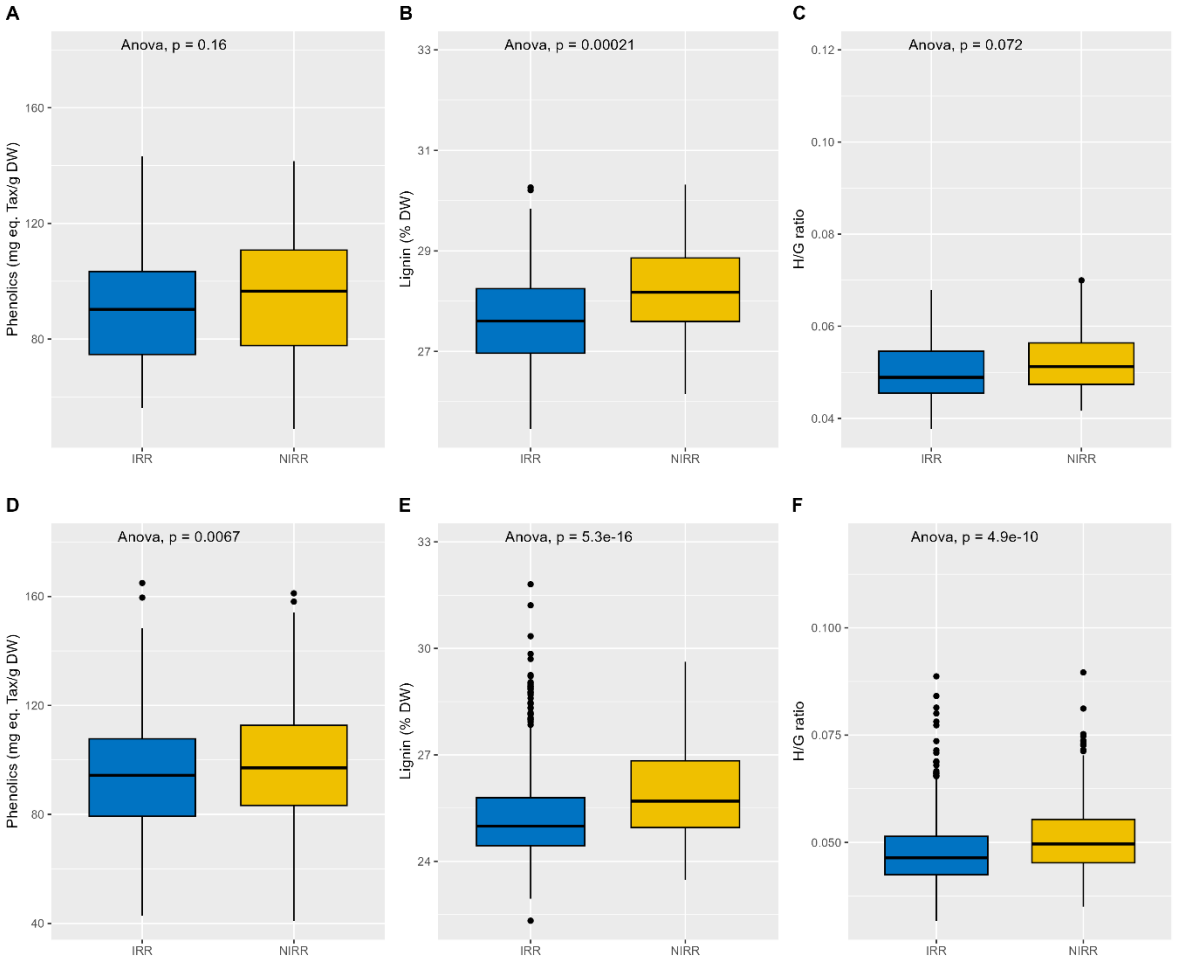


Fig S2: B Boxplot showing Traits in two watering treatments with ANOVA test ($p < 0.05$ (A–C) Measured traits: Phenolics(A), Lignin (B), and H/G ratio (C). (D–F) Predicted traits: Phenolics (D), Lignin (E), and H/G ratio (F). IRR: irrigated; NIRR: not irrigated

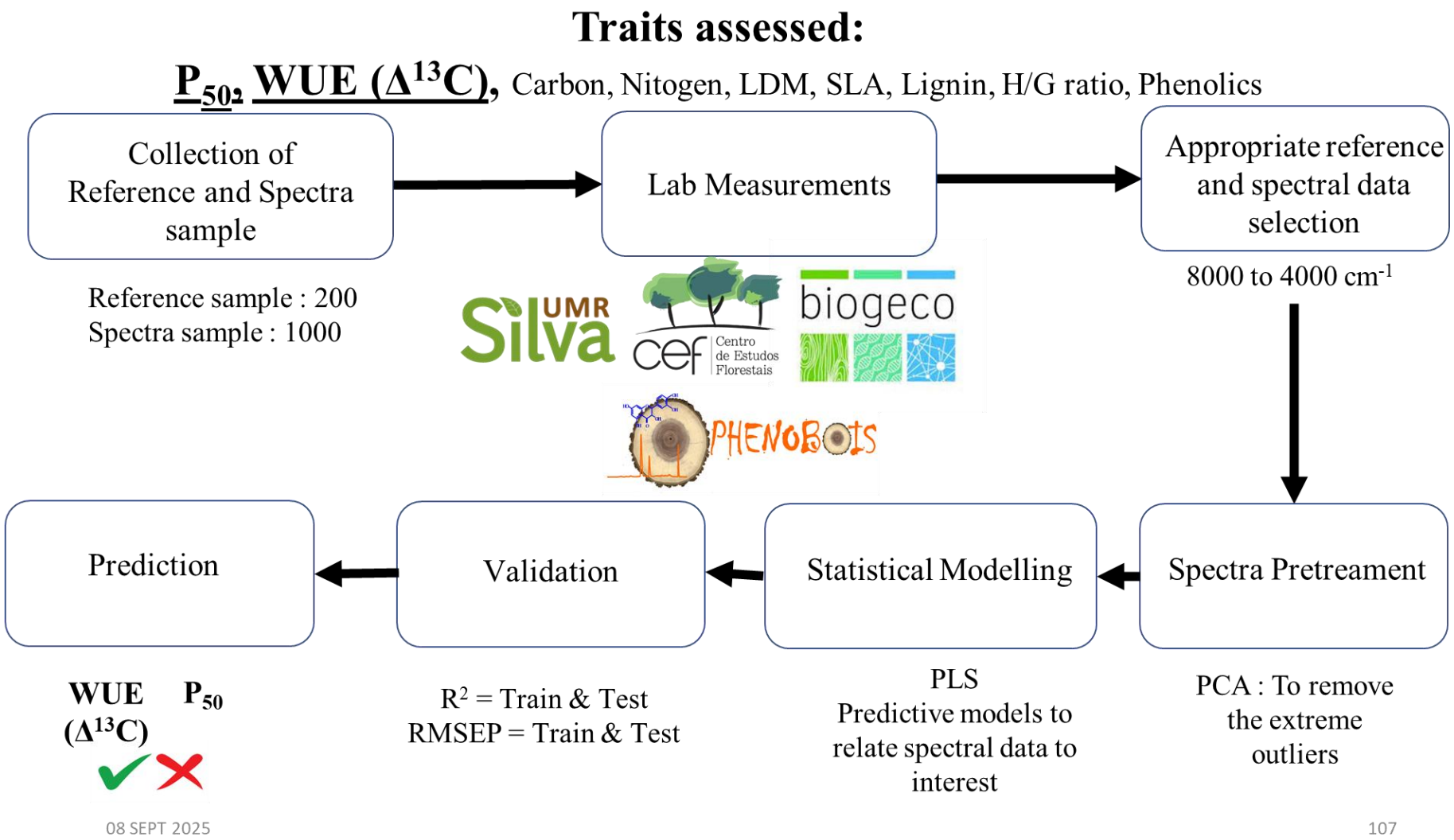


Fig S3: The figure illustrates the methodology underlying the NIRS-based modeling process, covering all steps from sample collection to validation and indicating the traits involved.

4.3. **Insights from Chapter II**

Chapter II demonstrated the potential of Near-Infrared Spectroscopy (NIRS) as a high-throughput, efficient tool for predicting a range of functional traits in larch. Model performance was reliable with good to moderate predictive powers for Lignin, H/G ratio, phenolics, C, N, and $\Delta^{13}C$. However, models for specific leaf area (SLA) and vulnerability to cavitation (P_{50}) performed poorly, in line with previous studies that highlighted the difficulty of capturing structural or hydraulic properties with NIRS due to their complex, multiscale nature and weaker spectral signal. Notably, while previous experiments often used raw branches for NIRS calibration (Guet 2015; Jansen et al. 2009; Tixier et al. 2014), our approach was based on powdered samples, which may have further limited model performance. Consequently, for these poorly predicted traits, only the values from the reference (training) dataset are available.

Moreover, the traits predicted from Chapter II will serve as a valuable complement to the growth and resilience analysis presented in the next chapter.

By integrating these predicted functional and structural traits with growth performance, we aimed to gain a more 'mechanistic' basis of drought response and adaptive potential in larch taxa and hybrids. However, due to prediction limitations, our analysis across the full experimental sample will remain restricted to a subset of traits. Further sampling—across sites, years, seasons, and genotypes—alongside improved modeling efforts will be necessary to enhance trait predictability.

In summary, Chapter II laid the groundwork for a trait-based understanding and demonstrated how high-throughput phenotyping methods like NIRS can play a central role in linking physiology, genetics, and performance in forest species, where the experiments could be constrained by scale, cost, or sampling and lab efforts.

5. Chapter III: Expression of heterosis for growth under contrasted soil watering treatments and over time in interspecific larch hybrids (*Larix x eurolepis*): Are fast-growing genotypes and taxa more vulnerable to water constraints?

5.1. Conceptualization

In Chapter I, we observed how the three larch trees reacted to differential soil water availability, where Japanese Larch was most vulnerable and had higher mortality levels than Hybrid Larch and European Larch. We also observed that trees that eventually had mortality underwent growth reduction in the preceding year of mortality. The late spring period experienced higher vapor pressure deficit (VPD) and temperatures, indicating hotter and drier conditions. These stressful periods likely triggered some changes in physiological mechanisms or growth strategies, which may explain why some species succumbed to mortality while others were able to survive. This led us to a key question that guides the next part of our work and has been a topic of discussion for a few decades (Reich 2014): Does fast growth come at a cost? Could the very trait that makes hybrids attractive and in demand—rapid growth—also make them more vulnerable when water is limited? This was also an underlying question as to why we worked on Chapter II to integrate an array of functional traits to discuss this broader question. In Chapter II, we used Near-Infrared Spectroscopy (NIRS) to predict, among others, traits like water-use efficiency and resistance to cavitation (like P_{50}). These traits could help explain how trees survive stress.

So, from climate stimulation in Chapter I and a technical solution in Chapter II, the last research chapter advances in evaluating the performance of larch across time, space, and climatic conditions. It explores whether hybrid vigor is consistent or context-dependent and whether fast growth leads to trade-offs regarding drought resilience, growth stability, or physiological cost.

5.1.1. Breeder's view: Interspecific Hybridization and Heterosis

For over a century, the concept of heterosis or hybrid vigor has been central to plant breeding, basically due to its potential to boost performance in key traits like growth and yield (Shull 1948). However, among forest trees, this study on heterosis is historically a bit lagging behind plants due to their long generation time. Still, interspecific hybridization in trees like *Larix* × *eurolepis*—a cross between European larch (*L. decidua*) and Japanese larch (*L. kaempferi*)—was initiated decades ago with the promise of combining rapid juvenile growth and resistance to diseases (Sylvestre-Guinot et al. 1999) from the Japanese parent with the superior form of the European parent.

However, why is it necessary to revisit this concept of "heterosis" now? With increasing climatic instability, particularly drought stress, there is renewed interest in whether vigor remains stable over time and across variable environments. This is changing the traditional paradigm of breeding and interspecific hybridization. While early breeding programs focused on maximizing growth, our study returns to the growth question with updated tools like trade-off information (Climent et al. 2024) and a broader perspective—one that considers not just performance but also sustainability.

5.1.2. Ecological view: Resilience and Trade-offs

Ecologically, fast and slow growth is being questioned as to whether these genotypes are actually able to balance the resources available. The idea that "grow fast, or grow safe," or “to fight or flight,” reflects a central trade-off in plant strategy theory: resource acquisition and conservation (Lauder et al. 2019). This is where the concept of trade-off rightly comes into play.

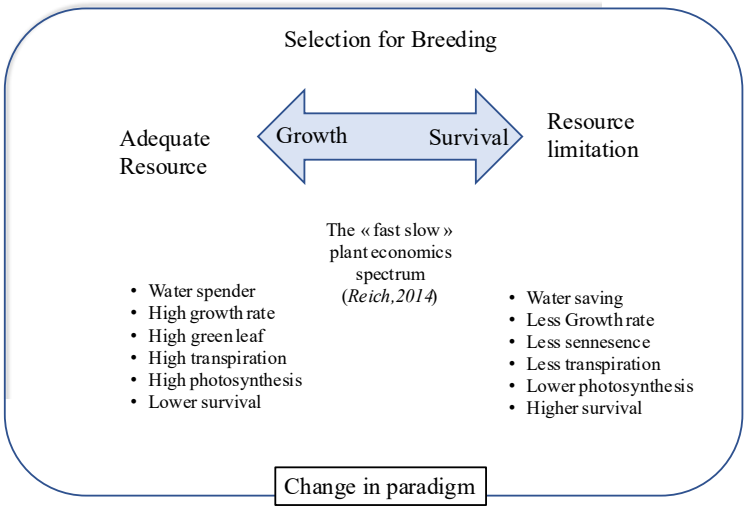


Fig. 30 The change in paradigm in tree breeding as inclusion of fast-slow economic spectrum inspired by Reich 2014.

Trade-offs in plants become especially apparent when performance is evaluated across environmental conditions, species, or genotypes. A comprehensive study (Tao et al. 2024) analyzing tree-ring data from 32 species (including gymnosperms like *Larix decidua* (European larch), *Picea abies* (Norway spruce), and angiosperms like *Quercus robur* (oak).) across the Northern Hemisphere found that fast-growing trees exhibited lower drought resistance but higher recovery rates, indicating a trade-off between growth rate and drought resilience. Similarly, research on aspen (*Populus tremuloides*) demonstrated that selection for rapid growth can compromise herbivore defense mechanisms, highlighting a trade-off between growth and defense traits (Cope et al. 2021). These findings emphasize the need to account for ecological context and trait interactions when evaluating tree adaptation and survival strategies.

This ecological lens emphasizes that resilience is multifaceted: it includes the ability to resist stress (resistance), recover after stress (recovery), and maintain function over time (stability). Evaluating larch through this framework allows us to ask not only how much it grows but also how well it withstands the environmental realities of changing climates. This perspective complements the breeder's view, encouraging the design of future breeding programs that aim for both productivity and sustainability.

5.2. Methodological Considerations

Although the chapter explains most of the methodology in detail, some methods were part of the prework and represented essential steps in the study.

1. Selection of Combinations

To evaluate heterosis, resilience, and plasticity in larch taxa, careful selection of hybrid and parental combinations was essential. The study prioritized combinations representing a balanced number of individuals, in particular families with ample parental crosses. This allowed for consistent comparison of hybrid (*Larix* × *eurolepis*) performance against both European larch (*L. decidua*) and Japanese larch (*L. kaempferi*) parents under identical experimental settings. There were also four combinations with ample European Larch parent individuals, but with not enough Japanese Larch parents, and to be uniform, we subjected the analysis to 8 families to remain uniform throughout.

2. Methods of spatial corrections

Spatial heterogeneity in the experimental field can introduce bias in growth measurements due to microenvironmental variation (like soil moisture, nutrient availability). To account for this, spatial correction methods are necessary. We used the "spline" model in the breedR package in R to account for this: we tested different sets of knots and kept the model with the lowest AIC. Due to the relatively high and scattered mortality of trees in the non-irrigated part of the experiment, we wanted to account for individual tree competition as well. We computed a competition index (Heygyi 1984) based on the distances of a tree to its neighbors and their diameters and used them as a covariate in our model. These steps ensured that residual variation attributed to field layout was minimized, improving the accuracy of performance estimates.

3. Space x time for phenotypic plasticity

Phenotypic plasticity for growth was assessed through the interaction with spatial (site-level irrigation regimes) and temporal (year-to-year) variation in climatic variables. For this study, we restricted the climatic gradient to soil water availability, as most specifically, Larch is known to be reactive to soil water. Also, between the two irrigated and non-irrigated plots, almost all climatic variables, such as VPD and temperatures, were considered a priori similar, the only difference being soil water availability.

By analyzing repeated growth measures across multiple years under both irrigated and non-irrigated conditions, we could quantify plasticity as the responsiveness of each taxon and combination to changing environmental conditions. This required careful structuring of longitudinal models that included fixed effects for treatment and year and their interactions, enabling us to detect patterns of genotype × environment interaction over time and space.

4. Using the growth reduction between IRR and NIRR as a method to analyze resilience

Resilience to drought was assessed by comparing growth performance under irrigation (IRR) versus no irrigation (NIRR). Specifically, we calculated the relative reduction in annual increment (apical and radial) between IRR and NIRR conditions, which served as a proxy for stress response. These reductions were computed annually and used to derive resilience indices (e.g., resistance = growth during drought / pre-drought growth), providing insight into both immediate and 'long-term' responses to stress across taxa and genotypes.

Abstract in French : Expression de l'hétérosis pour la croissance en fonction du temps et des conditions hydriques du sol chez l'hybride interspécifique (*Larix x eurolepis*) : les taxa et les génotypes les plus vigoureux sont-ils plus vulnérables aux contraintes hydriques ?

Résumé

L'hybridation interspécifique constitue une stratégie prometteuse pour améliorer les performances de croissance des arbres forestiers, mais ses implications sur la résilience au stress demeurent incertaines. Cette étude a évalué l'hétérosis pour la croissance, la réponse à la sécheresse, les compromis entre croissance, résilience et d'autres caractères physiologiques chez le mélèze hybride (*Larix* × *eurolepis*) comparé à ses espèces parentes (mélèze d'Europe (*L. decidua*) et mélèze du Japon (*L. kaempferi*)). Sur une période de dix ans, nous avons mesuré les croissances annuelles apicales et radiales dans un test clonal issu d'un plan de croisements diallèle intra-/inter-spécifique installé sur deux parcelles soumises à des régimes hydriques contrastés (irrigation vs. non-irrigation). Le mélèze hybride présente une forte hétérosis de croissance, particulièrement accentuée en conditions sèches (non irriguées). Avec le mélèze du Japon, l'hybride montre une forte réactivité à la disponibilité en eau du sol pour la croissance contrairement au mélèze d'Europe qui apparaît peu plastique et des réponses variables face à la sécheresse. Les mélèzes hybride et du Japon affichent une résistance plus faible à la sécheresse notamment pour la croissance radiale que le mélèze d'Europe mais une forte capacité de récupération après sécheresse, suggérant un compromis potentiel entre performance et tolérance au stress. Le mélèze d'Europe montre une croissance plus conservatrice et stable en conditions de sécheresse. Les mélèzes du Japon et hybride sont caractérisés par une croissance plus élevée, associée à une meilleure efficacité d'utilisation de l'eau (WUE), mais avec une plus forte vulnérabilité à la cavitation (P_{50}). Cependant une forte variabilité pour ce caractère existe entre combinaisons chez l'hybride. Ces résultats suggèrent que certaines familles de mélèze hybride pourraient combiner forte productivité, efficacité d'utilisation de l'eau et sécurité hydraulique. Ces résultats soulignent l'importance de l'évaluation à long terme et en milieux contrastés de caractères multiples pour sélectionner des hybrides alliant productivité et potentiel adaptatif face au changement climatique.

Mots-clés : Hybridation, Mélèze, Vigueur, Résilience, Plasticité, Hétérosis, Sécheresse, Trade-off, Efficience de l'utilisation de l'eau, Cavitation

5.3. **Expression of heterosis for growth under contrasted soil watering treatments and over time in interspecific larch hybrids (*Larix x eurolepis*): Are fast-growing genotypes and taxa more vulnerable to water constraints?**

Abstract

Interspecific hybridization is a promising strategy for enhancing growth performance in forest trees, yet its implications for stress resilience remain uncertain. This study assessed growth heterosis, drought response, growth-resilience, and other traits trade-offs in hybrid larch (*Larix* × *eurolepis*) compared to its parental species—European larch (*L. decidua*) and Japanese larch (*L. kaempferi*). Over ten years, we evaluated annual height, diameter, and ring area increments (for only resilience parameters) under contrasting soil water availability (irrigated vs. non-irrigated). Hybrid larch showed significant heterosis for growth, especially more improved under non-irrigated conditions, but displayed variable drought responses. Hybrid and Japanese Larch displayed high recovery but low resistance, especially at the diameter level, indicating a potential trade-off between performance and stress tolerance. Parental species demonstrated more conservative and stable growth under drought, especially European larch. Japanese and hybrid larch exhibited better water-use efficiency (WUE) and were also associated with greater growth while maintaining some vulnerability to cavitation (P_{50}). This suggests that hybrid larch may combine efficient water use and high productivity with moderate hydraulic safety, as it exhibited lower cavitation vulnerability compared to JL. These findings underscore the importance of long-term, multi-trait evaluations in selecting hybrids that balance productivity and adaptive potential under climate change.

Keywords: Hybridization, Larch, Vigor, Plasticity, Heterosis, Drought, Trade-off, WUE, Cavitation

1. Introduction

Inter-specific and inter-population hybrids have long been recognized as valuable in forest trees (Pâques 1989; Dungey 2001), owing to their potential to combine favorable traits from both parents and, in some cases, to express heterosis or hybrid vigor. Several hybridization breeding programs are currently underway, involving various broadleaf and coniferous species. Hybrids are already deployed on a large scale for commercial afforestation in several parts of the world (Lacrocque et al. 2013 - Poplar, Li et al. 1993 - Aspen, Cappa et al. 2013 - sub-tropical pines, Potts and Dungey 2014 - *Eucalyptus*).In Europe and North America, Hybrid Larch (*Larix* × *eurolepis*), resulting from the cross between European Larch (*L. decidua* Mill.) and Japanese Larch (*L. kaempferi*), is well known for its vigorous growth (Greenwood et al. 2015; Marchal et al, 2017; Maass et al. 2020). Hybrid larches such as *L. gmelinii* × *L. kaempferi* hybrids (Fujimoto et al, 2006; Watanabe et al. 2011) are also prominent in Asia. *Larix* × *eurolepis* inherits disease resistance traits, particularly against *Meria laricis* and larch canker (*Lachnellula willkommii*), from its Japanese parent (Sylvestre-Guinot et al., 1999), and straighter stems from its European parent (Pâques, 2004). Regarding wood properties, the hybrid is usually intermediate or superior to either parent species (Pâques, 1989, 1992, 2004, 2009; Greenwood et al. 2015; Marchal et al. 2017). Its faster growth rate also results in a higher proportion of heartwood, the most valuable component of larch timber (Pâques, 2015).

However, the level of expression of hybrid vigor may change with temporal and environmental conditions. In the early literature on hybrid larch, it was suspected that expression of hybrid

superiority was limited to the juvenile growth phase (Scamoni 1977, Gothe et al. 1980, Keiding 1980), with a strong reduction afterwards, but this was not observed by Bastien and Keller (1980). At a finer scale, on a yearly basis, the resulting level of heterosis in hybrid combinations showed different expression trajectories over the growing season in line with phenology and growth rhythm (Pâques 2009). Whereas it is well documented in crop plants (Gallais, 2009) that hybrids perform mostly better in harsh environments (e.g., corn, wheat), little is known about this aspect in the forestry literature. Several results in larch indicate that hybrids are better at buffering environmental site variation. Pâques (2002) showed that for several traits (growth, stem form, phenology), interspecific hybrid progenies were less interactive with the environment than parental species progenies. In a survey of progeny trials in France (Pâques et al. 2013, Marchal et al. 2017, 2019), hybrids were revealed to maintain superiority over their parent species across a wide range of ecologically contrasted environments. Marchal et al. (2019) illustrated the hybrid's overall better growth and more plastic response along a water availability gradient.

A better understanding of the differential growth patterns of hybrid larch and its parents over time and across environments, and thereby of the expression of heterosis, is becoming crucial in a context of more frequent and severe climatic accidents. This should help highlight the most efficient breeding strategy for larch (hybrid-oriented vs. parent-oriented) and provide the best conditions for deploying improved *Larix* material.

Selection for fast growth has consistently been a primary objective since the development of breeding programs worldwide, regardless of species. The success of interspecific hybridization programs also largely depends on growth heterosis. However, in a context of increasingly frequent and severe climatic stresses, such as higher temperatures, reduced precipitation, and rising pest and disease pressures (Cook et al.2018; Donat et al. 2013; Halofsky et al. 2020; Sturrock et al. 2011), the strategy of selecting and deploying fast-growing species or genotypes may need to be reconsidered. Indeed, fast growth usually involves the rapid consumption of resources in an exhaustive manner, while slow growth is characterized by resource use that is more conservative (Atkinson et al. 2012; Signori-Müller et al. 2022; Zhu et al. 2018). If the hypothesis of competition for resources making fast-growing trees more vulnerable to biotic and/or abiotic factors is verified, this would drastically reorient selection goals and breeding strategies. However, little is known about the impact of fast growth on traits linked to environmental adaptation, such as tolerance to abiotic factors. Hybrid larch and its parents, representing contrasted growth potentials, look thus as a good tree model to study possible growth-adaptation trade-offs.

In a clonal experimentation combining the three larch taxa evaluated in plots with contrasted soil water availability, we aimed to answer the following questions: (a) How important is heterosis for growth: how does it vary with soil water availability, and how does it develop over time? (b) Do EL, JL, and HL taxa differ in their growth responses to drought and, more globally, to soil water availability, as quantified by resilience parameters and phenotypic plasticity? (c) Is there a trade-off between growth and adaptive traits in larch taxa: does fast growth come at a cost?

2. Materials and Methods

2.1. Genetic Material and Study Location

The study is based on a subset of progenies from an intra-/inter-specific diallel mating design created between 1988 and 1994 between 9 European larches (*Larix decidua*, (EL), Sudetan origin) and 9 Japanese larches (*L.kaempferi* (JL), various origins from Hondo Island, Japan) selected from one of the four progeny trials planted in the forest (St-Saud, Dordogne) (Pâques 2002). Progenies were chosen based on the level of their heterotic performance for growth, classified as high, medium, and low heterotic families. The subset included 12 full-sib (FS) families of Hybrid Larch and 12 related FS families for both EL and JL, sharing common parental clones. Each family was represented by about 12 cloned individuals (ortets), with 15 copies (ramets). The ramets were produced through grafting on a common rootstock (Lavercantière hybridization seed orchard, characterized by its narrow genetic basis) in the spring of 2010.

	European larch									Japanese larch								
	104	106	109	166	214	221	222	242	284	3179	3180	3183	3190	3193	3194	3200	3203	3217
104	x	x	x		x	x	x		x				x	x	x	x		
106		x	x	x		x	x	x	x		o		x	x	x	x		
109			x				o											
166																		
214					x													
221						x									x	x		
222							x			o	o			o	x	x		
242								x										
284									x									
3179										x	o				x	x		
3180											x		x	x	x	x		
3183												x			x	x		
3190													x					
3193														x				
3194															x		x	x
3200																x	x	x
3203																	x	
3217																		x

Table 1: Subset of the (9 + 9) diallel mating design: x = the 12 full-sib families per taxon plus the parental clones (on diagonals) planted in the trial. (Note: o = extra material, including some individuals that were mislabeled, as revealed by post-plantation molecular marker analysis; in blue, the 8 hybrid larch 'priority' combinations used for some studies)

For heterosis analysis, we focus our work on eight hybrid combinations for which we could compute parental breeding values: 104×3194, 104×3200, 106×3194, 106×3200, 221×3194, 221×3200, 222×3194, and 222×3200. Each combination follows a naming convention: 104×3194 is referred to as HL, with 104 as the EL female parent and 3194 as the JL male parent.

This naming format is consistently used throughout the text, tables, and figures for a particular combination.

This material was kept at INRAe-Orléans nursery for two years before being planted in the winter of 2013. The material was split into two sets with the identical genotypes planted on two sites: one in a region recommended for larch plantation (Beaumont-du-Lac, Massif central) – not included in this study- and one close by to Orléans at INRAe nursery (Latitude: 47.83 N, Longitude: 1.91 E, Altitude of 108 m asl.). Orléans nursery site is marked by a low-altitude flat area with a poor soil composed of coarse sand and gravel, characterized by a low water-holding capacity (35 mm); it receives low precipitation, less than 700 mm annually. These severe restrictions on water availability make this site unsuitable for the reforestation with Larch. However, this site is considered ideal for testing the growth potential of larch against water limitations. In order to compare larch behavior in optimal and stressful conditions, the experimental site was divided into two plots ('Treatments')—an irrigated (IRR) plot, which has been drip irrigated daily from May to October since 2015, and a non-irrigated (NIRR) plot, which was deprived of any additional irrigation, creating natural "drought" stress conditions (**Fig 1, 3**).

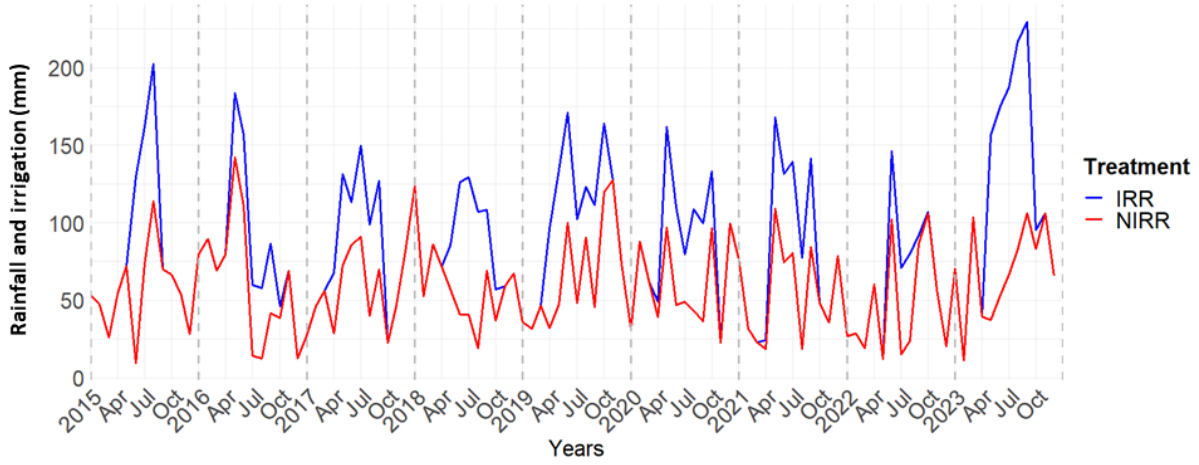


Fig 1: Total amount of water received by trees in the two plots of the experimental site: Irrigated (IRR, blue: rain + irrigation) and non-irrigated (NIRR, red; rain) over the years 2015 to 2023.

At the Orléans site, trees were planted at a 3 x 2.5 m spacing between and within rows following a split-plot design with 25 blocks across the experiment and 13 blocks per treatment. Each taxon was planted in bands across the experiment to minimize competition among the three taxa. Clones from each taxon were randomly distributed in bands. During the experimentation (from 2015 to 2023), weed control was regularly applied by mowing grass and shrubs to limit understory weed competition.

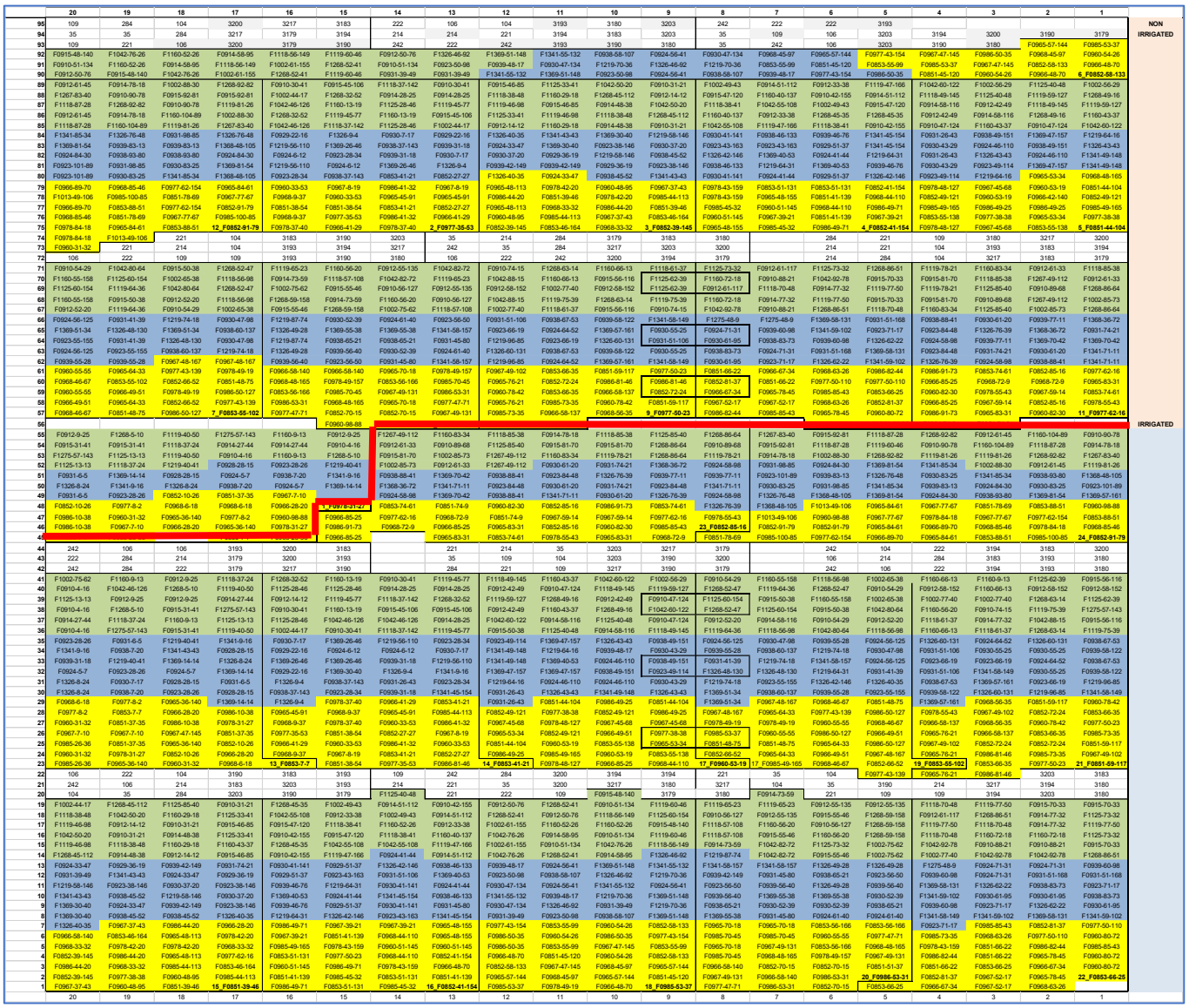


Fig 2. Map of the experimental trial at INRAe-Orléans nursery with the two treatments (non-irrigated and irrigated); taxa are planted across Blocks in bands: yellow = hybrid larch; blue = Japanese larch; green = European larch; white = clonal parents. Each boxed cell represents the Identification of the respective tree.

2.2. Climatic data over the years

Climatic data from 2015 to 2023 were obtained from a Météo-France meteorological station within a few hundred meters of the experimental site in the INRAE-Orléans nursery. These data include daily records of maximum, minimum, and average temperature (°C), precipitation (mm), wind speed (m/s), solar radiation (W/m²), and relative humidity (%). This dataset was used to characterize annual climatic variability and to identify drought years. Vapor Pressure Deficit (VPD, kPa) was calculated following Brice and Hall (2020), using relative humidity and average air temperature. In 2015, six soil moisture probes (TDR CS616 and WM 257L) were installed at 24 cm and 60 cm depths in IRR and NIRR plots to monitor soil water content and tension. Additionally, the BILJOU water balance model (Granier et al. 1999; https://appgeodb.nancy.inrae.fr/biljou/fr/) was used to estimate Relative Extractable Water (REW), integrating local stand (e.g. phenology, Leaf area index) and soil characteristics (e.g. depth, extractable water, rooting), and climate data. Given the challenges of monitoring LAI, especially in the heterogeneous NIRR sites, LAI was fixed arbitrarily at 5 to consider a close canopy. REW served as a comparative index of soil water constraints across years and treatments. In the context of our experiment, drought is characterized by higher soil water tension in summer compared to other seasons and is amplified in the NIRR sites.

The REW dynamics from 2015 to 2024 reveal strong seasonal fluctuations in soil water availability, with a consistent pattern of depletion during the summer months followed by recovery during the autumn and winter (**Fig. 3, top panel**). The middle panel (**Fig. 3**) illustrates the seasonal and interannual variation in soil matric potential from 2015 to 2023 under irrigated (IRR) and non-irrigated (NIRR) treatments. Soil matric potential was consistently higher under NIRR, indicating lower soil moisture availability and greater water stress. Sharp peaks were observed during the summer months, reflecting increased evapotranspiration demand and limited rainfall. These peaks were most pronounced in drought years such as 2017, 2019, and 2022, where values under NIRR exceeded -300 kPa, signaling severe stress conditions. In contrast, IRR plots showed buffered soil moisture conditions, with lower and less frequent peaks rarely surpassing -150 to -200 kPa, demonstrating the mitigating effect of irrigation. **Fig 3** (bottom panel) shows the relationship between mean annual vapor pressure deficit (VPD, kPa) and temperatures from 2013 to 2023 in the summer period. Higher VPD values correspond to drier air conditions, increasing the likelihood of extreme heat events. The figure highlights a clear pattern where years with higher VPD had more extreme heat. The most extreme years were 2019, 2022, 2018, and 2023, which had both the highest VPD and the greatest hot temperatures. These years indicate drier and hotter conditions. In contrast, 2014 and 2021 had the lowest VPD and the fewest extreme heat, reflecting milder climate conditions. Over time, the data show an upward trend in VPD and the hotter temperatures, suggesting that recent years have experienced increasing heat stress.

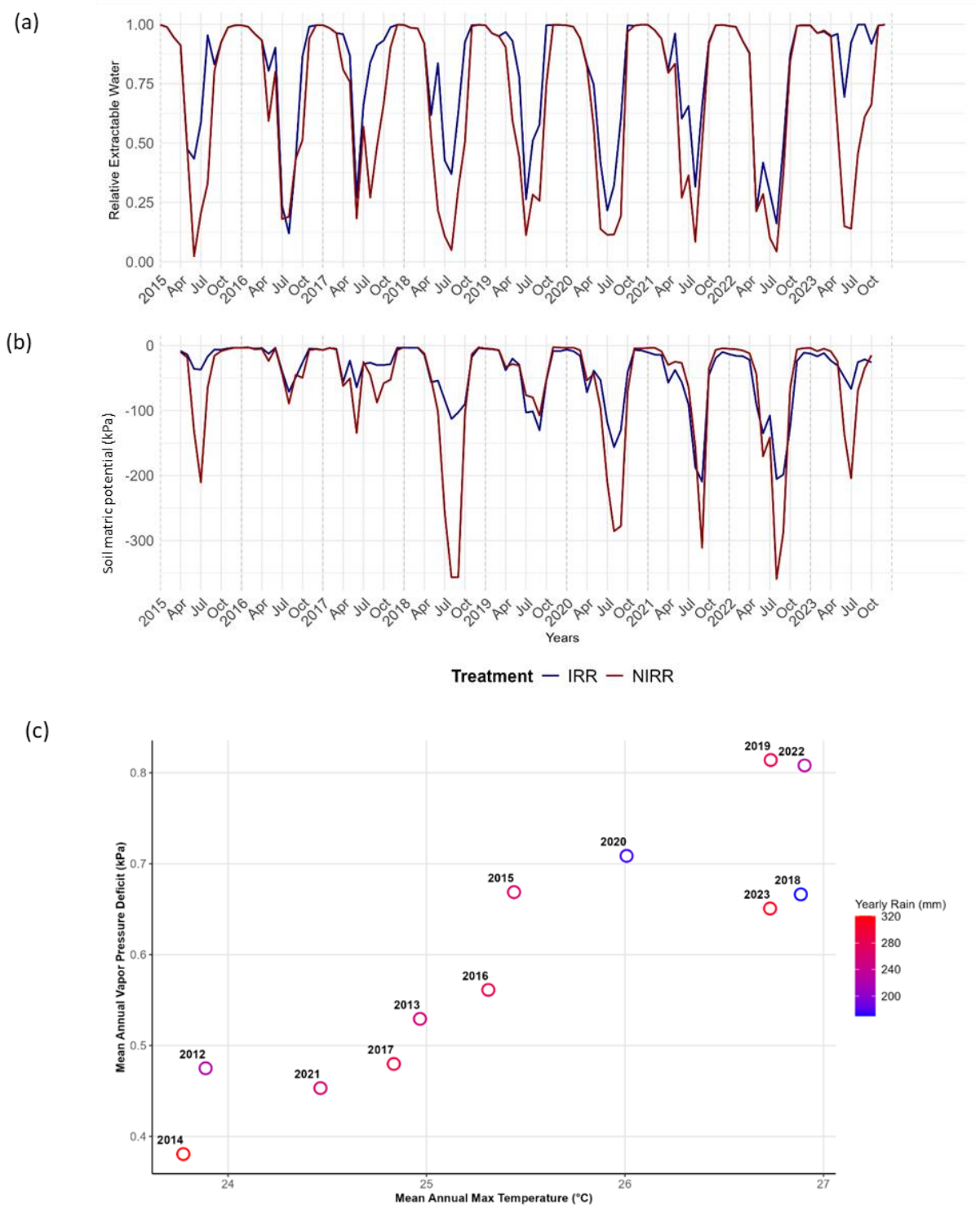


Fig Title see next page

Fig 3: a) Relative extractable water in NIRR part of experiment b) Temporal variation of soil matric potential in irrigated (IRR) and non-irrigated (NIRR) plots over years (at 24 cm depth) and c) scatterplot

of maximum temperature and Vapor Pressure deficit for years 2013 to 2023: each point represents a specific year, with colors corresponding to level of precipitation.

2.3. Field trait Measurements

The traits employed for this study were mainly ascertained for growth and development, i.e., total height, BH diameter, stem volume, height, and diameter annual increments. Total height (HT) was measured annually from 2013 to 2023 with a pole and later on with a Vertex, and diameter (D) was measured with a caliper at 1 m from the ground in two opposite directions, and in the last years with a tape. These measurements were done at the end of each year. Total stem volume (V) was computed based on Pauwels and Rondeux (1999) allometric relationship involving diameter and tree height. For annual development through the years, height increments or 'growth units' were computed as the living part of annual increments, effectively contributing to the final height. This was preferred to classical annual height increments (difference in height between years n and n-1) because of the frequent stems with top-dieback in the NIRR plot.

2.4. Sample collection and laboratory analysis

Annual Ring sizes: microdensitometry

Diametrical increment cores were collected around 1 m from the ground in early 2022 (January to March) with the help of an increment borer (5 mm diameter). In total, 1202 trees were selected based on common clones and families from the three taxa across the two plots of the experimental site. If any core was found with a defect or a damaged core during processing, a second collection was made for that particular tree in winter 2023 (October). These cores were sent to the INRAE Phénobois platform (Pierroton), where the X-ray images were produced from 2 mm-thick sawn boards. Images from scanned X-ray films were used to get microdensitometric profiles under WinDENDRO^{TM} (Regent Instruments Inc., 2008) at INRAe BioForA. Profiles were then checked for damages (breaks), and ring limits were ascertained and corrected when needed using R-scripts developed at INRAe-BioForA (R Core team 2024). Rings from 2014 and 2021 that were common to most trees were kept for further analysis. We converted ring widths to ring areas to better represent how much wood the trees produced each year and to reduce the influence of tree age on growth patterns. Diameter measurements were necessary to reconstruct core lengths when some rings around the pith were missing.

Water use efficiency and Vulnerability to xylem cavitation

In order to evaluate tree hydraulic wood properties, namely water use efficiency (WUE) and vulnerability to cavitation, which could directly or indirectly contribute to the differential growth behavior of the three taxa and thus to hybrid superiority, additional sampling and laboratory measurements were conducted. Due to the complexity, cost, and labor required to evaluate these two traits, a common approach was followed as described in Chapter II:

i) Tree sampling and laboratory analysis of a reference sample set of trees (training set) covering as large as possible phenotypic variability (based on *a priori* selection of trees from the three taxa and from families with distinct pedigrees, from the two plots (IRR and NIRR) for WUE and only one (IRR) for resistance to cavitation;
ii) At the same time, sampling of trees from the whole trial (prediction set);

iii) NIRS spectra acquisition on powder from all samples;
iv) Development of NIRS calibration models from the training set and
v) If the calibration model was reliable, the prediction of phenotypes on the full prediction set.

To assess the vulnerability to cavitation, branches were collected from 137 trees between January and February 2024. These branches measured approximately 10 to 15 cm long, with a basal diameter of over 10 to 15 mm. The selection was restricted to trees from the irrigated (IRR) plot to limit cavitation risk, most probably encountered in trees from the non-irrigated (NIRR) plot. Immediately after collection, each twig was properly labeled and wrapped in water-soaked tissue to prevent dehydration. The samples were then placed inside sealed plastic bags and stored in a cold room to preserve them until analysis. This storage method ensured that the samples remained hydrated. The cavitation analysis was performed at the Phenobois platform in Bordeaux using cavitrons equipped with a 14 cm rotor diameter. Cavitrons utilize centrifugal force to simulate increasing levels of water stress by progressively reducing the water potential within the xylem, allowing for precise measurements of hydraulic failure (Delzon et al. 2010). By gradually exposing the branches to higher levels of negative pressure, the instrument monitored water conductivity loss in response to stress. The data obtained from the cavitation experiments were analyzed using the "ftplc" package in R, developed by Duursma and Choat (2017). This package allowed for modeling the water conductivity loss as a function of increasing pressure. From these fitted curves, the P_{50} value—defined as the pressure at which 50% of maximum xylem conductivity is lost—was extracted (**Fig.4**).

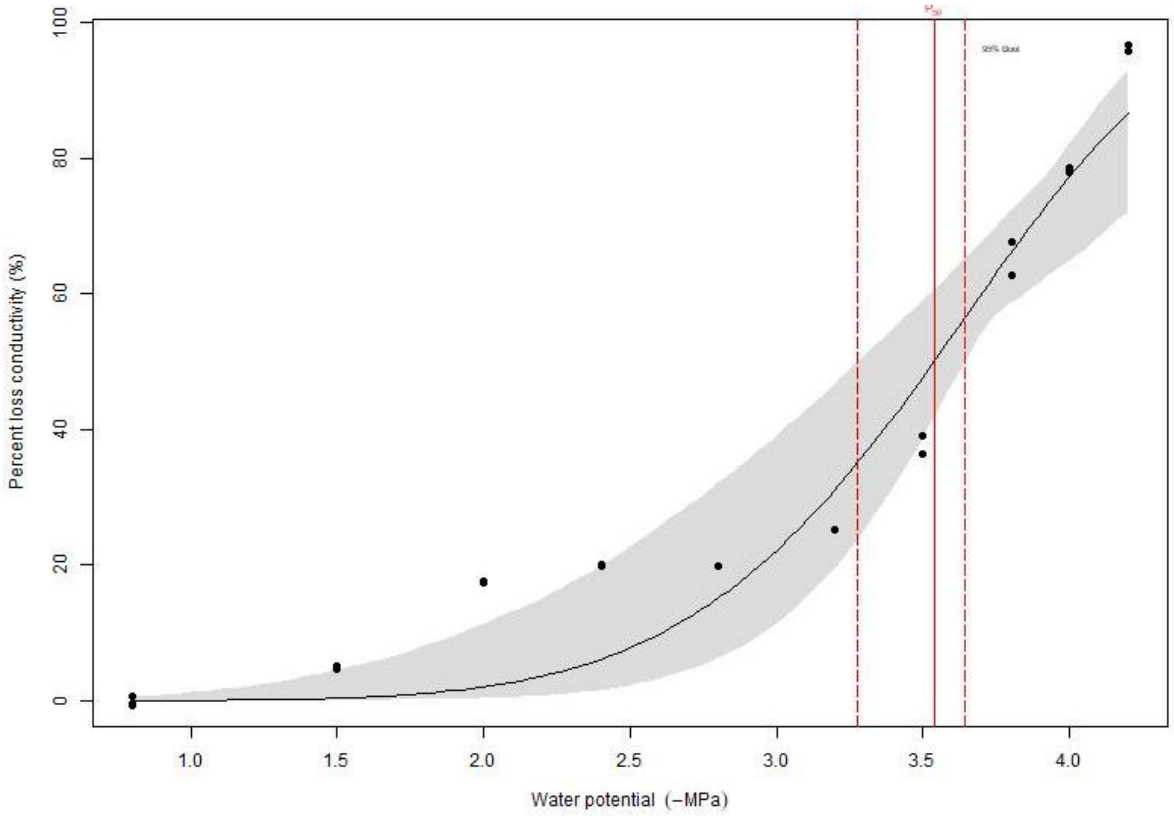


Fig 4: Vulnerability curve illustrating the relationship between percent loss of conductivity (PLC) and water potential (−MPa) for Sample 1. The black dots represent measured data points. The shaded gray region indicates the 95% confidence interval from bootstrapping. The red vertical solid line represents P_{50}, the water potential at which 50% loss of conductivity occurs, a key indicator of cavitation resistance.

For $\Delta^{13}C$ (which is considered a proxy for water use efficiency, measuring carbon isotope discrimination, Churakova et al. 2019), needle collection was made in late August of 2023 on 200 trees used as 'reference' and another collection set, which were supplementary trees for NIRS prediction. The trees were sampled to best represent the priority families and similar clones in both irrigated (IRR) and non-irrigated (NIRR) conditions. Needles were collected from five brachyblasts sampled in the upper part of the trees (sunshine needles). They were then lyophilized (freeze-dried) and initially stored at -70°C coolers. Subsequently, they were ball-milled at the Phenobois platform of INRAe Orléans and stored in glass vials with 1 g of powder. Carbon isotope discrimination ($\Delta^{13}C$, hereafter) was assessed using isotope ratio mass spectroscopy, which was done at the INRAe SILVATECH analytical platform in Nancy, France (Silvatech, INRAE, 2018. Structural and functional analysis of tree and wood Facility, doi: 10.15454/1.5572400113627854E1). These values were measured ($\delta^{13}C$) in ‰ relative to the isotope ratio of the Vienna Pee Dee Belemnite (VPDB) standard and were converted to carbon isotope discrimination, which is an indirect proxy for water use efficiency (Craig 1957, Farquhar and Richards 1984) using the formula:

$$\Delta^{13}C = \frac{\delta_{air} - \delta_{plant}}{1 + \left(\frac{\delta_{plant}}{1000}\right)}‰$$

where δ_{air} was assumed to be -8‰

The milled material (branches and needles powders) was also used to obtain near-infrared (NIRS) spectra using an FT-NIR spectrometer (Frontier Perkin Elmer, Waltham, MA, USA), with 64 scans conducted over a spectral range of 10,000 to 8,000 cm^{-1}. This was performed on the entire set, including both reference and supplementary samples, to develop reliable predictive models at the Phenobois platform INRAe Orléans (PHENOBOIS, INRAE, 2018. Platform for Phenotyping Hydraulic and Physicochemical Properties of Wood from Woody Genetic Resources. Resources. https://doi.org/10.15454/1.5572410490640864E12).

Resilience to drought

We were interested in assessing resilience in both apical (height) and radial (ring area) growth. Data were first standardized following Helama et al (2015) method, which allows the removal of ontogenetic growth trends while preserving climatic signals expressed in actual growth units. For height increments, annual data from 2013 to 2023 were used. Based on Helama et al. 2015's methodology, the time series was divided into three periods: 2013–2016, 2017–2020, and 2021–2023, with the last period (2021–2023) serving as the reference age class. Within each period, individuals were ranked based on their growth performance, and for the first two periods, the ranks were substituted by the growth performances of individuals with the corresponding ranks from the reference period. For ring area increments, data from 2015 to 2021 were used, and the series were similarly divided into three periods: 2015–2016, 2017–2018, and 2019–2021. The last period (2019–2021) was selected as the reference age class, and

ranks assigned within the earlier periods were replaced by the corresponding performance values from the reference period.

Secondly, the computation of resilience parameters requires identifying years with strong growth reduction directly linked to abiotic (or biotic) stresses (here, 'drought') and 'normal' years preceding and following the year with a stressful event. Classically, individual tree reaction is analyzed across favorable and stressful years. However, in our experimentation, trees in the non-irrigated plot (NIRR) are constantly submitted to (severe) water shortages, whereas trees in the IRR plot receive constant water (no or little water shortages), so clear identification of 'dry' years impact on growth is not visible in both cases. Therefore, we adopted another approach suggested by animal breeders (Berghof et al. 2019), evaluating resilience from the deviation between observed production and estimated production potential. In this study, we took advantage that the same genotypes (clones) were assessed in both 'optimal' (IRR) and 'drought-stress' (NIRR) conditions to assess resilience based on deviations between observed (due to drought disturbances in NIRR) and expected (in 'optimal' conditions in IRR) annual performances of an individual (**Fig 5** for the details).

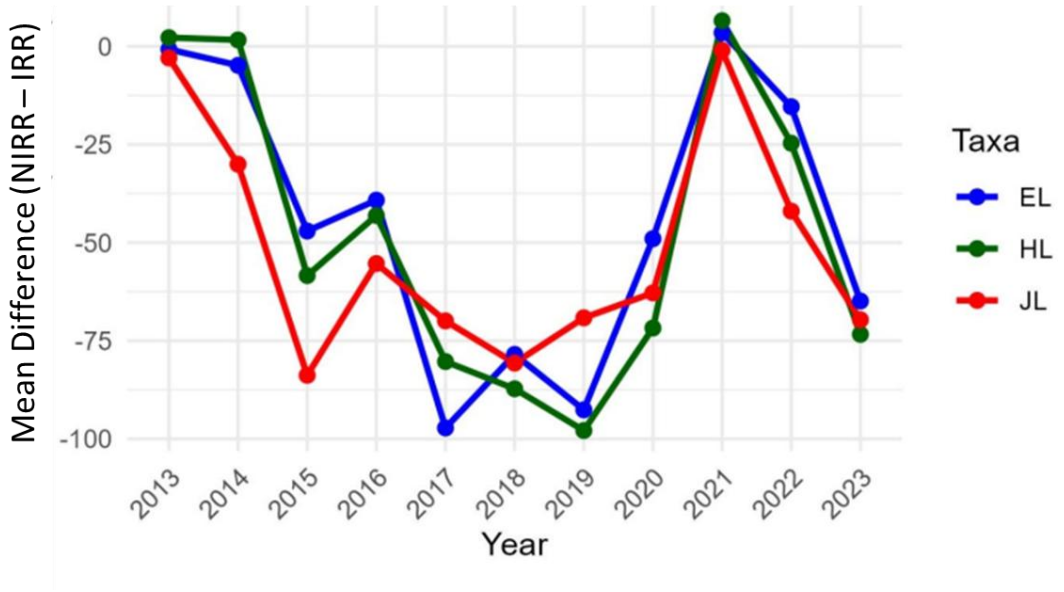


Fig 5 Example of growth deviation for height between NIRR and IRR treatments.

Based on these deviations, we identified three distinct growth phases including pre-stress, stress (drought), and post-stress periods. The stress period ranged from 2016 to 2020 (ring area) and 2016 to 2021 (height increment), with a peak of growth reduction in 2018-2019; 2014 and 2017 were identified as the pre-stress period, and 2021, with a strong recovery, was recognized as the post-stress period. Lloret et al. (2011) parameters were then computed. Indices include:

i) Resistance = Growth during stressful years/Growth during Pre-stress years
ii) Recovery = Growth during Post-stress years / Growth during the stress years, and
iii) Resilience = Growth during Post-stress years / Growth during Pre-stress years.

Finally, we quantified average growth reduction (AGR) during the drought period following Schwarz et al (2020). They defined AGR as the ratio of total growth reduction divided by the length of the recovery period. We adapted this concept as annual mean of deviations between growth under irrigation (IRR, representing optimal conditions) and observed growth under non-

irrigation (NIRR, drought conditions). This method allows us to isolate and quantify the mean cumulative effect of repeated water stress while accounting for growth potential.

2.5. Statistical Analysis

All analyses have been done on R software (R Core Team, 2024).

In a preliminary step, the data were adjusted for environmental effects, including block effects and micro-environmental variation, and individual tree competition. The competition index (CI) for each tree was calculated following Hegyi (1974) as the sum of the ratios of a neighboring tree's diameter to the product of the target tree's diameter and the distance between them, considering up to eight neighboring trees. Missing trees (due to mortality) were assigned a value of zero for competitiveness but kept at their original positions and distances. The computed competition index values were centered by standardization before being used for spatial correction. A spatial correction was performed using the spatial module in the R package "breedR" (developed at BioForA, Muñoz and Sánchez. 2015, R Core Team, 2024). The model included block as a random effect and CI as a covariate with a knot configuration of "9 x 9" for the non-irrigated site and "12 x 12" for the irrigated site (these knots were tested and chosen based on min AICc while comparing the models with knots). All available trees, including both clonal parents and family sibs (and some controls) of the three taxa, were used for spatial correction. Adjustments were performed separately for irrigated (IRR) and non-irrigated (NIRR) plots, which were treated as two distinct sites. However, for subsequent analyses, only intra- and interspecific families were considered.

The effects of taxa (3), watering treatments (2), and their interaction were analyzed using Analysis of Variance (ANOVA) on adjusted data. Post hoc comparisons were performed using Tukey's test to identify significant differences. The statistical model was as follows:

$$Y_{ijk} = \mu + T_i + W_j + (TxW)_{ij} + \varepsilon_{ijk}$$

where:

Y_{ijk} is the adjusted phenotype of individual k of taxon i in watering treatment j,
μ is the overall mean,
T_i *is* the effect of taxon,
W_j is the effect of the watering treatment,
$(TxW)_{ij}$ is the interaction between taxa and watering treatment,
ε_{ijk} is the residual error

The breeding values of parental clones (EL and JL) of hybrid combinations within each plot were estimated using a mixed model, which accounted for environmental variation and genetic effects, using the REML function of "breedR with an animal model.

Heterosis for growth traits (height, diameter, and volume) in 2023 and across multiple years (height and diameter increments) was estimated for each combination. Heterosis was calculated as the difference between hybrid means and either the mid-parent mean (mid-parent heterosis) or best-parent mean (best-parent heterosis) and was expressed in absolute and relative terms. This analysis was conducted separately for IRR and NIRR plots to assess heterosis expression

across watering treatments. The significance of heterosis was tested using a t-test, with standard errors obtained from models developed by Soehendi and Srinives (2005):

$$H = \frac{F_1 - MP}{S_H}$$

where,

F_1 =mean observation of the F_1 progenies from the total of n_1 individuals
MP = mean observation of both parents from $n_2 + n_3$ individual
S_H = Standard error of estimates of Heterosis

$$S_H = \left\{ \sqrt{\frac{VF_1}{n_1} + \frac{VP_1}{n_2} + \frac{VP_2}{n_3}} \right\}$$

where,

- VF_1 = Variance of F1
- VP_1= Variance of parent 1 (P_1)
- VP_2 = Variance of parent 2 (P_2)
- n_1, n_2, n_3 = Number of plants used to calculate means for F_1, P_1, and P_2, respectively

When the genotype-by-environment (G×E) interaction was present, it was further analyzed to determine the relative contribution of each genotype to the interaction term. This was done using: (i) Wricke's ecovalence parameter (Wricke 1962), which quantifies stability by assessing the proportion of variance of each genotype's in the interaction term, (ii) Hühn's non-parametric parameter S1 parameter (Huhn 1990), which evaluates rank changes across sites to identify the most stable or highly interactive genotypes.

To quantify phenotypic plasticity of height and diameter increments across an environmental gradient (water availability), we fitted linear mixed-effects models with the function "lmer" in the "lme4" package in R (Valladares et al. 2006) separately for each taxon. Several linear mixed-effects models differing in the structure of fixed (quadratic) and random (intercepts, slopes, and curvatures) effects were compared based on AIC, with the best model including a quadratic fixed effect of cRR and random intercepts and slopes for genotype to capture baseline growth differences and plasticity across the water gradient. The response variable was a standardized increment modeled as a quadratic function of mean-centered environmental conditions (specifically, relative soil water availability across sites and year). Random intercepts were included for both genotype and site to account for repeated measures and spatial structure. 95% confidence intervals were obtained via parametric bootstrapping with 1,000 simulations via "bootMer" in R that accompanies "lmer" (Lovell and McKay 2015).

$$\boldsymbol{Y_{ijk} = \beta_0 + \beta_1 . cRR + \beta_2 . cRR^2 + b_{0i} + b_{1i} . cRR_k + u_j + \varepsilon_{ijk}}$$

where:

Yijk: increment (height or diameter) for genotype *i*, at site *j*, under environmental condition *k*
β_0: overall intercept
cRR: centered water availability gradient
β_1: fixed effect of environment (cRR)
β_2: fixed effect of environment (cRR^2)
b_{0i}: random intercept for genotype *i*
b_{1i}: random slope for genotype *i*
u_j: random effect for site *j*
ε_{ijk}: residual error

To further explore broader growth strategies and assess potential trade-offs between fast growth and adaptive traits, we conducted phenotypic correlation analyses at both the family and clonal levels. First, we evaluated Pearson and Spearman correlations between individual tree growth parameters (absolute and relative growth in height, diameter, and volume) and stability indices (Huhn's stability and ecovalence), as well as heterosis, to test whether faster growth was associated with greater stability or increased heterosis.

Next, we performed Pearson correlations between mean family or clone values for height and diameter and key adaptive traits, including vulnerability to cavitation (P_{50}), intrinsic water use efficiency ($\Delta^{13}C$), and resilience components (Resistance, Recovery, Resilience, and Average Growth Reduction). Correlations involving cavitation were assessed only at the family level due to limited clonal replication. Correlations were performed using growth data and adaptive traits measured in the same years; for example, total height in 2021 was correlated with resilience indices (RRR) from the main drought period, and total height and diameter in 2023 were correlated with cavitation resistance measured in 2023.

All analyses were based on phenotypic (observed) correlations using individual family or clone levels, depending on the trait and sample structure. While these correlations do not disentangle genetic from environmental components, they provide valuable insights into functional trait associations and potential trade-offs influencing growth performance. In particular, they help identify whether high-performing genotypes also exhibit favorable physiological profiles, or whether growth is achieved at the expense of adaptive traits.

3. Results

3.1. Growth and Stability

Growth at age 10

At age 10, all three taxa exhibited significant differences in growth traits across irrigation treatments (**Table 2**). Under irrigated conditions (IRR), hybrid larch (HL) showed consistently superior performance in height, but for diameter, Japanese Larch was significantly superior to the hybrid. For stem volume, their performances were not significantly different. European larch (EL) showed the lowest performance across traits. Under non-irrigated conditions (NIRR), overall growth was reduced across all taxa, confirming an effect of water supply. Nonetheless, HL maintained a growth advantage in height, while JL matched HL in diameter and volume. Notably, the drop in mean volume from IRR to NIRR was sharpest in JL (-87.4 dm^3) – but similar in relative value (around 65%) to other taxa, highlighting its higher absolute growth potential but limitation in water-limited environments. Mortality was higher in the non-irrigated part of the experiment, and specifically in Japanese Larch (46.9%, Chapter I).

Table 2: Summary statistics for total height, BH diameter, and stem volume in Hybrid larch (HL), Japanese larch (JL), and European larch (EL) under irrigated (IRR) and non-irrigated (NIRR) conditions. Different letters after the means indicate significant differences (at $p < 0.05$) among taxa (Tukey post hoc test).

		IRR						NIRR					
Trait	**Taxa**	**no**	**Mean (SD)**	**Min**	**Max**	**CV (%)**	**Mortality rate (%)**	**no**	**Mean (SD)**	**Min**	**Max**	**CV (%)**	**Mortality rate (%)**
Height (cm)	HL	319	1311a (190)	298	1620	14.5	5.1	229	790a (167)	216	1175	21.2	33.2
	JL	282	1265b (113)	782	1540	8.93	6.7	185	738b (144)	291	984	19.6	46.9
	EL	284	1213c (175)	404	1560	14.4	3.1	186	716b (176)	207	1126	24.6	35.2
Diameter (mm)	HL	319	144.3b (29.9)	16.6	213.1	20.7		229	106.1a (23.8)	41.4	164.0	22.4	
	JL	284	154.9a (23.8)	61.5	219.4	15.4		185	1076a (27.0)	25.5	165.0	25.1	
	EL	282	127.0c (26.9)	27.4	192.0	21.2		186	94.5b (25.1)	15.3	168.5	26.6	
Volume (dm^3)	HL	319	119a (52.6)	0.328	265	44.1		229	40.2a (22.0)	2.22	119	54.7	
	JL	284	127a (43.5)	11.8	266	34.4		185	39.6a (22.2)	0.885	104	56.0	
	EL	282	85.5b (40.7)	1.22	217	47.6		186	30.4b (19.7)	0.237	118	64.8	

Due to differences in mortality rates (Chapter I) affecting mostly JL in the NIRR plot, mean growth levels mentioned above do not necessarily reflect the yield potential of the three taxa: production will rely on fewer trees, for example, in JL, which was submitted to less competition due to high mortality and could enhance individual tree growth. We then calculated the yield per ha (**Fig. 6**) for the three taxa. In the IRR plot, JL yield (nearly 17 m^3/ha/yr) slightly exceeded that of HL (16 m^3/ha/yr) and of EL (11.5 m^3/ha/yr) at age 10. In the NIRR plot, the yield gap between HL and JL narrowed (around 5.0 m^3/ha/yr), consistent with the non-significant difference in their individual volume means. Yield of EL remained the lowest at 4.0 m^3/ha/yr.

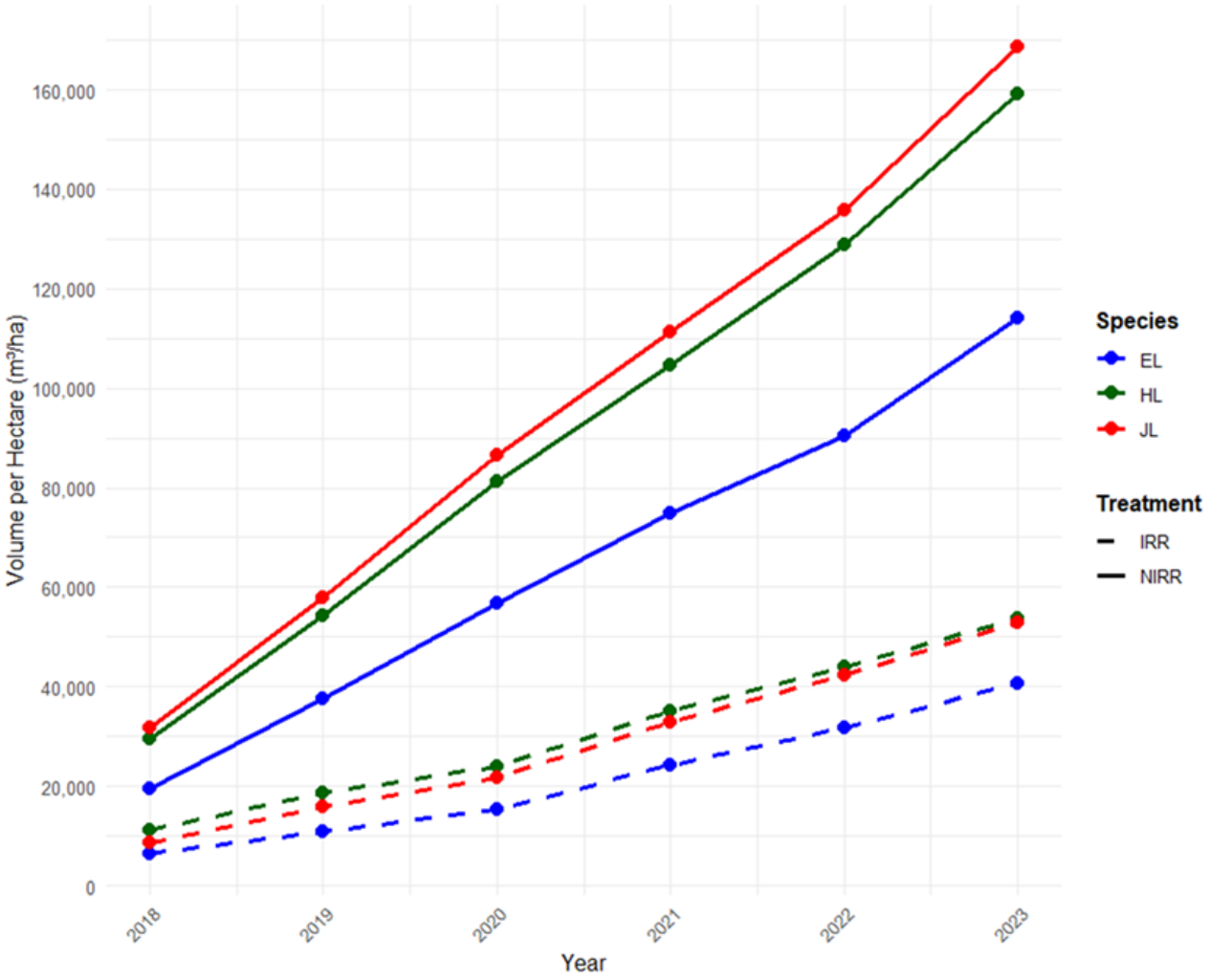


Fig 6: Estimated yield per hectare over the years (2018-2023) for European Larch (blue), Hybrid Larch (green), and Japanese Larch (red) in Irrigated (solid lines) and non-irrigated plots (dashed lines).

Comparison of taxa within combinations

Although taxa were represented by progenies not chosen at random, based on their contrasted levels of heterosis in the forest site where they were selected, taxa trends mainly remained the same at the individual combination level, with a few exceptions. To further explore within-taxon variability, we examined performance among individual families/combinations.

Under IRR conditions, HL exhibited superior performance in height across all combinations (except one 221x3194), consistently outperforming both EL and JL (**Fig. 7(A)**). For diameter, HL combinations were all intermediate except for the 104x3194 combination, which showed significantly higher growth over its parents (**Fig. 7(B)**). For volume, JL parents outperformed HL combinations except for two combinations: 104x3194 and 106x3200 (**Fig. 7(C)**). Under NIRR conditions, all individual HL combinations except one (221x3194) maintained their superiority over JL and EL for height (**Fig. 7(A)**). For diameter, half of the HL combinations (104x3194, 106x3194, 222x3194, and 222x3200) showed a significantly larger size than JL, but all were superior to the EL progenies (**Fig. 7(B)**). For stem volume, only two HL combinations out of eight were significantly less performant than their best parent (JL) (**Fig. 7(C)**).

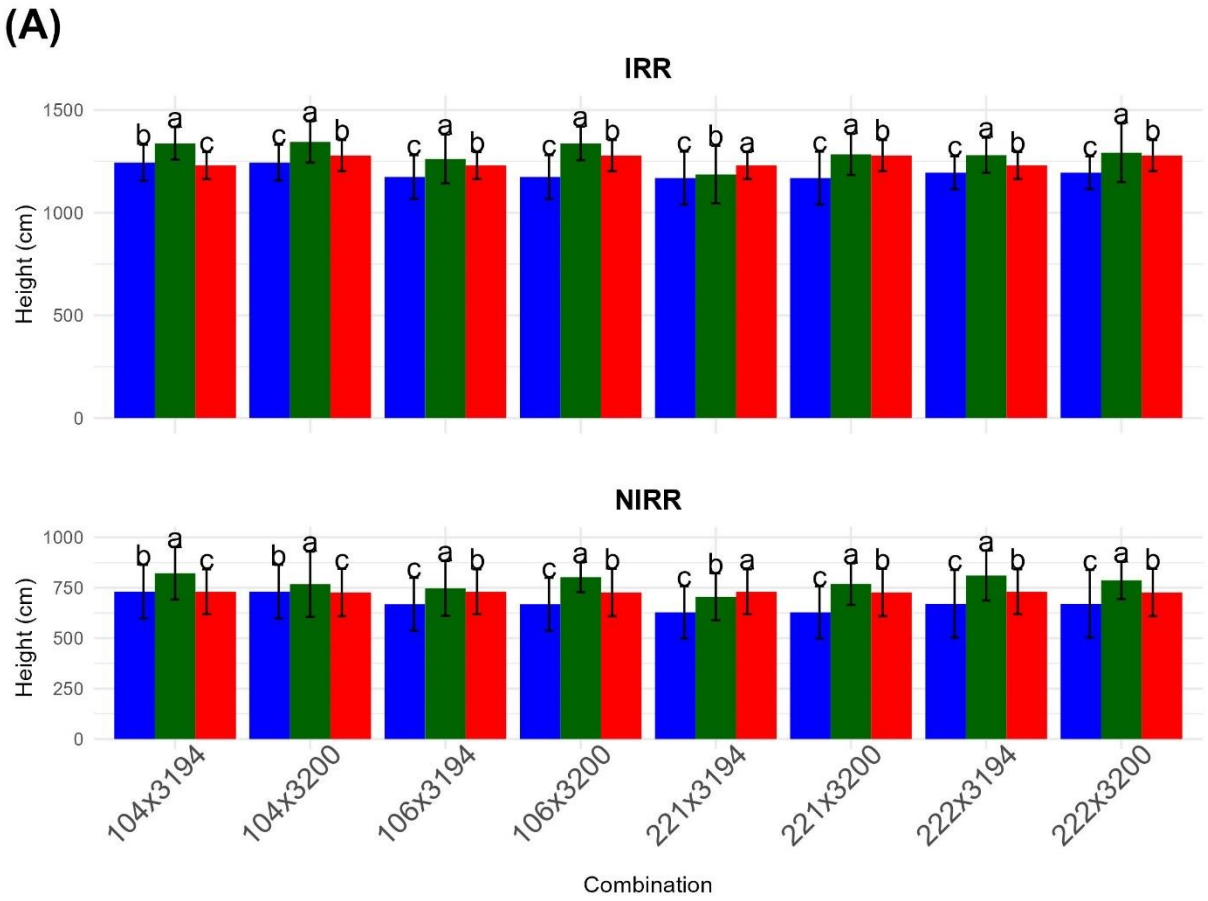


Fig 7 (A): Mean Total Height of European larch (EL, blue), Hybrid larch (HL, green), and Japanese larch (JL, red) across different combinations under irrigated (IRR) and non-irrigated (NIRR) conditions. *Different letters indicate significant differences at (p <0.05)*

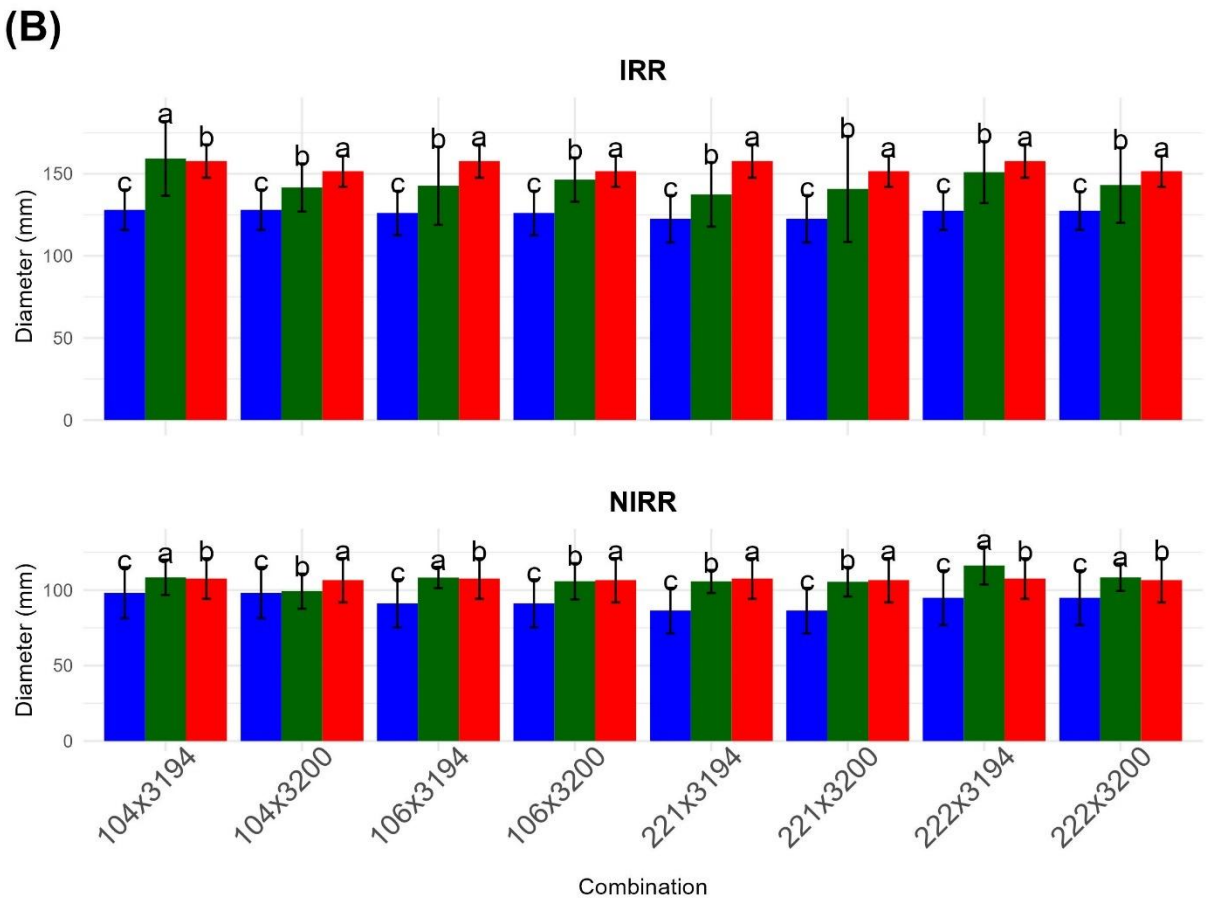


Fig 7 (B): Mean BH Diameter of European larch (EL, blue), Hybrid larch (HL, green), and Japanese larch (JL, red) across different combinations under irrigated (IRR) and non-irrigated (NIRR) conditions. *Different letters indicate significant differences at ($p < 0.05$)*

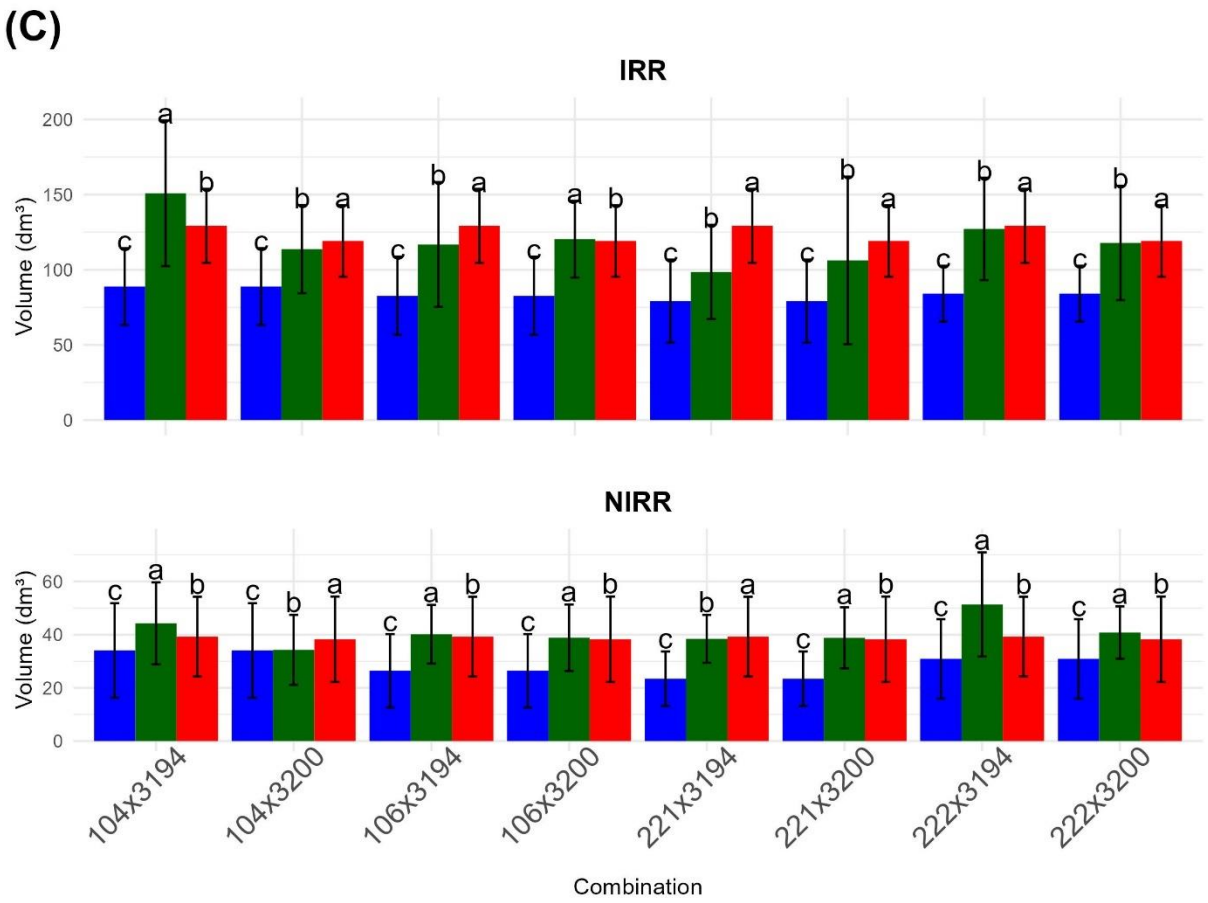


Fig 7(C): Mean stem volume of European larch (EL, blue), Hybrid larch (HL, green), and Japanese larch (JL, red) across different combinations under irrigated (IRR) and non-irrigated (NIRR) conditions. *Different letters indicate significant differences at (p <0.05)*

Growth over time

To better understand within-hybrid variability and its implications for long-term performance, we ranked HL combinations by height and diameter at age 10 under both IRR and NIRR conditions. Clear differences emerged among combinations (ANOVA, $p < 0.05$), allowing us to classify them into three performance groups. Under irrigation, combinations 104×3194, 104×3200, and 106×3194 ranked highest for both height and diameter, forming a high-performing group, while 221×3194 and 222×3194 fell in the low-performing group, with the others being intermediate. A similar classification was observed under NIRR, although performance gaps were generally narrower. Notably, 222×3194 stood out in NIRR plots with consistently superior height growth over both parental lines across all years, suggesting strong growth potential under limited water availability. Forming these groups also allowed us to track growth trends within each combination and explore whether different taxa maintained similar relative performances across years, a point that would be further analyzed in later sections. The high, medium, and low rankings observed at the forest site (Saint-Saud) from where the combinations were selected, were only partially conserved at the Orléans site. Some families maintained similar relative performance patterns, such as 106×3194, which consistently remained among the top performers, and 104×3194, which remained in the low-performing group. In contrast, 222×3194, which performed at an intermediate level at Saint-Saud, emerged as one of the best combinations under NIRR conditions at Orléans, suggesting a strong response to reduced water availability.

Annual growth trajectories over time are represented in **Fig S1, S2** for **annual increments for** height and diameter. Height growth patterns across the three taxa fluctuated over the years, with changes in performance between EL and JL. Under irrigation (IRR), JL outgrew EL in the early years (2014–2016) and again in 2022. However, from 2017 to 2020, particularly in 2017, EL showed superior growth. Growth levels between EL and JL converged in 2021 and 2023. Under non-irrigated conditions (NIRR), the performance gap between EL and JL was narrower, with both taxa showing overlapping growth levels in most years and no consistent dominance across the timeline. Under IRR, HL generally maintained a height advantage over both parents. In most combinations, HL displayed the highest increments, particularly in crosses involving the JL 3200 parent (e.g., 104x3200, 106x3200, 222x3200), where it frequently outperformed both EL and JL. JL also exhibited strong growth under IRR in several combinations, often comparable to HL, while EL consistently lagged behind. Under NIRR, however, patterns became more variable, and taxa rankings shifted over time. HL showed higher or comparable growth to both parents in early years, especially in combinations like 222x3194 and 221x3200. Yet, from 2020 onward, HL's performance under drought often declined relative to EL, and in several cases (e.g., 106x3194, 222x3200), HL growth converged with or fell below EL's.

Annual trajectories of diameter growth for different taxa under irrigation (IRR) and non-irrigation (NIRR) conditions are illustrated in Supplementary **Fig. S2**. These figures reveal distinct patterns among European Larch (EL), Hybrid Larch (HL), and Japanese Larch (JL) across multiple combinations. In most combinations, HL and JL outperformed EL, with JL generally exhibiting the highest growth, particularly in combinations involving the JL 3194 parent (106x3194, 221x3194, 222x3194), where JL's diameter increments consistently surpassed those of HL. Still, HL maintained a growth advantage over EL throughout. Under NIRR conditions, however, temporal variability in performance became more pronounced. While HL initially showed superior or comparable growth to both parents, especially in the 222x3194 combination (where it outperformed both EL and JL across all years), a decline in

HL's relative advantage was observed from 2020 onward in most combinations. In some cases, HL growth converged with or fell below EL's performance.

To determine whether hybrid superiority is recurrent or punctual, we examined the number of years (2015–2023) in which Hybrid Larch (HL) was superior or at least equal to the best-performing parent (EL or JL), under both treatments for height and diameter increments. For height increment, in some combinations, such as 104×3200 and 106×3200, HL repeatedly ranked in the top under irrigated conditions. In contrast, other combinations, like 221×3194 or 222×3200, exhibited HL superiority only in isolated years, indicating a punctual expression of superiority likely driven by specific climatic conditions or interactions with water availability. For diameter increments, under irrigation, HL showed relatively few years of superiority in most combinations. For instance, 106×3200 and 222×3200 expressed punctual HL dominance in only one to two years (2018 and 2022). In contrast, under NIRR conditions, HL superiority became more apparent and more frequent, especially in combinations like 106×3200, 221×3200, and 222×3194, where HL exhibited strict superiority in up to four or five years.

Annual growth trends across years with contrasting climates revealed clear differences among European Larch (EL), Hybrid Larch (HL), and Japanese Larch (JL). For height, HL generally outperformed both parents under irrigated conditions, but under drought (NIRR), its advantage was affected, particularly in hot, dry years like 2018, 2019, and 2022, where HL's performance converged with or dropped below EL in several combinations. In contrast, JL maintained strong height growth in dry years, while EL performed better in cooler, wetter years such as 2016 and 2021. For diameter, HL and JL typically surpassed EL under irrigation, with JL often leading, especially in combinations with JL 3194. Under drought, however, HL's advantage weakened over time, with performance gaps narrowing or disappearing.

Spatial and temporal stability

Growth differences between combinations across water supply treatments in 2023 (**Fig. 8)** revealed that overall, most genotype-by-environment (G×E) interactions were driven by scale changes rather than rank shifts. Due to irrigation, height, diameter, and volume increased across all taxa. Hybrid Larch (HL) combinations—particularly 104×3194 and 222×3194—achieved the highest gains. European Larch (EL) combinations showed moderate but consistent increases, while some Japanese Larch (JL) genotypes exhibited larger shifts, occasionally involving rank changes.

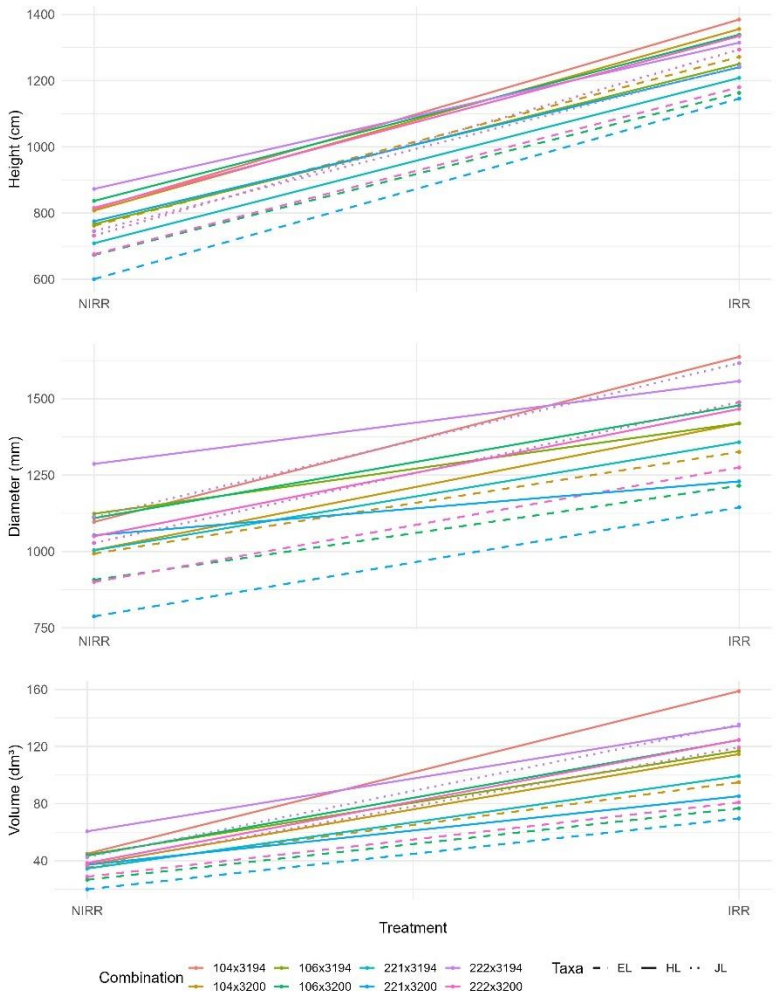


Fig 8. Height, diameter, and volume in 2023 for the different combinations across the two water supply treatments (non-irrigated: NIRR, irrigated: IRR): each color represents a different combination and traits the taxa (-- European Larch, ─ Hybrid Larch, and ··· Japanese Larch).

At age 10, stability analyses using Wricke Ecovalence and Hühn Stability Rank (**Fig. 9)** confirmed taxa-level differences in G×E across traits. HL combinations—especially 222×3194 and 104×3194—displayed the highest relative ecovalence values (up to ~40%) and Hühn scores (up to ~5), reflecting strong G×E interaction comprising both a change of relative magnitude in growth between NIRR and IRR plots, and a change of ranks. For example, for height increment, 222×3194 shifted from rank 2 under NIRR to rank 1 under IRR, while 104×3194 dropped from 3 to 6, indicating a dynamic reshuffling of performance. In contrast, EL combinations such as 221 and 222 had low relative ecovalences (<10%) and Hühn values (<2), indicating stable performance across plots. JL showed intermediate stability levels, with some genotypes like 3194 being more interactive, particularly for diameter, where its ecovalence exceeded 20%, and Hühn S1 reached nearly 3. This suggests that while ELs maintained consistent growth, HLs were more responsive to environmental variation, and JLs fell in between in terms of stability and interaction.

Stability analyses of annual height and diameter increments over the period 2013–2023 within each treatment (IRR and NIRR) revealed clear species-level differences in year-to-year performance dynamics (**Fig. 10**). For height increment, hybrid larch (HL) combinations—particularly 104×3194 and 222×3194—showed the highest temporal Ecovalence Percentages (up to ~12% in IRR and ~18% in NIRR) and Hühn S1 values (up to ~6), indicating pronounced interannual variation in both growth magnitude and ranking. These patterns reflect strong genotype-by-year (G×Y) interactions, especially under non-irrigated (NIRR) conditions, where hybrids responded variably to fluctuating climate over the decade. In contrast, European larch (EL) genotypes, such as 104 and 221, displayed low ecovalence (<10%) and Hühn scores (<4). Japanese larch (JL) genotypes, notably 3194 and 3200, were more variable under IRR. For diameter increment (bottom row), similar trends emerged: HL genotypes like 222×3194 exhibited strong G×Y effects, with ecovalence values peaking at ~30% in IRR and S1 values exceeding 3 in both sites. EL genotypes again showed the most stable performance, while JL responses varied depending on site, with greater instability under non-irrigation. Overall, these findings highlight that HL combinations, though often high-performing, exhibit substantial temporal interaction in growth, whereas EL maintains consistent year-to-year performance. JL genotypes appear more reactive under conditions.

Zooming into combination-level patterns, specific HL genotypes like 104×3194 and 222×3194 consistently showed greater instability than both of their parental lines. For example, 104×3194 exceeded its parents (104 and 3194) in both Ecovalence and Hühn, indicating more fluctuating performance across years. Similarly, 222×3194 was one of the most temporally responsive combinations, particularly for diameter under IRR, where its ecovalence exceeded 30%. These findings underscore that within the HL group, not all combinations behave equally - some hybrids are far more variable than others, depending on their specific parentage. Overall, while global taxa-level trends suggest that HLs are the most responsive and ELs the most stable, significant variation exists within each taxa, particularly among HL combinations.

Higher-performing HL combinations, such as 104×3194 and 222×3194 (from previous findings), tended to be moderately stable, showing strong G×E and G×Y interactions with high ecovalence and Hühn values. In contrast, lower-performing combinations and EL genotypes were more stable across environments and years.

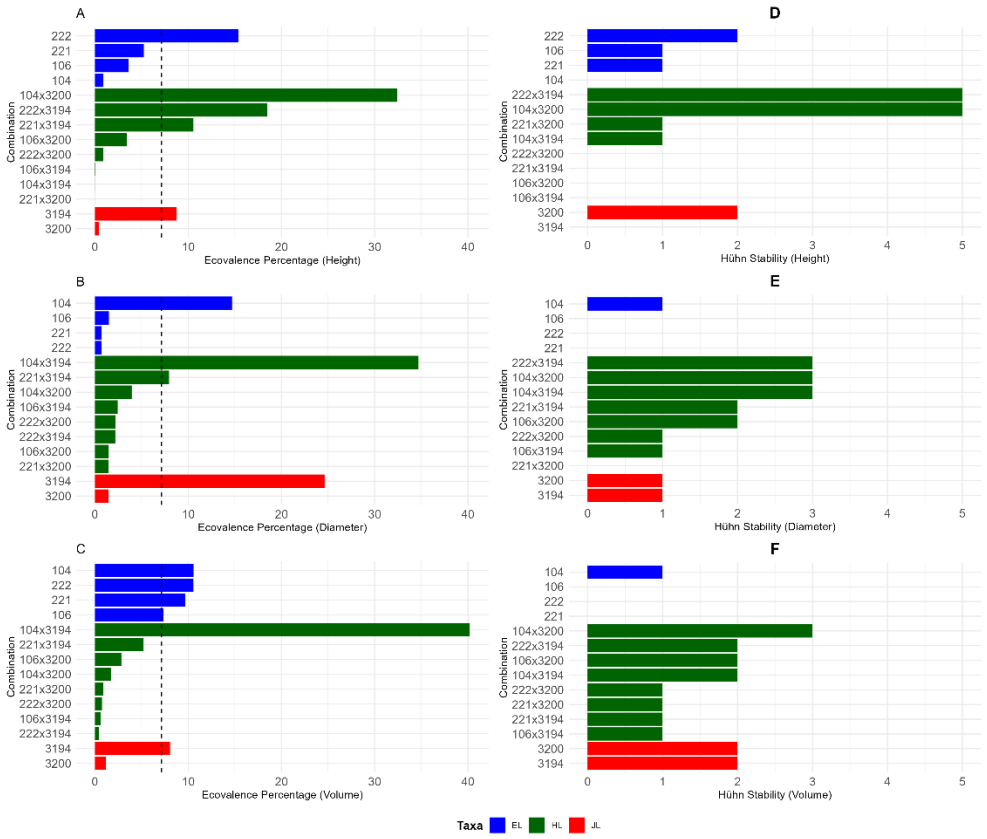
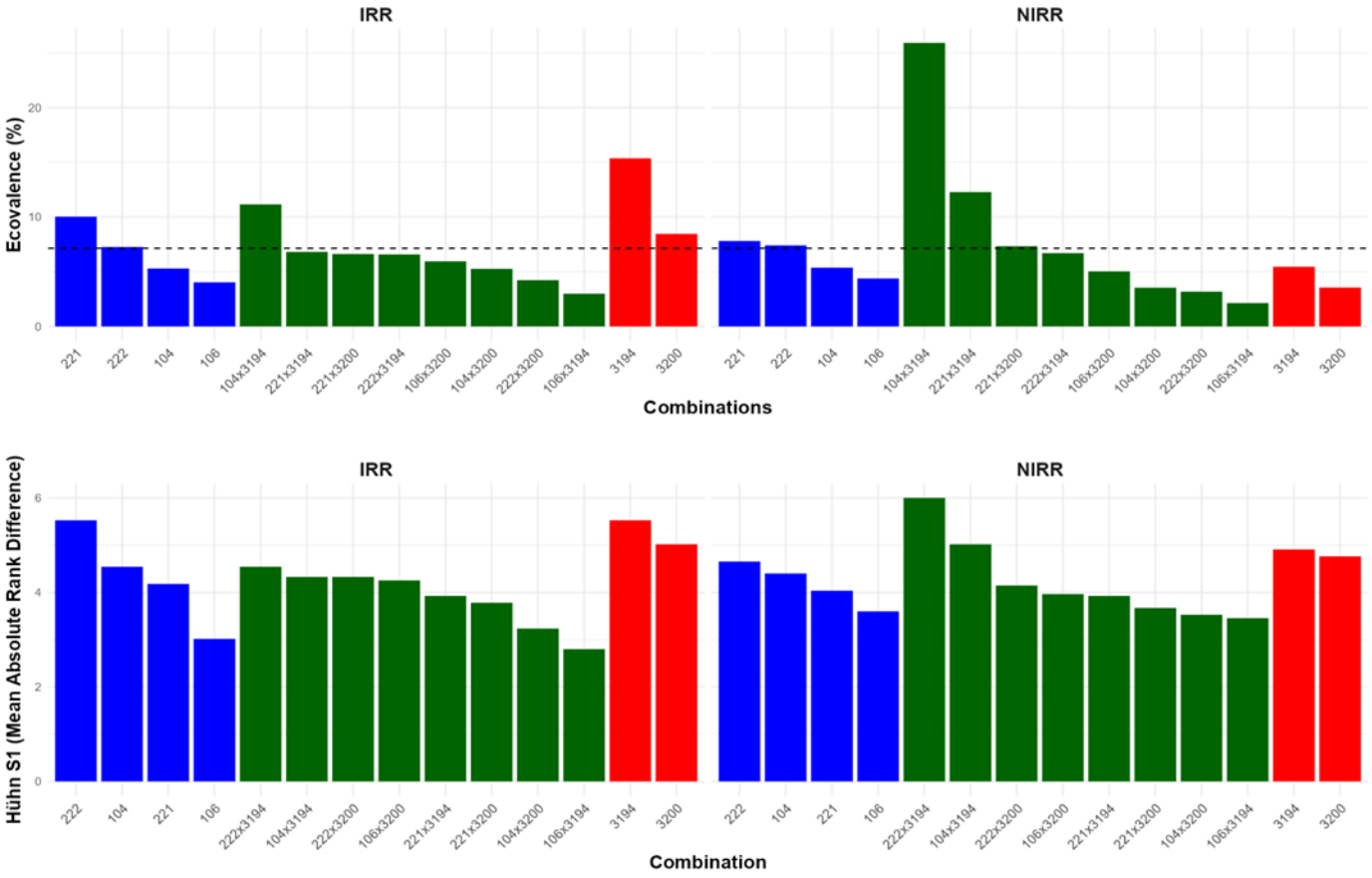


Fig 9: Wricke relative ecovalence (A-C) and Hühn S1 stability parameter (D-F) for total height, BH diameter, and stem volume for hybrid larch (HL) – green, Japanese larch (JL) - red, and European larch (EL) – blue, combinations. The combinations are sorted from most unstable to most stable across watering treatments per taxa. The dashed lines in A-C represent the threshold above which genotypes are considered unstable.

(A) Height increment (2013-2023)



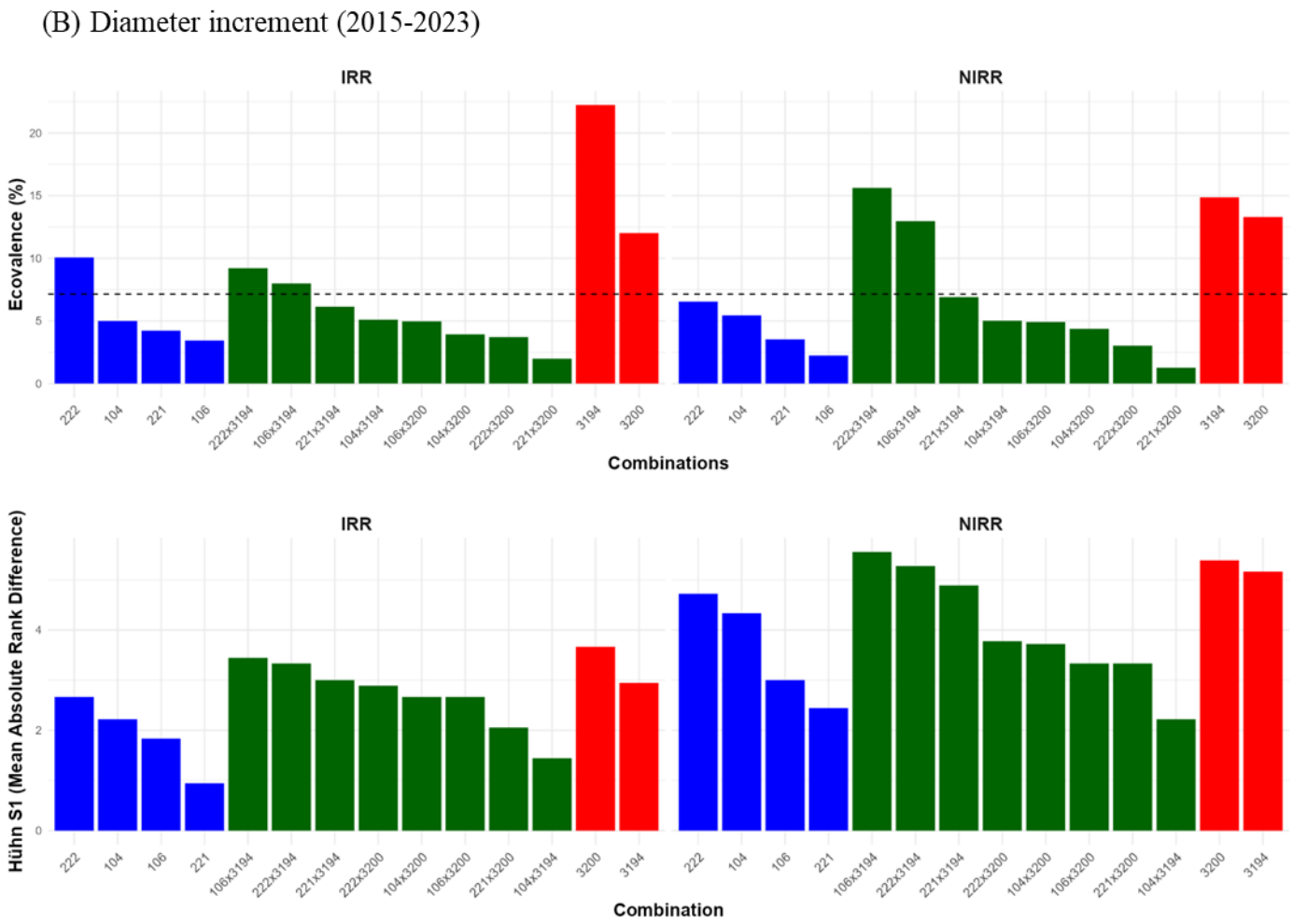


Fig 10. Temporal stability over years of combinations for the two watering treatments (IRR and NIRR): (A) Height increment and (B) Diameter increment: (top) Wricke relative ecovalence and (bottom) Hühn's S1 statistic, representing the mean absolute rank differences across years. Combinations are color-coded: red - Japanese Larch (JL), blue - European Larch (EL), dark green - Hybrid Larch (HL)

Table 3: Heterosis in Absolute and Relative values for the eight hybrid combinations in Irrigated (IRR) and Non-irrigated (NIRR) plots of experiment for Height, Diameter, and Volume. *Bold are values when Heterosis is significant (p<0.05)*

Trait	Combination	IRR **Absolute heterosis**	IRR **Relative Heterosis (%)**	NIRR **Absolute heterosis**	NIRR **Relative Heterosis (%)**
Height (cm)	104x3194	**68.27**	**5.38**	86.87	11.83
	104x3200	**75.64**	**5.96**	8.37	1.10
	106x3194	**79.06**	**6.69**	74.60	11.09
	106x3200	**155.88**	**13.19**	**104.70**	**15.01**
	221x3194	5.03	0.43	59.46	9.21
	221x3200	**103.39**	**8.76**	97.81	14.59
	222x3194	60.79	4.99	93.44	13.03
	222x3200	**73.34**	**6.02**	44.61	6.01
Diameter (mm)	104x3194	**1.63**	**11.41**	0.58	5.61
	104x3200	-0.03	-0.24	-0.65	-6.15
	106x3194	0.31	2.24	**1.03**	**10.52**
	106x3200	0.79	5.70	0.47	4.69
	221x3194	-0.55	-3.83	**1.29**	**13.95**
	221x3200	-0.11	-0.75	0.96	10.00
	222x3194	0.89	6.24	**1.48**	**14.57**
	222x3200	0.21	1.50	0.38	3.66
Volume (dm3)	104x3194	**40.80**	**37.07**	7.70	21.07
	104x3200	6.54	6.10	-5.42	-13.65
	106x3194	13.45	13.01	8.32	26.14
	106x3200	**19.82**	**19.72**	3.86	11.05
	221x3194	-8.73	-8.14	11.67	43.62
	221x3200	1.80	1.72	8.91	29.81
	222x3194	19.29	17.87	**16.63**	**47.91**
	222x3200	12.81	12.19	2.92	7.70

Mid-parent Heterosis

At age 10, clear differences in growth performance were observed among larch taxa across irrigation treatments (**Table 2**). Hybrid larch (HL) combinations consistently outperformed their parental species in mean height, diameter, and volume. Under irrigation (IRR), HLs reached average heights around 1300 cm, exceeding Japanese larch (JL, 1200 cm) and European larch (EL, 1150 cm). Similar trends were observed under non-irrigated (NIRR) conditions, where HLs maintained a higher mean height (900 cm) compared to JL (850 cm) and EL (800 cm). Diameter and volume followed almost the same ranking, with HLs leading under both treatments. Variation within taxa was also notable: HL combinations showed the variable range in performance, with some genotypes like 104×3194 and 222×3194 ranking among the best, while others (e.g., 221×3194) fell behind.

Heterosis analysis reinforced these patterns (**Table 3**). Under IRR, mid-parent heterosis (MPH) for height ranged from 0.43% (221×3194) to 13.19% (106×3200), while for volume it peaked at 37.07% (104×3194). Under NIRR, heterotic gains were even more pronounced: 222×3194 exhibited the highest MPH for volume (+47.91%), followed by 221×3194 and 221×3200, both of which outperformed their parents under drought despite weak performance under irrigation. Notably, 106×3200 showed consistently high MPH across all traits and treatments, reflecting stable expression. In contrast, some combinations, like 221×3194, displayed condition-specific heterosis, performing poorly under IRR but gaining markedly under stress. For diameter, heterosis was limited. Under IRR, only 104×3194 showed significant MPH (11.41%). However, under NIRR, combinations such as 221×3194, 221×3200, and 222×3194 achieved MPH values above 10%.

In Table 4, the effect of years and watering treatments on the annual level of heterosis on annual height and diameter increments was tested. Heterosis for height increment varied significantly across years ($p < 0.001$) from -3 to +25 %, but treatment had no overall effect. However, a significant Year × Treatment interaction ($p < 0.001$) was observed, indicating stronger heterosis in specific years under water stress. For diameter increment, heterosis varied significantly across years, with a strong Year × Treatment interaction ($p < 0.001$), indicating substantial temporal G×E. While irrigation had a minor overall effect ($p = 0.028$), heterotic responses differed markedly by year.

Post hoc analysis revealed year-to-year variation in height increment heterosis under both treatments (**Table 5**). Within the IRR treatment, 2017 stood out with the highest estimated marginal mean (16.64 cm,23.30%). Years such as 2016 and 2018 also showed above-average heterosis (9.39–11.38 cm), while 2022 had the lowest (-6.07 cm). Under NIRR, 2016 and 2017 also showed high heterosis (>14 mm). For diameter increment, differences between years were less marked under IRR, with most years showing no significant differences (all in Tukey group "a"), and absolute values close to zero. Under NIRR, 2016 (3.07 mm) years like 2015 and 2017 showed higher means (1.3–1.9 mm).

This revealed that mid-years, particularly 2016 and 2017, had a stronger influence on the annual expression of heterosis in both height and diameter.

Table 4: Analysis of Variance (ANOVA) of watering treatment and years effects on Absolute heterosis over the years, for Height and diameter increments.

Height increment					
Year * Treatment		**df**	**F value**	**Pr(>F)**	**Significance**
	Year	10	9.7434	1.65E-12	***
	Treatment	1	0.2625	0.6092	ns
	Year: Treatment	10	3.923	9.00E-05	***
Diameter increment					
Year*Treatment		**df**	**F value**	**Pr(>F)**	**Significance**
	Year	8	7.1653	8.551e-08	***
	Treatment	1	4.942	0.02799	*
	Year: Treatment	8	9.736	1.868e-10	***

Table 5: Estimated marginal means (emmean) of absolute heterosis for height and diameter increment per year under irrigated (IRR) and non-irrigated (NIRR) treatments. Values followed by different letters indicate significant differences between years within each treatment based on Tukey-adjusted post hoc comparisons ($p < 0.05$).

Years	Height increment		Diameter increment	
	Average: IRR	Average: NIRR	Average: IRR	Average: NIRR
2013	10.716^{bc}	13.06^{bcd}	-	-
2014	5.59^{abc}	25.26^{d}	-	-
2015	0.11^{ab}	7.16^{abc}	-0.18^{a}	1.34^{cd}
2016	9.39^{bc}	14.83^{cd}	-0.15^{a}	3.07^{d}
2017	16.64^{c}	19.17^{cd}	-0.03^{a}	1.95^{cd}
2018	11.38^{bc}	8.78^{abc}	0.09^{a}	0.49^{bc}
2019	5.806^{abc}	-6.33^{a}	-0.066^{a}	0.78^{bc}
2020	8.923^{abc}	-3.39^{a}	-0.313^{a}	-1.41^{a}
2021	-0.16^{ab}	2.902^{abc}	0.77^{a}	-0.96^{ab}
2022	-6.066^{a}	-1.680^{ab}	0.92^{a}	-0.55^{ab}
2023	2.77^{abc}	-6.54^{a}	-0.54^{a}	-0.62^{ab}

Across years, significant heterosis was observed in specific years and combinations for height increment, often with higher values in non-irrigated (NIRR) conditions (Tables **S1 and S2**). For instance, 222×3200 in 2014 showed 75.04% relative heterosis in NIRR, significantly higher than 3.41% in IRR, indicating a stronger heterosis under water stress. Similarly, 222×3194 in 2016 had 49.91% mid-parent heterosis in NIRR, much greater than 10.02% in IRR. In contrast, 104×3194 in 2017 exhibited a significant 30.85% heterosis in NIRR despite being non-significant in IRR. Significant heterosis for annual diameter increment followed a similar pattern to height, with hybrid combinations showing enhanced performance, particularly under drought conditions. For example, 222×3194 showed levels of heterosis in NIRR with 25.69% in 2017 and 13.33% in 2018, far exceeding values observed under IRR, which remained non-significant. Similarly, 106×3194 expressed 45.26% and 25.14% relative heterosis in NIRR during 2016 and 2017, while remaining non-significant under irrigation. These findings highlight a trend of heterosis, where hybrid vigor is more strongly expressed under limited water availability and with interannual variation indicating that certain years, especially mid-years 2016 and 2017, amplify the expression of heterosis.

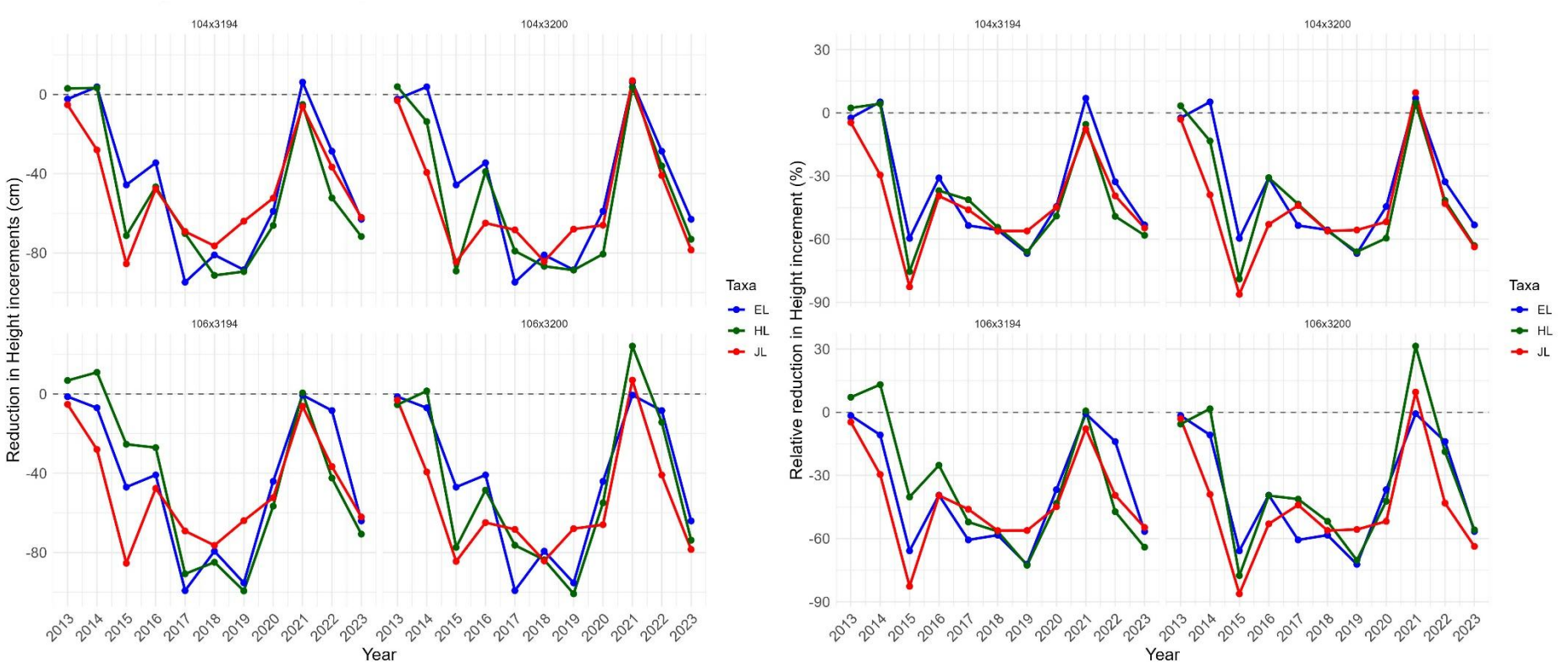
3.2. Taxa Response to Drought

Growth reduction under drought over the years

To characterize the impact of drought on the three taxa, we computed the absolute and relative growth reductions for annual height and diameter increment between the IRR and NIRR treatments.

For height increment, JL growth, compared to that of other taxa, strongly declined right after the start of the experiment in 2015, but this decline was not worsened with subsequent drought years; instead, overall reduction is maintained at a lower level than EL and HL (**Fig 11**). At worst, the reduction in height growth reached -80 to -90 cm (relative value: -60 to -85 %) in 2015. EL resisted better the first years of drought (2015-2016), but then was heavily affected by subsequent droughts. Later, the maximum reduction was observed in 2017 with an absolute reduction of -85 to -95 cm (relative value: -50 % to -60 %) and in 2019 with -85 to -95 cm (relative value: -70 % to -75 %). HL behavior was more variable according to combinations, but the impact of drought was more progressive, with the heaviest reduction being observed later than for the parental species: in 2018-2019, growth was reduced down to -85 to -95 cm (relative value: -70 % to -75 %).

For diameter increment, JL showed the earliest and strongest reduction, with sharp drops already in 2015–2016, reaching absolute decreases of up to -90 to more than -100 mm and relative losses around -50% to -60% during those early years (**Fig 12**). EL was initially less affected, maintaining higher radial growth during the early phase (2015–2016), but experienced more severe reductions in later years. In particular, EL showed maximum reductions in 2018–2019, with absolute values of -80 to -90 mm and relative reductions of -50 % to -55 %. HL displayed a more delayed and buffered response: although some combinations showed early reductions, the most impacts occurred later, especially from 2020 to 2022. In those years, HL reductions reached similar levels to the parental species and even greater (-90 to -100 mm; -55% to -70%), but the timing and magnitude varied between combinations. This progressive reduction pattern suggests that HL may have had slightly improved radial growth maintenance in the early years.


104x3194
104x3200
106x3194
106x3200
Reduction in Height incerements (cm)
Relative reduction in Height increment (%)
Year
Taxa
EL
HL
JL

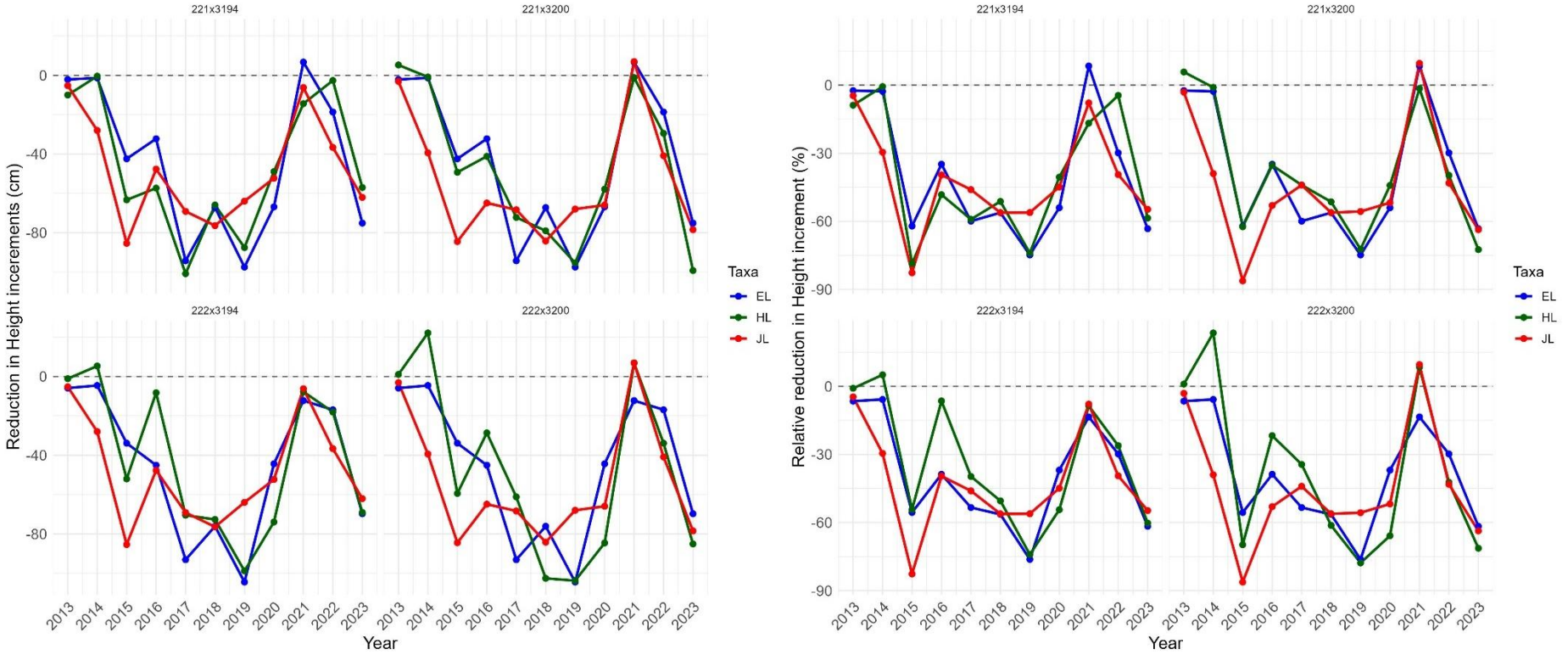
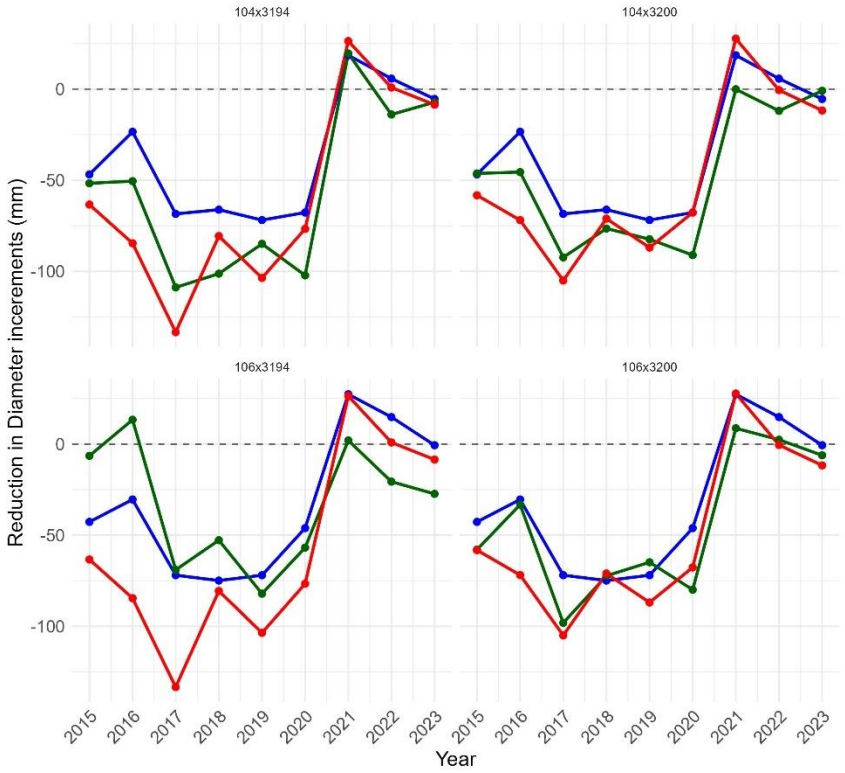
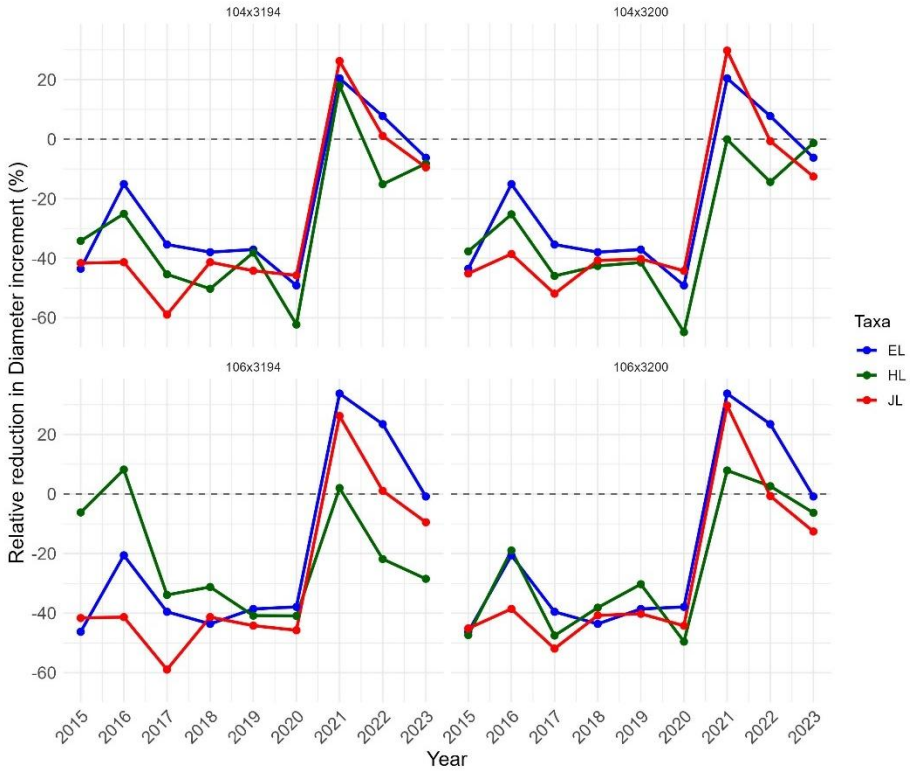


Fig 11. Absolute reduction and Relative reduction in Height increment over the years for the eight combinations: for each, the blue, red, and green curves correspond to European, Japanese, and hybrid larches, respectively.


104x3194
104x3200
106x3194
106x3200
Reduction in Diameter incerements (mm)
0
-50
-100
2015
2016
2017
2018
2019
2020
2021
2022
2023
Year
Taxa
EL
HL
JL


104x3194
104x3200
106x3194
106x3200
Relative reduction in Diameter increment (%)
20
0
-20
-40
-60
2015
2016
2017
2018
2019
2020
2021
2022
2023
Year
Taxa
EL
HL
JL

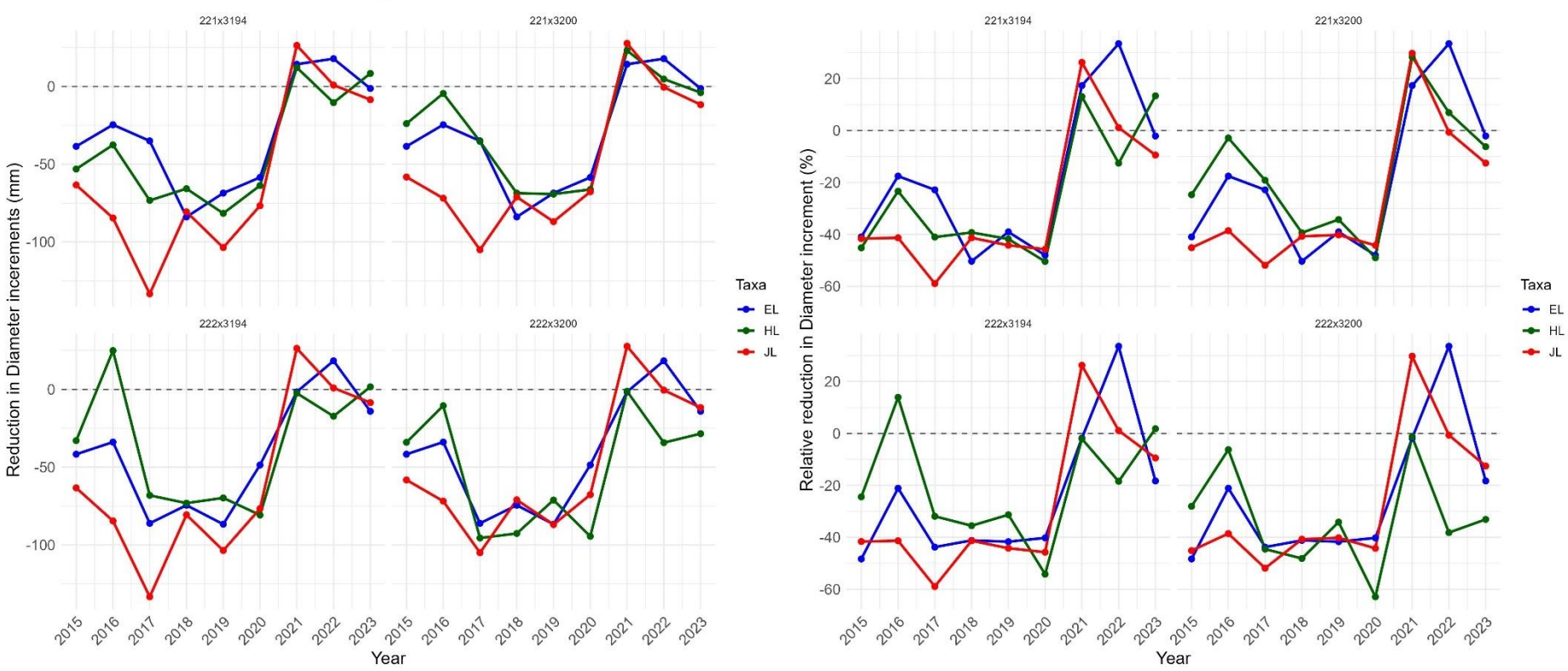


Fig 12. Absolute reduction and Relative reduction in diameter increment over the years for the eight combinations: for each, the blue, red, and green curves correspond to European, Japanese, and hybrid larches, respectively.

Interestingly, combinations classified as high-performing under irrigation (e.g., 104×3194, 106×3194) often exhibited the largest growth reductions under drought, especially in the early years. In contrast, some lower- or intermediate-performing combinations (e.g., 221×3194, 222×3200) maintained more stable growth across treatments and years, potentially reflecting more stable drought responses

Growth resilience under drought

Growth resilience to drought over the 2015–2020 period was assessed using four indicators: the average growth reduction in apical and radial increments between irrigated and non-irrigated conditions, along with the three classical resilience indices proposed by Lloret et al. 2011 (resistance, recovery, and resilience) (**Fig. 13**).

The three taxa demonstrated distinct drought-response patterns in terms of height increment (**Fig. 11**). European larch (EL) consistently exhibited the lowest resistance to the cumulated drought observed in 2018-2019 (0.4-0.6) among the three taxa, but with moderate average growth reductions across combinations (typically 5.5–6.0 cm/year) intermediate between JL and HL. In most combinations, Japanese larch (JL) showed the best resistance (up to 0.65–0.75) to the cumulated drought in 2018-2019, but with varying levels of average growth reduction (~5.2–6.2 cm/year) over the period 2015-2020, above or below those of other taxa. Its recovery capacity (~1.7–2.2) was usually lower than EL's. Overall, its resilience was the highest among the three taxa. Hybrid larch (HL) displayed a broader spread in both average growth reduction during 2015-2020 and resilience metrics to drought in 2018-2019, with average reductions between 5.0 and 6.5 cm/year, depending on the combination. While its resistance was generally similar to EL (0.5–0.6), HL showed recovery values between 2.0 and 2.5, highlighting its capacity for post-stress compensation.

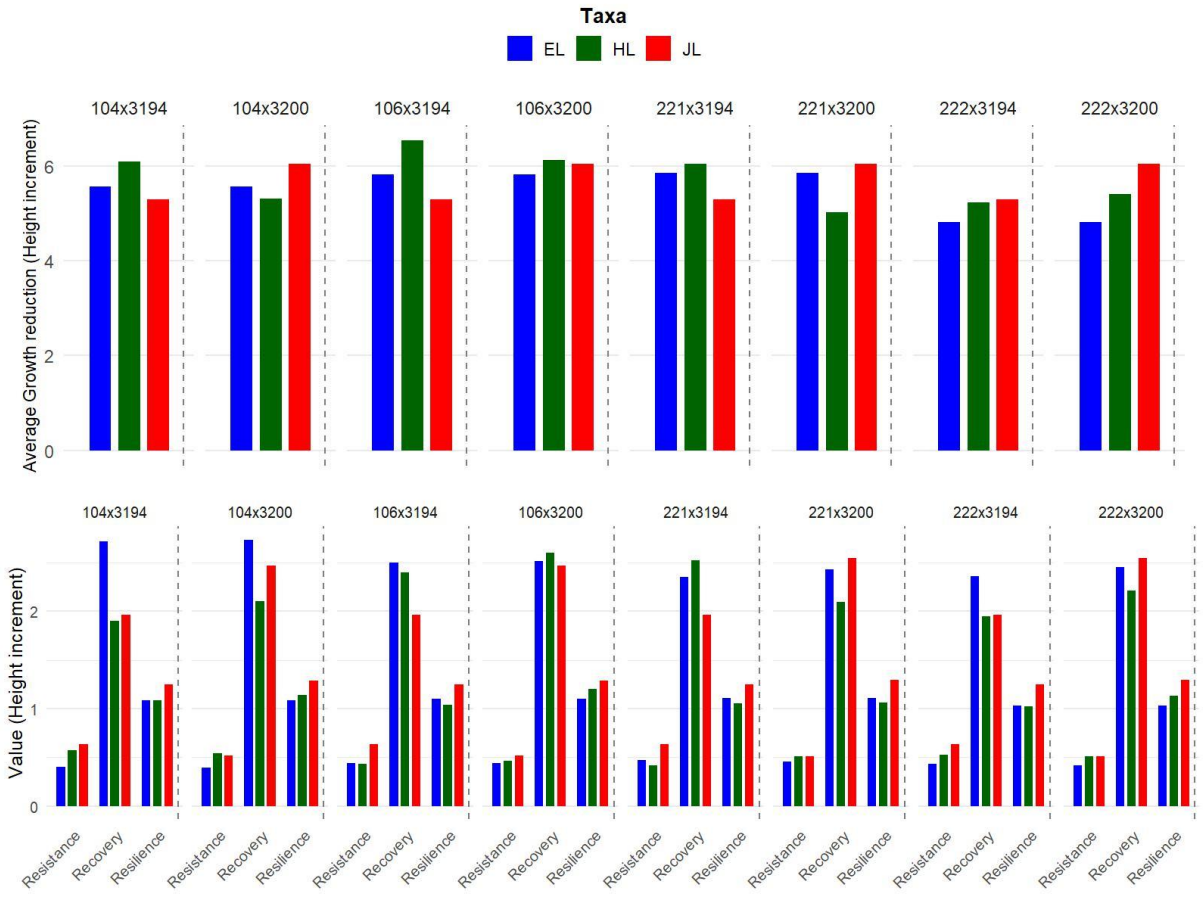


Fig 13 (A). Bar plots showing the Average growth reduction during 2015-2020 (top panel) and resilience parameters (bottom panel) of height increment across the three taxa (EL: blue, HL: green, and JL: red) within respective combinations.

For radial growth, **Fig. 13(B)** presents bar plots with the average growth reduction (2015–2020) and resilience parameters—resistance, recovery, and resilience—at the ring area level. In the top panel, which depicts average growth reduction, EL generally experienced moderate to high reductions, ranging from approximately 130 mm to 155 mm across combinations. JL showed slightly lower reductions in some combinations (e.g., 104×3194, 221×3194), with values ranging from 125 mm to 150 mm, though this trend was not consistent across all crosses. HL displayed greater variability, with reductions ranging from about 100 mm (e.g., 106×3200) to more than 160 mm (e.g., 106×3194, 222×3200). This suggests that while some HL combinations were more drought-affected in terms of radial growth, others-maintained reductions comparable to or even lower than those of their parent.

In the bottom panel of Fig 13(B), taxa responses to drought are illustrated through three resilience parameters: resistance, recovery, and resilience, assessed at the ring area level. EL showed the highest resistance values across most combinations, particularly in 104×3194, 221×3194, 221×3200, 222×3194, and 222×3200, with values frequently in the 0.6–0.8 range. This indicates EL's strong ability to maintain growth under drought, reflecting a conservative drought-tolerance strategy. In contrast, JL exhibited low resistance, often below 0.5, indicating greater initial growth suppression under drought stress. HL displayed intermediate resistance, typically in the range of 0.5–0.7. Regarding recovery, JL consistently showed the highest values, often exceeding 2.0, especially in combinations such as 104×3194, 221×3194, and 222×3194. This pattern highlights JL's tendency to adopt a compensatory growth strategy,

making up for earlier drought impacts through accelerated post-stress growth. EL, by contrast, exhibited lower recovery values, generally below 2.0, aligning with its strategy of damage minimization rather than compensation. HL again stood out with consistently good recovery, ranging from 1.8 to 2.2, across most combinations. As a result, resilience, which reflects the overall capacity to return to pre-drought growth levels, was often highest in HL, especially in combinations like 222×3200 and 106×3194. This reflects HL's capacity to balance moderate resistance with strong recovery, combining the strengths of both parents:

At the combination level, patterns in resilience traits align with performance rankings to some level. The high-performing combinations under IRR-104×3194, 104×3200, and 106×3194 - generally showed lower to moderate annual growth reductions, moderate to high resistance (0.6–0.8), and especially strong resilience and recovery, particularly in HL and JL taxa. For instance, in 106×3194, HL showed both the highest resilience and recovery in height and ring area, confirming its robust rebound capacity after stress. In contrast, low-performing combinations under IRR like 221×3194 and 222×3194 showed higher reductions and lower resistance in EL, though HL and JL sometimes compensated with better recovery. Interestingly, under NIRR, 222×3194—a low IRR performer—became a top performer, with HL showing high resilience and recovery values, supporting an adaptive response.

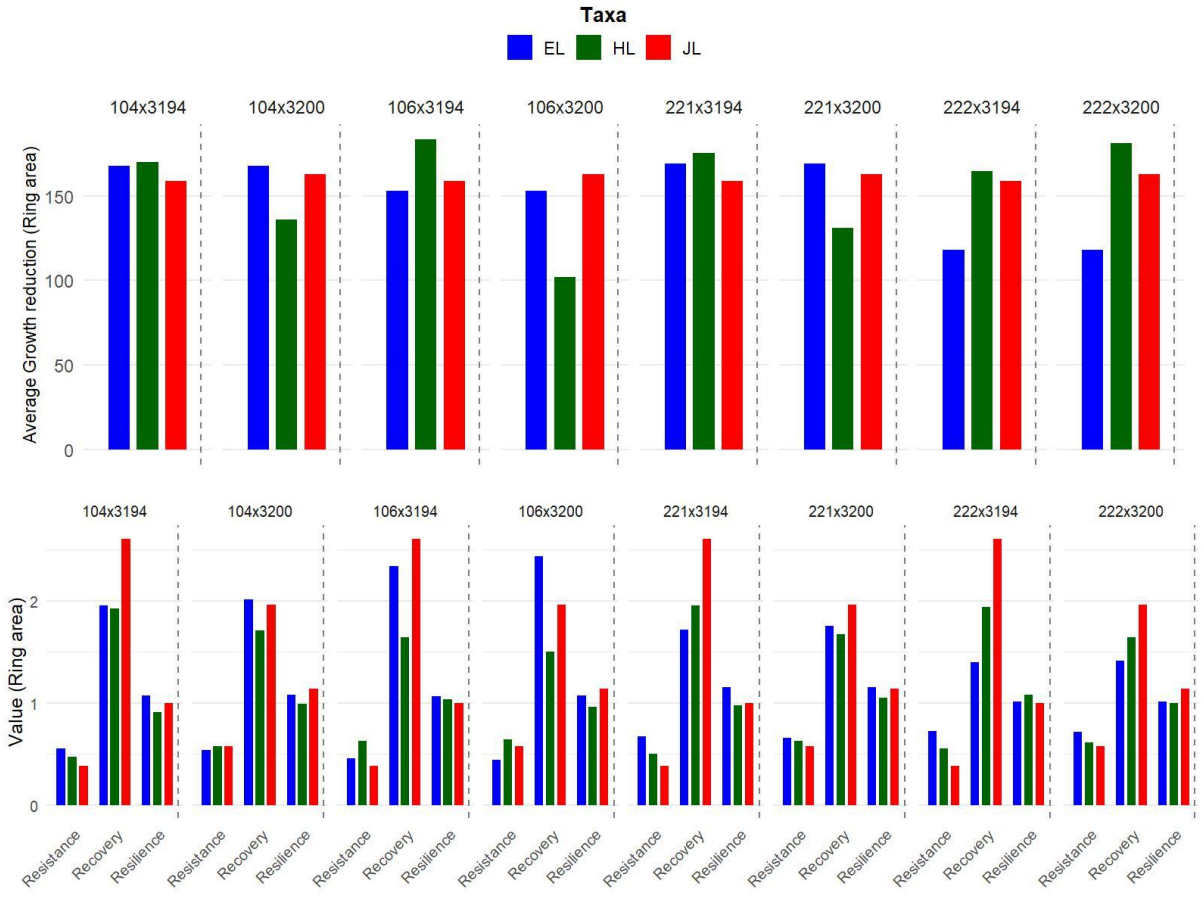


Fig 13 (B). Bar plots showing the Average Growth reduction during 2015-2020 (top panel) and resilience parameters (bottom panel) of ring area across the three taxa (EL: blue, HL: green, and JL: red) within respective combinations.

Phenotypic Plasticity of growth along a water availability gradient (irrigation + rainfall)

Building on the previous stability analysis, which revealed significant growth variation between irrigated and non-irrigated plots, we further investigated genotype-specific annual growth responses to a continuous gradient of water availability, defined as the total water received each year in both irrigated (rain + irrigation) and non-irrigated (rain) plots. For this analysis, we focused only on clones present in both watering treatments to enlarge the range of water availability gradient. Across all combinations, height increment increased non-linearly with water input, rising from approximately 45–60 cm at 300 mm to 120-130 cm at around 700-mm, from where growth began to plateau (**Fig. 14A**). Hybrid larch (HL) consistently exhibited the highest plasticity, particularly in combinations such as 104×3194, 106×3200, 222×3194, and 222×3200. These genotypes showed a pronounced response in the intermediate range of water availability (400–750 mm), with peak increments at around 700 mm, reaching up to 130 cm and clearly surpassing both parents. Beyond 700-750 mm, the height increment decreased more rapidly than EL and JL. European larch (EL) showed the lowest height gains across the gradient, particularly under drier conditions (300–600 mm), rarely exceeding 110–115 cm. Japanese larch (JL) showed intermediate to high increments, approaching HL levels under favorable conditions but generally exhibiting less plasticity.

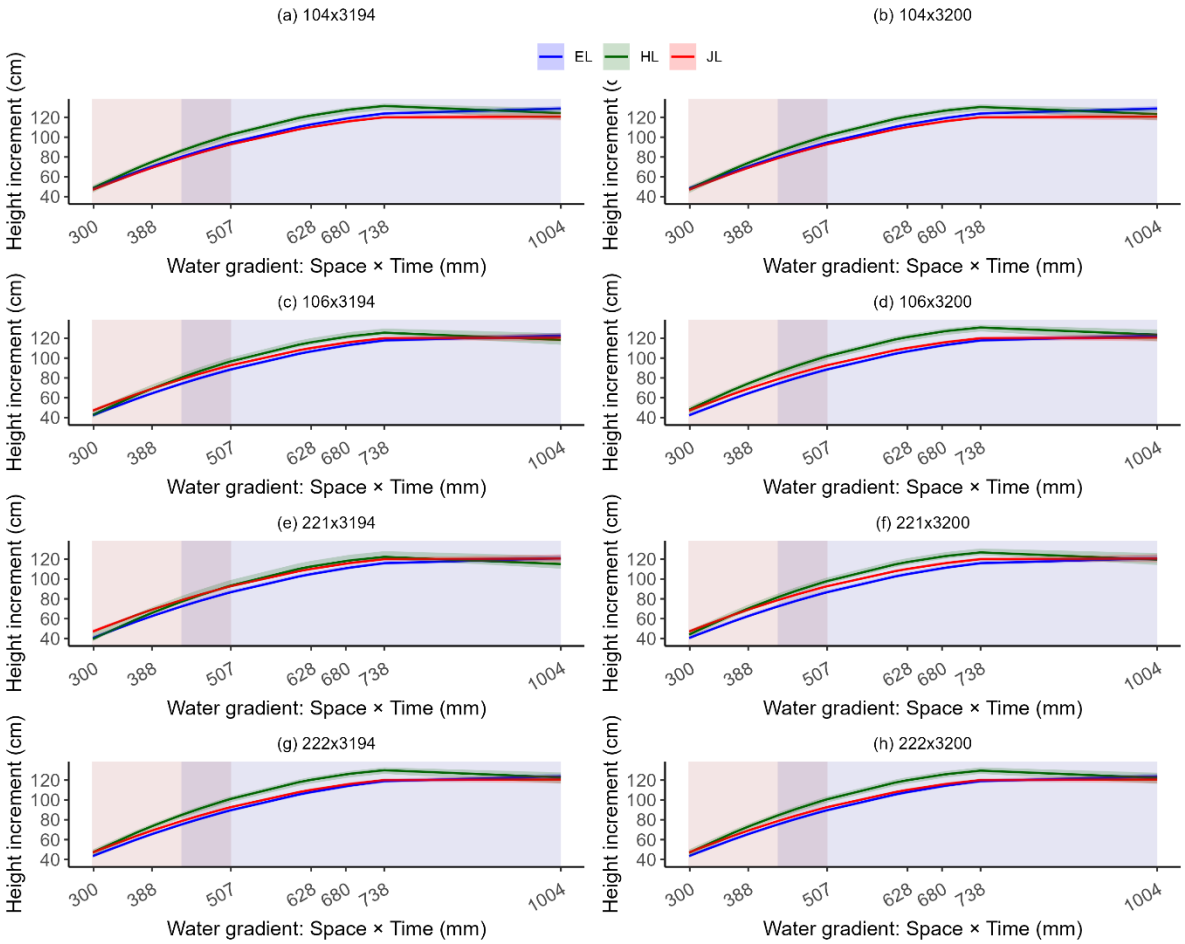


Fig 14 (A). Reaction norms for height increment along a water supply gradient. For each hybrid combination, the mean responses of clones of hybrid larch (HL, green lines) and of its parents (European larch (EL, blue lines) and Japanese larch (JL, red lines)) are represented. Shaded areas represent 95% confidence intervals around the predictions. The water gradient levels are represented by the shaded area in the plot: light red: NIRR and light blue: IRR, and a darker shade represents the overlapping gradient.

Across combinations, EL consistently exhibited lower diameter increments, particularly in low to intermediate water conditions (300–507 mm) (**Fig. 14 (B)**). For example, in combination 104×3194, EL reached a peak diameter increment of 13 mm around 680–738 mm, whereas JL exceeded 15 mm under the same conditions. JL's curve typically rose more steeply with increasing water availability, peaking around 16–17 mm in combinations like 104×3200 and 221×3194, reflecting more plasticity or flexibility. However, JL also showed sharper declines under drought (≤388 mm), suggesting higher sensitivity than EL, which maintained a more gradual, buffered curve with less variation.

HL's growth curves were generally intermediate but often overlapped with or surpassed parental lines under moderate to high water conditions. For instance, in 106×3200 and 222×3200, HL reached 16–17 mm at peak availability (738–1004 mm), similar to JL, while showing higher growth than EL (364–507 mm). In 106×3194, HL's performance declined less steeply than JL under drought but matched JL at peak water availability.

At the combination level, high-performing genotypes under irrigation, such as 104×3194, 104×3200, and 106×3194, also showed greater plasticity (green lines), with steep growth

increases across the water gradient at high availability. In contrast, lower performers like 221×3194 and 222×3194 had flatter response curves and lower/mid overall growth, reflecting limited plasticity.

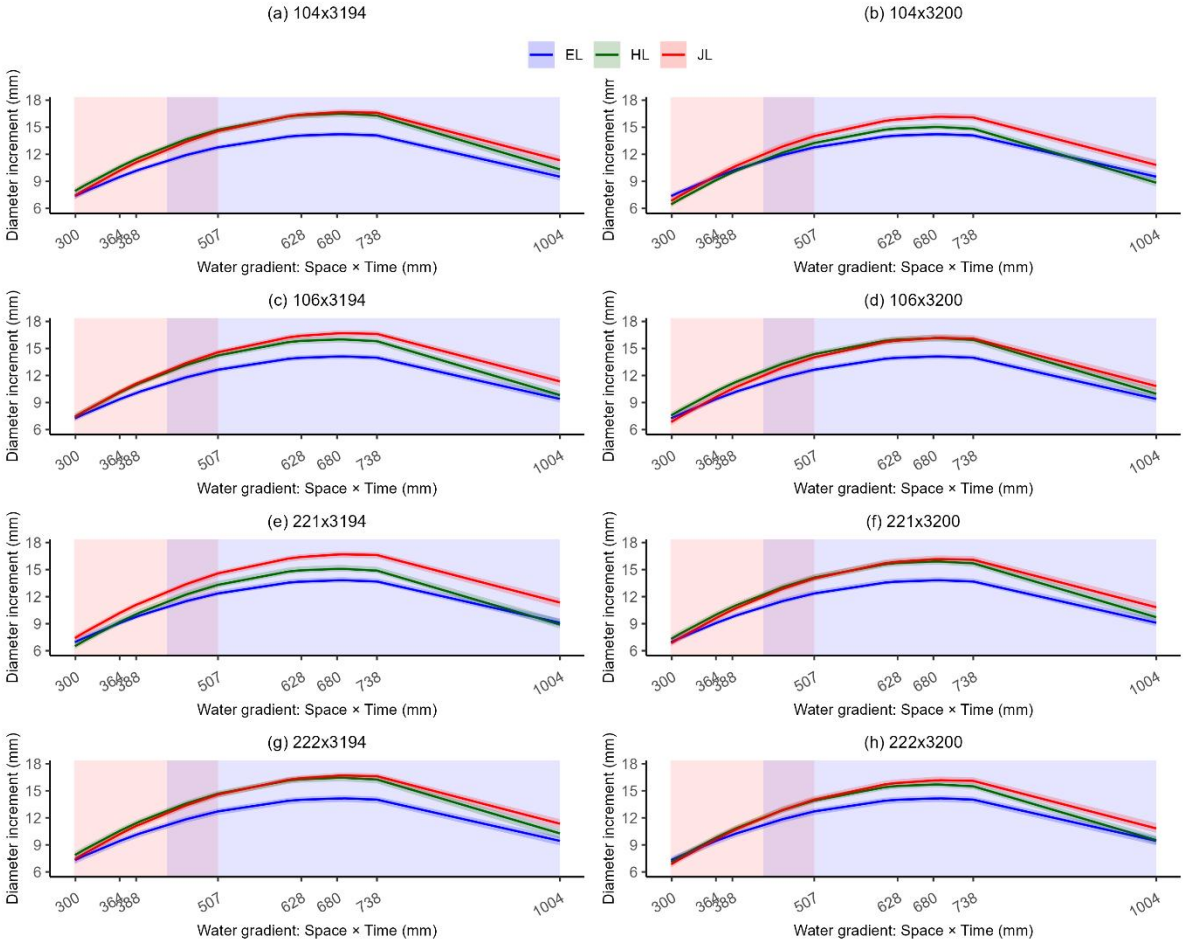


Fig 14 (B). Reaction norms for diameter increment along a water supply gradient. For each hybrid combination, the mean responses of clones of hybrid larch (HL, green lines) and of its parents (European larch (EL, blue lines) and Japanese larch (JL, red lines)) are represented. Shaded areas represent 95% confidence intervals around the predictions. The water gradient levels are represented by the shaded area in the plot: light red: NIRR and light blue: IRR, and a darker shade represents the overlapping gradient.

Table 6 presents the model-estimated trends in soil water availability and their effects on height and diameter increments across larch taxa. For height increment, HL exhibited the steepest positive trend (0.912), significantly higher than EL ($p < 0.01$) and JL ($p < 0.01$), indicating greater plasticity in response to soil moisture. EL and JL did not differ significantly. For diameter increment, JL showed the highest positive trend (0.122), while EL had a negative trend (−0.156), significantly lower than both HL and JL. HL was intermediate (0.043) but still differed significantly from both parents.

Table 6: Model - estimated species-specific trends in height increment and diameter increment over the covariate and pairwise comparisons of slope differences. The first column presents the model-estimated linear trends (± standard error) for each species. These reflect the rate of change in height increment over the covariates (year and environmental variable). The second part lists pairwise contrast, showing the difference in slope between species along with standard errors. Asterisks (*) indicate statistically significant differences ($p < 0.05$); "ns" indicates non-significant differences.

Height increment		**Estimated trend (SE)**	**Pairwise contrast**	**Difference in slope**	**Significance**
	EL	0.826 (0.192)	EL-HL	-0.086(0.029)	*
	HL	0.912 (0.192)	EL-JL	0.026(0.032)	ns
	JL	0.8 (0.192)	HL-JL	0.112(0.032)	*
Diameter increment		**Estimated trend (SE)**	**Pairwise contrast**	**Difference in slope**	**Significance**
	EL	-0.1562 (0.147)	EL-HL	-0.1997(0.014)	*
	HL	0.0432 (0.147)	EL-JL	-0.27(0.015)	*
	JL	0.1227 (0.147)	HL-JL	-0.0795(0.015)	*

Water-use strategy traits: P_{50} and WUE

One of the key growth responses under drought is water-use strategy; therefore, we assessed traits such as P_{50} (vulnerability to cavitation) and WUE (water-use efficiency) to understand taxa-specific adaptations.

Table 7: Anova (F-test for Taxa (EL=European Larch, JL=Japanese Larch, and HL=Hybrid Larch) descriptive statistics for vulnerability to cavitation P_{50}, and Tukey-test: different letters represent statistically significant differences between groups at $p < 0.05$.

ANOVA	Factor	F value	Pr(<F)	Taxa	no	Mean	SD	Min	Max	CV (%)
	Taxa	8.77	0.000263***	EL	39	-3.85 [b]	0.37	-4.7	-3.29	9.72
				HL	77	-3.70 [a]	0.38	-4.5	-2.3	10.14
				JL	21	-3.45 [a]	0.24	-3.9	-2.99	6.84

P_{50} values - a measure of xylem vulnerability to cavitation, ranged from -3.92 to -2.99 MPa across taxa (**Table 7**). Highly significant differences were observed between taxa: European larch (EL) combinations showed the most negative P_{50} values (≤ -3.9 MPa), indicating greater safety, but were associated with the lowest growth values in both height and diameter. In contrast, Japanese larch (JL) genotypes exhibited less negative P_{50} values (~ -3.4 MPa), reflecting higher vulnerability to cavitation, but they achieved superior growth, especially in diameter (Fig 15). Hybrid larch (HL) combinations occupied an intermediate position, with moderate P_{50} values and development, suggesting a functional compromise between hydraulic safety and performance. HL showed the highest coefficient of variation, i.e., 10.14%.

Table 8: ANOVA (F-test for Treatment (irrigated and non-irrigated plot) and Taxa (European Larch, Japanese Larch, and Hybrid Larch)) and descriptive statistics for Water use efficiency ($\Delta^{13}C$: carbon isotope discrimination), and Tukey-test**:** different letters represent statistically significant differences between groups at $p < 0.05$.

ANOVA		**Factor**	**F value**	**Pr(<F)**
		Treatment	1152.92	<2.2e-16***
		Taxa	2.56E+01	1.314e-11***
		Treatment: Taxa	2.36	0.09ns

Treatment	**Taxa**	**No**	**Mean (‰)**	**SD**	**Min**	**Max**	**CV (%)**
IRR	EL	261	22.79[a]	0.58	20.7	24.91	2.56
	HL	276	22.78[a]	0.56	20.99	24.33	2.45
	JL	257	22.48[b]	0.51	20.65	23.8	2.27
NIRR	EL	142	21.58[a]	0.57	19.93	2354	2.63
	HL	169	21.54[a]	0.64	19.47	23.53	2.97
	JL	112	21.42[a]	0.6	19.49	22.69	2.78

$\Delta^{13}C$ values showed significant differences between taxa and treatments, but with no significant interaction **(Table 8)**. JL displayed the lowest $\Delta^{13}C$ values (mean IRR: 22.48‰; NIRR: 21.42‰), indicating higher intrinsic water-use efficiency (WUE). EL had the highest $\Delta^{13}C$ (IRR mean: 22.79‰; NIRR: 21.58‰), reflecting lower WUE. HL displayed intermediate $\Delta^{13}C$ values (IRR mean: 22.78‰; NIRR: 21.54‰).

3.3. Growth vs other traits

Growth versus Heterosis and Stability

Correlation analyses were conducted to assess the relationships between mean growth, heterosis, and stability parameters at the family level in hybrid larch **(Table 9 A and B),** using both Pearson and Spearman coefficients. Under irrigation (IRR), mean growth showed strong and significant correlations with both absolute and relative heterosis for diameter ($r = 0.97 – 0.98$) and volume ($r = 0.95 – 0.98$), indicating that fast-growing combinations tended to express strong heterosis under favorable water supply. For height, however, growth and heterosis were positively correlated but not significantly. Under drought conditions (NIRR), the correlation between mean growth and heterosis was generally weaker and non-significant, except for diameter ($r = 0.75$, Pearson), suggesting that heterosis under stress does not always translate into superior growth.
Notably, stability was never significantly correlated with either heterosis or mean growth, indicating that faster-growing or more heterotic genotypes are not necessarily less stable across sites than slower-growing ones. Spearman correlations were used alongside Pearson to account for small sample size and to capture consistent ranking patterns beyond trends.

Table 9 (A): Pearson correlations between Mean Growth, Relative Heterosis, Huhn Stability, and relative ecovalence for Height, Diameter, and Volume at age 10. *asterisk when correlation is significant* ($p<0.05$).

			Mean	Absolute Heterosis	Huhn Stability	Ecovalence Percentage
		Mean	1	0.69	0.51	0.33
		Absolute Heterosis		1	0.12	-0.14
	IRR	Huhn Stability			1	0.87 *
		Ecovalence Percentage				1
Height		Mean	1	0.37	0.62	0.62
		Absolute Heterosis		1	0.12	0.24
		Relative Heterosis			0.07	0.17
	NIRR	Huhn Stability			1	0.87 *
		Ecovalence Percentage				1
		Mean	1	0.97 *	0.21	0.42
		Absolute Heterosis		1	0.09	0.33
		Huhn Stability			1	0.92 *
	IRR	Ecovalence Percentage				1
		Mean	1	0.75 *	0.01	0.1
Diameter		Absolute Heterosis		1	0.17	0.15
	NIRR	Huhn Stability			1	0.92 *
		Ecovalence Percentage				1
		Mean	1	0.98 *	0.34	0.73 *
	IRR	Absolute Heterosis		1	0.26	0.67
		Huhn Stability			1	0.56
Volume		Ecovalence Percentage				1
		Mean	1		0.55	0.21
	NIRR	Absolute Heterosis		1	0.64	0.05
		Huhn Stability			1	0.56
		Ecovalence Percentage				1

Table 9 (B): Spearman correlations between Mean Growth, Relative Heterosis, Huhn Stability, and relative ecovalence for Height, Diameter, and Volume at age 10. asterisk when correlation is significant ($p<0.05$).

			Mean	Absolute Heterosis	Huhn Stability	Ecovalence Percentage
		Mean	1	0.33	0.4	0.24
		Absolute Heterosis		1	-0.03	-0.33
	IRR	Huhn Stability			1	0.9 *
		Ecovalence Percentage				1
Height		Mean	1	0.48	0.6	0.45
		Absolute Heterosis		1	0.23	-0.05
		Huhn Stability			1	0.9 *
	NIRR	Ecovalence Percentage				1
		Mean	1	0.98 *	-0.1	0.21
		Absolute Heterosis		1	-0.04	0.24
	IRR	Huhn Stability			1	0.88 *
		Ecovalence Percentage				1
		Mean	1	0.38	0	0.31
		Absolute Heterosis		1	0.2	0.29
Diameter	NIRR	Huhn Stability			1	0.88 *
		Ecovalence Percentage				1
		Mean	1	0.95 *	0.29	0.17
	IRR	Absolute Heterosis		1	0.19	0.12
		Huhn Stability			1	0.38
Volume		Ecovalence Percentage				1
		Mean	1	0.29	0.59	0.07
		Absolute Heterosis		1	0.44	-0.21
	NIRR	Huhn Stability			1	0.38
		Ecovalence Percentage				1

Following our analysis of growth performance, heterosis, and stability, we further investigated how growth relates to adaptive traits. We first examined the relationship between growth and water-use strategy traits (P_{50} and WUE) in hybrid and parental larch combinations. We then extended this analysis to include other adaptive traits, such as resistance, recovery, and resilience, exploring their association with growth both across taxa and within taxa through correlations. This approach aimed to identify potential trade-offs or synergies that could inform breeding for both productivity and drought adaptation

Growth versus other traits across taxa

To better understand the relationship between growth and other traits, we explored growth–trait associations across taxa at two levels: the family-group and the clonal-group level. Supplementary Table 3 provides the number of clones and families included in this analysis, both at the family and clonal levels.

At the mean family level for 'priority' hybrid and parental larch combinations

Figs 15 and 16 illustrate the relationships between growth (height and diameter in 2023) and two traits: vulnerability to cavitation (P_{50}) and intrinsic water-use efficiency ($\Delta^{13}C$) at the mean combination (family) level.

We observed a general trend across taxa where genotypes with less negative P_{50} values (i.e., lower cavitation resistance) tended to exhibit greater height and diameter in 2023 **(Fig. 15).** Notably, Japanese larch (JL) clones, such as JL-3200 and JL-3194, which had the highest growth values, also showed the least negative P_{50}. In contrast, European larch (EL) showed more negative P_{50} values, with higher cavitation resistance but lower growth. Hybrid larch (HL) combinations displayed intermediate to high growth, generally balancing between moderate P_{50} values and performance.

We found that Japanese larch (JL) and hybrid larch (HL) generally exhibited higher intrinsic water-use efficiency (WUE), as indicated by their lower $\Delta^{13}C$ values (closer to 22.0‰) **(Fig. 16).** These also tended to have higher growth (both height and diameter) in 2023. In contrast, European larch (EL) displayed higher $\Delta^{13}C$ values (around 22.6‰), indicating lower WUE, and showed lower growth.

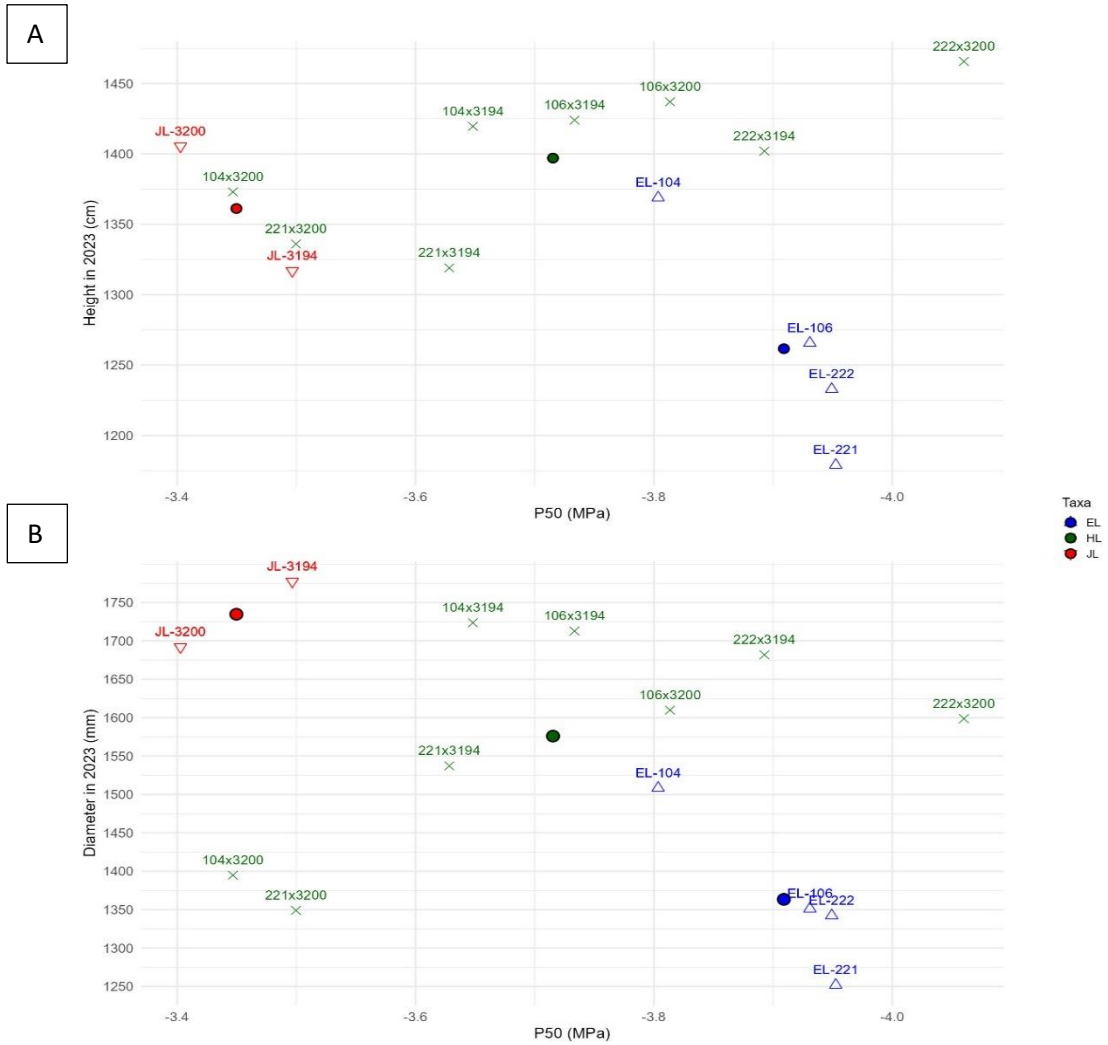


Fig 15. Relationship between Vulnerability to cavitation (P_{50}) and A) tree height and B) diameter for European Larch (EL, blue), Japanese Larch (JL, red), and their hybrid (HL, green) combinations. Each point represents the mean value of a combination (family) with its genotype codes (female x male). Circles with black borders are for their respective taxa means.

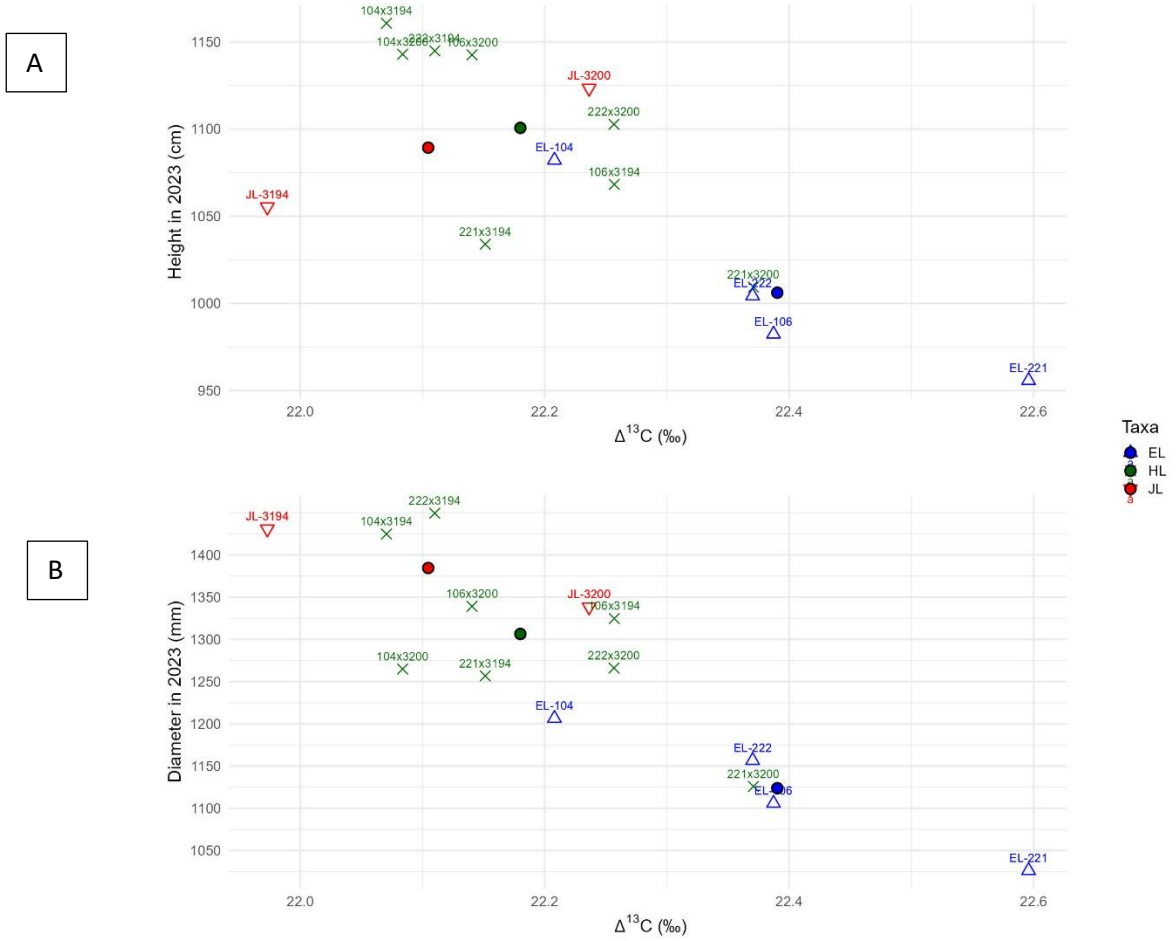


Fig 16. Relationship between Δ^{13}Cand A) tree height and B) diameter in 2023 for European Larch (EL), Japanese Larch (JL), and their hybrid (HL) combinations. Each point represents the mean value of a combination, with hybrid combinations labeled by their parental taxa, and parental taxa labeled by EL and JL genotype numbers. Circles with black borders are for their respective taxa means.

At the mean family level

Under irrigated conditions (IRR), clear relationships emerged between diameter growth and several traits (Fig. 17). Diameter was significantly negatively correlated with both water-use efficiency (WUE; $r = -0.778$, $p < 0.05$) and vulnerability to cavitation (P_{50}; $r = 0.554$, $p < 0.05$), suggesting that trees maintaining high water-use efficiency also achieved greater radial growth, despite increased hydraulic vulnerability. Additionally, diameter was significantly and positively correlated with average growth reduction ($r = 0.394$, $p < 0.05$) but not with other resilience parameters. Height was also positively correlated with P_{50} ($r = 0.375$, $p < 0.05$), though less strongly than diameter. Interestingly, correlations between height and resilience components (resistance, recovery, resilience) were weak and non-significant, suggesting that under IRR, height was not tightly linked to immediate or post-stress growth dynamics.

Under non-irrigated conditions (NIRR), correlations were generally weaker and fewer were significant, indicating more variable genotypes responses under water-limited conditions. However, diameter still maintained a significant correlation with average growth reduction ($r = 0.333$, $p < 0.05$) and moderate positive associations with recovery ($r = 0.287$) but with no significance. Height, by contrast, did not show significant correlations with any trait under NIRR, although its relationship with WUE ($r = -0.527$) was still moderate with no significance. This pattern suggests that diameter growth remained more sensitive to physiological and resilience traits than height when water was limited.

At the mean clonal level

At the clonal level, correlation patterns between growth and resilience traits again varied by treatment, though overall associations were weaker and more trait-specific than at the family level (Fig 18). Under irrigated conditions (IRR), diameter growth showed significant positive correlations with multiple traits: most notably with recovery ($r = 0.578$, $p < 0.05$), resilience ($r = 0.267$, $p < 0.05$), and average growth reduction ($r = 0.385$, $p < 0.05$). A weak negative correlation with WUE ($r = -0.282$, $p < 0.05$) was also observed. In contrast, diameter was negatively associated with resistance ($r = -0.372$, $p < 0.05$), reflecting maintaining growth and tolerating immediate impacts. For height, correlations were generally weak and mostly non-significant, with the exception of a slight negative correlation with resistance ($r = -0.151$, $p < 0.05$).

Under non-irrigated conditions (NIRR), all correlations became weaker, but some remained significant for diameter. Specifically, diameter correlated positively with recovery ($r = 0.221$, $p < 0.05$), resilience ($r = 0.209$, $p < 0.05$), and average growth reduction ($r = 0.329$, $p < 0.05$) but with weak values.

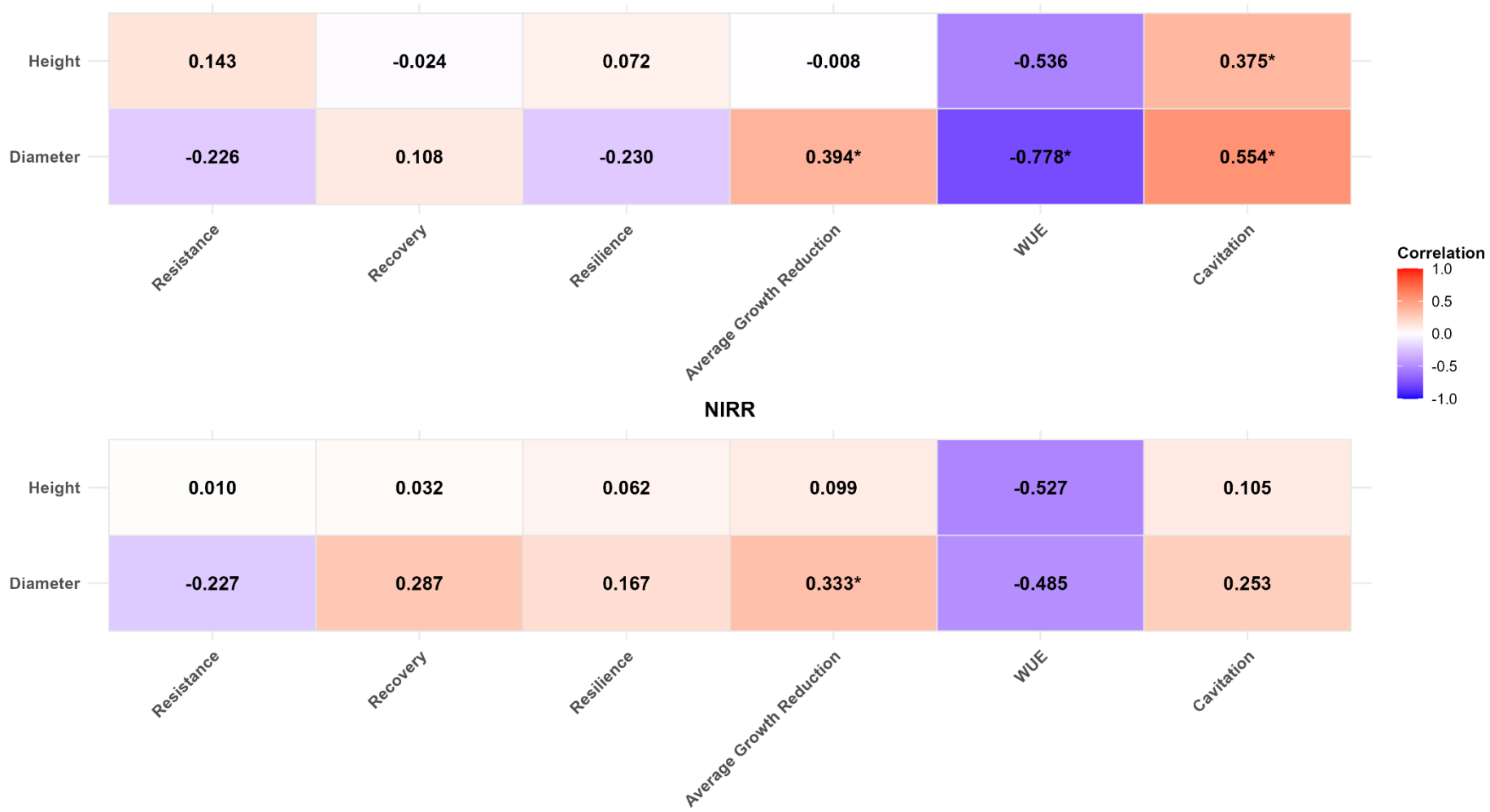


Fig 17. Correlation heatmaps between growth traits (height and diameter) and other traits (resistance, recovery, resilience, average growth reduction, water use efficiency, and cavitation) at the Family level under irrigated (IRR, top) and non-irrigated (NIRR, bottom) conditions across taxa. Color gradients represent the strength and direction of Pearson correlation coefficients (red = positive, blue = negative), with asterisks (*) indicating statistically significant correlations ($p < 0.05$).

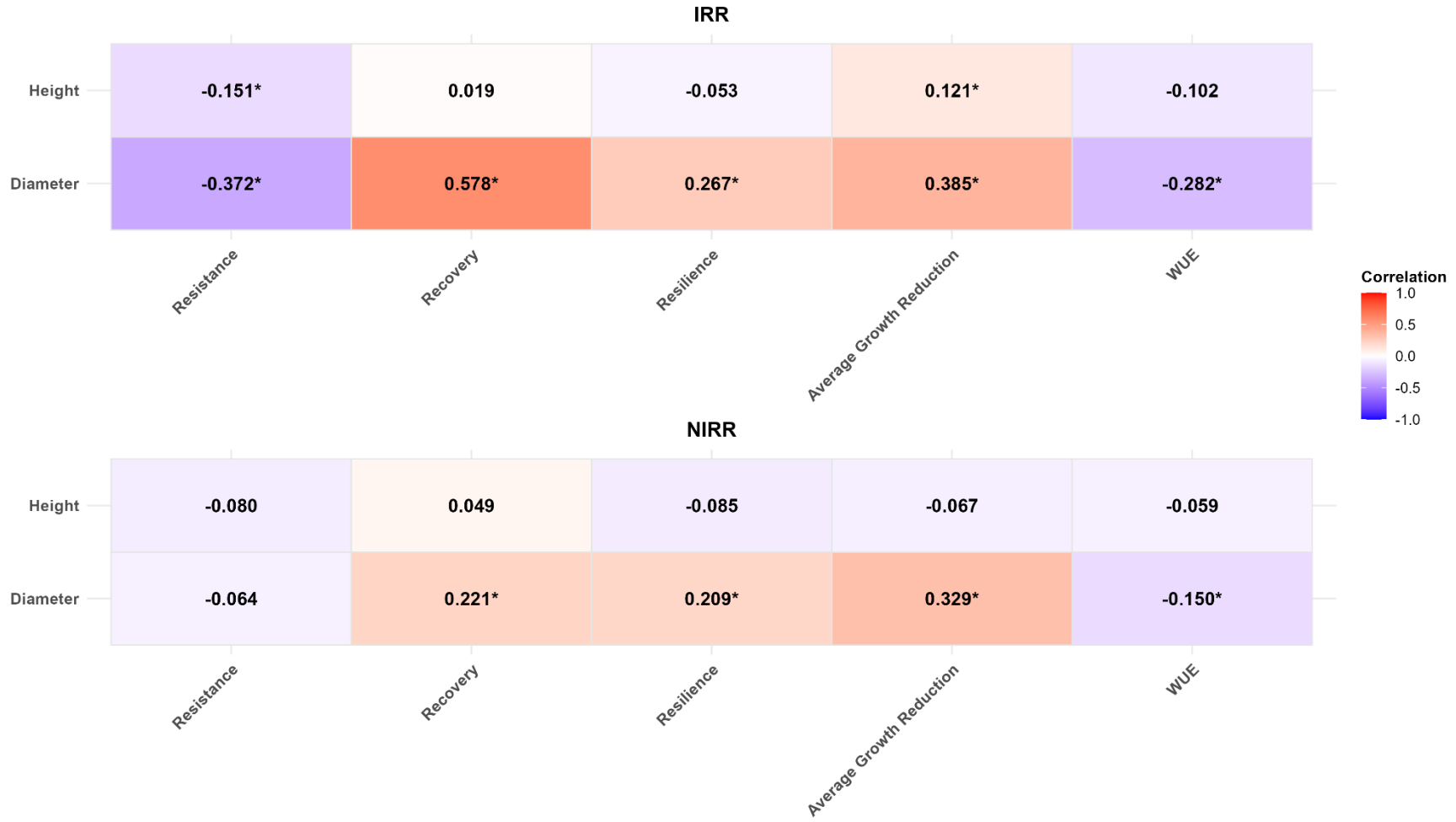


Fig 18. Correlation heatmaps between growth traits (height and diameter) and other traits (resistance, recovery, resilience, average growth reduction, water use efficiency, and cavitation) at the Clonal level under irrigated (IRR, top) and non-irrigated (NIRR, bottom) conditions across taxa. Color gradients represent the strength and direction of Pearson correlation coefficients (red = positive, blue = negative), with asterisks (*) indicating statistically significant correlations ($p < 0.05$).

Growth versus other *traits within taxa*

At the mean family level

Growth in irrigated conditions (IRR), showed significant relationships between diameter growth and other traits, with notable taxa-specific patterns **(Fig. 19).** In all taxa, diameter was negatively correlated with water-use efficiency (WUE), significantly so in Hybrid Larch (HL; $r = - 0.890$, $p < 0.05$), followed by Japanese Larch (JL; $r = - 0.495$) and European Larch (EL; $r = - 0.586$, $p < 0.05$). Diameter was positively associated with vulnerability to cavitation (P_{50}) in EL ($r = 0.477$) and JL ($r = 0.229$), but negatively in HL, although these were not significant. Additionally, a clear positive correlation was observed between diameter and average growth reduction in JL ($r = 0.624$, $p < 0.05$) and in EL ($r = 0.422$ ns), indicating in particular that high-performing JL families were more sensitive to stress. By contrast, no link was observed in HL ($r = 0.048$ ns).

For height growth in IRR, negative correlations with WUE were observed in EL ($r = - 0.660$) and HL ($r = 0.689$), but was positive in JL ($r = 0.114$ ns) while P_{50} was moderately positively linked to height in EL ($r = 0.428$) and JL ($r = 0.561$) but negatively in HL ($r = -0.196$ ns). Correlations between height and resilience traits (resistance, recovery, resilience) were generally weak or inconsistent. In EL, a significant negative correlation was found between height and recovery ($r = –0.662$, $p < 0.05$), suggesting a trade-off between apical growth and recovery capacity.

For growth in non-irrigated conditions (NIRR), correlations were generally weaker and fewer reached significance, reflecting more variable responses under drought. Diameter in JL maintained strong positive correlations with average growth reduction ($r = 0.740$, $p < 0.05$) and recovery ($r = 0.737$, $p < 0.05$), indicating that fast-growing JL families were also the most affected during drought but exhibited strong rebound potential. In EL, diameter showed moderate correlations with WUE ($r =- 0.566$, $p<0.05$) and recovery ($r = 0.234$), though only the WUE–height correlation remained significant ($r = 0.616$, $p < 0.05$). In HL, diameter was moderately correlated with WUE ($r = - 0.415$) and resilience ($r = 0.373$), while height in HL showed a significant positive correlation with resilience ($r = 0.570$, $p < 0.05$), suggesting some capacity to maintain height growth while coping with drought. Across species, height showed minimal or no significant correlation with other traits, highlighting once again that diameter growth is more closely linked to water and resilience traits under both favorable and stressful conditions.

At the mean clonal level

For growth under IRR **(Fig. 20),** EL diameter growth was significantly correlated with WUE ($r = - 0.456$, $p < 0.05$) and recovery ($r = 0.544$, $p < 0.05$) and negatively with resistance ($r = - 0.292$, $p < 0.05$) Similar patterns were seen with resilience ($r = 0.359$, $p < 0.05$) and average growth reduction (AGR, $r = 0.308$, $p < 0.05$), indicating a strong link between growth and variable reduction. In Hybrid Larch (HL), the association was significant between diameter and recovery ($r = 0.491$, $p < 0.05$), as well as with AGR ($r = 0.319$, $p < 0.05$). JL displayed positive correlations between diameter and all resilience-related traits, including resistance (-0.28, $p<0.05$), recovery ($r = 0.603$, $p <0.05$), resilience ($r = 0.278$, $p<0.05$), and AGR ($r = 0.395$) (all $p < 0.05$). For height, EL was positively correlated with AGR (0.227, $p<0.05$) and negatively with WUE (- 0.332, $p <0.05$) and negatively with resistance, while no significant correlations

with any traits was observed in HL. JL remained significantly correlated with recovery (0.320, $p<0.05$), resilience (0.257, $p<0.05$), AGR (0.350, $p<0.05$), and WUE (0.339, $p<0.05$)

When growth is considered in non-irrigated conditions (NIRR), overall correlations between growth and adaptive traits weakened, refocusing patterns observed at the family level. However, some significant relationships persisted. In EL, diameter remained significantly correlated with recovery ($r = 0.441$, $p < 0.05$), resilience ($r = 0.389$, $p < 0.05$), and AGR ($r = 0.422$, $p < 0.05$). In contrast, HL and JL showed much weaker or non-significant associations. For instance, in HL, no traits showed significant correlations with height, and only AGR was weakly linked to diameter ($r = 0.230$, $p < 0.05$). In JL, diameter was only weakly correlated with AGR ($r = 0.292$, $p < 0.05$), and other traits showed little association with growth. Still, consistent with family-level trends, WUE showed diminishing relevance under drought, as most correlations with growth in NIRR were weak within taxa, especially in HL and JL.

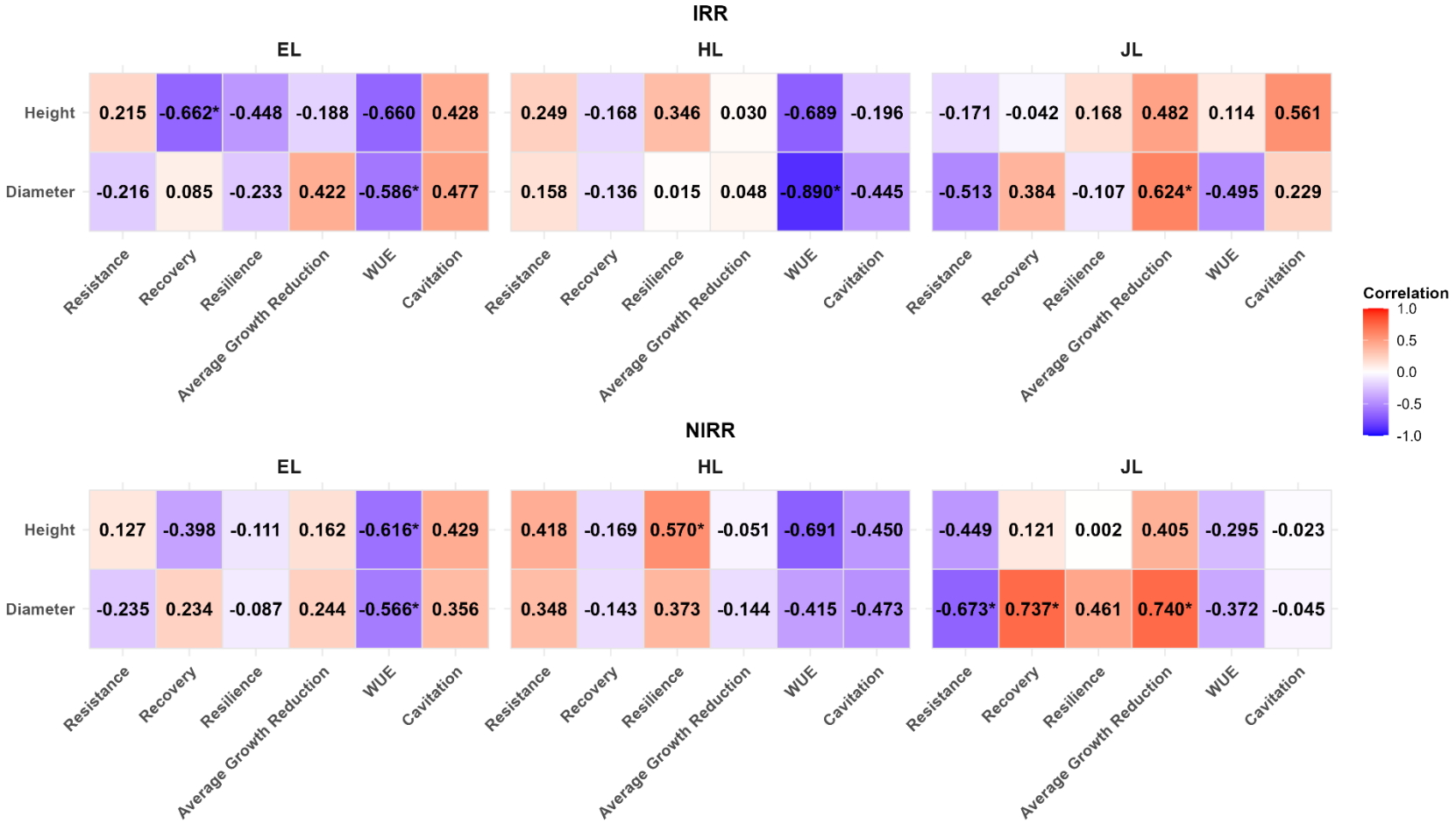


Fig 19. Correlation heatmaps between growth traits (height and diameter) and other traits (resistance, recovery, resilience, average growth reduction, water use efficiency and cavitation) at the Family level for European Larch (EL), Hybrid Larch (HL), and Japanese Larch (JL) under irrigated (IRR, top) and non-irrigated (NIRR, bottom) conditions within respective taxa. Color gradients represent the strength and direction of Pearson correlation coefficients (red = positive, blue = negative), with asterisks (*) indicating statistically significant correlations ($p < 0.05$)

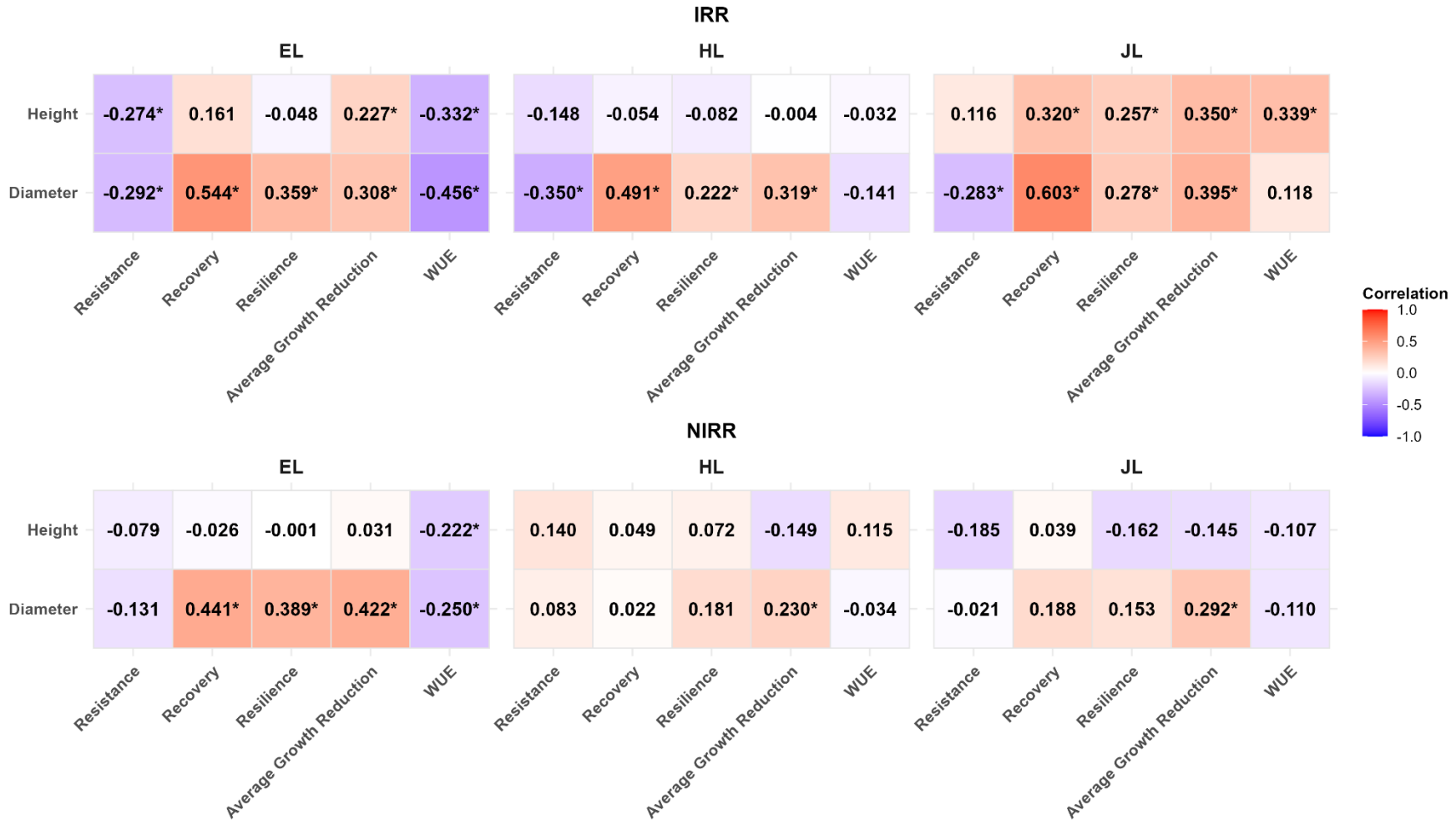


Fig 20. Correlation heatmaps between growth traits (height and diameter) and other traits (resistance, recovery, resilience, average growth reduction, and water use efficiency) at the Clonal level for European Larch (EL), Hybrid Larch (HL), and Japanese Larch (JL) under irrigated (IRR, top) and non-irrigated (NIRR, bottom) conditions within respective taxa. Color gradients represent the strength and direction of Pearson correlation coefficients (red = positive, blue = negative), with asterisks (*) indicating statistically significant correlations ($p < 0.05$)

4. Discussion

Tree breeding faces increasing challenges as climate variability intensifies with more frequent and severe drought and heat-wave episodes (Cook et al. 2018), and the demand for high-performing but also more resilient genotypes to abiotic and biotic threats is growing (Reich 2014; Kissoudis et al. 2014). Traditionally, breeding and selection have focused primarily on growth and yield. Some quality traits (like stem form or wood density) have been added in a second phase, together with resistance to some pests or diseases, in a relatively low number of species. Adaptation to environmental conditions has been roughly approached through survival and growth performances across multisite testing and *GxE* interaction analysis. Except in some Nordic countries with strong climatic gradients, environmental effects are actually appreciated as the global site effects, confounding topography, soil, and climate parameters. Depending on the climatic context and breeding goals, the strategy has then been either to select and deploy a limited number of broadly adapted genotypes, as it is mostly the case in Europe, or to use specialized genotypes tailored to specific climatic gradients (e.g., heat-sum/thermal time) as practiced in Nordic countries. With the new threats, it is increasingly evident that breeding strategies must evolve to become more flexible and better cope with the latest environmental and socio-economic challenges. For Climent et al. (2024), it is clear that breeding strategies must now evolve beyond productivity-focused selection to incorporate multi-trait integration, explicitly targeting resilience to both abiotic and biotic stresses under changing environmental conditions.

In most species, particularly conifers, simple recurrent selection is used to increase, cycle after cycle, the frequency of favorable genes for the target traits, and is also the most common method to preserve the favorable trait. Interspecific hybridization offers a complementary pathway, recombining distinct gene pools from two parent species: it potentially accelerates genetic gains accumulation through complementation of traits and “heterosis” or hybrid vigor - a term coined by Shull in 1914 (Shull 1914, 1948).

Expression of heterosis

While heterosis is well-documented and widely exploited in agricultural crops (Birchler 2003; Gallais 2009), its expression in forest trees is less consistently reported and appears more variable, depending on species and the traits assessed (Brewbaker and Sun 1999). The scarcity of appropriate mating designs and long-term experimentation primarily constrains the study of heterosis in trees. Most often, hybrids are compared to commercial varieties of parental species. In conifers, including larch, complementation of favorable traits has been documented (Pâques 2009; Dungey 2001) and largely contributes to hybrid merits. In addition, hybrid vigor in larch has been put in evidence in the early years (before age 15–20) (Greenwood et al. 2015; Marchal et al. 2017), but according to Keiding (1980) it would often plateau or diminish with age, leading him to hypothesize that it is primarily a juvenile trait. Marchal et al. (2017) reported that mid-parent heterosis for growth in hybrid larch was significant during juvenile stages but less marked later in life. Baltunis and Greenwood (1999) attributed hybrid vigor in larch to extended shoot elongation periods in hybrids compared to their parental species, a trait expressed during the juvenile stage. Similar age-dependent patterns of heterosis have been observed in other forest tree species. In poplars (*Populus* spp.), for instance, heterosis for stem growth is most pronounced during the first few years of development and tends to fade as trees mature (Mohrdiek 1979).

Our findings up to age 10 confirm earlier studies (Pâques 2009; Marchal et al. 2017) showing that interspecific hybridization in larch confers heterotic effects during early growth stages. At the global taxon level, we observed mid-parent heterosis (MPH) at age 10 for height (up to +14%), diameter (+11%), and especially for stem volume (+37%). We observed considerable variation in heterosis for example for volume across specific combinations, ranging from –8% to +37% under irrigation and –13.65% to +47.9% under drought. For height, heterosis ranged from +5% to +13% in IRR and +1% to +15% in NIRR, while for diameter it ranged from –0.2% to +11.4% in IRR and –+6% to +15% in NIRR. This highlights that heterosis strongly depends on the specific hybrid combinations. It was also observed that best-parent heterosis (BPH) was less frequent than mid-parent heterosis, BPH was observed more frequently under non-irrigated (NIRR) conditions, especially for volume. The presence of mid-parent heterosis without best-parent heterosis is a common trend in conifers and is often interpreted as a result of additive genetic effects, especially for traits with high heritability and low dominance variance (Dungey 2001; Falconer and Mackay 2009; Marchal et al. 2017).

Importantly, heterosis tended to be higher (in relative value) under non-irrigated (NIRR) conditions. This pattern highlights the differential sensitivity of the two parental species to water availability, with European and Japanese larches responding differently to water stress. Under NIRR conditions, growth differences are more strongly expressed, likely amplifying the hybrid's advantage, resulting in higher MPH or BPH values. Enhancement of heterosis under harsh environments is a common trend in crop plants, as discussed by Gallais (2009). This context-dependent expression, which is often amplified under environmental stress conditions, is also supported by the few studies available in the forestry literature. In poplar, Zanewich et al. (2018) showed that hybrid cottonwoods (*Populus* × *acuminata*) expressed stronger heterosis for root and leaf growth under cooler, suboptimal temperatures, while parental species showed growth declines. The cooler temperature is not too lethal, but it can limit growth in poplar. In pine, Nikles (2000) reported that *Pinus elliottii* × *P. caribaea* hybrids outperformed their parents on nutrient-poor and poorly drained soils, making hybrid vigor particularly useful in challenging plantation environments. Similarly, in larch, Pâques (1989) observed that hybrid advantages were most pronounced where at least one parent species performed poorly, especially under less favorable site conditions. Dungey (2001) also emphasized that heterosis in pines and other forest trees tends to manifest more strongly when environmental conditions are suboptimal.

Our findings show that heterosis is not consistent over time but it varies significantly across years, especially under water-limited conditions. Strong Year × Treatment interactions for both height and diameter increments indicate that the hybrid advantage depends on annual conditions and the relative susceptibility of taxa to annual growth conditions. Notably, years like 2016 and 2017 showed the highest levels of heterosis, particularly under non-irrigated conditions, suggesting that stress may enhance hybrid relative performance. Growth variability over time appears to be associated with genetic heterozygosity. Work on *ponderosa* pine, aspen, and lodgepole pine indicated that growth variability over time is associated with genetic heterozygosity, suggesting that more genetically diverse trees may exhibit enhanced or more flexible performance across years (Mitton et al. 1981). As well, Arcade et al. (1996) found that the performance of hybrid larches for growth was positively correlated with parental genetic distances and levels of heterozygosity, but that these correlations evolved with tree age with the effect being strongest during specific developmental windows (age 6). These results indicate that heterosis should not only be viewed as a static trait but as a dynamic and context-dependent trait, influenced by both genotype and environment.

It can be clearly sourced from our trial that across multiple growing seasons (2013 to 2023) and sites (two differential watering treatments), particularly during drought-impacted years, hybrid larch was consistently among the best-growth performing taxa (for traits like height, diameter, annual increments) while parental species - European Larch and Japanese Larch often showed greater variability or decline. The greater stability of hybrid performances across sites was also previously evidenced for larch by Pâques (2002) and Marchal et al. (2017). This supports the concept of "heterosis by homeostasis" (Knight 1973), where hybrid performance benefits not only from an elevated growth but also a greater temporal and environmental stability.

Growth Response under a gradient of soil water availability

Beyond heterosis, our results clearly highlight the context-dependent nature of hybrid relative performance, emphasizing that environmental factors - particularly here soil water availability - are essential to consider. In our experiment, although several climatic drivers were at play on tree development, soil water availability emerged as the critical limiting factor, as evidenced by the markedly higher mortality and frequency of top-dieback in the non-irrigated (NIRR) treatment as described in Chapter I. Several studies also support this factor as critical in larch. Across an extensive field evaluation network across France (17 sites), Cazaux et al. (1993) identified soil water capacity as a key factor for hybrid larch survival and growth, much more important than soil fertility. Escobar - Sandoval, 2020 also found that daily radial growth recorded by automatic dendrometers were best explained by precipitation and relative extractable water (REW) ($r \leq 0.38$) than by other climatic factors, whereas radial stem expansion and contraction traits were mainly associated with mean temperature ($r = 0.47–0.63$) and atmospheric humidity (VPD - RH; $r = 0.41–0.56$). Beyond growth reduction and mortality, water limitation was shown to favor top-dieback symptoms (see Chapter I) and to increase the frequency of stem cracks in larch (Pâques et al. 2023).

<u>Phenotypic plasticity of growth along a water availability gradient-</u>

Our experimentation combining IRR and NIRR treatments allowed us to benefit from a large gradient of water availability (rain + irrigation (or not)) during the growing season, with yearly values ranging from a minimum of 299 mm in NIRR to a maximum of 1009 mm in IRR. This allowed us to build up norms of reaction for apical and radial growth and to evaluate phenotypic “plasticity” across combinations and taxa. Most studies on plasticity with trees either consider *individual tree* growth (predominantly radial) responses across a climatic factor gradient (Shrestha et al. 2015) or *groups of trees* (population, family) responses across a site gradient, like different elevations (Escobar-Sandoval et al. 2021). In contrast, our design benefits from having identical genotypes (clones) tested under both optimal (IRR) and stressful (NIRR) conditions and monitored for both apical and radial growth over a relatively long period (around 10 years). This allowed us to get access to a wide spatio-temporal gradient of soil water availability, strengthening inferences about plastic responses.

Across the full gradient of water availability, hybrid larch (HL) and Japanese larch (JL) consistently were more responsive than European larch (EL) in both height and diameter increments. The differences among taxa were more pronounced for diameter than height. While height increments increased steadily with water availability for all taxa, the gap between HL, JL, and EL remained relatively narrow. In contrast, radial growth (diameter increment) showed a much clearer separation, with JL achieving the highest values, followed closely by HL, and EL consistently showing the lowest performance. This indicates a stronger differentiation in

radial than apical growth. In terms of phenotypic plasticity, reflected by the steepness of the reaction norms across the water gradient, JL and HL demonstrated greater plasticity than EL. Their growth responded more strongly to increasing water availability, particularly in diameter, suggesting higher environmental responsiveness. EL, by contrast, exhibited a lower plasticity, with flatter growth curves and a more stable behaviour across the gradient. This implies that HL and JL adopt more responsive (plastic) strategies. These patterns align with findings from Marchal et al. (2019), who used random regression models to characterize reaction norms of hybrid and parental larches across a soil water availability, showing that hybrids generally exhibited higher plasticity in response to environmental variation, particularly in traits related to radial development.

At the dry end of our gradient (300–388 mm), all taxa experienced reduced growth, but the decline was more gradual in EL. In contrast, JL and HL showed sharper slopes, consistent with their higher plasticity and water dependence. At the wettest end of the gradient (>738 mm), growth trends were contrasted between traits: for height, growth tended to plateau for all taxa, indicating that further increases in water availability did not enhance anymore growth for height. A counter pattern was detected for diameter response: whilst diameter growth steadily increased with water availability from 300 mm to 680 mm, it then declined at the highest level of water supply (found in irrigated (IRR) conditions: after 738 mm). At the highest levels of water availability, the needs of all three taxa were likely (over-)satisfied, depicted by the fall in the radial growth curves after 680-738 mm of soil water. This may suggest that when water is no longer limiting, competition, particularly for space, may become a dominant constraint, suppressing radial expansion (but not height) despite favorable moisture conditions. In both mixed and pure species stands, competition on high-productivity sites can shift growth allocation and limit overall growth potential, as reviewed by Forrester (2014).

Resilience parameters for growth under water stress

We then examined specifically the growth responses of the three larch taxa when water availability was constraining to evaluate taxa resilience to soil water limitation (drought). Inspired by approaches in animal breeding, where variation in traits like lactation yield is compared between ‘optimal’ and ‘non-optimal’ conditions to evaluate resilience (Berghof et al. 2019), we adapted a similar framework, taking advantage of our design with the same clones tested in the two treatments. Our analysis was based on the growth reduction between ‘optimal’ growth in irrigated conditions (IRR) and growth under stress in non-irrigated (NIRR) conditions. This contrast can serve as a proxy for drought sensitivity. One reason behind this approach -compared to the more classical approach used for forest trees- was the lack of strong inter-annual variation within both treatments to actually differentiate harsh years, which restricts our ability to model dynamic, year-to-year resilience patterns. Nonetheless, using identical genotypes across both treatments provides a robust basis for comparing performances under ‘optimal’ versus ‘stressful’ conditions. We examined both apical and radial growth under water limitation using two complementary methods: (1) resilience parameters as proposed by Lloret et al. (2011), and (2) the average growth reduction (AVG) across the whole period, which reflects long-term performance under chronic water deficit.

For radial growth, European larch (EL) was characterized by a higher resistance, maintaining growth more steadily during drought, and experiencing the lowest average growth reduction. In contrast, Japanese larch (JL) exhibited stronger recovery, marked by a sharper post-drought rebound in growth. Hybrid larch (HL) generally held an intermediate position for resistance and recovery, with its performance varying widely across combinations. Resilience, which integrates resistance and recovery, showed more contrasting patterns: despite lower resistance,

JL often achieved high resilience scores. EL showed moderate resilience, primarily driven by high resistance but limited recovery. HL, again, showed combination-dependent resilience, occasionally matching or exceeding parental values, particularly when both resistance and recovery were moderately expressed. For height, the trends were broadly consistent but less pronounced. EL maintained relatively stable height growth under drought (moderate to some reduction), reflecting resistance. JL and HL showed higher height growth reductions on average, yet their capacity for height recovery varied with the combination. Importantly, HL displayed high reduction and recovery in some combinations, leading to moderate-to-high resilience scores overall. This further highlights the hybrid's ability to buffer stress effects through compensatory growth.

There has not been much work on the detailed physiological response to drought of the larch parental species and their hybrids to explain what could be behind these differential responses. Recent findings by Sasani et al. (2021) on seedlings suggest that EL possesses a hydraulically safer xylem, characterized by higher cell wall-to-lumen ratios and smaller pit cavities, which could underlie its superior resistance to drought stress compared to JL and HL. EL is currently classified as an anisohydric species (Anfodillo et al. 1998; Swidrak et al. 2013; Klein 2014; Leo et al. 2014; Sasani et al. 2021), which can keep growing when water is limiting. On the other side, Sasani et al. 2021 observed that JL displayed an isohydric-like strategy with a stronger stomata regulation, reducing transpiration early to conserve water better, but also thereby growth: JL showed the highest transpiration efficiency (TE) and $\delta^{13}C$ enrichment under drought, reflecting a strong stomatal regulation. During drought, JL also reduced its needle surface area, further minimizing water loss. These traits point to a conservative, water-saving response, allowing JL to recover more effectively when conditions improve. HL exhibited intermediate patterns, with evidence of both parental strategies.

These physiological strategies reflect a broader trade-off between resistance (growth maintenance during stress) and recovery (growth rebound after stress), a phenomenon widely observed in tree species. Tao et al. (2024) highlighted in a study based on 32 different species different trade-offs for resistance and recovery, showing that the relationship is differently related with growth rate - species with high growth tend to have low resistance, but high recovery (resistance, $r = -0.17$, $p < 0.001$; recovery, $r = 0.21$, $p < 0.01$). Overall, resilience to drought represents a synthetic parameter that integrates both components, capturing the overall ability of a tree to absorb and recover from drought-related disturbances. In our trial, HL was more closely associated with resilience, reflecting its balanced performance between resistance and recovery.

Can traits like P_{50} and WUE help predict resilience for drought conditions?

If a better resilience can reflect an "adaptive" response to drought, identifying traits linked to resilience parameters can guide further selection. In our context, "adaptation" is the process, action, or capacity of an individual or system to modify its inherent genetic or behavioral characteristics to better cope with change (inspired by Zhai and Lee (2024)). In our study, hybrid larch (HL), which appeared more resilient, also exhibited favorable (at intermediate position: **Figs 15 and 16**) values for P_{50} and WUE. This raises the question: can traits like P_{50} and WUE help predict resilience for drought conditions? When examining resilience parameters alongside water-use traits, clear taxon-level patterns emerge. European larch (EL), which showed the highest resistance, also had the most negative P_{50} values, indicating that resistance may be linked to a lower vulnerability to cavitation. In contrast, Japanese larch (JL) exhibited a lower resistance but a stronger recovery and higher overall resilience, and was also associated with a lower resistance to cavitation but a better water-use efficiency (WUE). This

suggests that while P_{50} may serve as a useful indicator of drought resistance, particularly in conservative species like EL, WUE appears more closely associated with resilience, especially when recovery is a major component. Taken together, P_{50} and WUE capture distinct yet complementary aspects of drought response—resistance and recovery—and may therefore be valuable physiological predictors for selecting genotypes with resilience. This opens the door to integrating them into breeding strategies to improve resilience, not just in performance but in long-term survival under climate stress.

For breeders, an ideal trait for selection should be variable among tested genotypes, heritable, and not unfavorably correlated with other desirable characteristics like growth (Sampaio Filho et al. 2023). In this study, the observed phenotypic coefficients of variation (CV%) for P_{50} were moderately high, with coefficients ranging from 9.7% to 10.1 % across taxa, with the highest one in HL, thus, supporting the potential of P_{50} as a meaningful and variable trait for selection. For water-use efficiency, CV ranged from 2.2 to 2.5% in IRR and 2.6 to 2.9% in NIRR, indicating a low variability, commonly reported in the literature (Guet 2015). Low values for WUE were also observed in Sassani et al. (2021) study with larch, with coefficients of variation (recalculated) ranging from 3.5% to 3.9% across taxa. However because the measures of $\delta^{13}C$ depend on the underlying standard used, their CVs can hardly be compared to those of other traits (Brendel 2014).

Prior studies support the breeding relevance of these traits: P_{50} has shown moderate to high heritability in *Pinus pinaster* ($h^2 = 0.40$) with substantial population-level variation (Lamy et al. 2014). Heritability for $\delta^{13}C$ reached 0.29 in the same species (Marguerit et al. 2014) Similarly, the resilience components - resistance, recovery, and resilience - have demonstrated moderate heritability in *Picea glauca*, especially under extreme drought years ($h^2 = 0.112–0.352$; Depardieu et al. 2020). While our study observed some associations between resilience parameters and physiological traits like P_{50} and WUE, we did not estimate heritability directly due to the limited number of combinations. Nonetheless, these findings remain important: they suggest that these traits are not only functionally relevant but also promising candidates for selection in future breeding programs targeting drought resilience. Yet, incorporating such traits into selection programs invites a fundamental question in ecology and genetics: even though these traits are variable or heritable, can they be associated with growth performance?

Does Fast growth come with a cost?

This brings us to a broader question relevant to ecology and tree breeding: how do these physiological/ ecological traits associated with a more efficient or safer water use relate to growth potential, and does fast growth inherently come at a cost, *i.e*. be detrimental to other favourable traits? The concept of "fast growth" is increasingly questioned in breeding and ecological contexts, particularly regarding potential trade-offs with traits like wood quality, drought resilience, and long-term mortality (Lamy et al. 2014; Anderegg et al. 2016). There has been a shift toward valuing slower-growing species or genotypes that may be more conservative in resource use and more resilient under stress (Reich 2014). In our study, "fast growth" was defined not as a rate measured annually over time (growth speed), but by the attained size at a fixed age, a cumulative outcome that integrates growth and survival. This pragmatic definition aligns with how growth is assessed in many operational breeding programs, where final height, diameter, or volume at a target age (like 10 years) serves as a proxy for productivity (Zobel and Talbert, 1984). In our study, the relationship between growth and other traits revealed context-

dependent and trait-specific patterns, suggesting that the costs of fast growth are not universal, but may emerge under specific environmental or genetic contexts.

Ecological view: Across taxa

To explore whether fast growth comes with a cost under drought, we analyzed how correlations between growth (height and diameter) and other traits change across water treatments, from irrigated (IRR) to non-irrigated (NIRR). This can provide an ecologist-level understanding of general functional trade-offs.

WUE showed consistently negative correlations with growth traits, ranging from r = - 0.12 to -0.77 at the family and clonal level, particularly under irrigation. These trends suggest that genotypes with higher WUE tend to grow more, likely because they can assimilate more carbon per unit of water lost. P_{50} also showed positive correlations with growth (r = 0.25 to 0.55 at the clonal and family level), indicating that higher growth might be associated with increased risk of cavitation; this clearly differentiated EL from JL genotypes. Resilience parameters were moderately associated with diameter growth, especially under IRR. The correlation between resistance and growth was consistently negative, especially for diameter (family: r = –0.226, clonal: r = –0.372) and average growth reduction (AGR) was also positively correlated with diameter at both levels (family: r = 0.394, clonal: r = 0.385, both $p < 0.05$), implying that the most productive ones could be variably impacted under drought. In contrast, recovery showed a weak but positive correlation at the family level (r = 0.108) and a stronger correlation at the clonal level (r = 0.578, $p < 0.05$), indicating that faster-growing genotypes may recover more strongly after stress. The correlation between resilience and diameter was r = 0.230 at the family level, but declined to r = –0.267 at the clonal level. Height, by contrast, was weakly or inconsistently related to resilience traits, with generally non-significant or weak associations across both levels, apart from a slight negative correlation with resistance under IRR at the clonal level (r = –0.151, $p < 0.05$) and also a positive one with AGR (r = 0.35). However, under non-irrigated conditions (NIRR), these positive associations weakened. For resilience parameters, fast-growing trees were linked to a lower resistance and a larger recovery, particularly in diameter (-0.064 to -0.22 for resistance and r = 0.221 to 0.287 for recovery at family or clonal level). A similar observation was made by Tao et al. 2024: resistance also had a negative relation with growth rate (r = -0.17, $p < 0.001$). Notably, this negative correlation between growth and resistance was already present - and even stronger - under irrigated conditions suggesting that this trade-off is not solely drought-induced but may reflect an inherent physiological cost of rapid growth.

This reinforces the idea of inherent trade-offs in resource allocation, whereby trees must balance growth and stress tolerance in response to environmental demands (Herms and Mattson, 1992; Poorter et al. 2010; Reich, 2014; Lauder et al. 2019). In Chapter I, it was also observed that during years of heavy stress, not only a higher mortality rate but also a strong stem growth reduction and frequent top dieback were observed: this suggests that resource reallocation away from stem growth may reflect a survival strategy prioritizing maintenance and repair.

Breeder view: Within taxa

For breeders, it is worth to consider separately links between growth and other traits within taxa across families and clones. Indeed, the final objective is to select genotypes within populations of each taxon. Ideally, genotypes combining fast growth and efficient water-use, less vulnerable to cavitation, and resilient to drought would be a kind of ideotype. A second objective would

be to compare correlations for HL with those of parental species to get some insights into the genetic source of correlation (pleiotropy or linkage disequilibrium).

At the family level under irrigation, diameter growth in the irrigated treatment was strongly linked with water-use efficiency (WUE) in all taxa, especially in HL ($r = -0.890$) and JL ($r = -0.495$, $p<0.05$), and also in EL ($r = -0.586$, $p<0.05$). P_{50} showed a positive association with diameter in EL and JL, but a negative trend in HL, suggesting that fast-growing HL families are less vulnerable to cavitation and may better face some risk. However, it is important to note that HL families represent diverse genetic backgrounds, and as shown in the scatterplots **(Figs. 15 and 16),** some HL families exhibited variable WUE and P_{50}, indicating that within-hybrid variability could offer opportunities to select more hydraulically safe yet productive genotypes. For height in IRR treatment, WUE remained negatively associated across the three taxa, but recovery showed more variable links. In EL, a strong negative correlation between height and recovery ($r = –0.662$, $p<0.05$) suggested a potential trade-off between apical growth and post-stress recovery.

Under non-irrigated conditions (NIRR), relationships weakened overall, though diameter-WUE correlations persisted in EL and HL. Notably, JL retained strong associations between diameter and both recovery ($r = 0.737$, $p<0.05$) and AGR ($r = 0.740$, $p<0.05$), reinforcing its rebound capacity under drought. Height correlations remained generally weak, though HL showed a moderate link with resilience ($r = 0.570$, $p<0.05$), and EL maintained its negative trend with recovery.

At the clonal level, within taxa, diameter growth consistently showed stronger and more consistent correlations with other traits than height, highlighting its value as a key selection trait. Diameter growth showed clear and consistent links with adaptive traits, particularly under irrigated conditions. In European larch (EL), diameter was negatively correlated with WUE ($r = -0.456$, $p <0.05$), positively with recovery ($r = 0.544$, $p<0.05$), resilience ($r = 0.359$, $p<0.05$), and AGR ($r = 0.308$, $p<0.05$), but negatively with resistance ($r = –0.292$, $p<0.05$), suggesting that EL clones can achieve good growth and water-use efficiency while recovering well after stress, though at the cost of reduced resistance. In hybrid larch (HL), strong associations were also found under IRR between diameter and recovery ($r = 0.491$), resilience ($r = 0.491$, $p<0.05$), and AGR ($r = 0.319$, $p<0.05$); however, these correlations declined under drought stress in NIRR, with recovery dropping to $r = 0.022$. Japanese larch (JL) showed the strongest link with diameter in IRR positively correlated with recovery ($r = 0.603$, $p<0.05$), resilience ($r = 0.278$, $p<0.05$), and AGR ($r = 0.395$, $p<0.05$), and a still-significant AGR correlation under NIRR ($r = 0.292$, $p<0.05$). Across all taxa, diameter in IRR was consistently and negatively associated with resistance (EL: –0.292, HL: –0.350, JL: –0.283), confirming a trade-off between maintaining growth and drought tolerance. In contrast, height showed weaker and less stable relationships, making diameter a more useful trait for identifying resilient, productive genotypes.

From a breeding standpoint, the central question is whether it is possible to select for fast growth without compromising adaptive traits. The results suggest that this is partially possible, but the outcome depends on the trait in question, the taxon, and the environment.

First, we observed substantial variation in growth and some adaptive traits (not in WUE: about 2%) at the family and clonal levels across all three taxa (EL, HL, JL), indicating that there is genetic variability to act upon. This is encouraging - it confirms that selection within taxa is

feasible, especially when targeting individuals that may escape unfavorable trade-offs. Second, the direction of correlations matters. Traits like WUE and recovery were often positively (favourably) or neutrally associated with diameter growth, particularly under irrigated conditions. This means that, in many cases, selecting for faster growth will not penalize— and may even assist in improving - certain adaptive traits. However, resistance to stress consistently showed negative correlations with growth. In our study, resilience, a composite indicator of stress resistance and recovery, often showed positive correlations with diameter, particularly in hybrids. This suggests that, in contrast to the expected trade-offs, hybrids with higher growth potential do not necessarily compromise their resilience, indicating the possibility of identifying genotypes that combine productivity and adaptive capacity.

Thirdly, iInterspecific hybridization can lead to new trait combinations but may also disrupt favorable ones seen in the parents. For example, while EL and JL showed a positive correlation (but not significant) at the mean family level between growth and P_{50}, suggesting that faster growers were less cavitation-resistant, this pattern was reversed in HL, where a negative correlation (but not significant) indicates that fast-growing genotypes tend to be less vulnerable. This suggests that, in HL, selecting for both high growth and hydraulic safety can be possible, as recombination between EL and JL appears to have broken non -beneficial trait linkages. In a similar way, interspecific recombination can also improve links between traits: for example, the strong unfavourable correlation between diameter and average growth reduction due to drought in both EL and JL ($r = 0.422$ and 0.624, respectively) becomes not significantly different from 0 in hybrids ($r = 0.048$). Such breakage of correlations through interspecific hybridization might indicate a linkage disequilibrium source for these correlations.

Importantly, when moving from irrigated (IRR) to non-irrigated (NIRR) conditions, correlations between growth and adaptive traits generally weakened, particularly for traits like recovery in JL, where the strength and even direction of associations shifted. This instability suggests that trait relationships observed under optimal conditions do not consistently translate to performance under stress. This aligns with the work of Sgrò and Hoffmann (2004), who reviewed those genetic correlations between traits and observed that they can shift significantly across environments, sometimes even changing sign.

Overall, selection for fast-growing and well-adapted genotypes is possible, but must be taxon-specific and trait-informed. Traits like WUE and recovery can improve alongside with growth, while resistance may need to be balanced carefully due to trade-offs. Moreover, given the environmental sensitivity of many adaptive traits, particularly under drought, it is clear that selection in optimal conditions alone is insufficient. To reliably capture drought resilience, testing and selecting under stressful conditions remain essential.

5. Conclusion

This study highlights the complex but promising role of interspecific hybridization in larch breeding under varying water availability. Hybrid Larch (HL) consistently demonstrated heterosis for growth, particularly in height and volume, though the degree of expression varied by trait, environment, and genetic combination. European Larch (EL) displayed stable but conservative responses, with strong drought resistance but limited recovery, whereas Japanese Larch (JL) showed high growth under irrigation but greater vulnerability under stress. Fast-growing combinations were not always the most resilient, underscoring the need for breeding programs to move beyond growth metrics and incorporate trait-based and context-sensitive

selection strategies. Although correlations between growth and other traits weakened at the clonal level, hybrids still maintained an intermediate position, making them valuable candidates for deployment.

In conclusion, hybrid larch offers a compelling path forward for afforestation and climate-adaptive forestry. However, its effective use requires careful evaluation of genotype performance across both traits and environments, emphasizing the integration of physiological screening, resilience assessment, and multi-environment trials in future breeding efforts.

6. Reference

Anderegg WRL (2015) Spatial and temporal variation in plant hydraulic traits and their relevance for climate change impacts on vegetation. New Phytol 205:1008–1014. https://doi.org/10.1111/nph.12907

Anderegg WRL, Klein T, Bartlett M, Sack L, Pellegrini AFA, Choat B, Jansen S (2016) Meta-analysis reveals that hydraulic traits explain cross-species patterns of drought-induced tree mortality across the globe. Proc Natl Acad Sci 113:5024–5029. https://doi.org/10.1073/pnas.1525678113

Anfodillo T, Rento S, Carraro V, Furlanetto L, Urbinati C, Carrer M (1998) Tree water relations and climatic variations at the alpine timberline: seasonal changes of sap flux and xylem water potential in *Larix decidua* Miller, *Picea abies* (L.) Karst. and *Pinus cembra* L. Ann Sci For 55:159–172. https://doi.org/10.1051/forest:19980110

Arcade A, Faivre-Rampant P, Le Guerroué B, Pâques LE, Prat D (1996) Heterozygosity and hybrid performance in larch. Theor Appl Genet 93:1274–1281

Atkinson RRL, Burrell MM, Osborne CP, Rose KE, Rees M (2012) A non-targeted metabolomics approach to quantifying differences in root storage between fast- and slow-growing plants. New Phytol 196:200–211. https://doi.org/10.1111/j.1469-8137.2012.04274.x

Baltunis BS, Greenwood MS (1999) Variation in lateral shoot elongation patterns and hybrid vigor in full-sib families and interspecific hybrids of larch. Tree Physiol 19:131–136. https://doi.org/10.1093/treephys/19.2.131

Bastien JK, Keller R (1980) Intérêts comparés du Mélèze hybride (*Larix x eurolepis* Henry) avec les deux espèces parentes. Revue forestière française.32(6):521-530. ff10.4267/2042/21438ff.ffhal-03397251ff

Berghof TVL, Poppe M, Mulder HA (2019) Opportunities to Improve Resilience in Animal Breeding Programs. Front Genet 9. https://doi.org/10.3389/fgene.2018.00692

Bigler C, Veblen TT (2009) Increased early growth rates decrease longevities of conifers in subalpine forests. Oikos 118:1130–1138. https://doi.org/10.1111/j.1600-0706.2009.17592.x

Birchler JA, Auger DL, Riddle NC (2003). In search of the molecular basis of heterosis. The Plant Cell, 15(10), 2236-2239.

Brendel O (2014)Is the coefficient of variation a valid measure for variability of stable isotope abundances in biological materials?, Rapid Commun. Mass Spectrom, 4:370-376. https://doi.org/10.1002/rcm.6791

Brewbaker JL, Sun WG (1999) Trees and Heterosis. In: Genetics and Exploitation of Heterosis in Crops. John Wiley & Sons, Ltd, pp 463–477

Brice T, Hall T (2020) US Dept of Commerce National Oceanic and Atmospheric Administration National Weather Service [WWW Document]. Vapor Pressure Calculator. URL: https://www.weather.gov/epz/wxcalc_vapor pressure

Cappa EP, Marcó M, Garth Nikles D, Last IS (2013) Performance of *Pinus elliottii*, *Pinus caribaea*, their F1, F2 and backcross hybrids and *Pinus taeda* to 10 years in the Mesopotamia region, Argentina. New For 44:197–218. https://doi.org/10.1007/s11056-012-9311-2

Cazaux JP, Chevalier R, Gilbert JM, Ginisty C (1993) Le mélèze hybride en plantation : résultats provisoires sur 17 sites en France. Inf Tech CEMAGREF 8

Churakova (Sidorova) OV, Lehmann MM, Siegwolf RTW, Saurer M, Fonti MV, Schmid L, Timofeeva G, Rinne-Garmston KT, Bigler C (2019) Compound-specific carbon isotope patterns in needles of conifer tree species from the Swiss National Park under recent climate change. Plant Physiol Biochem 139:264–272. https://doi.org/10.1016/j.plaphy.2019.03.016

Climent J, Alía R, Karkkainen K, Bastien C, Benito-Garzon M, Bouffier L, De Dato G, Delzon S, Dowkiw A, Elvira-Recuenco M, Grivet D, González-Martínez SC, Hayatgheibi H, Kujala S, Leplé J-C, Martín-Sanz RC, de Miguel M, Monteverdi MC, Mutke S, Plomion C, Ramírez-Valiente JA, Sanchez L, Solé-Medina A, Soularue J-P, Steffenrem A, Teani A, Westin J, Whittet R, Wu H, Zas R, Cavers S (2024) Trade-offs and Trait Integration in Tree Phenotypes: Consequences for the Sustainable Use of Genetic Resources. Curr For Rep 10:196–222. https://doi.org/10.1007/s40725-024-00217-5

Climent J, Prada MA, Calama R, Chambel MR, de Ron DS, Alía R (2008) To grow or to seed: ecotypic variation in reproductive allocation and cone production by young female Aleppo pine (*Pinus halepensis*, Pinaceae). Am J Bot 95:833–842. https://doi.org/10.3732/ajb.2007354

Cook BI, Mankin JS, Anchukaitis KJ (2018) Climate Change and Drought: From Past to Future. Curr Clim Change Rep 4:164–179. https://doi.org/10.1007/s40641-018-0093-2

Cope OL, Keefover-Ring K, Kruger EL, Lindroth RL (2021) Growth–defense trade-offs shape population genetic composition in an iconic forest tree species. Proc Natl Acad Sci 118:e2103162118. https://doi.org/10.1073/pnas.2103162118

Craig H (1957) Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim Cosmochim Acta 12:133–149. https://doi.org/10.1016/0016-7037(57)90024-8

Delzon S, Douthe C, Sala A, Cochard H (2010) Mechanism of water-stress induced cavitation in conifers: bordered pit structure and function support the hypothesis of seal capillary-seeding. Plant Cell Environ 33:2101–2111. https://doi.org/10.1111/j.1365-3040.2010.02208.x

Depardieu C, Girardin MP, Nadeau S, Lenz P, Bousquet J, Isabel N (2020) Adaptive genetic variation to drought in a widely distributed conifer suggests a potential for increasing forest resilience in a drying climate. New Phytol 227:427–439. https://doi.org/10.1111/nph.16551

Donat MG, Alexander LV, Yang H, Durre I, Vose R, Dunn RJH, Willett KM, Aguilar E, Brunet M, Caesar J, Hewitson B, Jack C, Klein Tank AMG, Kruger AC, Marengo J, Peterson TC, Renom M, Oria Rojas C, Rusticucci M, Salinger J, Elrayah AS, Sekele SS, Srivastava AK, Trewin B, Villarroel C, Vincent LA, Zhai P, Zhang X, Kitching S (2013) Updated analyses of temperature and precipitation extreme indices since the beginning of the twentieth century: The HadEX2 dataset. J Geophys Res Atmospheres 118:2098–2118. https://doi.org/10.1002/jgrd.50150

Dungey HS (2001) Pine hybrids—a review of their use performance and genetics. For Ecol Manag 148(1-3):243-258.

Duursma RA, Choat BA (2017) fitplc - an R package to fit hydraulic vulnerability curves. J Plant Hydraul 4:e002. https://doi.org/10.20870/jph.2017.e002

Escobar-Sandoval M (2020) Plasticité phénotypique inter et intra-annuelle de la croissance radiale et d'autres propriétés liées à la formation du cerne chez le mélèze. Doctoral dissertation, Institut agronomique, vétérinaire et forestier de France

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

Falconer DS, Mackay TF (1996) Introduction to quantitative genetics, 4th edn. Longman, Harlow

Farquhar G, Richards R (1984) Isotopic Composition of Plant Carbon Correlates With Water-Use Efficiency of Wheat Genotypes. Funct Plant Biol 11:539–552. https://doi.org/10.1071/PP9840539

Forrester DI (2014) The spatial and temporal dynamics of species interactions in mixed-species forests: From pattern to process. For Ecol Manag 312:282–292. https://doi.org/10.1016/j.foreco.2013.10.003

Fujimoto T, Kita K, Uchiyama K, Kuromaru M, Akutsu H, Oda K (2006) Age trends in the genetic parameters of wood density and the relationship with growth rates in hybrid larch (*Larix gmelinii* var. japonica × *L. kaempferi*) F1. J For Res 11:157–163. https://doi.org/10.1007/s10310-005-0200-9

Gallais A (2009) Hétérosis et variétés hybrides en amélioration des plantes. Quae, Versailles

Gothe H, Schober R,Bork H (1980) Ein Kreuzungsversuch mit *Larix europaea* D.C, Herkunft Schlitz, und *Larix leptolepis* Gord. Allg. Forstztg. 151 (6-7):101-112

Granier A, Bréda N, Biron P, Villette S (1999) A lumped water balance model to evaluate duration and intensity of drought constraints in forest stands. Ecol Model 116:269–283. https://doi.org/10.1016/S0304-3800(98)00205-1

Greenwood MS, Roth BE, Maass D, Irland LC (2015) Near rotation-length performance of selected hybrid larch in Central Maine, U.S.A. Silvae Genet 64:73–80. https://doi.org/10.1515/sg-2015-0006

Guet J (2015). Expression d'une variabilité génétique pour la phénologie de croissance, l'efficience d'utilisation de l'eau et la résistance à la cavitation au sein de populations naturelles de peuplier noir (*Populus nigra* L.) (Doctoral dissertation, Université d'Orléans).

Halofsky JE, Peterson DL, Harvey BJ (2020) Changing wildfire, changing forests: the effects of climate change on fire regimes and vegetation in the Pacific Northwest, USA. Fire Ecol 16:4. https://doi.org/10.1186/s42408-019-0062-8

Hegyi F (1974) A simulation model for managing jack-pine. RoyalColl. For, Res. Notes, 30: 74-90.

Huhn M (1990). Nonparametric measures of phenotypic stability. Part 1: Theory. Euphytica 47:189–1990.

Helama S (2015) Expressing Tree-Ring Chronology as Age-Standardized Growth Measurements. For Sci 61:817–828. https://doi.org/10.5849/forsci.14-139

Herms DA, Mattson WJ (1992) The Dilemma of Plants: To Grow or Defend. Q Rev Biol 67:283–335. https://doi.org/10.1086/417659

Johnson KM, Brodribb TJ (2023) Evidence for a trade-off between growth rate and xylem cavitation resistance in *Callitris rhomboidea*. Tree Physiol tpad037. https://doi.org/10.1093/treephys/tpad037

Kissoudis C, van de Wiel C, Visser RGF, van der Linden G (2014) Enhancing crop resilience to combined abiotic and biotic stress through the dissection of physiological and molecular crosstalk. Front Plant Sci 5. https://doi.org/10.3389/fpls.2014.00207

Keiding H (1980) Hybridlaerke vaekst og tilpasningsevne i forhold til de rene arter. Dans. Skovforen. Tidsskr. 65, 204-234

Klein T (2014) The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. Funct Ecol 28:1313–1320. https://doi.org/10.1111/1365-2435.12289

Knight R (1973) The relation between hybrid vigour and genotype-environment interactions. Theoret Appl Genetics 43:311–318. https://doi.org/10.1007/BF00275258

Lamy J, Delzon S, Bouche PS, Alia R, Vendramin GG, Cochard H, Plomion C (2014) Limited genetic variability and phenotypic plasticity detected for cavitation resistance in a Mediterranean pine. New Phytol 201:874–886. https://doi.org/10.1111/nph.12556

Larocque GR, DesRochers A, Larchevêque M, Tremblay F, Beaulieu J, Mosseler A, Major JE, Gaussiran S, Thomas BR, Sidders D, Périnet P, Kort J, Labrecque M, Savoie P, Masse S, Bouman OT, Kamelchuk D, Benomar L, Mamashita T, Gagné P (2013) Research on hybrid poplars and willow species for fast-growing tree plantations: Its importance for growth and yield, silviculture, policy-making and commercial applications. For Chron 89:32–41. https://doi.org/10.5558/tfc2013-009

Lauder JD, Moran EV, Hart SC (2019) Fight or flight? Potential tradeoffs between drought defense and reproduction in conifers. Tree Physiol 39:1071–1085. https://doi.org/10.1093/treephys/tpz031

Leo M, Oberhuber W, Schuster R, Grams TEE, Matyssek R, Wieser G (2014) Evaluating the effect of plant water availability on inner alpine coniferous trees based on sap flow measurements. Eur J For Res 133:691–698. https://doi.org/10.1007/s10342-013-0697-y

Li B, Wyckoff GW, Einspahr DW. (1993). Hybrid aspen performance and genetic gains. North. J. Appl. For. 10(3), 117-122.

Lloret F, Keeling EG, Sala A (2011) Components of tree resilience: effects of successive low-growth episodes in old ponderosa pine forests. Oikos 120:1909–1920. https://doi.org/10.1111/j.1600-0706.2011.19372.x

Lovell JT, McKay JK (2015) Ecological genetics of range size variation in *Boechera* spp. (Brassicaceae). Ecol Evol 5:4962–4975. https://doi.org/10.1002/ece3.1746

Maass DI, Irland LC, Anderson JL, Laustsen KM, Greenwood MS, Roth BE (2020) Reassessing Potential for Exotic Larch in Northern United States. J For 118:124–138. https://doi.org/10.1093/jofore/fvz066

Marchal A, Muñoz F, Millier F, Sánchez L, Pâques LE (2017) Hybrid larch heterosis: for which traits and under which genetic control? Tree Genet Genomes 13:92. https://doi.org/10.1007/s11295-017-1177-1

Marchal A, Schlichting CD, Gobin R, Balandier P, Millier F, Muñoz F, Pâques LE, Sánchez L (2019) Deciphering Hybrid Larch Reaction Norms Using Random Regression. G3 GenesGenomesGenetics 9:21–32. https://doi.org/10.1534/g3.118.200697

Marguerit E, Bouffier L, Chancerel E, Costa P, Lagane F, Guehl J-M, Plomion C, Brendel O (2014) The genetics of water-use efficiency and its relation to growth in maritime pine. J. Exp. Bot. 65:4757–4768. https://doi.org/10.1093/jxb/eru226

Mitton JB, Knowles P, Sturgeon KB, Linhart YB, Davis M (1981). Associations between heterozygosity and growth rate variables in three western forest trees. Proc Symp Isozymes North Am For Trees For Insects USDA For Serv Washington: US Forest Service. 27-34.

Mohrdiek O (1979) Juvenile-mature and trait correlations in some aspen and poplar [*Populus*] trials. Silvae Genet Ger.107-111

Muñoz F, Sánchez L (2015) breedR: statistical methods for forest genetic resources analysts. URL https://github.com/famuvie/breedR, R package

Nikles,DG (2000). Experience with some *Pinus* hybrids in Queensland, Austrlalia.

Pâques L (1992) Performance of vegetatively propagated *Larix decidua*, *L kaempferi* and *L laricina* hybrids. Ann Sci For 49:63–74. https://doi.org/10.1051/forest:19920106

Pâques LE (1989) A critical review of larch hybridization and its incidence on breeding strategies. Ann Sci For 46:141–153. https://doi.org/10.1051/forest:19890204

Pâques LE (2004) Roles of European and Japanese larch in the genetic control of growth, architecture and wood quality traits in interspecific hybrids (*Larix x eurolepis* Henry). Ann For Sci 61:25–33. https://doi.org/10.1051/forest:2003081

Pâques LE (2009) Growth Rhythm Parameters as Components of Hybrid Vigour in Young Seedlings of Hybrid Larch (*Larix decidua x L. kaempferi*). Silvae Genet 58:42–53. https://doi.org/10.1515/sg-2009-0006

Pâques LE (ed) (2013) Forest Tree Breeding in Europe: Current State-of-the-Art and Perspectives. Springer Netherlands, Dordrecht

Pâques LE, Charpentier J-P (2015) Perspectives for genetic improvement in heartwood size and extractive content in relation to natural durability and aesthetics in interspecific hybrid larch (*Larix* × *eurolepis*). Eur J For Res 134:857–868. https://doi.org/10.1007/s10342-015-0895-x

Pâques LE, Lejeune V, Veisse D (2022) Do biomass partitioning and growth efficiency contribute to growth heterosis in inter-specific hybrid larch *Larix x eurolepis*? For Int J For Res 95:466–476. https://doi.org/10.1093/forestry/cpac004

Pâques LE, Millier F, Veisse D (2023) Longitudinal stem cracks in larch: what makes trees vulnerable? New For 54:813–832. https://doi.org/10.1007/s11056-022-09942-w

Pauwels D, Rondeux J (1999). Stand volume tables for larches in southern Belgium.

Poorter L, McDonald I, Alarcón A, Fichtler E, Licona J, Peña-Claros M, Sterck F, Villegas Z, Sass-Klaassen U (2010) The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. New Phytol 185:481–492. https://doi.org/10.1111/j.1469-8137.2009.03092.x

Potts BM, Dungey HS Interspecific hybridization of *Eucalyptus*: key issues for breeders and geneticists. New Forests, 27(2):115-138

R Core Team (2024) R: a language and environment for statistical computing. URL https://www. R-project.org/

Reich PB (2014) The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. J Ecol 102:275–301. https://doi.org/10.1111/1365-2745.12211

Sampaio Filho JS, Olivoto T, Campos MDS, Oliveira EJD (2023) Multi-trait selection in multi-environments for performance and stability in cassava genotypes. Front Plant Sci 14:1282221. https://doi.org/10.3389/fpls.2023.1282221

Sasani N, Pâques LE, Boulanger G, Singh AP, Gierlinger N, Rosner S, Brendel O (2021) Physiological and anatomical responses to drought stress differ between two larch species and their hybrid. Trees 35:1467–1484. https://doi.org/10.1007/s00468-021-02129-4

Scamoni A. (1977) Uber Larchenhybriden aus freier Bestaubung und über die weitere Entwicklung gelenkter Larchenkreuzungen. Beitr. Forstwirtsch. 11 (1), 21-26

Schwarz J, Skiadaresis G, Kohler M, Kunz J, Schnabel F, Vitali V, Bauhus J (2020) Quantifying Growth Responses of Trees to Drought—a Critique of Commonly Used Resilience Indices and Recommendations for Future Studies. Curr For Rep 6:185–200. https://doi.org/10.1007/s40725-020-00119-2

Sgrò CM, Hoffmann, A A. (2004). Genetic correlations, tradeoffs and environmental variation. Heredity, 93(3), 241-248.

Shrestha KB, Hofgaard A, Vandvik V (2015) Tree-growth response to climatic variability in two climatically contrasting treeline ecotone areas, central Himalaya, Nepal. Can J For Res 45:1643–1653. https://doi.org/10.1139/cjfr-2015-0089

Shull GH (1914) Duplicate genes for capsule-form in Bursa bursa-pastoris. Zeitschrift für induktive Abstammungs-und Vererbungslehre. 12:97.

Shull GH (1948) What Is "Heterosis"? Genetics 33:439–446

Signori-Müller C, Oliveira RS, Valentim Tavares J, Carvalho Diniz F, Gilpin M, de V. Barros F, Marca Zevallos MJ, Salas Yupayccana CA, Nina A, Brum M, Baker TR, Cosio EG, Malhi Y, Monteagudo Mendoza A, Phillips OL, Rowland L, Salinas N, Vasquez R, Mencuccini M, Galbraith D (2022) Variation of non-structural carbohydrates across the fast–slow continuum in Amazon Forest canopy trees. Funct Ecol 36:341–355. https://doi.org/10.1111/1365-2435.13971

Soehendi R, Srinives P (2005). Significance of heterosis and heterobeltiosis in F~ 1 hybrid of mungbean (*Vigna radiata* (L.) Wilczek) for hybrid seed production. Sabrao J. Breed. Genet. 37(2):97.

Sturrock RN, Frankel SJ, Brown AV, Hennon PE, Kliejunas JT, Lewis KJ, Worrall JJ, Woods AJ (2011) Climate change and forest diseases. Plant Pathol 60:133–149. https://doi.org/10.1111/j.1365-3059.2010.02406.x

Swidrak I, Schuster R, Oberhuber W (2013) Comparing growth phenology of co-occurring deciduous and evergreen conifers exposed to drought. Flora - Morphol Distrib Funct Ecol Plants 208:609–617. https://doi.org/10.1016/j.flora.2013.09.004

Sylvestre-Guinot G, Pâques LE, Delatour C (1999) Résistance du mélèze hybride inoculé par *Lachnellula willkommii*. Ann For Sci 56:485–492. https://doi.org/10.1051/forest:19990605

Tao W, He J, Smith NG, Yang H, Liu J, Chen L, Tao J, Luo W (2024) Tree growth rate-mediated trade-off between drought resistance and recovery in the Northern

Hemisphere. Proc R Soc B Biol Sci 291:20241427. https://doi.org/10.1098/rspb.2024.1427

Valladares F, Sanchez-Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J Ecol 94:1103–1116. https://doi.org/10.1111/j.1365-2745.2006.01176.x

Watanabe M, Watanabe Y, Kitaoka S, Utsugi H, Kita K, Koike T (2011) Growth and photosynthetic traits of hybrid larch F1 (*Larix gmelinii var. japonica x L. kaempferi*) under elevated CO2 concentration with low nutrient availability. Tree Physiol 31:965–975. https://doi.org/10.1093/treephys/tpr059

Wricke O 1962 Uber eine Method Zur Erffassung derkologi schen strenb reite in Feldversuchen. Z pflan zen zuchtung 47:92 – 96

Zanewich KP, Pearce DW, Rood SB (2018) Heterosis in poplar involves phenotypic stability: cottonwood hybrids outperform their parental species at suboptimal temperatures. Tree Physiol 38:789–800. https://doi.org/10.1093/treephys/tpy019

Zhai L, Lee J-E (2024) Investigating Vulnerability, Adaptation, and Resilience: A Comprehensive Review within the Context of Climate Change. Atmosphere 15:474. https://doi.org/10.3390/atmos15040474

Zhu Y, Queenborough SA, Condit R, Hubbell SP, Ma KP, Comita LS (2018) Density-dependent survival varies with species life-history strategy in a tropical forest. Ecol Lett 21:506–515. https://doi.org/10.1111/ele.12915

Zobel B, Talbert J (1984). Applied Forest Tree Improvement. New York: Wiley.

Supplementary Material

S Table 1: Heterosis in Absolute and relative for eight combinations in Irrigated site of experiment for Height increment across years. ** when significant, p<0.05*

Year	Combination	IRR Absolute Heterosis(cm)	Relative Heterosis(%)	Significance	NIRR Absolute Heterosis(cm)	Relative Heterosis(%)	Significance
	104x3194	26.71	26.04	*	36.06	37.63	*
	104x3200	17.77	17.85		18.67	18.27	*
	106x3194	-3.20	-3.24		7.77	8.34	
2013	106x3200	2.56	2.67		-5.34	-5.37	
	221x3194	17.09	17.81		12.26	13.35	
	221x3200	-1.06	-1.14		-1.07	-1.09	
	222x3194	18.72	17.83		27.32	28.85	*
	222x3200	7.14	7.00		8.83	8.74	
	104x3194	-9.87	-11.69		6.73	9.40	
	104x3200	14.80	17.22		12.01	16.48	
	106x3194	19.78	28.08	*	24.08	36.19	
2014	106x3200	22.11	30.73	*	25.27	37.30	*
	221x3194	-16.30	-19.07		23.22	43.08	
	221x3200	0.78	0.90		25.30	45.90	
	222x3194	10.34	11.63		34.73	52.25	*
	222x3200	3.08	3.41		50.81	75.04	*
	104x3194	5.45	6.19		2.68	12.99	
	104x3200	21.74	24.71	*	1.03	4.68	
	106x3194	-14.86	-16.88	*	17.49	84.81	*
2015	106x3200	13.01	14.78		3.93	17.85	
	221x3194	-9.99	-11.35		2.36	11.42	
	221x3200	-7.56	-8.59		6.43	29.21	
	222x3194	-3.73	-4.24		18.75	90.92	*
	222x3200	-3.17	-3.60		4.63	21.05	
	104x3194	7.71	6.73		7.48	10.38	
	104x3200	4.63	3.91		9.82	13.11	
2016	106x3194	3.73	3.40		12.38	18.02	
	106x3200	15.71	13.80		10.46	14.62	
	221x3194	9.06	8.30		4.80	7.48	
	221x3200	10.51	9.28		10.46	15.59	
	222x3194	11.28	10.02		35.37	49.91	*
	222x3200	12.56	10.77		27.90	37.83	*
	104x3194	-2.93	-1.76		24.47	30.85	*
	104x3200	16.31	10.06		22.26	28.08	*
	106x3194	18.71	12.08		3.44	4.49	
2017	106x3200	35.12	23.30	*	30.22	39.42	*
	221x3194	12.82	8.15		-3.44	-4.65	
	221x3200	14.24	9.30		16.31	22.02	
	222x3194	15.58	10.00		22.47	28.75	
	222x3200	23.31	15.37		37.65	48.18	*

	104x3194	22.32	15.91	*	17.05	28.76	*
	104x3200	9.82	6.65		6.57	11.08	
	106x3194	12.79	9.51		3.39	5.71	
2018	106x3200	18.68	13.18	*	16.38	27.64	*
	221x3194	-4.50	-3.26		2.41	4.06	
	221x3200	8.60	5.92		14.63	24.69	*
	222x3194	5.99	4.33		7.10	11.97	
	222x3200	17.39	11.95		2.77	4.67	
	104x3194	6.84	5.50		3.53	7.77	
	104x3200	4.96	3.87		0.90	1.97	
	106x3194	11.72	9.42		-6.93	-15.45	
2019	106x3200	14.20	11.07	*	-7.47	-16.70	
	221x3194	-4.44	-3.57		-10.78	-24.11	
	221x3200	4.76	3.71		-5.44	-12.20	
	222x3194	5.31	4.27		-7.71	-17.07	
	222x3200	3.10	2.42		-16.77	-37.25	*
	104x3194	5.51	4.34		2.06	3.04	
	104x3200	10.31	8.28		-19.08	-28.16	*
	106x3194	6.12	4.94		2.32	3.42	
2020	106x3200	10.01	8.22		4.30	6.34	
	221x3194	0.24	0.20		9.07	13.38	
	221x3200	19.01	15.77		7.13	10.52	
	222x3194	12.45	10.35		-10.12	-14.94	
	222x3200	7.74	6.55		-22.87	-33.76	*
	104x3194	4.04	4.75		5.27	5.97	
	104x3200	-4.75	-5.61		-5.37	-6.18	
	106x3194	-1.68	-2.06		1.12	1.42	
2021	106x3200	-4.93	-6.10		16.95	21.87	
	221x3194	5.25	6.50		-3.33	-4.18	
	221x3200	3.20	3.99		5.60	7.14	
	222x3194	1.78	2.09		-1.95	-2.30	
	222x3200	-4.24	-5.01		4.93	5.90	
	104x3194	1.31	1.29		-2.39	-4.24	
	104x3200	-10.14	-10.53		-6.49	-11.36	
	106x3194	-2.78	-3.28		-3.82	-7.71	
2022	106x3200	-1.48	-1.86		10.04	19.99	
	221x3194	-17.14	-21.52		0.96	1.89	
	221x3200	4.33	5.82		-3.80	-7.36	
	222x3194	-16.50	-19.28		-5.94	-11.23	
	222x3200	-6.13	-7.63		-2.00	-3.72	
	104x3194	1.35	1.16		-0.64	-1.24	
	104x3200	-0.65	-0.54		-9.64	-18.75	
	106x3194	-2.02	-1.73		-7.78	-16.06	
2023	106x3200	12.35	10.48		4.19	8.66	
	221x3194	-7.40	-6.68		-5.92	-12.37	
	221x3200	27.64	24.62		-9.26	-19.33	

222x3194	-3.17	-2.71	-9.43	-18.64	
222x3200	-5.92	-4.99	-13.91	-27.51	*

S Table 2: Heterosis in Absolute and relative for eight combinations in Irrigated site of experiment for Diameter increment across years. * *when significant, p<0.05*

Year	Combination	IRR Absolute Heterosis(mm)	Relative Heterosis(%)	Significance	NIRR Absolute Heterosis(mm)	Relative Heterosis(%)	Significance
	104x3194	1.09	8.57		2.58	41.49	*
	104x3200	0.43	3.68		-0.06	-0.80	
	106x3194	-0.35	-2.73		2.89	46.59	*
2015	106x3200	0.37	3.17		-0.07	-1.01	
	221x3194	-1.92	-15.17		1.39	22.45	
	221x3200	-0.38	-3.25		0.54	7.39	
	222x3194	-0.80	-6.32		2.86	46.04	*
	222x3200	0.12	1.01		0.63	8.58	
	104x3194	1.27	7.29		2.35	19.25	*
	104x3200	0.03	0.19		1.07	8.73	
2016	106x3194	0.27	1.55		5.54	45.26	*
	106x3200	0.45	2.60		3.53	28.86	*
	221x3194	-1.17	-6.69		0.89	7.24	
	221x3200	-0.42	-2.42		2.72	22.20	*
	222x3194	-0.75	-4.31		5.23	42.74	*
	222x3200	-0.88	-5.08		3.30	26.94	*
	104x3194	0.97	4.60		2.65	25.04	*
	104x3200	-1.22	-5.83		0.66	6.23	
	106x3194	1.29	6.40		2.66	25.14	*
2017	106x3200	0.56	2.81		1.17	11.04	
	221x3194	-1.63	-7.80		0.70	6.66	
	221x3200	-0.28	-1.38		3.38	31.96	*
	222x3194	-0.27	-1.30		2.72	25.69	*
	222x3200	0.30	1.50		1.69	15.98	*
	104x3194	0.32	1.69		0.41	4.00	
	104x3200	-0.05	-0.30		0.05	0.43	
	106x3194	-1.46	-7.69		0.72	6.89	
2018	106x3200	0.60	3.34		0.90	8.30	
	221x3194	-1.58	-8.19		0.93	9.11	
	221x3200	0.00	0.02		0.52	4.84	
	222x3194	1.27	6.83		1.41	13.33	
	222x3200	1.65	9.32		-1.01	-9.14	
	104x3194	-0.30	-1.38		2.27	18.41	*
	104x3200	-0.46	-2.25		-1.29	-10.47	
	106x3194	-0.24	-1.13		-0.50	-4.03	
2019	106x3200	0.91	4.64		1.55	12.55	
	221x3194	-1.39	-6.30		-0.20	-1.63	
	221x3200	-0.22	-1.05		1.22	9.88	
	222x3194	0.36	1.72		1.81	14.68	
	222x3200	0.81	4.09		1.41	11.39	
	104x3194	0.15	0.94		-1.18	-15.16	
	104x3200	-1.21	-7.89		-2.95	-37.81	*

	106x3194	-0.30	-2.04		-0.02	-0.22	
2020	106x3200	1.18	8.08		-0.89	-11.18	
	221x3194	-0.82	-5.66		-1.63	-20.56	
	221x3200	-0.47	-3.22		-0.52	-6.50	
	222x3194	-0.80	-5.32		-1.97	-24.93	
	222x3200	-0.24	-1.61		-2.14	-27.05	*
	104x3194	1.21	12.96		1.46	13.10	
	104x3200	-0.29	-3.08		-3.05	-26.48	*
	106x3194	0.88	9.75		-1.52	-13.59	
2021	106x3200	1.09	11.77		-0.80	-6.95	
	221x3194	0.06	0.62		-0.53	-4.74	
	221x3200	0.05	0.50		-0.78	-6.81	
	222x3194	2.72	31.47	*	-0.85	-7.65	
	222x3200	0.47	5.35		-1.65	-14.36	
	104x3194	1.45	18.92		-0.19	-2.46	
	104x3200	-0.10	-1.17		-0.97	-12.34	*
	106x3194	2.09	29.15	*	-0.59	-7.68	
2022	106x3200	1.42	18.33	*	0.79	10.19	
	221x3194	0.76	10.10		-0.55	-7.05	
	221x3200	0.35	4.25		-0.29	-3.73	
	222x3194	1.27	16.17		-1.10	-14.10	
	222x3200	0.19	2.24		-1.52	-19.46	*
	104x3194	-0.02	-0.26		-0.27	-3.36	
	104x3200	-2.78	-28.27	*	-1.81	-22.29	*
	106x3194	1.96	25.93	*	-1.01	-13.05	
2023	106x3200	0.97	10.91		0.62	8.02	
	221x3194	-1.73	-21.11		-0.30	-3.92	
	221x3200	-1.98	-20.79		-1.49	-19.43	
	222x3194	0.47	5.69		0.80	10.01	
	222x3200	-1.22	-12.76		-1.51	-19.03	

Table S3: Number of Families and clones used for the correlation study at Family and Clonal levels.

		IRR				NIRR			
Family		Growth, Resilience parameters and WUE	Resilience parameters	WUE	P_{50}	Growth, Resilience parameters and WUE	Resilience parameters	WUE	P_{50}
	EL	14	14	14	13	14	14	14	13
	JL	15	15	15	10	15	15	15	10
	HL	17	17	17	8	17	17	17	8
		Growth	Resilience parameters	WUE		Growth	Resilience parameters	WUE	
Clones	EL	120	98	120	-	98	98	95	-
	JL	112	90	110	-	81	80	76	-
	HL	132	112	126	-	115	112	111	-

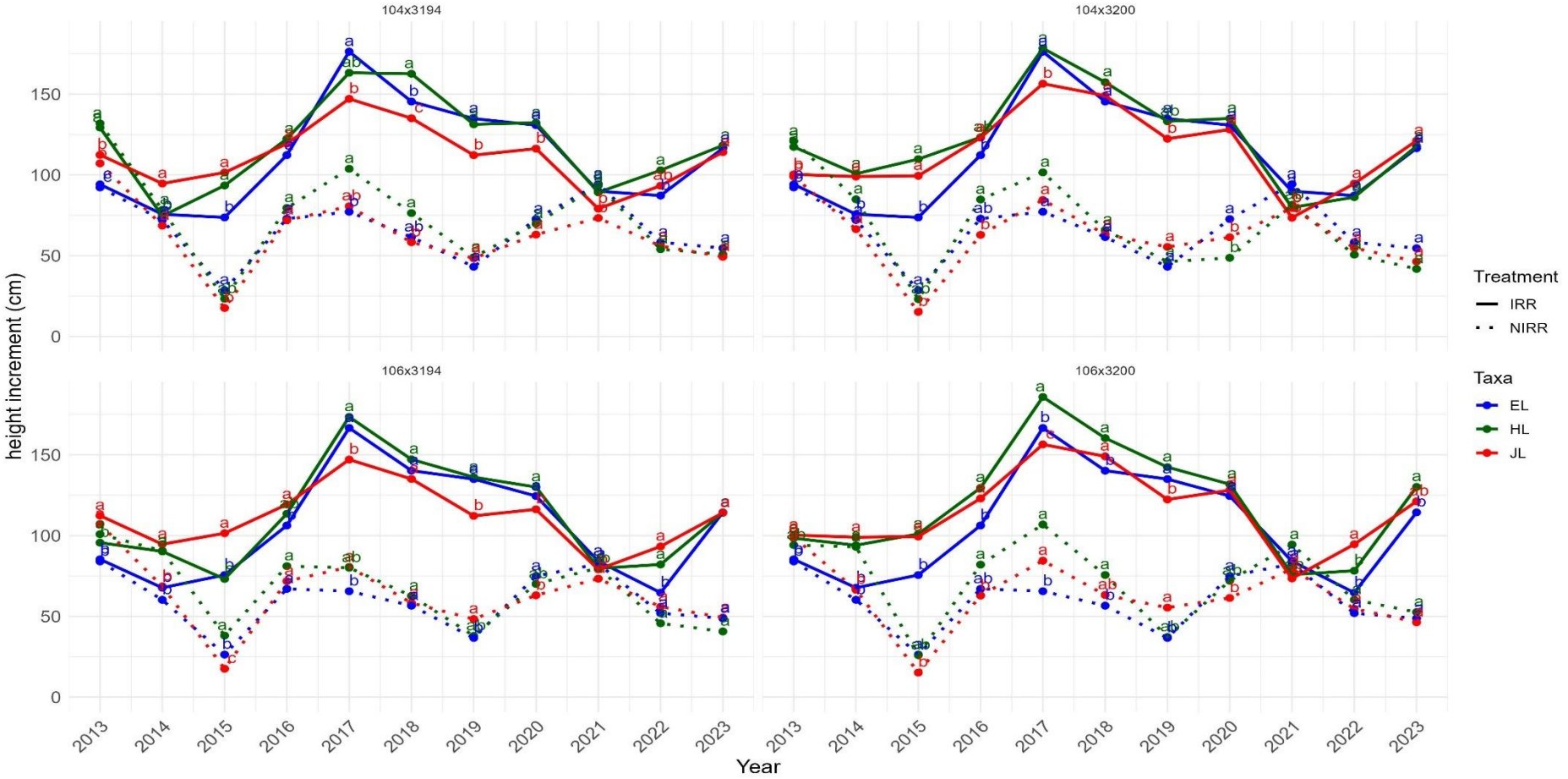


Fig S1 (A): Mean height increment (2013-2023) for European Larch (EL), Japanese Larch (JL), and Hybrid Larch (HL) under irrigated (IRR, solid line) and non-irrigated (NIRR, dotted line) conditions for combinations 104x3194,104x3200, 106x3194 and 106x3200. Different letters indicate significant differences among taxa within each year based on Tukey's HSD test ($p < 0.05$

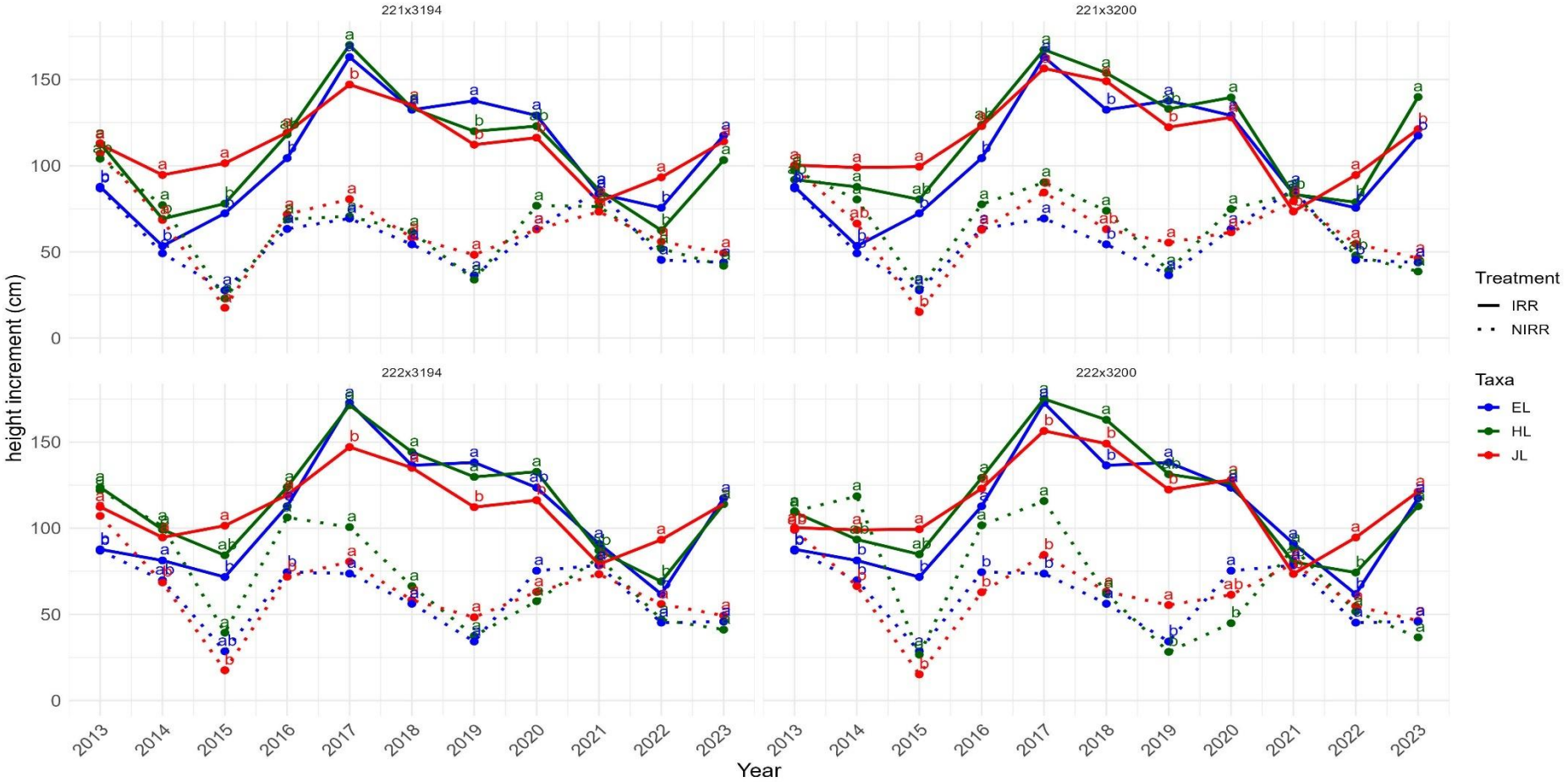


Fig S1 (B): Mean height increment (2013-2023) for European Larch (EL), Japanese Larch (JL), and Hybrid Larch (HL) under irrigated (IRR, solid line) and non-irrigated (NIRR, dotted line) conditions for combinations 221x3194,221x3200, 222x3194 and 222x3200. Different letters indicate significant differences among taxa within each year based on Tukey's HSD test ($p < 0.05$

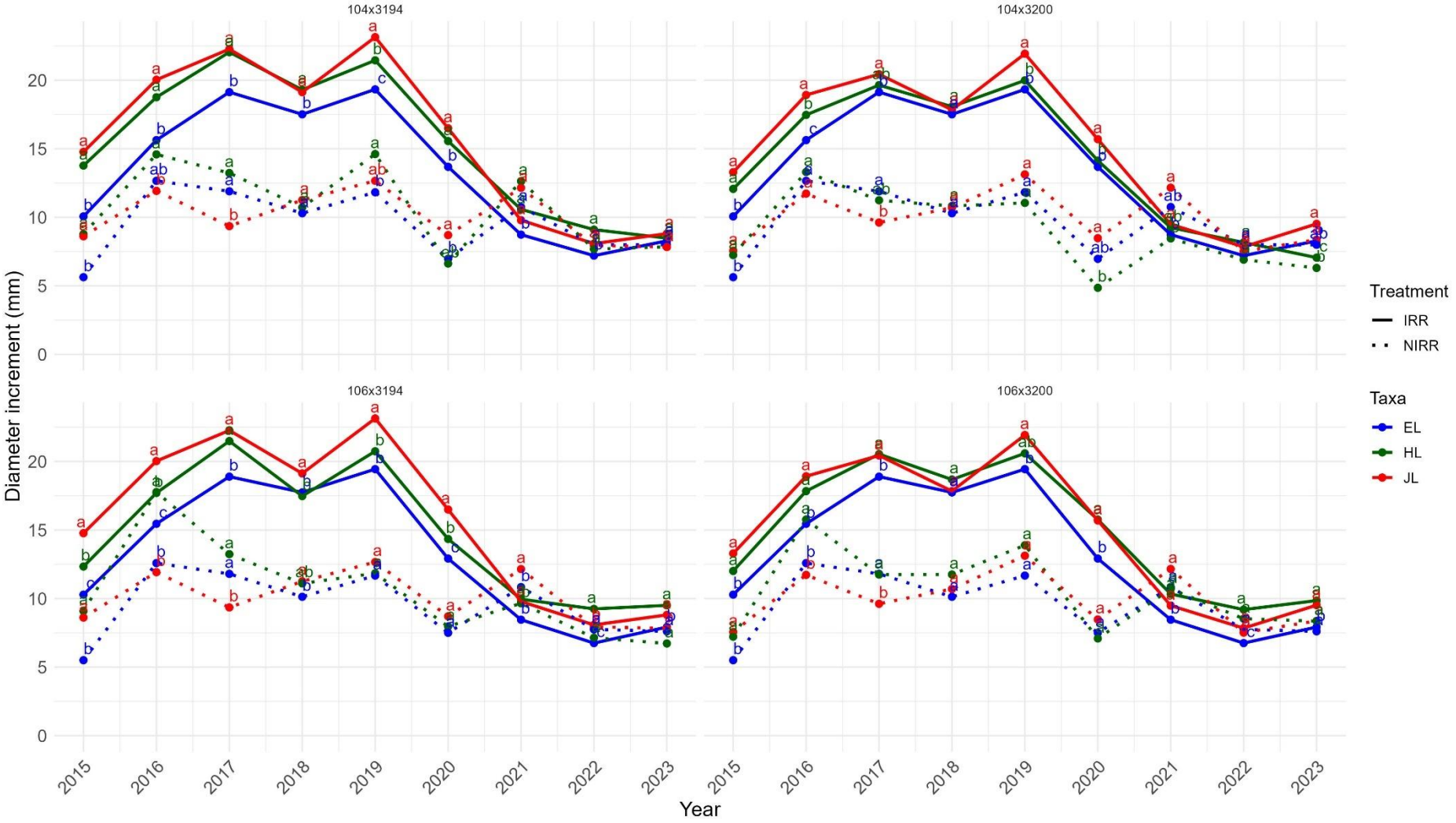


Fig S2 (A): Mean Diameter increment (2015-2021) for European Larch (EL), Japanese Larch (JL), and Hybrid Larch (HL) under irrigated (IRR, solid line) and non-irrigated (NIRR, dotted line) conditions for combinations 104x3194,104x3200, 106x3194 and 106x3200. Different letters indicate significant differences among taxa within each year based on Tukey's HSD test ($p < 0.05$

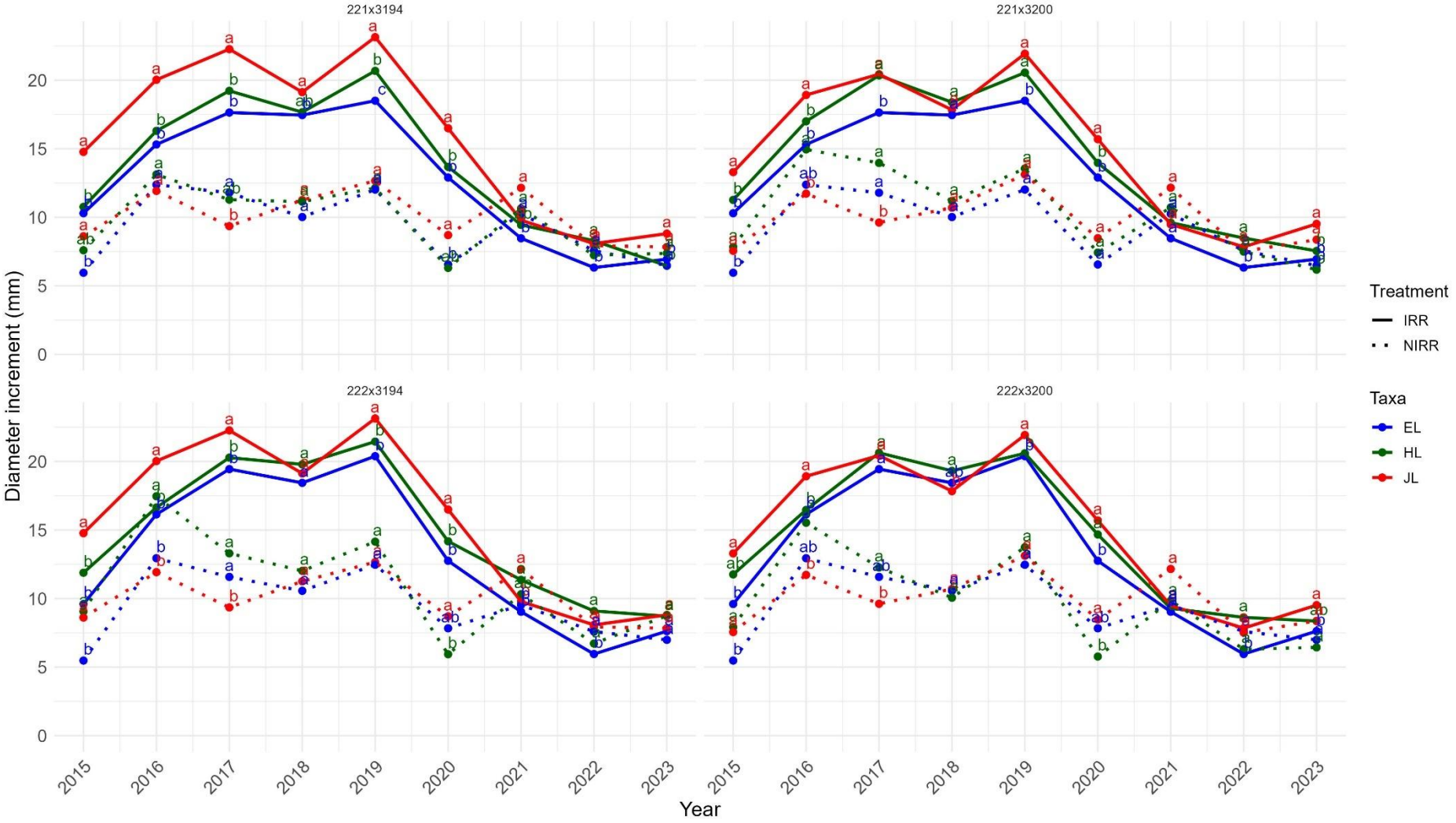


Fig S2 (B): Mean Diameter increment (2015-2021) for European Larch (EL), Japanese Larch (JL), and Hybrid Larch (HL) under irrigated (IRR, solid line) and non-irrigated (NIRR, dotted line) conditions for combinations 104x3194,104x3200, 106x3194 and 106x3200. Different letters indicate significant differences among taxa within each year based on Tukey's HSD test ($p < 0.05$)

5.4. Insights from Chapter III

The main aim of this chapter was to contribute incrementally to the existing knowledge on evolving breeding potential, using a limited but insightful set of traits. These included both breeder-focused traits, such as heterosis and the mechanisms underlying it, and ecologist-oriented traits, reflecting potential trade-offs, with resilience parameters (RRR), cavitation resistance (P_{50}), and water-use efficiency (WUE) with growth.

The results demonstrated that heterosis was not uniformly expressed across environments or time, highlighting its dynamic and trait-dependent nature. Hybrid combinations often showed superior growth under irrigated conditions, but this advantage shifted or even amplified under drought, suggesting that the expression of heterosis is conditional and may involve different underlying mechanisms.

Regarding adaptability, hybrid genotypes exhibited considerable plasticity, adjusting their growth responses according to water availability. Furthermore, species-specific differences emerged in growth–resilience relationships. While HL genotypes tended to recover strongly after stress, their resistance during drought was often lower, indicating a compensatory but potentially resilient pattern.

Physiological traits such as water-use efficiency (WUE), vulnerability to cavitation (P_{50}), and resilience were moderately linked to growth traits, particularly, offering clues into the mechanisms supporting adaptation. Collectively, these findings suggest that fast growth may come at a cost but with resilience and underscore the importance of considering trade-offs and trait integration in breeding programs. For breeders, this implies that hybrid larch holds promise, but its deployment should be informed by both performance potential and resilience traits to ensure long-term sustainability under climate variability.

6. Discussion

6.1. Context: breeding in an uncertain world

The central thread running through this thesis is the climate-driven paradigm shift in tree breeding. Traditionally, breeding programs prioritized traits like volume growth and stem form, often focusing on fast-growing genotypes and high-yielding short-rotation species such as conifers. However, increasing climatic uncertainty—manifested in more frequent droughts, heatwaves, and pest outbreaks (Allen et al. 2010; Sturrock et al. 2011; Cook et al. 2018; Hammond et al. 2022) — has challenged this growth-centric model. As a result, there is now a pressing demand for species that are not only productive but also functionally adapted to stress, sparking discussion between different schools of thought: Foresters and Ecologists. From the forester's perspective, the goal remains clear: access to species or genotypes that are better adapted and can still deliver productivity under future climate scenarios. This has led to exploring exotic species, assisted migration, and selective breeding using intra- and interspecific hybrids. In contrast, ecologists advocate for a return to slower-growing and resource-conservative species (Reich 2014; Sartori et al. 2019; Colesie et al. 2020), which they insist are better suited to long-term survival under stress with their resource-saving strategies (Atkinson et al. 2012; Eller et al. 2018; Signori-Müller et al. 2022). This thesis was built on the idea of reconciling these two views through a balanced, trait-integrative breeding model. To implement such a model, it is essential to harness the considerable genetic variability commonly available in forest trees, particularly through provenances and interspecific combinations, which provide a rich resource for identifying genotypes that combine desirable traits across environments. Yet, within this wealth of variability, a key challenge emerges: Can we truly combine fast growth with high resilience to climatic threats?

Addressing this kind of question requires conceptual clarity and empirical evidence. Fast-growing species and particularly pioneer ones like larches are associated with acquisitive strategies - to prioritize resource uptake and biomass accumulation, which makes them more competitive, but sometimes at the expense of survival. As such, breeders must ensure that selection for growth does not compromise adaptive traits - a risk particularly pertinent when functional trade-offs are not yet fully understood or quantified (Climent et al. 2024). These trade-offs are not always straightforward. Traits - such as vulnerability to cavitation (P_{50}), water-use efficiency (WUE), growth resilience, or mortality - often interact in ways that are context-dependent and may vary across species, environments, or developmental stages (Anderegg 2015; Keen et al. 2020). Like the work on ponderosa pine during the extreme 2012–2015 California drought, it was observed that slow-growing individuals were more prone to mortality, whereas faster-growing trees were more likely to survive, indicating that the intrinsic growth rate (Keen et al. 2020) might contribute to survival. Root establishment might have played a role in survival, as observed in hybrid larches, which developed finer roots facilitating water absorption (Pâques et al. 2022). This complexity makes it difficult to predict whether selecting for one trait, such as rapid growth, will inadvertently reduce performance under stress.

This thesis tried to address this challenge by evaluating how growth traits relate to key adaptive traits across differing soil water availability (two treatments) and over time (ten years). It used a mating design combining intra- and interspecific crosses between European and Japanese larches, so that the three taxa (European, Japanese, and hybrid larches), connected by their pedigrees and represented by the identical clones in the two treatments, can be more precisely evaluated and compared. Our aim was not only to identify superior taxa but also to understand how trait interactions contribute to performance under stress, and whether heterosis for growth (fast growth) is linked to some underlying adaptive potential.

6.2. The Thesis Framework: From Concept to Experiment

At the outset of this thesis, we envisioned a comprehensive approach aimed at understanding tree performance through a broader lens. As illustrated in **Fig. 31**, our objective was to go beyond traditional growth traits and explore a wide array of physiological, anatomical, structural, and defense-related traits, each tied to core life history functions: growth, survival, and reproduction. As highlighted by Hilty et al. 2021, growth is a complex, multifactorial process that combines cell production, tissue expansion, biomass accumulation, and resource allocation, each operating at different spatial and temporal scales. For instance, in trees, radial growth (wood formation) depends not only on photosynthesis supplying carbon but also on water availability controlling cell expansion, on hormonal signaling coordinating cell division, and storage dynamics buffering seasonal fluctuations. An event (stress) may not immediately reduce photosynthesis, but can limit growth by restricting cell expansion due to reduced turgor. This illustrates why growth cannot be seen as a direct output of carbon assimilation and why integrating growth with more traits is necessary when evaluating performance.

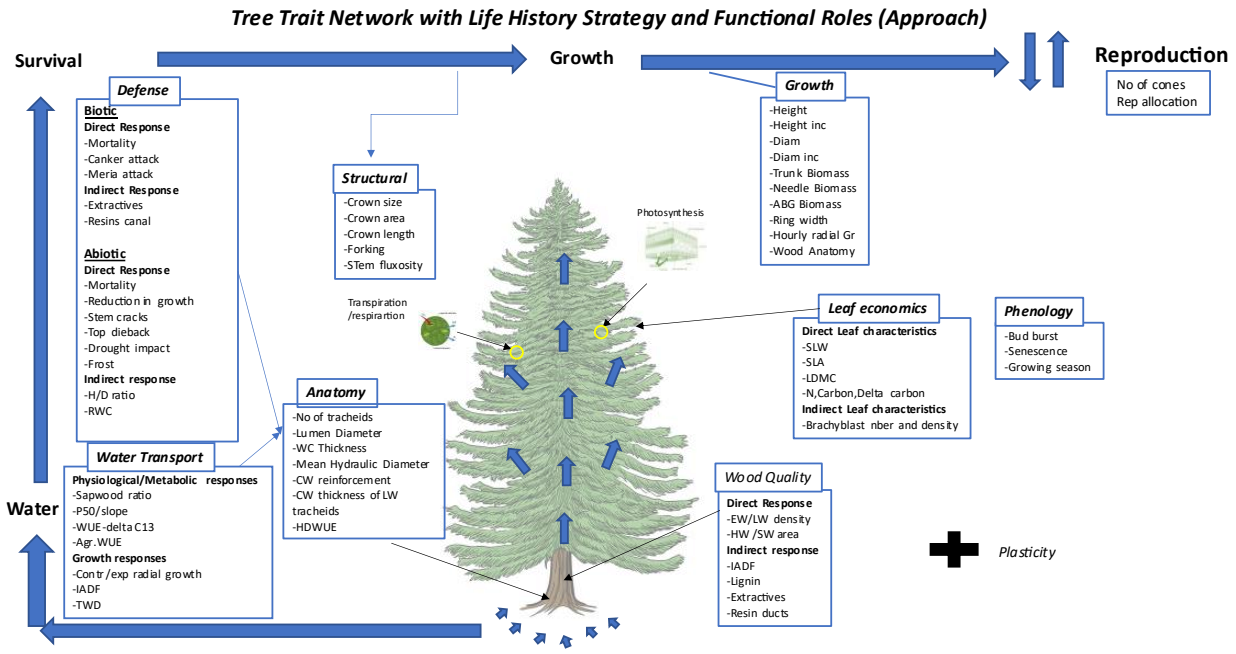


Fig 31. List of traits assessed since 2013 in INRAE-Orléans trial (irrigated and non-irrigated plots). Rooting was addressed in another nursery trial (Pâques et al. 2022). The tree trait network approach integrates life-history strategies and functional roles. This is the conceptual framework that guided our approach at the beginning of this thesis

Therefore, the framework of the thesis was grounded in the idea that tree growth performance cannot be understood in isolation but must be interpreted through interactive trait networks, shaped by both environmental context and developmental stage. This approach was inspired by concepts such as the Growth Differentiation Balance Model (Herms and Mattson 1992), the fast – slow continuum (Reich 2014) and frameworks explaining species survival or mortality under stress (McDowell et al. 2008; Lauder et al. 2019; Trugman et al. 2021). The figure highlights key trait categories such as water transport efficiency, wood and leaf economics, defense mechanisms, phenology, and reproduction, all of which contribute to how trees acquire, use, and conserve resources. These traits were mapped onto axes of survival, growth, reproduction, and water management, with plasticity acting as a modulator of these responses across space and time. Although the experimental scope of this thesis later narrowed to focus more directly on mortality, growth, and its resilience under drought, the original systems-level perspective was not abandoned. Instead, it was translated into a more tractable framework focused on key areas—initially mortality in a space–time–environment view, followed by growth responses, resilience traits, and phenotypic plasticity across taxa. The refined focus still supports the initial vision: to understand how trait interactions govern performance, and whether superior genotypes can be identified not only for their productivity but also for their functional robustness in the face of climatic stress.

Another key element was using “established drought” (Orléans INRAE nursery, by the nature of its soil and its climate, with low precipitation, is prone to summer drought) as an experimental lever

to test how parents and hybrid combinations responded over time to water stress. The experimental design, based on a structured mating design and replicated across two soil water availability levels, allowed the examination of growth expression, plasticity, and trait correlations across hybrid (HL) and parental (EL, JL) Larches. We try to be more "environmentally realistic" from highly controlled experimentation (e.g., young potted trees in a greenhouse): most climatic and soil interannual variation only controls soil water availability in our trial, which can assist in future climate simulations. **Fig. 32** shows the future prediction of compatibility and incompatibility areas (year 2050, scenario: optimistic RCP 4.5, indicators: Annual Water deficit, Annual Minimum Temperatures, and annual sum of degree days: https://climessences.fr/) where European Larch and Japanese Larch may face risks of dieback or incompatibility in the red zones. It is visible that these areas are projected to expand in the coming years, particularly for Japanese Larch and, to a lesser extent, for European Larch. This situation can likely be approached in Orléans or drier parts of France. Following the climessences climate analogy tool (scenario: optimistic, RCP 4.5), climate in regions recommended for larch forest deployment such as Western part of Limousin and its plateau (where a twin trial, Beaumont-du-Lac, is located) could be similar in 2050 to the one met today in Orléans, a place definitely not appropriate for larch cultivation. Results from a trial like ours, conducted under more natural conditions over a longer time span, are more readily transferable to operational forestry practice.

By restricting access to water under more natural settings (drought conditions in NIRR plot), the trial provided a valuable framework for examining not only the differential response of these taxa to water limitation in comparison to a more optimal water supply (IRR plot) but, more importantly, the potential of interspecific hybridization as a breeding strategy—a long-standing goal in forest genetics aimed at combining complementary traits from different species. In particular, we examined whether the observed heterosis for growth in hybrids was consistent across watering treatments and whether it extended beyond growth to include adaptive traits such as resilience and water use traits. Whereas interspecific hybridization has been widely used and promoted in forest trees, heterosis—or hybrid vigor—has been little documented due to a lack of appropriate mating designs and long-term, well-designed field trials. However, available results show that its expression is often context-dependent, influenced by environmental conditions and the traits under consideration in species like Pine and Larch (Dungey 2001; Marchal et al. 2017). Heterosis can be seen not only as a boost in growth potential but also as a phenomenon that varies across different environments. The interaction between heterosis expression and environmental context has been demonstrated in several forest tree species. For example, in *Populus* × *acuminata* (a hybrid of *P. angustifolia* × *P. deltoides*), mid-parent heterosis for growth (approximately 20% for height) was particularly expressed under suboptimal, cooler temperatures (15–24°C) in controlled growth chamber conditions (Zanewich et al. 2018). Similarly, in *Pinus elliottii* × *Pinus caribaea* (F_1 hybrid), superior growth was observed under field conditions where the hybrid outperformed even the best-performing parent for height and volume on a poorly drained site in Queensland, Australia (Nikles 2000). In conifers, it has also been discussed in a review of Pines and Larch that hybrid vigor might amplify under constraints (Pâques 1989; Dungey 2001).

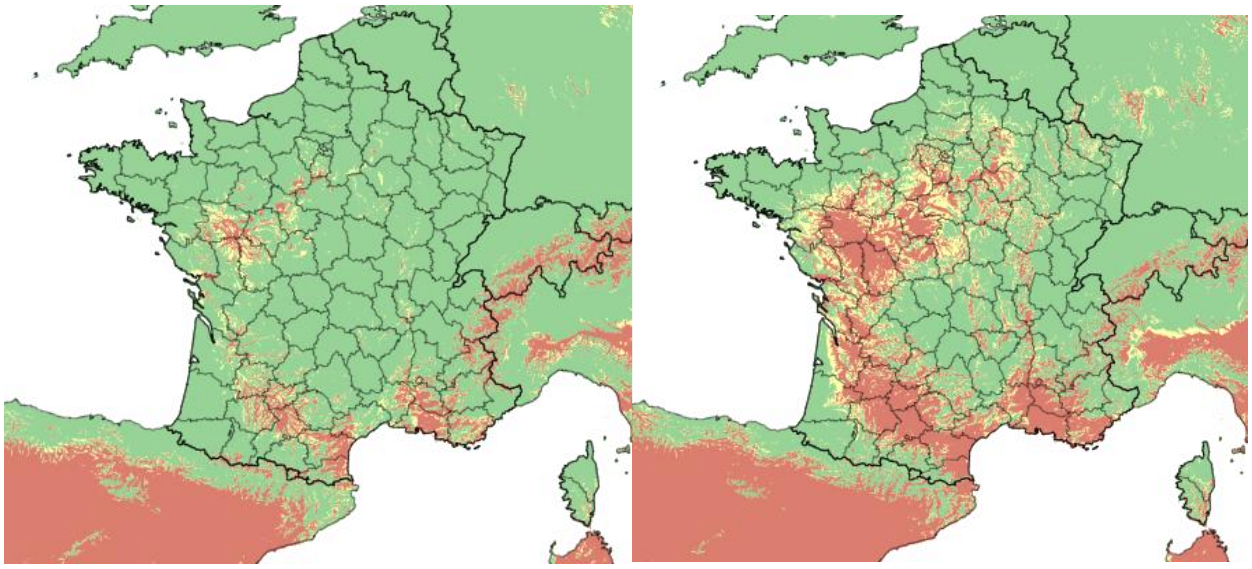


Fig 32 (A): Species compatibility map with current climate (left) and the future prediction (right) for European larch (https://climessences.fr). The green area shows compatible areas for a threshold of 97.5%, the yellow area shows areas compatible with a 99% threshold, and the red area shows areas incompatible with a 99% threshold

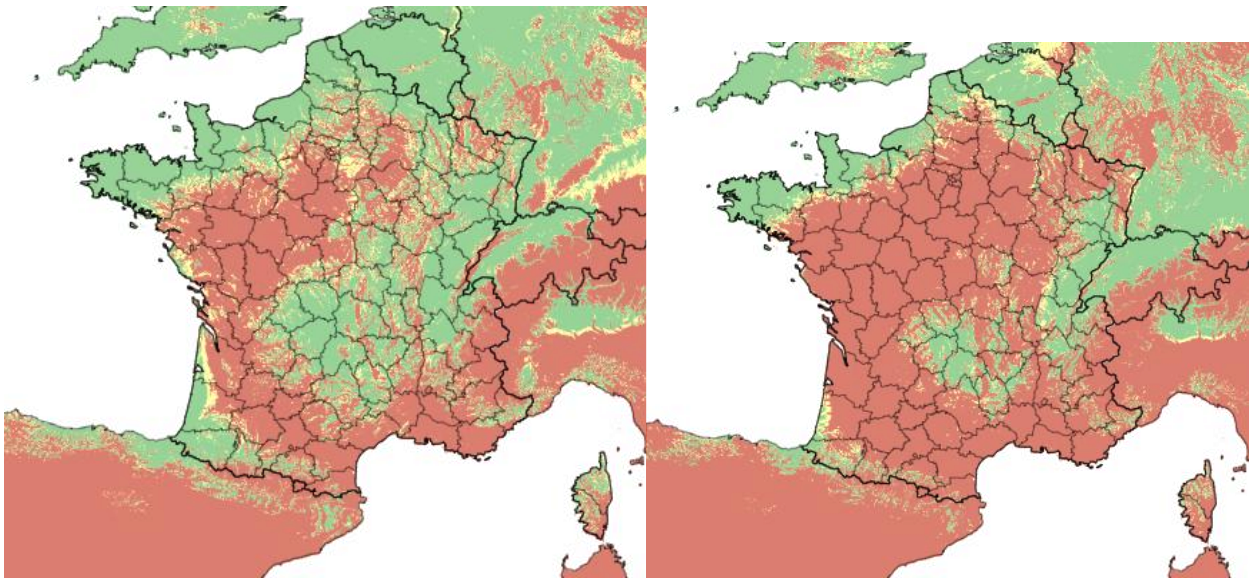


Fig 32 (B): Species compatibility map with current climate (left) and the future prediction (right) for Japanese larch (https://climessences.fr). The green area shows compatible areas for a threshold of 97.5%, the yellow area shows areas compatible with a 99% threshold, and the red area shows areas incompatible with a 99% threshold

Such observations suggest that heterosis may not only reflect simple additive growth advantages but also enhanced homeostasis — the hybrid's ability to buffer environmental fluctuations by integrating complementary physiological mechanisms from both parents. As demonstrated by Knight (1973), the expression of heterosis is not always uniform across environments. Under moderate (intermediate) environmental conditions, hybrids may outperform their parents and exhibit best-parent heterosis; however, under more extreme or stressful conditions, hybrids often do not exceed the best parent but still maintain performance closer to or above the average of both parents.

Our goal was not to dissect the genetic origin of heterosis. This was previously achieved in a previous thesis (Marchal 2018) based on performances in three field trials testing the whole mating design (18 x 18) material, from which a subset was derived for our study. Instead, our study aimed to determine whether HL outperformed their parents and how consistently each parental taxon (EL and JL) performed across soil water conditions. We were particularly interested in phenotypic responses to drought and their association with adaptive traits. For instance, we examined whether higher WUE or lower vulnerability to cavitation (P_{50}) could help explain faster growth - i.e., whether the hybrid's or parents' superior performance had a functional foundation? From a breeder's perspective, an important question is whether traits like P_{50}, WUE, resilience indices, growth, or observed survival can be predictive indicators for selecting genotypes better adapted to future drought conditions. The observed variability across genotypes for these traits provides an essential basis for selection. However, variability alone is not sufficient; heritability and the strength and direction of correlations between growth and adaptive performance (e.g., under drought) are equally critical. Heritability evaluation was a limitation in our study, as we did not have a sufficient number of combinations and individuals to estimate heritability properly or to statistically compare correlation differences between taxa

6.3. Interpretation of Results

From the broader perspective of this thesis, we aimed to adopt a systematic approach, starting from the most immediate trait of interest—mortality—progressing through growth performance and differences in drought response, and finally exploring whether superior performance under drought is linked to key adaptive traits. This integrated framework allowed us to connect survival, growth, and adaptive characteristics better to understand the basis behind fast growth and drought adaptation.

From Chapter I

- It was observed that Japanese Larch was most prone to mortality, especially under constrained water availability over the decade-long period of observation, with European Larch being less affected and Hybrid Larch occupying an intermediate position. In addition to soil water shortages, the climate variables most explicitly associated with this mortality were vapor pressure deficit (VPD) and maximum

temperature, with late spring, corresponding to the growth linear phase, as the most critical period. Following this critical growth phase period of intense metabolic activity under strong constraints, top dieback was frequently observed and was attributed to cavitation. Mortality either directly followed or was delayed by one year.

- Whereas risks and impacts of drought are actually confounded with high temperature stresses (heat-waves), our experimental design (where only water supply differed between IRR and NIRR plots) allowed to demonstrate that extreme temperatures alone (up to 32 to 34°C) if compensated by water supply may be not or less harmful than drought itself to tree survival.
- The relationship between growth and mortality was evaluated by modeling the probability of mortality to a function of growth, first at the individual tree ring level and subsequently at the clonal level. In all three taxa, reduced radial growth in the year preceding mortality was significantly associated with a higher risk of mortality. At the clonal level, mean long-term growth (ten years) was negatively correlated with mortality: i.e., the faster growing clones had a higher probability of surviving.

Fast growth can provide an early advantage, particularly during the juvenile phase, by promoting rapid establishment and resource acquisition. However, this benefit may diminish with age or become disadvantageous under prolonged resource limitation, as the physiological costs of sustaining fast growth may increase vulnerability to drought-induced stress. While some studies have reported lower mortality among fast-growing clones, likely due to efficient water use and deeper rooting (Giovannelli et al. 2007; Kaczmarek et al. 2013), other investigations present contrasting evidence where highly productive genotypes exhibit greater mortality under severe drought (Monclus et al. 2006; Bennett et al. 2015). This conflicting body of evidence suggests that the relationship between growth and survival is complex and context-dependent, influenced by species-specific physiological limits, stress intensity, and ontogenetic stage. In our study, positive growth–survival associations were mostly observed at the clonal level, but the species-specific patterns—higher mortality in Japanese larch, lower in European larch, and intermediate in the hybrid—underscore the possibility that higher growth potential may, in certain taxa, come at the cost of increased hydraulic vulnerability under drought. Nevertheless, some taxa or genotypes may achieve a favorable combination of vigorous growth and tolerance to climatic stress.

From Chapter III

- Our results revealed that HL combinations frequently outperformed both parents regarding volume and height. Heterosis was more pronounced in stressful non-irrigated conditions, which aligns with previous observations suggesting that heterosis is often expressed more strongly under stress. However, not all hybrid combinations were equal—variability among combinations was high, with some performing better than others.
- HL showed quite consistently a greater phenotypic plasticity for apical growth along a spatio-temporal water availability gradient than JL and EL. For diameter growth, JL showed the greatest plasticity, with that of HL being either similar or intermediate according to combinations. For both radial and apical growth, EL was the least plastic. Interestingly, norms of reaction for height and for diameter showed different patterns:

at water availability levels exceeding approximately 650 – 700 mm, height growth plateaued, whereas diameter growth started to decline, suggesting that competition might be the underlying cause.

- All taxa showed a reduction in growth under non-irrigated (NIRR) conditions compared to irrigated (IRR) conditions, but the magnitude varied. Japanese larch (JL) generally exhibited the strongest growth reduction, especially for diameter increments. Hybrid larch (HL) showed intermediate reductions, while European larch (EL) displayed the smallest reduction, particularly for height and in the early years.
- Resilience components (resistance, recovery, and resilience) revealed species-specific strategies. EL maintained higher resistance, especially for radial growth, but showed moderate recovery. HL displayed consistently higher recovery and resilience indices for height, and intermediate to high recovery also for diameter, though resistance for diameter remained lower compared to EL. JL also had generally lower resistance and moderate resilience. The hybrid thus combined a capacity for recovery with moderate resilience, pointing to buffering against drought impacts. The average growth reduction parameter was higher in JL and HL, and lower in EL.
- Stability analyses based on Wricke's ecovalence and Hühn's stability ranks showed differences among taxa. EL consistently exhibited stability across environments for both height and diameter, with low ecovalence values and favorable Hühn's stability ranks. HL, despite its higher mean growth, displayed intermediate stability, suggesting some sensitivity to soil water variation. JL generally showed the lowest stability, with higher ecovalence values and poor Hühn's ranks.
- The hydraulic traits considered in this study were P_{50} as a proxy for vulnerability to cavitation and $\Delta^{13}C$ as a proxy for intrinsic water use efficiency. JL appeared as the most vulnerable to cavitation (P_{50} =-3.45 MPa), HL was on average intermediate (P_{50} = -3.70 MPa) but with mean family values ranging from close to those of JL or to those of EL, and EL was the least vulnerable (P_{50} = -3.85 MPa). In contrast, for WUE, both JL and HL, the faster growing taxa, showed on average higher water-use efficiency (JL: 21.42‰ and HL: 21.54 ‰) than EL (21.58‰).
- Using P_{50} and resilience parameters as selection criteria to enhance drought tolerance can be a step ahead, as these traits displayed sufficient phenotypic variability at the phenotypic level (P_{50}: CV: 6-10%, Resistance: CV: 13-15%, Recovery: CV: 6-11.5%, Resilience: CV:4.0-8.5% and AGR:CV: 8.1-16.4%), and are also reported in the literature to be at least moderately heritable (Depardieu et al. 2020, h2 = 0.112-0.352 for resilience parameters), and showed moderate favorable associations with growth (i.e., better recovery or resilience tended to accompany growth rates; P_{50}, showed moderate favorable associations with growth in EL and JL, but not in HL).
- Fast growth did not necessarily come at a cost, as WUE and recovery parameters were often positively associated with diameter growth. Negative associations were also observed between growth and resistance traits, indicating that increased diameter may come at the cost of reduced resistance.
- When examining these correlations with growth in hybrids versus in the parental species, we observed that hybrid may sometimes exhibit more favorable links with growth than its parents (e.g., with Average Growth Reduction, where correlations in HL were lower than in its parents), but could also manifest more unfavorable links (e.g., with P_{50}). These 'broken' correlations might illustrate some examples of correlations

originating from linkage disequilibrium generated by complementary mating (interspecific hybridization) (Klápště et al. 2022b).

The stronger expression of heterosis under stressful (non-irrigated) conditions observed in this study parallels findings reported in other plant species (e.g., maize (Tollenaar and Lee 2002)). In *Arabidopsis* hybrids, Fiévet et al. 2010 proposed through theoretical modeling that hybrids may benefit from greater flexibility in metabolic networks, enabling them to better stabilize physiological functions when exposed to environmental stress. Such buffering capacity may help explain why heterosis becomes more pronounced under drought conditions, where parental physiological limitations are more strongly expressed. In conifers, while heterosis is well documented for growth traits, its interaction with environmental stress remains poorly studied. Studies in larch (Marchal et al. 2019; Pâques et al. 2022) suggest that hybrid larch can combine vigorous growth with adaptive plasticity, potentially offering advantages under drought. Yet when water availability was getting limited (D1rew < 0.2), hybrid growth performances did not significantly differ from those of its parents. The stronger expression of heterosis under non-irrigated conditions in our experiment may reflect both the release of some hidden genetic variation under stress and the ability of hybrids to integrate complementary physiological mechanisms inherited from both parents.

In this study, intrinsic water-use efficiency and vulnerability to cavitation were selected as proxy traits representing key aspects of drought response. In many studies, proxy traits are often used to capture complex physiological processes that are difficult to measure directly, allowing efficient assessment of functional responses to environmental stress (Violle et al. 2007). P_{50} was selected as the key indicator of hydraulic vulnerability, as it represents the water potential at which 50% of hydraulic conductivity is lost, providing a robust and widely used metric to compare drought sensitivity across taxa. Although thresholds such as P_{12} or P_{88} can provide information on early or near-complete xylem embolism formation, P_{50} can be a more standardized reference point for sensitivity to cavitation, especially in conifers (Delzon et al. 2010). $\Delta^{13}C$ was chosen as a proxy for intrinsic water-use efficiency, as it integrates how trees balance carbon assimilation and water loss (Farquhar and Richards 1984). While this study focused on vulnerability to cavitation (P_{50}) and water-use efficiency (WUE) traits to assess drought adaptation, other physiological mechanisms may likely contribute to the observed variation in growth and resilience among taxa and clones. Traits such as stomatal conductivity, osmotic adjustment, non-structural carbohydrate (NSC) storage, root-to-shoot allocation, and hormonal regulation (e.g., ABA signaling) play critical roles in plant responses to water limitation but were not directly assessed here. Compared to the latter, P_{50} and $\Delta^{13}C$ remain technically rather accessible for screening a few tens or even a few hundred trees, potentially many more through the development of NIRS predictive models, which made them attractive for this study.

From Chapter II

- Near-infrared spectrometry (NIRS) predictive models were developed for several functional traits needed to explore complex traits like growth. NIRS proved to be useful as a high-throughput technique and powerful to screen large sets of genotypes (nearly

2000 in this study) as needed in genetic studies and breeding: in particular, we found moderate to good predictive powers for some biochemical traits like N and C content (needles), phenolic content (needles), $\Delta^{13}C$ (needles) and, lignin and H/G ratio (wood). On the contrary, results were disappointing for traits like P_{50} (twigs), technically complex to assess and involve hydraulic and structural properties, and SLA or LDM (needles) expressed as ratios of physical dimensions (area and weight).

We did not obtain good prediction models for P_{50}, which may be partly explained by the fact that NIRS was performed on milled (powdered) wood samples, resulting in the loss of the structural wood features (intact conduits, pit structure) where cavitation actually occurs. In contrast, P_{50} has been more successfully predicted in some studies using NIRS on wood blocks or cubes, where anatomical structures remain preserved and can explain vulnerability more (Tixier et al. 2014). Although SLA was recognized as a major trait to characterize plant's strategies along a resource use axis, it was recognized by Wilson et al. 1999 to suffer from several drawbacks (variability among replicates, influenced by leaf thickness); in this respect, LDM appears less sensitive. Yet the poor model performance for both traits likely reflects the fact that these are primarily structural or dimensional traits, not directly or only partially related to chemical composition, which limits the predictive power of NIRS, which primarily captures chemical signatures rather than physical dimensions.

6.4. Which taxa and for what?

As discussed in the Introduction, one motivation for this thesis and its approach was to contribute to better defining the respective place of European and Japanese larches and their hybrids for deployment in the forest and their potential concerning ongoing climatic changes. This issue is also relevant for the breeding and selection programme of *Larix sp.* oriented towards genetic improvement for low- to middle elevation lands reforestation: it is still currently active in intraspecific breeding of European larch (2nd-generation) but with a strong inflection from the 80's on towards interspecific hybridization, and more recently with advanced hybridization (composite breeding). The operational question is: Do we need to continue investing in selecting pure European larch varieties? or, said in another way, can we rely solely on hybrids?

At the end of the thesis, here is a summary of traits by taxon:

- European Larch (EL) showed good survival under drought, with low mortality observed in highly water-constraining conditions (non-irrigated plot, NIRR). Its growth was generally lower than that of other taxa with a lower water-use efficiency (WUE), but progressively increased over time. EL exhibited limited phenotypic plasticity to water availability, indicating a lower capacity to adjust growth under changing environmental conditions. Physiologically, it showed strong resistance traits to drought (resistance parameter, less Average growth reduction, and vulnerability to cavitation P_{50}) with the lowest occurrence of top-dieback during dry years. Overall, EL appears well-suited for afforestation in dry or drought-prone areas where survival is the main priority at the

expense of yield, and can also contribute valuable drought survival traits (e.g., conduit wall reinforcement that enhances hydraulic safety under water stress, (Sasani et al. 2021) in breeding programs.

- Japanese larch (JL) showed good diameter growth with a high level of plasticity along a water availability gradient and a good recovery after drought. It demonstrated a high water-use efficiency in both soil water conditions. Nevertheless, it exhibited higher mortality rates under drought stress. It was characterized by a low resistance to cavitation (P_{50}), making it more vulnerable to hydraulic failure under severe water deficit, supported by the observation of the highest frequency of top-dieback during dry years. JL was particularly sensitive to abrupt drought events. Therefore, it may be better suited for afforestation in moisture sites where water limitation is less critical. Its use in dry or drought-prone areas is not recommended.

- Hybrid larch (HL) displayed the highest overall growth, particularly for height and volume, and showed high plasticity in response to environmental variation. It was comparable to EL with a good survival and less frequent top-dieback occurrence in the most stressful condition (NIRR plot). On average, HL exhibited intermediate values for both cavitation resistance (P_{50}) and water-use efficiency (WUE), but showed a wide range of values among combinations, ranging along the two pure species extremes. HL exhibited greater resilience than both parental species, EL and JL. It is therefore possible to find combinations with WUE close to the most efficient parent (JL) or combinations with P_{50} close to the less vulnerable to cavitation parent (EL). For example, combination 106×3200 demonstrated rapid height growth combined with high WUE and good cavitation resistance, while 222×3194 similarly combined vigorous diameter growth with favorable hydraulic traits. Its drought response was intermediate, with some years showing more abrupt declines like JL. These traits make HL particularly suitable for breeding programs that combine high productivity with moderate drought adaptation. For afforestation, HL (but with better genotypes) can be a good option. Careful monitoring is recommended as its drought response can vary depending on severity and timing of stress.

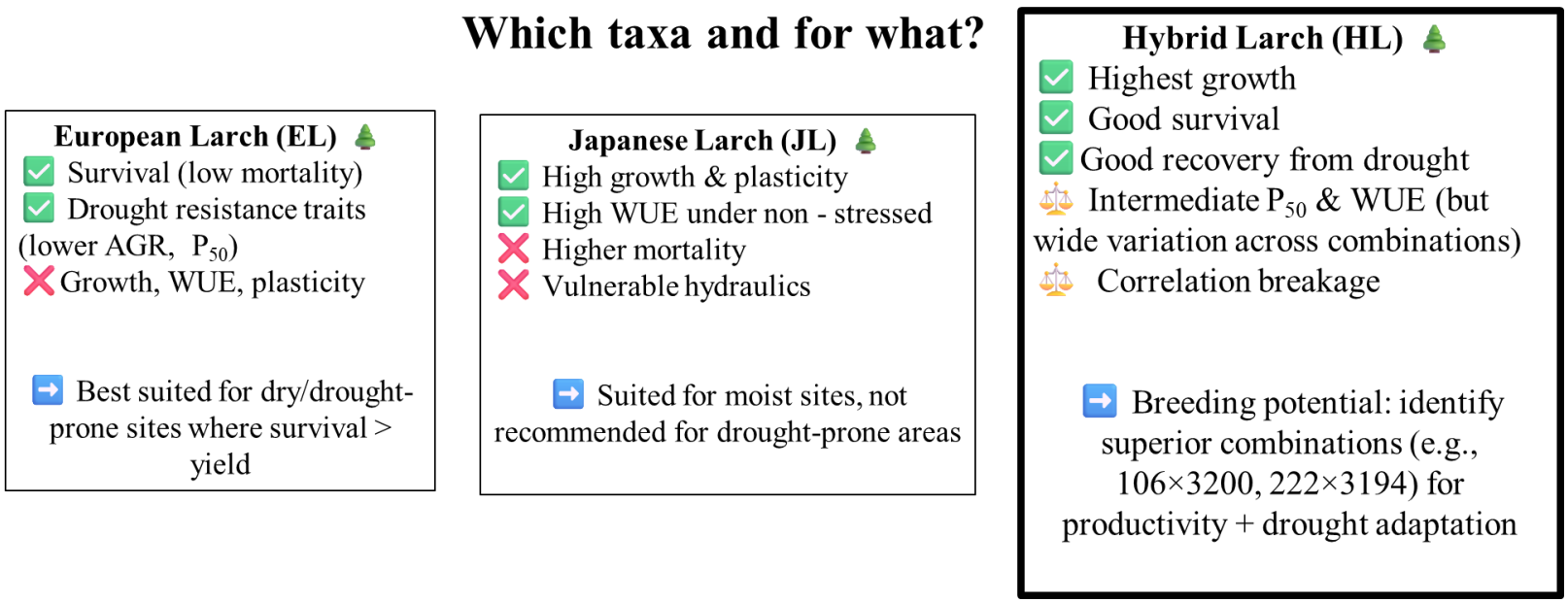


Fig 33. Comparison of functional profiles and recommended site uses for European Larch (EL), Japanese Larch (JL), and Hybrid Larch (HL).

Although EL and JL originate from very different parts of the world (East Asia and Central Europe), they are surprisingly similar in terms of their morphology (Pâques et al. 2006) and phylogenetically close (Semerikov and Lascoux 2001). Studies like Bobrov (1973) have shown in fact that these two species are part of the same larger group of closely related larches that spread across Eurasia. Because of this close relationship, they can easily cross with each other, both through controlled crosses in breeding programs or when planted near each other in nature. Indeed, many natural hybrids and intermediate forms have been observed where their ranges meet. This means that EL and JL are not fully separate species in a strict sense, but are part of a '*species complex'* where boundaries between species are flexible and mixing can occur. However, EL and JL showed clear differences in several traits, including height and diameter growth, SLA, N content, lignin content, WUE, plasticity, resistance to drought-induced growth reduction, recovery capacity in response to soil water availability, resistance to xylem embolism and, phenolics content. In addition to these trait averages, the relationships between traits also appeared to differ between species. For example, in JL, growth was more closely linked to water-use efficiency and resilience, while in EL, these traits were less strongly connected. This suggests that the two species have evolved different trait combinations to cope with their environments. These differences likely result from long-term adaptation to contrasting conditions: EL evolved in colder, drier, and more continental climates, while JL comes from regions with milder and wetter weather patterns, like those found in Hondo Island in Japan. Such environmental differences have shaped their distinct strategies—not only in growth and drought tolerance, but also in traits we did not measure here, like resistance to pests or some wood quality properties. Interspecific hybridization, as seen in HL, allows the mixing of these contrasting trait sets. This can help breeders combine the fast growth and efficiency of JL with the stress resistance of EL, though the outcome depends on which parental traits are inherited. In our study, EL and JL each showed distinct strategies- one with higher WUE and a better recovery to drought, the other with a lower vulnerability to cavitation and a better resistance to drought, while HL generally displayed intermediate values, suggesting a mix of both traits.

Further consideration is required to fully explain the contrasting behaviors observed between these taxa and the respective traits studied:

Beyond the general patterns of growth and mortality discussed earlier, recent studies have offered more detailed insights into the physiological mechanisms underlying the growth–survival balance in different taxa. These mechanisms are often closely linked to water transport efficiency and safety, which are critical for tree performance under drought. In HL and JL, faster growth is frequently associated with better overall performance. This pattern reflects the classical efficiency–safety trade-off: JL combines high growth and high hydraulic efficiency with a lower resistance to cavitation, and some HL combinations (e.g., 104×3200) show a similar profile. This vulnerability can be closely linked to hydraulic architecture—particularly conduit diameter—which governs water transport efficiency but also influences susceptibility to failure. Hydraulic conductivity is

largely determined by the diameter of conduits or tracheids within the sapwood, and overall conductivity reflects how permeable the sapwood is to water flow (Hacke 2006). All features within the sapwood are interconnected and collectively influence water conductance, from the structure and porosity of pit membranes, including their thickness, to the proportion of air bubbles present. It is also observed that different tree species are associated with varying anatomical features, which makes them more or less vulnerable to cavitation. In line with this, Sasani et al. (2021) found that the $(t/b)^2$ ratio—representing cell wall thickness to span measured in the tangential direction—was higher in EL than in HL and JL, suggesting greater resistance to cavitation in EL. While traits like P_{50} and WUE provide useful indicators of hydraulic vulnerability and water-use strategy, they offer only a partial view of a species' overall drought resistance. These metrics capture specific points in the hydraulic pathway but overlook the broader structural and functional complexity of water transport. There is a clear need to adopt a more integrative approach that combines anatomical traits (e.g., pit structure, conduit density), physiological responses, and whole-tree performance. This integrated perspective has been effectively demonstrated in recent comparative studies. Wang et al. (2024) highlighted the classic efficiency–safety trade-off in xylem hydraulics in two temperate species with contrasting growth strategies. The fast-growing hybrid *Populus alba* × *P. berolinensis* exhibited significantly greater radial growth, hydraulic conductivity (Ks and Kl), and leaf photosynthetic capacity than the slow-growing *Acer truncatum*. These functional differences were underpinned by contrasting xylem anatomical traits: *Populus* had larger vessel diameters (12.16 µm vs. 8.27 µm) and lower wood density (0.41 vs. 0.50 g cm^{-3}), enhancing water transport but reducing mechanical strength. In contrast, *Acer's* denser wood and smaller vessels contributed to superior resistance to embolism (P_{88} = –3.12 MPa vs. –2.49 MPa) and a wider hydraulic safety margin. Structural traits at the pit level (e.g., smaller membrane area and lower pit aperture fraction) further reinforced *Acer's* safety strategy. This contrast illustrates how fast-growing species prioritize hydraulic efficiency to support carbon gain and rapid growth, while slow-growing species invest in anatomical reinforcement to maintain hydraulic safety under stress, reflecting the broader fast–slow plant economics spectrum. Such findings emphasize the importance of evaluating drought vulnerability through an integrated lens that includes wood density, vessel anatomy, and pit membrane characteristics alongside metrics like P_{50} and WUE.

While such efficiency–safety trade-offs are often discussed at the taxa level, our findings reveal that they can be just as pronounced within taxa, particularly among HL combinations. Many studies, and even parts of this thesis, tend to treat HL, EL, and JL as uniform groups. However, our results reveal substantial intraspecific variation, especially within HL, where some combinations showed high resilience while others performed poorly. This highlights the need to move beyond species-level comparisons and focus on clonal or family-level analyses to better capture the genetic diversity that underlies adaptive potential.

It is no longer sufficient to assess genotypes based only on their mean growth or overall resilience scores across years. What truly matters is whether growth performance can be modeled and understood in relation to specific climatic extremes. In our study, several combinations, particularly in HL and in taxa JL, maintained strong growth under moderate soil water restrictions but experienced abrupt reductions in performance during years of extreme drought, especially in observable years of mortality (2015 and 2019: late spring) and mid years for heterosis development. Identifying when these breakdowns occur and which traits fail under stress is critical for anticipating future risks. This represents a step beyond classical trait screening: it pushes us to ask whether we can predict growth collapse as a function of climate dynamics, rather than retrospectively describing it. Addressing this challenge requires integrating time-sensitive climate indicators with performance metrics and exploring the synergies among multiple traits, not just hydraulic safety or WUE in isolation, but how they interact with root allocation, stomatal behavior, or growth timing. Such modeling remains rare but has recently been applied to European larch, comparing the effects of climate and altitude using cluster models. The results showed that altitude acted as a stronger limiting factor in tree-ring development than climate alone (Danek et al. 2022). Moving toward such predictive, trait-based climate modeling is essential if we aim to select genotypes that are not just high-yielding, but consistently reliable in an increasingly variable climate.

Taken together, these findings suggest that the benefits of early fast growth may decline over time as mechanical and hydraulic limitations accumulate, particularly under recurring or prolonged drought episodes. However, the high variability observed among hybrid larch combinations in our study indicates that some genotypes may partly overcome these constraints, combining high growth performance with favorable hydraulic properties and water-use efficiency. However, such favorable combinations appear not rare but limited and highlight the importance in a short-term: to screen large sets of combinations with a multi-trait selection approaches; in a mid-term: to properly screen and choose genitors from both species to benefit from complementation of traits and in a longer-term: to genetically improve parental species breeding populations for key traits through recurrent selection, the final objective being to better balance growth and survival in future breeding programs.

7. Conclusion

This thesis provides a step forward toward integrating growth, physiological traits, and resilience into a structured, trait-based approach for climate-resilient breeding in long-lived forest species. While significant heterosis for growth was observed in hybrid larch, particularly under non-irrigated conditions, our results also highlighted important variability among hybrid combinations and taxa in their drought response strategies. The contrasting performances of the parental species—Japanese larch, characterized by fast early growth but higher mortality, and European larch, exhibiting slower growth but superior survival—illustrate the potential merit of hybrids that combine favorable attributes of both parents. The capacity of certain hybrid combinations to associate fast growth with improved cavitation resistance (P_{50}) and higher water-use efficiency (WUE) suggests that specific genotypes may partially escape the classical growth–resilience trade-offs.

Yet, further work is needed to identify which trait constellations most reliably predict performance across variable environments and through ontogeny. The extensive trait dataset collected in this trial (over 1900 trees and about more than 70 traits measured initially related to survival, growth, and reproduction) offers the opportunity to deepen our understanding of trait interdependencies and causal relationships through advanced multivariate methods, such as path analysis or structural equation modeling. Importantly, when antagonisms between target traits are revealed, breeding strategies will require careful consideration of the relative "economic" value assigned to each trait, balancing productivity, resilience, and long-term survival. In this context, interspecific hybrids often represent promising compromises, with globally superior performance profiles compared to their parental species.

Expression of heterosis for growth was also shown to fluctuate across years, likely influenced by climate variability. Similar modeling approaches as those applied in the mortality analyses could help to disentangle which climatic factors (e.g., timing and intensity of drought episodes) most strongly modulate heterosis expression over time. Our plasticity analyses already indicated that growth differences between hybrids and parents were maximal under intermediate water availability (around 600–700 mm), but diminished under both extreme drought and highly favorable (wet) conditions. As this study focused on the first ten years of development, it remains unclear whether heterosis for growth is primarily a juvenile trait, as suggested by some previous studies, or whether these hybrid advantages persist over the full lifespan of the trees. Long-term trials will be necessary to clarify these dynamics.

Looking forward, the considerable variability observed among hybrid combinations points to the need for large-scale screening efforts to identify superior genotypes. To effectively proceed with selection, integrating multi-trait indices that account for growth, hydraulic safety, plasticity, and survival—while also considering potential trade-offs—will be essential. Incorporating tools such as genomic selection, predictive modeling, and functional trait networks could further optimize breeding decisions, supporting the development of climate-smart forest plantations.

Terminology

- Adaptation: The process, action, or capacity of an individual or system to modify its inherent genetic or behavioral characteristics to better cope with change (inspired by (Zhai and Lee 2024))
- Adaptive Traits: Adaptive traits enhance a tree's ability to survive, grow, and reproduce under specific environmental conditions, particularly under stress or changing environments. *In the context of this thesis, adaptive traits refer to physiological, morphological, or phenological features (e.g., vulnerability to cavitation P_{50}, $\Delta^{13}C$, water-use efficiency (WUE), resilience parameters) that contribute to improved tolerance to drought and variable soil water availability.*
- Additive effect: Genetic effects where the contribution of individual alleles at different loci adds up to determine the trait value. These effects are directly transmissible from parents to offspring and drive response to selection.
- Average growth reduction: *We quantified average growth reduction during the drought period using Schwartz et al (2020) as the annual mean of deviations between growth under irrigation (optimal) and observed growth under non-irrigated (drought) divided by the number of years under stress.*
- Breeding value: An estimate of how much a parent can pass on a trait to its offspring. It depends on the parent's genetic quality for that trait.
- Cavitation: It is the formation of air bubbles (embolisms) in the water-conducting xylem vessels (conduit pits), which occurs when water tension becomes too high (like during drought). These air bubbles block the continuous column of water, reducing the plant's ability to transport water from roots to leaves, which can lead to reduced photosynthesis, growth decline, and potentially mortality if severe (inspired from explanations by (Delzon et al. 2010)). *In this thesis, vulnerability to cavitation is assessed by P_{50}, the xylem pressure at which 50% loss of hydraulic conductivity occurs, serving as a standardized and widely used indicator of a genotype's resistance to drought-induced embolism, particularly in conifers.*
- Clonal variation: Differences in traits observed between genetically identical clones, usually caused by environmental differences.

- Dominance: Expression where one allele at a locus mask or overrides the effect of the alternative allele (Falconer and Mackay 1996)
- Drought: It is generally defined as a prolonged period of insufficient rainfall, especially during the growing season. *In this study, we specifically define drought as the seasonal decline in soil matric potential during summer, especially under non-irrigated (NIRR) conditions.*
- Embolism: It refers to the blockage of water transport in the xylem caused by the formation and expansion of air bubbles (gas-filled voids) within the water column.
- Functional Traits: They are measurable characteristics of an organism that influence its growth, survival, or reproduction, and reflect its ecological strategy or physiological functioning in response to environmental conditions.
- Genetic architecture: The overall genetic basis of a trait, encompassing the number of genes involved, their effect sizes, allele frequencies, and the nature of genetic interactions (additive, dominance, epistasis).
- Genotype: The genetic information or set of allelic variants of an individual (Mason and Batley 2015)
- Heritability: Heritability measures how much of the variation in a trait is due to genetic differences that can be passed on to offspring.
- Heterosis (hybrid vigor): It is the increase in stature, biomass, and fertility that characterizes the progeny of crosses between diverse parents such that the F1 is superior to the better of the two parents (Birchler et al. 2006).
- High-throughput phenotyping: It refers to the application of automated and scalable methods for the rapid, non-invasive measurement of plant traits, enabling large-scale screening of genetic and environmental interactions (Fiorani and Schurr, 2013).
- Hydraulic safety: It is the plant's ability to avoid or resist loss of water transport capacity (hydraulic conductivity) under stress
- Hydraulic traits: *In this study, hydraulic traits refer to functional traits related to water transport and use, including cavitation resistance (P_{50}) and water-use efficiency (WUE), which reflect a plant's ability to maintain hydraulic function and optimize water use under drought.*

- Near-infrared spectroscopy: rapid, non-destructive analytical technique that measures the absorption of near-infrared light (typically 780–2500 nm) by molecular bonds such as C–H, N–H, and O–H, allowing the prediction of chemical and physical properties of a sample.
- Non-additive gene effect: Genetic effects arising from interactions between alleles, including dominance (within a locus) and epistasis (between loci).
- Non-destructive phenotyping: Techniques that allow measurement of plant traits without damaging the organism, enabling repeated assessments on the same individual (e.g., imaging, remote sensing, NIR spectroscopy).
- Overdominance: heterozygous individuals (with two different alleles at a locus) perform better than either homozygous parent (Falconer and Mackay 1996)
- Phenotype: The expression of genetic information as mediated by the environment and which can be measured; e.g. visible traits (Inspired by (Mason and Batley 2015)).
- Plasticity: The capacity of a given genotype to render different phenotypic values for a given trait under different environmental conditions (Valladares et al. 2006)
- Proxy: *It is an indirect measure or indicator used to estimate or infer a value that is difficult or impossible to observe directly. In this thesis, $\Delta_{13}C$ is used as a proxy for intrinsic water use efficiency.*
- Recovery: The ability to recover relative to the damage experienced during disturbance, and it is estimated as the ratio between performance after and during disturbance (following Lloret et al. 2011).
- Resilience: It is the capacity to reach pre-disturbance performance levels, and is estimated as the ratio between the performance after and before disturbance (following Lloret et al. 2011).
- Resistance: It is considered the reversal of the reduction in ecological performance during disturbance, and it is estimated as the ratio between the performance during and before the disturbance (following Lloret et al. 2011)
- Sensitivity: The degree to which a plant's performance or function is affected by a given stress. A highly sensitive plant will show strong negative responses (e.g. growth reduction, physiological decline) even under mild stress.

- Susceptibility: The likelihood or predisposition of a plant or genotype to suffer damage when exposed to a stressor. A susceptible plant has low intrinsic capacity to resist or avoid harm.
- Tolerance: The capacity of a plant to maintain function, growth, or survival despite the presence of stress. A tolerant genotype experiences minimal loss of performance even under adverse conditions.
- Trait: It is defined as any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization (Violle et al. 2007)
- Vulnerability: It reflects the inherent sensitivity or adaptive capacity of the system. *Here, that includes all factors/traits that make trees more sensitive to the stress: e.g. genotype, size and growth rate of trees, age, soil characteristics. The modeling of mortality as a function of growth, genetics (taxa and clones), and environmental conditions comes directly into this vulnerability concept* (inspired by (Sergent 2011).
- Water use efficiency (WUE): The amount of biomass or carbon assimilated (e.g., via photosynthesis) per unit of water lost through transpiration. It reflects how effectively a plant converts water into growth or productivity. *In this thesis, $\Delta^{13}C$ values in needles as been used as a proxy for intrinsic WUE*.

8. References

Agelet LE, Hurburgh CR (2010) A Tutorial on Near Infrared Spectroscopy and Its Calibration. Crit Rev Anal Chem 40:246–260. https://doi.org/10.1080/10408347.2010.515468

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH (Ted), Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim J-H, Allard G, Running SW, Semerci A, Cobb N (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manag 259:660–684. https://doi.org/10.1016/j.foreco.2009.09.001

Alley R, Berntsen T, Bindoff NL, Chen Z, Chidthaisong A, Friedlingstein P, Gregory J, Hegerl G, Heimann M, Hewitson B, Hoskins B, Joos F, Jouzel J, Kattsov V, Lohmann U, Manning M, Matsuno T, Molina M, Nicholls N, Overpeck J, Qin D, Raga G, Ren J, Rusticucci M, Solomon S, Somerville R, Stocker TF, Stott P, Whetton P, Wood RA, Wratt D, Arblaster J, Brasseur G, Christensen JH, Denman K, Fahey DW, Forster P, Jansen E, Jones PD, Knutti R, Treut HL, Lemke P, Meehl G, Mote P, Randall D, Stone DA, Willebrand J, Zwier (2007). Summary for policymakers. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC. Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds). Cambridge Univ Press.

Alves A, Schwanninger M, Pereira H, Rodrigues J (2006) Analytical pyrolysis as a direct method to determine the lignin content in wood. J Anal Appl Pyrolysis 76:209–213. https://doi.org/10.1016/j.jaap.2005.11.004

Anderegg WRL (2015) Spatial and temporal variation in plant hydraulic traits and their relevance for climate change impacts on vegetation. New Phytol 205:1008–1014. https://doi.org/10.1111/nph.12907

Anderegg WRL, Klein T, Bartlett M, Sack L, Pellegrini AFA, Choat B, Jansen S (2016) Meta-analysis reveals that hydraulic traits explain cross-species patterns of drought-induced tree mortality across the globe. Proc Natl Acad Sci USA 113:5024–5029. https://doi.org/10.1073/pnas.1525678113

Atkinson RRL, Burrell MM, Osborne CP, Rose KE, Rees M (2012) A non-targeted metabolomics approach to quantifying differences in root storage between fast- and slow-growing plants. New Phytol 196:200–211. https://doi.org/10.1111/j.1469-8137.2012.04274.x

Attia Z, Domec J-C, Oren R, Way DA, Moshelion M (2015) Growth and physiological responses of isohydric and anisohydric poplars to drought. J Exp Bot 66:4373–4381. https://doi.org/10.1093/jxb/erv195

Ayari A, Khouja ML (2014) Ecophysiological variables influencing Aleppo pine seed and cone production: a review. Tree Physiol 34:426–437. https://doi.org/10.1093/treephys/tpu022

Bartlett MK, Scoffoni C, Sack L (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecol Lett 15:393–405. https://doi.org/10.1111/j.1461-0248.2012.01751.x

Baudouin L, Baril C, Clément-Demange A, Leroy T,Paulin, D. (1997). Recurrent selection of tropical tree crops. Euphytica 96:101-114.

Bennett AC, McDowell NG, Allen CD, Anderson-Teixeira KJ (2015) Larger trees suffer most during drought in forests worldwide. Nat Plants 1:1–5. https://doi.org/10.1038/nplants.2015.139

Birchler JA, Yao H, Chudalayandi S (1996) Unraveling the genetic basis of hybrid vigor. Proc Natl Acad Sci 103:12957–12958. https://doi.org/10.1073/pnas.0605627103

Bobrov EG (1973) Introgressive Hybridisation, Sippenbildung und Vegetationsänderung. Feddes Repertorium, 84(4): 273-293.

Boisvenue C, Running SW (2006) Impacts of climate change on natural forest productivity – evidence since the middle of the 20th century. Glob Change Biol 12:862–882. https://doi.org/10.1111/j.1365-2486.2006.01134.x

Bolund E (2020) The challenge of measuring trade-offs in human life history research. Evoland Hum Behav 41:502–512. https://doi.org/10.1016/j.evolhumbehav.2020.09.003

Bower AD, Frerker KL, Pike CC, Labonte NR, Palik BJ, Royo AA, Anderson SM, Ferreira AR, Brandt LA (2024) A practical framework for applied forestry assisted migration. Front For Glob Change 7. https://doi.org/10.3389/ffgc.2024.1454329

Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate Forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. Ann For Sci 63:625–644. https://doi.org/10.1051/forest:2006042

Brice T, Hall T (2020) US Dept of Commerce National Oceanic and Atmospheric Administration National Weather Service [WWW Document]. Vapor Pressure Calculator. URL: https://www.weather.gov/epz/wxcalc_vapor pressure

Brodribb TJ, Powers J, Cochard H, Choat B (2020) Hanging by a thread? Forests and drought. Science 368:261–266. https://doi.org/10.1126/science.aat7631

Brunner I, Herzog C, Galiano L, Gessler A (2019) Plasticity of Fine-Root Traits Under Long-Term Irrigation of a Water-Limited Scots Pine Forest. Front Plant Sci 10:701. https://doi.org/10.3389/fpls.2019.00701

Brendel O (2014). Is the coefficient of variation a valid measure for variability of stable isotope abundances in biological materials?. *Rapid Commun Mass Spectrom*, *28*(4), 370-376.

Byram TD, Myszewski JH, Gwaze DP, Lowe WJ (2005) Improving wood quality in the western gulf forest tree improvement program: the problem of multiple breeding objectives. Tree Genet Genom1:85–92. https://doi.org/10.1007/s11295-005-0017-x

Camarero JJ (2021) The drought–dieback–death conundrum in trees and forests. Plant Ecol Diver 14:1–12. https://doi.org/10.1080/17550874.2021.1961172

Camarero JJ, Gazol A, Sangüesa-Barreda G, Oliva J, Vicente-Serrano SM (2015) To die or not to die: early warnings of tree dieback in response to a severe drought. J Ecol 103:44–57. https://doi.org/10.1111/1365-2745.12295

Cameron A, Orr D, Clark J (2017) Variation in the incidence and severity of drought crack in three conifer species in North East Scotland. Scand J For Res 32:658–662. https://doi.org/10.1080/02827581.2017.1360936

Carron LT, Aken KM, Australian Centre for International Agricultural Research (eds) (1992) Breeding technologies for tropical acacias: proceedings of an international workshop held in Tawau, Sabah, Malaysia, 1-4 July 1991. ACIAR, Canberra

Carson SD, Carson MJ (1989) Breeding for Resistance in Forest Trees-A Quantitative Genetic Approach. Annu Rev Phytopathol 27:373–395. https://doi.org/10.1146/annurev.py.27.090189.002105

Chakhchar A, Haworth M, El Modafar C, Lauteri M, Mattioni C, Wahbi S, Centritto M (2017) An Assessment of Genetic Diversity and Drought Tolerance in Argan Tree (Argania spinosa) Populations: Potential for the Development of Improved Drought Tolerance. Front Plant Sci 8. https://doi.org/10.3389/fpls.2017.00276

Charru M, Seynave I, Morneau F, Bontemps J-D (2010) Recent changes in forest productivity: An analysis of national forest inventory data for common beech (*Fagus sylvatica* L.) in north-eastern France. For Ecol Manag 260:864–874. https://doi.org/10.1016/j.foreco.2010.06.005

Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE (2009) Towards a worldwide wood economics spectrum. Ecol Lett 12:351–366. https://doi.org/10.1111/j.1461-0248.2009.01285.x

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought — from genes to the whole plant. Functional Plant Biol 30:239. https://doi.org/10.1071/FP02076

Choat B, Brodribb TJ, Brodersen CR, Duursma RA, López R, Medlyn BE (2018) Triggers of tree mortality under drought. Nature 558:531–539. https://doi.org/10.1038/s41586-018-0240-x

Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Feild TS, Gleason SM, Hacke UG, Jacobsen AL, Lens F, Maherali H, Martínez-Vilalta J, Mayr S, Mencuccini M, Mitchell PJ, Nardini A, Pittermann J, Pratt RB, Sperry JS, Westoby M, Wright IJ, Zanne AE (2012) Global convergence in the vulnerability of forests to drought. Nature 491:752–755. https://doi.org/10.1038/nature11688

Christensen JH, Hewitson B, Busuioc A, Chen A, Gao X, Held I, Jones R, Kolli RK, Kwon WT, Laprise R, Magaña RV, Mearns L, Menéndez CG, Räisänen J, Rinke A, Sarr A, Whetton P (2007) Regional Climate Projections. Climate Change 2007: the physical science basis 847–940

Clarke PJ, Lawes MJ, Midgley JJ, Lamont BB, Ojeda F, Burrows GE, Enright NJ, Knox KJE (2013) Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. New Phytol 197:19–35. https://doi.org/10.1111/nph.12001

Climent J, Alía R, Karkkainen K, Bastien C, Benito-Garzon M, Bouffier L, De Dato G, Delzon S, Dowkiw A, Elvira-Recuenco M, Grivet D, González-Martínez SC, Hayatgheibi H, Kujala S, Leplé J-C, Martín-Sanz RC, de Miguel M, Monteverdi MC, Mutke S, Plomion C, Ramírez-Valiente JA, Sanchez L, Solé-Medina A, Soularue J-P, Steffenrem A, Teani A, Westin J, Whittet R, Wu H, Zas R, Cavers S (2024) Trade-offs and Trait Integration in Tree Phenotypes: Consequences for the Sustainable Use of Genetic Resources. Curr For Rep 10:196–222. https://doi.org/10.1007/s40725-024-00217-5

Climent J, Prada MA, Calama R, Chambel MR, de Ron DS, Alía R (2008) To grow or to seed: ecotypic variation in reproductive allocation and cone production by young female Aleppo

pine (*Pinus halepensis,* Pinaceae). Am J Bot 95:833–842. https://doi.org/10.3732/ajb.2007354

Colesie C, Stangl ZR, Hurry V (2020) Differences in growth-economics of fast vs. slow growing grass species in response to temperature and nitrogen limitation individually, and in combination. BMC Ecol 20:63. https://doi.org/10.1186/s12898-020-00333-3

Cook BI, Mankin JS, Anchukaitis KJ (2018) Climate Change and Drought: From Past to Future. Curr Clim Change Rep 4:164–179. https://doi.org/10.1007/s40641-018-0093-2

Cope OL, Keefover-Ring K, Kruger EL, Lindroth RL (2021) Growth–defense trade-offs shape population genetic composition in an iconic forest tree species. Proc Natl Acad Sci USA 118:e2103162118. https://doi.org/10.1073/pnas.2103162118

Cortés AJ, Restrepo-Montoya M, Bedoya-Canas LE (2020) Modern Strategies to Assess and Breed Forest Tree Adaptation to Changing Climate. Front Plant Sci 11:583323. https://doi.org/10.3389/fpls.2020.583323

Craig H (1957) Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochimica et Cosmochimica Acta 12:133–149. https://doi.org/10.1016/0016-7037(57)90024-8

Crausbay SD, Ramirez AR, Carter SL, Cross MS, Hall KR, Bathke DJ, Betancourt JL, Colt S, Cravens AE, Dalton MS, Dunham JB, Hay LE, Hayes MJ, McEvoy J, McNutt CA, Moritz MA, Nislow KH, Raheem N, Sanford T (2017) Defining Ecological Drought for the Twenty-First Century. Bull Am Meteorol Soc 98:2543–2550. https://doi.org/10.1175/BAMS-D-16-0292.1

Danek M, Danek T (2022) Recent changes in the climate-growth response of European larch (*Larix decidua* Mill.) in the Polish Sudetes. Trees 36:803–817. https://doi.org/10.1007/s00468-021-02251-3

Delzon S, Douthe C, Sala A, Cochard H (2010) Mechanism of water-stress induced cavitation in conifers: bordered pit structure and function support the hypothesis of seal capillary-seeding. Plant, Cell Envir 33:2101–2111. https://doi.org/10.1111/j.1365-3040.2010.02208.x

Depardieu C, Girardin MP, Nadeau S, Lenz P, Bousquet J, Isabel N (2020) Adaptive genetic variation to drought in a widely distributed conifer suggests a potential for increasing forest resilience in a drying climate. New Phytol 227:427–439. https://doi.org/10.1111/nph.16551

Ditmarová L, Kurjak D, Palmroth S, Kmeť J, Střelcová K (2010) Physiological responses of Norway spruce (*Picea abies*) seedlings to drought stress. *Tree physiology*, *30*(2): 205-213.

Dobbertin M (2005) Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. Eur J Forest Res 124:319–333. https://doi.org/10.1007/s10342-005-0085-3

Donat MG, Alexander LV, Yang H, Durre I, Vose R, Dunn RJH, Willett KM, Aguilar E, Brunet M, Caesar J, Hewitson B, Jack C, Klein Tank AMG, Kruger AC, Marengo J, Peterson TC, Renom M, Oria Rojas C, Rusticucci M, Salinger J, Elrayah AS, Sekele SS, Srivastava AK, Trewin B, Villarroel C, Vincent LA, Zhai P, Zhang X, Kitching S (2013) Updated analyses of temperature and precipitation extreme indices since the beginning of the twentieth

century: The HadEX2 dataset. JGR Atmospheres 118:2098–2118. https://doi.org/10.1002/jgrd.50150

Dosio A, Mentaschi L, Fischer EM, Wyser K (2018) Extreme heat waves under 1.5 °C and 2 °C global warming. Environ Res Lett 13:054006. https://doi.org/10.1088/1748-9326/aab827

DungeyHS (2001). Pine hybrids—a review of their use performance and genetics. For ecol mana, 148(1-3):243-258.

Eller C, De V. Barros F, R.L. Bittencourt P, Rowland L, Mencuccini M, S. Oliveira R (2018) Xylem hydraulic safety and construction costs determine tropical tree growth: Tree growth vs hydraulic safety trade-off. Plant Cell Environ 41:548–562. https://doi.org/10.1111/pce.13106

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

Falconer DS, Mackay TF (1996) Introduction to quantitative genetics, 4th edn. Longman, Harlow

Farquhar G, Richards R (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Funct Plant Biol 11:539–552

Fiévet JB, Dillmann C, De Vienne D (2010) Systemic properties of metabolic networks lead to an epistasis-based model for heterosis. Theor Appl Genet 120:463–473. https://doi.org/10.1007/s00122-009-1203-2

Fiorani F, Schurr U (2013) Future Scenarios for Plant Phenotyping. Annu Rev Plant Biol 64:267–291. https://doi.org/10.1146/annurev-arplant-050312-120137

Firth D (1993) Bias reduction of maximum likelihood estimates. Biometrika 80:27–38. https://doi.org/10.1093/biomet/80.1.27

Fririon V, Davi H, Oddou-Muratorio S, Lebourgeois F, Lefèvre F (2023) Within and between population phenotypic variation in growth vigor and sensitivity to drought stress in five temperate tree species. For Ecol Manag 531:120754. https://doi.org/10.1016/j.foreco.2022.120754

Fulé PZ, Cocke AE, Heinlein TA, Covington WW (2004) Effects of an Intense Prescribed Forest Fire: Is It Ecological Restoration? Restor Ecol 12:220–230. https://doi.org/10.1111/j.1061-2971.2004.00283.x

Garland T (2014) Trade-offs. Current Biology 24: R60–R61. https://doi.org/10.1016/j.cub.2013.11.036

Gazol A, Camarero JJ, Vicente-Serrano SM, Sánchez-Salguero R, Gutiérrez E, de Luis M, Sangüesa-Barreda G, Novak K, Rozas V, Tíscar PA, Linares JC, Martín-Hernández N, Martínez del Castillo E, Ribas M, García-González I, Silla F, Camisón A, Génova M, Olano JM, Longares LA, Hevia A, Tomás-Burguera M, Galván JD (2018) Forest resilience to drought varies across biomes. Glob Change Biol 24:2143–2158. https://doi.org/10.1111/gcb.14082

Gebreselassie MN, Ader K, Boizot N, Millier F, Charpentier J-P, Alves A, Simões R, Rodrigues JC, Bodineau G, Fabbrini F, Sabatti M, Bastien C, Segura V (2017) Near-infrared spectroscopy enables the genetic analysis of chemical properties in a large set of wood

samples from *Populus nigra* (L.) natural populations. Ind Crops Prod 107:159–171. https://doi.org/10.1016/j.indcrop.2017.05.013

Gil-Pelegrín E, Corcuera L, Camarero JJ (2004) Effects of a severe drought on *Quercus ilex* radial growth and xylem anatomy. Trees - Struct Funct 18:83–92. https://doi.org/10.1007/s00468-003-0284-9

Giovannelli A, Deslauriers A, Fragnelli G, Scaletti L, Castro G, Rossi S, Crivellaro A (2007) Evaluation of drought response of two poplar clones (*Populus*×*canadensis* Mönch 'I-214' and *P. deltoides* Marsh. 'Dvina') through high resolution analysis of stem growth. J Exp Bot 58:2673–2683. https://doi.org/10.1093/jxb/erm117

Gorham E, Kelly J (2018) A History of Ecological Research Derived from Titles of Articles in the Journal "Ecology" 1925–2015. The Bull Ecol Soc Am 99:61–72. https://doi.org/10.1002/bes2.1380

Gower ST, Isebrands JG, Sheriff DW (1995) 7 - Carbon Allocation and Accumulation in Conifers. In: Smith WK, Hinckley TM (eds) Resource Physiology of Conifers. Academic Press, San Diego.217–254

Granier A, Bréda N, Biron P, Villette S (1999) A lumped water balance model to evaluate duration and intensity of drought constraints in forest stands. Ecol Model 116:269–283. https://doi.org/10.1016/S0304-3800(98)00205-1

Grattapaglia D, Kirst M (2008) *Eucalyptus* applied genomics: from gene sequences to breeding tools. New Phytol 179:911–929. https://doi.org/10.1111/j.1469-8137.2008.02503.x

Grattapaglia D, Silva-Junior OB, Resende RT, Cappa EP, Müller BSF, Tan B, Isik F, Ratcliffe B, El-Kassaby YA (2018) Quantitative Genetics and Genomics Converge to Accelerate Forest Tree Breeding. Front Plant Sci 9. https://doi.org/10.3389/fpls.2018.01693

Gray LK, Hamann A, John S, Rweyongeza D, Barnhardt L, Thomas BR (2016) Climate change risk management in tree improvement programs: selection and movement of genotypes. Tree Genet Genomes 12:23. https://doi.org/10.1007/s11295-016-0983-1

Greenwood MS, Roth BE, Maass D, Irland LC (2015) Near rotation-length performance of selected hybrid larch in Central Maine, U.S.A. Silvae Genetica 64:73–80. https://doi.org/10.1515/sg-2015-0006

Groover A, Holbrook NM, Polle A, Sala A, Medlyn B, Brodersen C, Pittermann J, Gersony J, Sokołowska K, Bogar L, McDowell N, Spicer R, David-Schwartz R, Keller S, Tschaplinski TJ, Preisler Y (2025) Tree drought physiology: critical research questions and strategies for mitigating climate change effects on forests. New Phytol 245:1817–1832. https://doi.org/10.1111/nph.20326

Grubb PJ (1998) A reassessment of the strategies of plants which cope with shortages of resources. Perspect Plant Ecol Evol Syst 1:3–31. https://doi.org/10.1078/1433-8319-00049

Guisset C, Dendoncker M, Vincke C, Ponette Q (2024) Drought timing, intensity, and consecutiveness have more influence on Douglas fir growth response than site conditions and stand density in European temperate climate. For Ecol Manag 569:122177. https://doi.org/10.1016/j.foreco.2024.122177

Gustafson EJ, Kern CC, Miranda BR, Sturtevant BR, Bronson DR, Kabrick JM (2020) Climate adaptive silviculture strategies: How do they impact growth, yield, diversity and value in

forested landscapes? For Ecol Manag 470–471:118208. https://doi.org/10.1016/j.foreco.2020.118208

Hadfield JD (2010) MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. J Stat Softw 33:1–22. https://doi.org/10.18637/jss.v033.i02

Hammond WM, Williams AP, Abatzoglou JT, Adams HD, Klein T, López R, Sáenz-Romero C, Hartmann H, Breshears DD, Allen CD (2022) Global field observations of tree die-off reveal hotter-drought fingerprint for Earth's forests. Nat Commun 13:1761. https://doi.org/10.1038/s41467-022-29289-2

Hacke UG (2006) Scaling of angiosperm xylem structure with safety and efficiency. Tree Physiol. 26:689–701.

Helama S (2015) Expressing Tree-Ring Chronology as Age-Standardized Growth Measurements. For Sci 61:817–828. https://doi.org/10.5849/forsci.14-139

Herms DA, Mattson WJ (1992) The Dilemma of Plants: To Grow or Defend. Q Rev Biol 67:283–335. https://doi.org/10.1086/417659

Hilty J, Muller B, Pantin F, Leuzinger S (2021) Plant growth: the What, the How, and the Why. New Phytol 232:25–41. https://doi.org/10.1111/nph.17610

Hisamoto Y, and Goto S (2017) Genetic control of altitudinal variation on female reproduction in Abies sachalinensis revealed by a crossing experiment. J For Res 22:195–198. https://doi.org/10.1080/13416979.2017.1304863

Hogg E (Ted), Brandt JP, Kochtubajda B (2005) Factors affecting interannual variation in growth of western Canadian aspen forests during 1951-2000. Can J For Res 35:610–622. https://doi.org/10.1139/x04-211

Intergovernmental Panel On Climate Change (Ipcc) (2023) Climate Change 2022 – Impacts, Adaptation and Vulnerability: Working Group II Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, 1st edn. Cambridge University Press, https://www.ipcc.ch/site/assets/uploads/2018/03/SREX_Full_Report-1.

Ivković M, Gapare WJ, Abarquez A, Ilic J, Powell MB, Wu HX (2009) Prediction of wood stiffness, strength, and shrinkage in juvenile wood of radiata pine. Wood Sci Technol 43:237–257. https://doi.org/10.1007/s00226-008-0232-3

Janes J, Hamilton J (2017) Mixing It Up: The Role of Hybridization in Forest Management and Conservation under Climate Change. Forests 8:237. https://doi.org/10.3390/f8070237

Joshi J, Stocker BD, Hofhansl F, Zhou S, Dieckmann U, Prentice IC (2022) Towards a unified theory of plant photosynthesis and hydraulics. Nat Plants 8:1304–1316. https://doi.org/10.1038/s41477-022-01244-5

Kaczmarek DJ, Coyle DR, Coleman MD (2013) Survival and growth of a range of *Populus* clones in central South Carolina USA through age ten: Do early assessments reflect longer-term survival and growth trends? Biomass and Bioenergy 49:260–272. https://doi.org/10.1016/j.biombioe.2012.12.005

Karlman, L. (2010). Genetic variation in frost tolerance, juvenile growth and timber production in Russian larches (*Larix* Mill.). Acta Universitatis Agriculturae Sueciae, (2010: 30).

Keen RM, Voelker SL, Bentz BJ, Wang S-YS, Ferrell R (2020) Stronger influence of growth rate than severity of drought stress on mortality of large ponderosa pines during the 2012–2015 California drought. Oecologia 194:359–370. https://doi.org/10.1007/s00442-020-04771-0

Keenan, RJ (2015). Climate change impacts and adaptation in forest management: a review. Ann For Sci 72:145 - 167. https://doi.org/10.1007/s13595-014-0446-5

Kim J, Kim K-S, Kim Y, Chung YS (2021) A short review: Comparisons of high-throughput phenotyping methods for detecting drought tolerance. Sci agric (Piracicaba, Braz) 78:e20190300300. https://doi.org/10.1590/1678-992x-2019-0300

Klápště J, Jaquish B, Porth I (2022a) Building resiliency in conifer forests: Interior spruce crosses among weevil resistant and susceptible parents produce hybrids appropriate for multi-trait selection. PLoS ONE 17:e0263488. https://doi.org/10.1371/journal.pone.0263488

Klápště J, Telfer EJ, Dungey HS, Graham NJ (2022b) Chasing genetic correlation breakers to stimulate population resilience to climate change. Sci Rep 12:8238. https://doi.org/10.1038/s41598-022-12320-3

Koller S, Jedmowski C, Kamm K, Brüggemann W (2014) The South Hesse Oak Project (SHOP): Species- and site-specific efficiency of the photosynthetic apparatus of Mediterranean and Central European Oaks. Plant Biosyst - Int J Deal Asp Plant Biol 148:237–248. https://doi.org/10.1080/11263504.2012.762947

Kolström M, Lindner M, Vilén T, Maroschek M, Seidl R, Lexer MJ, Netherer S, Kremer A, Delzon S, Barbati A, Marchetti M, Corona P (2011) Reviewing the Science and Implementation of Climate Change Adaptation Measures in European Forestry. Forests 2:961–982. https://doi.org/10.3390/f2040961

Körner C (2019) No need for pipes when the well is dry—a comment on hydraulic failure in trees. Tree Physiol 39:695–700. https://doi.org/10.1093/treephys/tpz030

Kosmidis I (2022) Mean and median bias reduction: a concise review and application to adjacent-categories logit models. https://doi.org/10.48550/arXiv.2112.02621

Kremer A, Ronce O, Robledo-Arnuncio JJ, Guillaume F, Bohrer G, Nathan R, Bridle JR, Gomulkiewicz R, Klein EK, Ritland K, Kuparinen A, Gerber S, Schueler S (2012) Long-distance gene flow and adaptation of forest trees to rapid climate change. Ecol Lett 15:378–392. https://doi.org/10.1111/j.1461-0248.2012.01746.x

Kurinobu S, 2005. Forest Tree Breeding for Japanese larch. Eurasian J Res 8:127–134.

Lailvaux SP, Husak JF (2017) Predicting Life-History Trade-Offs with Whole-Organism Performance. Integr Comp Biol 57:325–332. https://doi.org/10.1093/icb/icx073

Laitinen RAE, Nikoloski Z (2024) Strategies to identify and dissect trade-offs in plants. Mol Ecol 33: e16780. https://doi.org/10.1111/mec.16780

Lauder JD, Moran EV, Hart SC (2019) Fight or flight? Potential tradeoffs between drought defense and reproduction in conifers. Tree Physiol 39:1071–1085. https://doi.org/10.1093/treephys/tpz031

Laughlin DC, Strahan RT, Huffman DW, Sánchez Meador AJ (2017a) Using trait-based ecology to restore resilient ecosystems: historical conditions and the future of montane forests in western North America. Restor Ecol 25:S135–S146. https://doi.org/10.1111/rec.12342

Lindner M, Fitzgerald JB, Zimmermann NE, Reyer C, Delzon S, Van Der Maaten E, Schelhaas M-J, Lasch P, Eggers J, Van Der Maaten-Theunissen M, Suckow F, Psomas A, Poulter B, Hanewinkel M (2014) Climate change and European forests: What do we know, what are the uncertainties, and what are the implications for forest management? J Env Manag146:69–83. https://doi.org/10.1016/j.jenvman.2014.07.030

Linsley Jr RK, Kohler MA, Paulhus JLH (1975) Hydrology for engineers

Lloret F, Keeling EG, Sala A (2011) Components of tree resilience: effects of successive low-growth episodes in old ponderosa pine forests. Oikos 120:1909–1920. https://doi.org/10.1111/j.1600-0706.2011.19372.x

Lloret F, Jaime LA, Margalef-Marrase J, Pérez-Navarro MA, Batllori E (2022) Short-term forest resilience after drought-induced die-off in Southwestern European forests. Sci Total Environ 806:150940. https://doi.org/10.1016/j.scitotenv.2021.150940

Lovell JT, McKay JK (2015) Ecological genetics of range size variation in *Boechera* spp. (Brassicaceae). Ecology and Evolution 5:4962–4975. https://doi.org/10.1002/ece3.1746

Luss S, Schwanninger M, Rosner S (2015) Hydraulic traits of Norway spruce sapwood estimated by Fourier transform near-infrared spectroscopy (FT-NIR). Can J For Res 45:625–631. https://doi.org/10.1139/cjfr-2014-0452

Malhi Y, Franklin J, Seddon N, Solan M, Turner MG, Field CB, Knowlton N (2020) Climate change and ecosystems: threats, opportunities and solutions. Phil Trans R Soc B 375:20190104. https://doi.org/10.1098/rstb.2019.0104

Marchal A, Muñoz F, Millier F, Sánchez L, Pâques LE (2017) Hybrid larch heterosis: for which traits and under which genetic control? Tree Genet Genomes 13:92. https://doi.org/10.1007/s11295-017-1177-1

Marchal A (2018). Larch hybrid vigor and role of the phenotypic plasticity in the construction of heterosis (Doctoral dissertation, Université d'Orléans).

Marchal A, Schlichting CD, Gobin R, Balandier P, Millier F, Muñoz F, Pâques LE, Sánchez L (2019) Deciphering Hybrid Larch Reaction Norms Using Random Regression. G3 Genes Genomes Genet 9:21–32. https://doi.org/10.1534/g3.118.200697

Marguerit E, Bouffier L, Chancerel E, Costa P, Lagane F, Guehl JM, Plomion C, Brendel O (2014). The genetics of water-use efficiency and its relation to growth in maritime pine. J Exp Bot, 65(17): 4757–4768. https://doi.org/10.1093/jxb/eru226

Mason AS, Batley J (2015) Creating new interspecific hybrid and polyploid crops. Trends Biotechnol 33:436–441. https://doi.org/10.1016/j.tibtech.2015.06.004

Mátyás C (1996) Climatic adaptation of trees: rediscovering provenance tests. Euphytica 92:45–54. https://doi.org/10.1007/BF00022827

Matyssek R, Schulze ED (1988) Carbon uptake and respiration in above-ground parts of a *Larix decidua* × *leptolepis* tree. Trees 2:233–241. https://doi.org/10.1007/BF00202378

Matyssek R, Schulze E-D (1987) Heterosis in hybrid larch (*Larix decidua x leptolepis*). Trees 1:219–224. https://doi.org/10.1007/BF01816819

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mortality during

drought: why do some plants survive while others succumb to drought? New Phytologist 178:719–739. https://doi.org/10.1111/j.1469-8137.2008.02436.x

Millar CI, Stephenson NL, Stephens SL (2007) Climate Change and Forests of the future: managing in the face of uncertainty. Ecol App 17:2145–2151. https://doi.org/10.1890/06-1715.1

Moles AT, Westoby M (2004) Seedling survival and seed size: a synthesis of the literature: *Seedling survival and seed size*. J Ecol 92:372–383. https://doi.org/10.1111/j.0022-0477.2004.00884.x

Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit J, Barbaroux C, Le Thiec D, Bréchet C, Brignolas F (2006) Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* × *Populus nigra*. New Phytol 169:765–777. https://doi.org/10.1111/j.1469-8137.2005.01630.x

Namkoong G (1979) Introduction to Quantitative Genetics in Forestry. U.S. Department of Agriculture, Forest Service

Nantongo JS, Potts B, Rodemann T, Fitzgerald H, Davies N, O'Reilly-Wapstra J (2021) Developing near infrared spectroscopy models for predicting chemistry and responses to stress in *Pinus radiata* (D. Don). J Infrared Spectrosc 29:245–256. https://doi.org/10.1177/09670335211006526

Negbi M (1995) Male and Female in Theophrastus's Botanical Works. J Hist Biol 28:317–332

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, Van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. Trends Plant Sci 15:684–692. https://doi.org/10.1016/j.tplants.2010.09.008

Nogués I, Llusià J, Ogaya R, Munné-Bosch S, Sardans J, Peñuelas J, Loreto F (2014) Physiological and antioxidant responses of *Quercus ilex* to drought in two different seasons. Plant Biosyst - Int J Deal Asp Plant Biol 148:268–278. https://doi.org/10.1080/11263504.2013.768557

Oleksyn J, Fritts HC (1991) Influence of climatic factors upon tree rings of *Larix decidua* and *L. decidua* × *L. kaempferi* from Pulawy, Poland. Trees 5:75–82. https://doi.org/10.1007/BF00227488

Padilla FM, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. Funct Ecol 21:489–495. https://doi.org/10.1111/j.1365-2435.2007.01267.x

Pâques LE (1989) A critical review of larch hybridization and its incidence on breeding strategies. Ann For Sci 46:141–153. https://doi.org/10.1051/forest:19890204

Pâques LE (2001). Genetic control of heartwood content in larch. Silvae Genet, 50(2), 69-74.

Pâques LE, Philippe G, Prat D (2006) Identification of European and Japanese Larch and Their Interspecific Hybrid with Morphological Markers: Application to Young Seedlings. Silvae Genet 55:123–134. https://doi.org/10.1515/sg-2006-0018

Pâques LE (2009) Growth Rhythm Parameters as Components of Hybrid Vigour in Young Seedlings of Hybrid Larch (*Larix decidua x L. kaempferi*). Silvae Genet 58:42–53. https://doi.org/10.1515/sg-2009-0006

Pâques LE (ed) (2013) Forest Tree Breeding in Europe: Current State-of-the-Art and Perspectives. Springer Netherlands, Dordrecht

Pâques LE, Lejeune V, Veisse D (2022) Do biomass partitioning and growth efficiency contribute to growth heterosis in inter-specific hybrid larch *Larix x eurolepis*? Forestry: An International J For Res 95:466–476. https://doi.org/10.1093/forestry/cpac004

Pâques LE, Millier F, Veisse D (2023) Longitudinal stem cracks in larch: what makes trees vulnerable? New For 54:813–832. https://doi.org/10.1007/s11056-022-09942-w

Pauwels D, Rondeux J (1999). Stand volume tables for larches in southern Belgium.

Peñuelas J, Boada M (2003) A global change-induced biome shift in the Montseny mountains (NE Spain). Global Change Biol 9:131–140. https://doi.org/10.1046/j.1365-2486.2003.00566.x

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico L, Pausas JG, de Vos AC, Buchmann N, Funes G, Quétier F, Hodgson JG, Thompson K, Morgan HD, ter Steege H, van der Heijden MGA, Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino S, Cornelissen, J H C (2016) Corrigendum to: New handbook for standardised measurement of plant functional traits worldwide. Aust J bot, *64*(8): 715-716.

Piya L, Maharjan KL, Joshi NP (2019). Socio-Economic Issues of Climate Change. Springer Nature, Singapore,10, 978-981.

Potts BM, Dungey HS (2004). Interspecific hybridization of *Eucalyptus*: key issues for breeders and geneticists. New For 27(2), 115-138.

Prohens J, Gramazio P, Plazas M, Dempewolf H, Kilian B, Díez M, Fita A, Herraiz F, Rodriguez Burruezo A, Soler S, Knapp S, Vilanova S (2017) Introgressiomics: a new approach for using crop wild relatives in breeding for adaptation to climate change. Euphytica 213. https://doi.org/10.1007/s10681-017-1938-9

Redmond MD, Davis TS, Ferrenberg SM, Wion AP (2019) Resource allocation trade-offs in a mast-seeding conifer: Piñon pine prioritizes reproduction over defense. AoB PLANTS plz070. https://doi.org/10.1093/aobpla/plz070

Reich PB (2014) The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. J Ecol 102:275–301. https://doi.org/10.1111/1365-2745.12211

Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB (2003) The Evolution of Plant Functional Variation: Traits, Spectra, and Strategies. Int J Plant Sci 164:S143–S164. https://doi.org/10.1086/374368

Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A (2006) Physiological Responses of Forest Trees to Heat and Drought. Plant Biol 8:556–571. https://doi.org/10.1055/s-2006-924084

Roff DA, Fairbairn DJ (2007) The evolution of trade-offs: where are we? J Evolution Biol 20:433–447. https://doi.org/10.1111/j.1420-9101.2006.01255.x

Ruotsalainen S (2014) Increased forest production through forest tree breeding. Scandinavian Journal of Forest Research 29:333–344. https://doi.org/10.1080/02827581.2014.926100

Ryan MG (2011) Tree responses to drought. Tree Physiol 31:237–239. https://doi.org/10.1093/treephys/tpr022

R Core Team (2024) R: a language and environment for statistical computing. URL https://www.R-project.org/

Sartori K, Vasseur F, Violle C, Baron E, Gerard M, Rowe N, Ayala-Garay O, Christophe A, Jalón LG de, Masclef D, Harscouet E, Granado M del R, Chassagneux A, Kazakou E, Vile D (2019) Leaf economics and slow-fast adaptation across the geographic range of *Arabidopsis* thaliana. Sci Rep 9:10758. https://doi.org/10.1038/s41598-019-46878-2

Sasani N, Pâques LE, Boulanger G, Singh AP, Gierlinger N, Rosner S, Brendel O (2021) Physiological and anatomical responses to drought stress differ between two larch species and their hybrid. Trees 35:1467–1484. https://doi.org/10.1007/s00468-021-02129-4

Schär C, Jendritzky G (2004) Hot news from summer 2003. Nature 432:559–560. https://doi.org/10.1038/432559a

Schwarz J, Skiadaresis G, Kohler M, Kunz J, Schnabel F, Vitali V, Bauhus J (2020) Quantifying Growth Responses of Trees to Drought—a Critique of Commonly Used Resilience Indices and Recommendations for Future Studies. Curr Forestry Rep 6:185–200. https://doi.org/10.1007/s40725-020-00119-2

Semerikov VL, Lascoux M (1999) Genetic relationship among Eurasian and American *Larix* species based on allozymes. Heredity 83 :62–70. https://doi.org/10.1038/sj.hdy.6885310

Sergent A-S (2011) Diversité de la réponse au déficit hydrique et vulnérabilité au dépérissement du douglas. Thèse de doctorat, Université d'Orléans

Shakoor N, Lee S, Mockler TC (2017) High throughput phenotyping to accelerate crop breeding and monitoring of diseases in the field. Curr Opin Plant Biol 38:184–192. https://doi.org/10.1016/j.pbi.2017.05.006

Shipley B (2010) From Plant Traits to Vegetation Structure: Chance and Selection in the Assembly of Ecological Communities. Cambridge University Press

Shull GH (1948) What Is "Heterosis"? Genet 33:439–446. https://doi.org/10.1093/genetics/33.5.439

Signori-Müller C, Oliveira RS, Valentim Tavares J, Carvalho Diniz F, Gilpin M, de V. Barros F, Marca Zevallos MJ, Salas Yupayccana CA, Nina A, Brum M, Baker TR, Cosio EG, Malhi Y, Monteagudo Mendoza A, Phillips OL, Rowland L, Salinas N, Vasquez R, Mencuccini M, Galbraith D (2022) Variation of non-structural carbohydrates across the fast–slow continuum in Amazon Forest canopy trees. Funct Ecol 36:341–355. https://doi.org/10.1111/1365-2435.13971

Slette IJ, Post AK, Awad M, Even T, Punzalan A, Williams S, Smith MD, Knapp AK (2019) How ecologists define drought, and why we should do better. Glob Change Biol 25:3193–3200. https://doi.org/10.1111/gcb.14747

Spiecker H (2003) Silvicultural management in maintaining biodiversity and resistance of forests in Europe—temperate zone. J Environ Manag 67:55–65. https://doi.org/10.1016/S0301-4797(02)00188-3

Stamp N (2004) Can the growth-differentiation balance hypothesis be tested rigorously? Oikos 107:439–448. https://doi.org/10.1111/j.0030-1299.2004.12039.x

Schmitz RF (1976). Status of larch casebearer research in the Northern Rocky Mountains [USA]. In Proceedings of the Western Forestry Conference Western Forestry and Conservation Association.

Shrestha KB, Hofgaard A, Vandvik V (2015) Tree-growth response to climatic variability in two climatically contrasting treeline ecotone areas, central Himalaya, Nepal. Can J For Res 45:1643–1653. https://doi.org/10.1139/cjfr-2015-0089

Stearns SC (1989) Trade-Offs in Life-History Evolution. Funct Ecol 3:259. https://doi.org/10.2307/2389364

Sturrock RN, Frankel SJ, Brown AV, Hennon PE, Kliejunas JT, Lewis KJ, Worrall JJ, Woods AJ (2011) Climate change and forest diseases. Plant Pathol 60:133–149. https://doi.org/10.1111/j.1365-3059.2010.02406.x

Subrahmanyam VP, Incidence and spread of continental drought, WMO/IHD Report No. 2, Geneva, 1967.

Sylvestre-Guinot G, Pâques LE, Delatour C (1999) Résistance du mélèze hybride inoculé par *Lachnellula willkommii*. Ann For Sci 56:485–492. https://doi.org/10.1051/forest:19990605

Tao W, He J, Smith NG, Yang H, Liu J, Chen L, Tao J, Luo W (2024) Tree growth rate-mediated trade-off between drought resistance and recovery in the Northern Hemisphere. Proc R Soc B 291:20241427. https://doi.org/10.1098/rspb.2024.1427

Taylor K (1999) New evidence shows that earth's climate can change dramatically in only a decade. Could greenhouse gases flip that switch? *American Scientist 87*(4), 320-327.

Tixier A, Herbette S, Jansen S, Capron M, Tordjeman P, Cochard H, Badel E (2014) Modelling the mechanical behaviour of pit membranes in bordered pits with respect to cavitation resistance in angiosperms. Ann Bot 114:325–334. https://doi.org/10.1093/aob/mcu109

Tollenaar M, Lee EA (2002) Yield potential, yield stability and stress tolerance in maize. Field Crops Research 75:161–169. https://doi.org/10.1016/S0378-4290(02)00024-2

Trugman AT, Anderegg LDL, Anderegg WRL, Das AJ, Stephenson NL (2021) Why is Tree Drought Mortality so Hard to Predict? Trends Ecol Evol 36:520–532. https://doi.org/10.1016/j.tree.2021.02.001

Valladares F, Sanchez-Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J Ecol 94:1103–1116. https://doi.org/10.1111/j.1365-2745.2006.01176.x

Violle C, Navas M-L, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E (2007) Let the concept of trait be functional! Oikos 116:882–892. https://doi.org/10.1111/j.0030-1299.2007.15559.x

Villellas J, García MB (2018) Life-history trade-offs vary with resource availability across the geographic range of a widespread plant. Plant Biol J 20:483–489. https://doi.org/10.1111/plb.12682

Vollenweider P, Menard T, Arend M, Kuster TM, Günthardt-Goerg MS (2016) Structural changes associated with drought stress symptoms in foliage of Central European oaks. Trees 30:883–900. https://doi.org/10.1007/s00468-015-1329-6

Wagner S, Liepelt S, Gerber S, Petit RJ (2015) Within-Range Translocations and Their Consequences in European Larch. PLoS ONE 10:e0127516. https://doi.org/10.1371/journal.pone.0127516

Wang A-Y, Lu Y-J, Cui H-X, Liu S-S, Li S-Q, Hao G-Y (2024) Xylem Hydraulics of Two Temperate Tree Species with Contrasting Growth Rates. Plants 13:3575. https://doi.org/10.3390/plants13243575

White TL (1987) A conceptual framework for tree improvement programs. New For 1:325–342. https://doi.org/10.1007/BF00031742

Wilhite DA, Glantz MH (1985) Understanding the Drought Phenomenon: The Role of Definitions. Water Int, 10(3):111-120.

Wilson PJ, Thompson K, Hodgson JG (1999) Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. New Phytol 143:155–162. https://doi.org/10.1046/j.1469-8137.1999.00427.x

Wold S, Sjöström M, Eriksson L (2001) PLS-regression: a basic tool of chemometrics. Chemom Intell Lab Sys 58:109–130. https://doi.org/10.1016/S0169-7439(01)00155-1

Wolfe BT, Sperry JS, Kursar TA (2016) Does leaf shedding protect stems from cavitation during seasonal droughts? A test of the hydraulic fuse hypothesis. New Phytol 212:1007–1018. https://doi.org/10.1111/nph.14087

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. Nature 428:821–827. https://doi.org/10.1038/nature02403

Wright SJ, Kitajima K, Kraft NJB, Reich PB, Wright IJ, Bunker DE, Condit R, Dalling JW, Davies SJ, Engelbrecht BMJ, Harms KE, Hubbell SP, Marks CO, Ruiz-Jaen MC, Salvador CM, Zanne AE (2010) Functional traits and the growth–mortality trade-off in tropical trees. Ecol 91(12), 3664-3674.

Xiong W, Reynolds M, Xu Y (2022) Climate change challenges plant breeding. Curr Opin Plant Biol 70:102308. https://doi.org/10.1016/j.pbi.2022.102308

York LM (2019) Functional phenomics: an emerging field integrating high-throughput phenotyping, physiology, and bioinformatics. J Exp Bot 70:379–386. https://doi.org/10.1093/jxb/ery379

Zhai L, Lee J-E (2024) Investigating Vulnerability, Adaptation, and Resilience: A Comprehensive Review within the Context of Climate Change. Atmosphere 15:474. https://doi.org/10.3390/atmos15040474

Zuo J, Pullen S, Palmer J, Bennetts H, Chileshe N, Ma T (2015) Impacts of heat waves and corresponding measures: a review. J Clean Prod 92:1–12. https://doi.org/10.1016/j.jclepro.2014.12.078

Naincy SAGAR

Trade-off de l'hétérosis pour la croissance chez le mélèze : de la vigueur, mais à quel prix ?

Le changement climatique oblige les améliorateurs forestiers à sélectionner des génotypes d'arbres capables non seulement de bien croître mais aussi de conserver leur capacité adaptative face aux stress environnementaux croissants. Certains écologues privilégient les espèces à croissance lente adoptant une stratégie conservatrice. L'hybridation interspécifique, souvent utilisée en sélection, permet un rendement élevé grâce à des génotypes à croissance rapide et à courtes révolutions, avantages générés chez les hybrides par la complémentarité des caractères et l'hétérosis. Cette thèse se situe au croisement de ces deux visions, combinant le potentiel de l'hybridation interspécifique avec la nécessité d'intégrer des caractères adaptatifs pour faire face aux défis climatiques futurs. Nous avons exploité un dispositif clonal installé à Orléans depuis 2013. Il combine des génotypes de trois espèces de mélèze : le Mélèze d'Europe (*Larix decidua* Mill.), du Japon (*Larix kaempferi* (Lamb.) Car.) et leurs hybrides (*Larix eurolepis* A. Henry), issus d'un plan de croisement intra/interspécifique (diallèle). Ce matériel génétique a été planté sur deux parcelles contrastées pour leur disponibilité en eau du sol (irriguée et non-irriguée). Le premier chapitre étudie la dynamique de mortalité pendant 10 ans : le Mélèze du Japon s'est montré le plus affecté, le Mélèze d'Europe le plus résistant, et l'hybride intermédiaire. Les températures maximales et le déficit de pression de vapeur fin de printemps/début d'été se sont révélés comme des facteurs critiques, en plus des déficits hydriques estivaux. La mortalité est apparue étroitement liée à la croissance radiale annuelle, avec une croissance réduite l'année précédant la mort. La vigueur clonale, quel que soit le taxon, est positivement associée à la survie. Pour approfondir nos connaissances sur la réponse des arbres face aux défis environnementaux, il est nécessaire d'évaluer un large éventail de caractères, notamment ceux liés à l'hydraulique des arbres. Néanmoins, ces mesures sont souvent longues, coûteuses et exigeantes en main-d'œuvre. Dans le Chapitre II, nous développons des modèles prédictifs via la spectroscopie proche infrarouge (NIRS) pour des caractères clés : contenus en N, C, lignine, H/G, et $\Delta^{13}C$ (efficience d'utilisation de l'eau, WUE). En s'appuyant sur les acquis environnementaux du Chapitre I et les avancées méthodologiques du Chapitre II, le dernier chapitre étudie la variabilité de croissance entre les trois espèces de mélèze et le lien potentiel avec des caractères adaptatifs. L'hybride surpasse souvent les espèces parentes en hauteur et en volume, avec un hétérosis accentué en conditions de sécheresse. Il montre une forte réactivité à la disponibilité en eau du sol, contrairement au Mélèze d'Europe, le moins plastique. Le Mélèze du Japon apparaît comme le plus vulnérable à la cavitation (P_{50}), l'hybride étant intermédiaire mais proche du Japonais pour certaines combinaisons, et l'Europe le plus résistant. WUE est plus élevé chez le Japonais et l'hybride comparé à l'Europe. Une forte vigueur ne s'est pas révélée systématiquement pénalisante, étant souvent associée positivement à WUE et à la récupération de croissance après sécheresse. Cependant certains compromis défavorables, notamment pour P_{50}, sont observés chez les hybrides. P_{50} et les caractères de résilience montrent une variabilité suffisante et des corrélations modérées avec la croissance, soutenant leur intérêt pour la sélection de génotypes combinant croissance et adaptation. A l'inverse, WUE, moins variable, offre un potentiel prédictif moindre. Le mélèze hybride présente ainsi un fort potentiel en combinant productivité élevée et capacité adaptative intermédiaire à bonne. Cette étude devrait être poursuivie en intégrant davantage de caractères structurels, phénologiques et physiologiques et en étudiant leurs inter-relations pour approfondir nos connaissances sur le développement de la supériorité hybride.

Mots clés : vigueur, adaptation, hétérosis, hybride, trade-off, séchresse

Trade-off of growth heterosis in Larch: Vigor at what cost?

Climate change challenges tree breeders to select tree genotypes that not only grow well but also maintain adaptive capacity under increasing environmental stresses. Ecologists with different schools of thought emphasize the use of slow-growing species with a conservative strategy. Interspecific hybridization, often favored for breeding, promotes high yield through fast-growing, short-rotation genotypes, further enhanced in hybrids by heterosis and trait complementation. This thesis sits at the center of these two visions, combining the promising tool of interspecific hybridization with the broader need to integrate adaptive traits for future climate challenges. We took advantage of a decade-long clonal experimental trial in Orléans combining genetic material of the three species of Larch (European (*Larix decidua* Mill.) and Japanese Larch (*Larix kaempferi* (Lamb.) Car.), and their hybrids (*Larix eurolepis* A. Henry), sampled from an intra-/inter-specific diallel mating design. The same genetic material (clones) was planted in two plots contrasted for their soil water availability (irrigated and non-irrigated). Our objective was to study the differential mortality and growth responses of the three taxa to drought and the possible trade-offs of growth with some adaptive traits. The first chapter explored mortality dynamics and found that Japanese Larch was the most affected, European Larch the least, and Hybrid Larch remained intermediate. Maximum temperature and VPD during late spring-early summer were the most critical parameters and time period, besides summer soil water shortages. Mortality was closely associated with growth at individual ring and clonal levels, but in different ways. Annual radial growth in the year preceding death was consistently lower in trees that eventually died. Importantly, we showed that clonal vigor was, whatever the taxa, positively associated with survival. Building on these findings, addressing environmental challenges requires assessing a broader range of traits, particularly those related to tree hydraulics. However, such evaluations are often time-consuming, costly, and labor-intensive. In Chapter II, we developed good to moderately reliable prediction models for key traits using a high-throughput phenotyping approach through NIRS. These include N, C and lignin contents, H/G ratio and and $\Delta^{13}C$ (water-use efficiency, WUE). Building on the environmental insights from Chapter I and the methodological advances from Chapter II, the last Chapter focused on the variability of expression of growth among the three *Larix* taxa, and possibly how adaptive traits could drive performance. Hybrid larch often outperformed growth of both parental species, with stronger relative heterosis observed under drought conditions. It was very sensitive to soil water availability in contrast to European larch, the least plastic species. Japanese larch was the most vulnerable to cavitation (P_{50}), hybrid larch was on average intermediate but with values close to Japanese larch for some combinations, and European larch was the most resistant. WUE was higher in Japanese and hybrid larches compared to European larch. Fast growth did not necessarily come at a cost, as it was generally positively associated with WUE and growth recovery after drought; however, some unfavorable trade-offs particularly for P_{50}, were observed in hybrids. P_{50} and resilience traits exhibited sufficient variability and moderate correlations with growth, supporting their value as predictive traits for selecting genotypes with growth and adaptation, whereas WUE showed limited variability and thus offered less predictive potential. Hybrid larch offers promising growth advantages by combining high productivity with intermediate to good adaptive capacities. This study should be continued through integration of more structural, phenological and physiological traits and the study of their interrelationships to get a more insight in heterosis development.

Keywords: vigor, adaptation, heterosis, hybrid, trade-off, drought

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