Universidad de Valladolid

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Máster en Ingeniería de Montes

Determinación de los costes de construcción de la corteza, carácter de adaptación al fuego, en una especie de pino mediterráneo

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Determination of bark production costs, a fire-adaptive trait, in a Mediterranean pine species

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Abstract

Bark thickness is a fire-adaptive trait in plants, influenced by environmental factors. In this study, we use *Pinus halepensis* to study the production costs of bark, because even though this species is an obligate seeder, recent studies showed that some populations could survive certain fire regimens. In dry continental climates, *P. halepensis* populations require more time and resources to develop the minimum basal bark thickness necessary for survival under moderately intense fires. However, these populations do not reach a critical bark thickness at breast height. This can increase the immaturity risk, dead by fire before establishing an aerial seed bank capable of ensuring recruitment. Understanding ecotypic patterns, phenotypic plasticity, and relative resource allocation to bark requires assessing the construction costs of bark and wood, which have not been previously studied in trees. To address this, we collected bark and wood samples at breast height and the tree base by a destructive analysis. The samples underwent carbon-nitrogen (C/N) ratio analysis and ash/nitrate determination, as carbon concentration is a reliable indicator of construction costs. Our results showed that, as expected, bark construction costs were higher than wood. The construction costs of bark were also significantly higher than wood at the tree base. Moreover, we observed significant differences among populations between the costs of wood and bark, and in some cases, between breast height and the tree base. These findings highlight the importance of studying resource allocation to key adaptive traits such as bark thickness. Furthermore, our results confirm the importance of bark thickness in *P. halepensis* so implementing silvicultural treatments to reduce immaturity risk under changing climatic conditions is essential for the conservation and management of its populations, ensuring their persistence.

Keywords: bark thickness, fire adaptations, life-history theory, *Pinus halepensis*, resources allocation, wood.

Resumen ampliado

La Teoría de Historia Vital (LHT) es un concepto fundamental en ecología evolutiva que explica cómo la selección natural modela las adaptaciones de los organismos para optimizar la asignación de energía y recursos entre funciones vitales (supervivencia y mantenimiento, crecimiento y reproducción). Dado que los recursos son limitados, surgen compensaciones en su asignación, lo que influye en los rasgos relacionados con la aptitud biológica. Estas compensaciones, especialmente entre crecimiento y reproducción, desempeñan un papel clave en la evolución de las estrategias de historia vital.

En las plantas, la asignación de recursos implica la distribución de carbono, nitrógeno y otros nutrientes entre distintas estructuras con funciones fisiológicas específicas, lo que impacta significativamente en su crecimiento y desempeño ecológico. Los costes de construcción se definen como la cantidad de glucosa necesaria para la creación de biomasa, varían entre especies y tejidos debido a las diferencias que presentan en su composición química y las rutas biosintéticas que utilizan. Para estimar estos costes se emplean diversas metodologías, siendo el análisis de carbono y nitrógeno la más utilizada por su sencillez.

La corteza representa entre el 9-15% del volumen de un tronco y el 13-21% de su peso seco. Su morfología es muy diversa y cumple funciones esenciales en los árboles. Se divide en dos componentes principales: la corteza interna (corteza viva) que transporta y almacena fotosintatos, y la corteza externa (corteza muerta), compuesta por células muertas que protegen al árbol de incendios, patógenos, herbívoros y condiciones climáticas adversas, además de reducir la pérdida de agua y proporcionar soporte estructural. En ecosistemas secos, la corteza interna tiende a ser más gruesa debido a su capacidad de almacenamiento de agua. A pesar de su importancia ecológica y su diversidad en anatomía, química y textura, la corteza ha sido poco estudiada en comparación con otras estructuras vegetales como las hojas.

En los últimos años, estos diferentes rasgos de la corteza han adquirido relevancia en estudios ecológicos, especialmente en relación con su espesor, ya que desempeña un papel clave en la adaptación, la protección y el aislamiento contra el fuego. Esta hipótesis relacionada con el fuego sugiere que una corteza más gruesa protege mejor el cambium en entornos propensos a incendios; sin embargo, otras hipótesis, como las relacionadas con la estabilidad biomecánica o la adaptación al clima, también podrían explicar la variabilidad en el espesor de la corteza. Comprender la variación en este carácter vital y su variabilidad es esencial para predecir la respuesta de las plantas a los cambios ambientales y mejorar las estrategias de conservación.

Numerosos estudios evolutivos han analizado el papel protector del espesor de corteza, especialmente en ecosistemas tropicales y en especies adaptadas al fuego con estrategias de supervivencia en la etapa adulta. Sin embargo, pocos trabajos han explorado la variación intraespecífica y la plasticidad en el espesor de la corteza, y aún menos han investigado los costes de construcción en términos de asignación de recursos, ni la comparación de esos costes entre la corteza y la madera. Ambos tejidos poseen diferentes propiedades físicas, químicas y biológicas, y tienen diferentes funciones. La corteza contiene reservas más concentradas y compuestos de alto coste energético, lo que sugiere que su producción podría ser más costosa que la de la madera. Estos altos costes implican compensaciones evolutivas (trade-offs) entre los beneficios de una corteza más gruesa y sus exigencias energéticas (cantidad de recursos asignados). Por ello, comprender esos costes es clave para determinar patrones de asignación de recursos. Su estimación puede realizarse mediante análisis de la relación carbono/nitrógeno y determinación de cenizas o nitratos, dado que la concentración de carbono es un buen indicador de los costes de construcción.

En este estudio hemos utilizado muestras de *Pinus halepensis* (pino carrasco) por su relevancia ecológica y su valor como modelo para estudiar la adaptación local y los rasgos de adaptación al fuego. Originario de Siria, pero ampliamente distribuido en el Mediterráneo occidental, su expansión hacia el oeste ha ocasionado la reducción de la diversidad genética, especialmente en poblaciones ibéricas. Su distribución está condicionada por su baja resistencia al frío y su alta tolerancia a la sequía, lo que le permite colonizar rápidamente suelos áridos. Es una especie pionera con un sistema radicular altamente ramificado y una capacidad de crecimiento rápida en ambientes semiáridos, lo que convierte a esta especie en una buena opción para la restauración ecológica y la reforestación. Además, su ciclo de vida, con una edad temprana de reproducción y la presencia de serotinia (carácter de adaptación al fuego), facilita el estudio retrospectivo de su reproducción (ya que las piñas, aunque estén abiertas, se quedan en el árbol gracias a su grueso pedúnculo). Esto hace de esta especie un modelo ideal para analizar estrategias adaptativas y cambios evolutivos frente a condiciones ambientales adversas, como los incendios.

Esta especie está considerada como una sembradora obligada, “obligate-seeder” en inglés, ya que el árbol adulto no suele sobrevivir al paso del fuego, pero se produce regeneración a partir de semillas provenientes de sus piñas serótinas en situaciones post-incendio, permitiendo así la persistencia de las poblaciones. Su nivel de serotinia es intermedio y variable, con conos que pueden abrirse en ausencia de fuego debido a la escasez de recursos, principalmente, escasez de agua, lo que podría poner en riesgo la supervivencia de las poblaciones de esta especie en un contexto de cambio climático. Debido a esta estrategia, *P. halepnesis* invierte pocos recursos en corteza para la supervivencia adulta, aunque estudios recientes han demostrado que algunas poblaciones pueden resistir incendios superficiales de baja o moderada intensidad gracias a una corteza basal más gruesa, que disminuye a lo largo del tronco. La corteza basal es clave para evitar la muerte por anillamiento en incendios superficiales y moderadamente intensos, antes de conseguir un banco aéreo de semillas suficiente para la persistencia de la población. Se ha encontrado una fuerte diferenciación genética en el espesor de corteza entre poblaciones de esta especie, con patrones ecotípicos claros a la altura del pecho, pero no en la base del árbol, lo que sugiere una selección estabilizadora en la base para garantizar un espesor mínimo que permita la persistencia de la población. Aunque el espesor de la corteza parece más importante de lo esperado para la supervivencia en *P. halepensis,* aún se desconocen aspectos fundamentales como la morfología de la corteza, su estructura interna y la tasa de reducción del espesor a lo largo del tronco, elementos clave para la adaptación y resistencia de la especie al fuego.

Los costes de construcción de la biomasa vegetal, es decir, la cantidad de glucosa necesaria para producir un gramo de biomasa, muestran poca variación entre especies y no parecen verse afectados por las condiciones ambientales. Aunque las hojas de especies herbáceas suelen tener costos ligeramente más altos que tallos o raíces, la mayoría de los estudios se han centrado en hojas, semillas y frutos, dejando otros tejidos como la corteza poco explorados. Este estudio se centra en los costes de construcción de la corteza y la madera. La asignación de recursos es clave en la ecología de las plantas y comprender los costes de construcción de la corteza y su comparación con los de la madera es fundamental para analizar patrones ecotípicos, plasticidad fenotípica y estrategias de distribución de recursos dentro y entre especies.

Este estudio se desarrolla en el contexto de la ecología evolutiva y su objetivo principal es la determinación de los costes de construcción de la corteza frente a los de madera en poblaciones de *P. halepensis*, para entender los patrones ecotípicos y la plasticidad fenotípica del espesor de corteza en esta especie, además de los patrones de asignación de recursos entre poblaciones. Con este fin se han estudiado poblaciones a lo largo de toda la distribución natural de la especie, considerando dos alturas para cada tipo de muestra (corteza y madera): la base del árbol y la altura del pecho. La finalidad de este trabajo es la obtención de información útil en el ámbito de la

gestión forestal sostenible, facilitando la selección de las regiones de procedencias y/o poblaciones más apropiadas y mejor adaptadas a cada escenario teniendo en cuenta el escenario de cambio climático en el que nos encontramos.

Las muestras que se han utilizado proceden de una parcela de la red de ensayos en ambiente común de la red nacional de ensayos genéticos forestales (GENFORED). Además, se ha desarrollado un protocolo de laboratorio a partir de técnicas ya existentes adaptándolas a los tipos de muestras que se hemos utilizado por falta de un procedimiento previo específico. Este protocolo consta de dos partes principales, la determinación del contenido en cenizas y su alcalinidad a través de calcinación y valoración por retroceso de NaOH, y la extracción por incubación de las muestras y determinación del contenido en nitratos con colorimetría de salicilato. Además de determinar el contenido total de C y N por combustión en seco en autoanalizador.

De acuerdo con los datos obtenidos podemos concluir que existen diferencias entre los costes de construcción de la madera y de la corteza a nivel de especie, siendo mayores en la corteza.

Hasta donde sabemos, este trabajo es el primero que determina los costes de construcción en la madera y la corteza de *Pinus halepensis*, por lo que puede aportar información muy útil para el manejo y conservación de sus masas y para posibles proyectos de mejora genética o migración asistida con esta especie.

Palabras clave: asignación de recursos, caracteres de adaptación, espesor de corteza, madera, *Pinus halepensis*, teoría de historia vital

1. Introduction

1.1. Climate change and new fire regimes

Climate change is modifying the behavior of species as well as the dynamic of the Earth's climate. Climatic extreme events like heavy rains, longer, more frequent and severe droughts, and the new fire regimes are more common every year. The changing pattern of bioclimatic conditions may unevenly alter the habitat suitability of different forest tree species with a potential further diversification impacts among plant communities and forest types (De Rigo et al., 2017).

Fire is a global ecosystem process which role in shaping the distribution of fauna and flora is widely underappreciated (Keeley et al., 2011). Over half of the Earth's land surface is considered to be fire-prone, with perhaps a third of the land mass experiencing frequent intensive burning (Chuvieco et al., 2008).

Historical records show that Mediterranean ecosystems have been always modified by fire, meaning that they are highly fire-prone environments, and thus, some species in theses habitats have developed strategies to survive fires. A key feature of Mediterranean-type climate regions is that precipitation exceeds potential evapotranspiration during the rainy season. This results in sufficient plant growth to produce contiguous fuel loads that are highly flammable during the summer drought (the most usual climatic factor in the Mediterranean climate). Consequently, these climate regions are highly flammable and prone to high-intensity fires on an annual basis (Keeley et al., 2011). However, since climatic conditions are changing so are fire regimens, affecting the development and survival of different plant species. Any plant species is 'fire adapted' but rather is adapted to a particular fire regime, in which, among other things, fire frequency, fire intensity and fuel consumption patterns should be considered (Keeley et al., 2011). Plants exhibit adaptive responses to the direct effects of fire and there may be specific selection for physiological or life history strategies in the post-fire environment (Schwilk & Ackerly, 2001). Not every species is adapted to every fire regimen, so they show specific fire-adaptive traits that are key to providing resilience under those particular fire regimes. Some typical fire adaptations in tree species are resprouting, serotiny, heat-shock triggered germination, the absence of self-pruning of dead branches, early female flowering or thick bark (Ne'eman et al., 2004; Schwilk & Ackerly, 2001).

Fire regimes play a significant role in shaping the genetic composition of a plant species over time by interacting with its life history traits, particularly those related to adaptations (Budde et al., 2017). Frequent fires can create population bottlenecks by killing individuals before the canopy seed bank is replenished, reducing both effective population size and genetic diversity (Whelan, 1995). Additionally, fire regimes act as selective pressures on adaptive traits, leaving detectable signatures on specific genes that underpin these traits (Parchman et al., 2012). Fire-mediated selection can also influence seed traits or dispersal mechanisms, altering the spatial distribution of related individuals or gene copies within a population (Saracino et al., 1997). Furthermore, local site conditions—such as shrub cover, aspect, slope, soil depth, ectomycorrhizal distribution, and the accumulation of dead branches—affect seedling establishment and survival after fire events (Pausas et al., 2004b; Buscardo et al., 2011). Together, these factors illustrate the complex interplay between fire and genetic dynamics in plant populations.

Empirical population genetic studies suggest that in seeder species, soil or canopy seed banks generally preserve genetic diversity within populations and provide a buffer against demographic fluctuations (Templeton and Levin, 1979; Barrett et al., 2005; Ayre et al., 2009; Budde et al., 2017). However, this buffering capacity is compromised if fire frequency is too high for the seed bank to recover between fire events (Bradstock et al., 1996). For example, *Pinus halepensis*, a typical obligate-seeder species, has been shown to maintain similar levels of genetic diversity in both unburnt stands and those that regenerated after a fire (Schiller et al., 1997).

While these empirical studies explored the population genetic effects of one or a few fire events, they did not specifically investigate the evolutionary responses of populations to frequent fires, highlighting the need to compare populations exposed to varying fire regimes to understand the evolutionary impact on genetic diversity and spatial structure (Budde et al., 2017).

Global change drivers, such as increased ignition events, land-use changes, global warming, are altering fire regimes—particularly by shortening the intervals between high-intensity crown fires—beyond the historical variability observed at local scales (Enright et al., 2015; Turner et al., 2019; Pausas and Keeley, 2021; Guiot & Pausas, 2023).

1.1. Life-History theory

Life History theory (LHT) (Stearns, 1989) is an important part of evolutionary ecology and biology. Evolutionary ecology focuses on the putative problems of species' adaptations, studying also natural selection and other genetic and stochastic evolutionary forces affecting populations and species (Krebs, 1972). Adaptive traits are those that provide a fitness advantage in a given environment (Keeley et al., 2011).

LHT studies the historical reasons why natural selection has favoured the adaptations that organisms now show (Krebs, 1972). LHT refers to the series of fitness-related events in the life of an organism that are modelled by natural selection to produce the maximum number of viable offspring (Roff, 1993). All organisms must optimize the energy and resources used for each vital function, i.e. maintenance, growth and reproduction, when facing different environmental limitations (biotic and abiotic). Since resources are finite, their use in a function means they will not be available for other functions.

Allocation is the central concept in LHT because resources allocated to one function are not available to other functions and this implies evolutionary trade- offs (Martín, 2018a; Mooney, 1972; Grace, 1997). Therefore, LHT predicts that the distribution of resources among vital functions may be subject to physiological and evolutionary trade-offs regulated by the balance between the costs and benefits in terms of fitness of each of these functions (Koricheva et al., 2004). For example, is very well known the existing trade-off between growth and reproduction in plants (Stearns, 1982; McConnaughay & Coleman, 1999; Weiner, 2004; Climent et. al, 2008; Santos-del-Blanco et. al, 2010). Trade-offs have played a central role in the development of life-history theory (Stearns, 1989). If there were no trade-offs, then selection would drive all traits correlated with fitness to limits imposed by history and design (Stearns, 1989). Life history theory postulates the existence of trade-offs between fitness functions that are expressed as costs in survival, growth and reproduction (Climent et al., 2024; Stearns & Stearns, 1998). These trade-offs represent the costs paid in the currency of fitness when a beneficial change in one trait is linked to a detrimental change in another (Stearns, 1989). Therefore, minimising or balancing such costs and benefits against each other are key issues behind the evolution of life history strategies. Trade-offs can be defined, and traits evolution can be predicted either by a genetic model or by assuming that selection maximizes some measurable metric that defines fitness (Roff, 1993). The importance of adaptive traits and their study relies on the possibility of adaptation to new climatic scenarios. The comparison of the genetic architecture of traits closely linked to fitness with that of other traits is scientifically challenging, since it deals with the interplay between past directional selection (reducing within-population variation and promoting differentiation between populations) and the availability of enough additive genetic variation that enables future evolutionary processes (Martín Sanz, 2018a; Merilä & Sheldon, 1999). There is clear evidence that the variation in several traits has an allometric component (i.e., the covariation of a given trait with body size) across different plant species, although the direction of this trend may vary among genera (Cowling and Lamont, 1985; Thanos and Daskalakou, 2000; Tonnabel et al., 2012; Santos-del-Blanco et al., 2013; Martín-Sanz et al., 2016, 2019, among others). Resource allocation in plants shifts along ontogenetic trajectories, making it crucial to distinguish environmental effects from purely developmental differences when studying plasticity in allocation (Poorter and Nagel, 2000; Wright and McConnaughay, 2002; Weiner, 2004). Although accounting for ontogenetic changes in complex organisms such as adult trees remain challenging, the concepts and theoretical framework of allometry provide the most robust tools currently available for this purpose.

Resource allocation in plants refers to the partitioning of essential elements such as carbon, nitrogen, and other nutrients among various structures that perform distinct physiological functions, including photosynthesis, nutrient acquisition, and reproduction (Bloom et al., 1985; Lovett-Doust, 1989). The pattern of resource distribution among different organs and metabolic processes significantly influences a plant's overall growth and performance within a specific ecological niche (Williams et al., 1987). One methodological approach to elucidate resource allocation patterns involves quantifying the energetic requirements for the construction and maintenance of specific tissues (Spencer et al., 1997). The concept of construction cost has been operationally defined as the quantity of glucose necessary to provide carbon skeletons, reducing power, and adenosine 5'-triphosphate (ATP) for the biosynthesis of organic compounds (Williams et al., 1987). These quantitative estimates establish a crucial link between carbon fixation and biomass accumulation (Chiariello et al., 1989; Spencer et al., 1997).

Plant species exhibit substantial heterogeneity in their chemical composition, encompassing both qualitative and quantitative variations (Poorter, 1994). This compositional diversity can be attributed to a combination of phenotypic plasticity in response to environmental factors (Waring et al. 1985, Diamantoglou et al. 1989) and genetic variation among species (e.g., Kramer 1979, Poorter & Bergkotte 1992).

The biosynthetic pathways responsible for producing the diverse array of chemical constituents in plants demonstrate considerable variability in their energetic requirements. Specifically, the demand for adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NAD(P)H), which serve as the primary energy currency and reducing power in cellular metabolism, respectively, can differ significantly among these pathways (Poorter, 1994). Furthermore, the carbon requirements for constructing the molecular skeletons of various compounds also exhibit notable variation. Consequently, the construction costs—defined as the total glucose input necessary to synthesize one gram of plant biomass—are subject to substantial variation among plant species and tissues (Poorter, 1994). This variation in construction costs reflects the underlying differences in biosynthetic pathways, energy requirements, and carbon allocation patterns among different plant species and organs. The balance between ATP and NADPH supply from photosynthesis and the demand from various metabolic pathways is crucial for efficient plant metabolism. Understanding these variations in chemical composition and construction costs is essential for elucidating plant adaptation strategies, resource allocation patterns, and the evolution of plant development across different species and environments (Poorter, 1994).

Multiple methodologies exist for quantifying the construction costs of plant tissues. The optimal approach would involve a comprehensive analysis of all chemical constituents within a plant and their respective biosynthetic pathways. However, this level of detail is extremely time-consuming, and researchers typically resort to estimating costs for broad categories of compounds (Poorter, 1994).

Penning de Vries et al. (1974) employed the estimation of costs for various classes of compounds to calculate the construction costs of a maize plant. They derived costs for various compound classes, including lipids, lignin, organic N-compounds, (hemi)cellulose/starch/sugars, organic acids, and minerals, based on the most probable biosynthetic pathways (Poorter, 1994). An alternative method, proposed by Williams et al. (1987), estimates construction costs by measuring nitrogen concentration, heat of combustion, and ash content of plant material (Poorter, 1994). Vertregt & Penning de Vries (1987) suggested a further simplified approach. Recognizing the correlation between the reduction state of different compounds and their carbon content, they posited that determining ash and carbon content of plant material provides a satisfactory estimate of construction costs. They demonstrated this method's efficacy using a range of fruits and seeds, which exhibit substantial variation in lipid content—the constituent with the highest carbon concentration and construction costs (Poorter, 1994).

According to recent studies, bark traits are considered as key functional traits in structuring communities and biomes (Pausas, 2015). Bark thickness is the most studied bark property so far, due to its correlation with cambial insulation and protection, especially against fires (Hoffmann et al., 2012). One of the crucial roles of bark is protection of the stem, along with many others, such as storage or insulation. Recently the correlation between bark thickness and fire regime (fire hypothesis) has attracted increasing interest by plant scientists (Pausas, 2015, 2017; Rosell, 2016). Thick bark is usually related to fire tolerator species that are characterised for adult survival as vital strategy, like *Pinus pinaster, P. ponderosa* or *P. palustris* among others (Jackson et al., 1999; Keeley & Zedler, 2000).

Researchers have proposed several hypotheses to explain the development of thick bark (Pausas, 2015). The biotic hypothesis in which bark protects the stem against harmful organisms (pathogens, pests or herbivores). The climate hypothesis where bark is the protective barrier against extreme climates. The biomechanical hypothesis shows bark as a mechanism to increase mechanical stability. However, there is not enough evidence to justify the variation in bark thickness produced by selective forces related to these three hypotheses. However, the fire hypothesis does not explain completely this bark variability either. Meaningfully, not all ecosystem types are burned naturally, and those that are fire-prone, usually burn more often than necessary (compared to plants' life cycle), being highly relevant at an evolutionary scale. Therefore, bark is an important trait in plants that plays a key role as insulation against fire (Martín-Sanz, 2018b).

1.2. Bark thickness as a fire-adaptive trait

Bark, which is defined as all the tissues external to and surrounding the vascular cambium, comprises about 9–15% of a typical log by volume or 13–21% on a dry weight basis (Harkin, 1971). Bark is a very morphologically diverse and functionally important part of stems (Romero, 2014), ranging from smooth to rough, scaly, spiny, furrowed, and tessellated (Rosell & Olson, 2014). Behind the apparent simplicity of this concept, there is a complex of tissues located outside the vascular cambium that includes live and dead cells (Pausas, 2017). As the outer layer of trees, the bark is made up for two main components with their own functions: phloem, or living inner bark, responsible for the transport and storage of photosynthates, and the dead outer bark that protects the tree from fires, pathogens and herbivores, reduces water loss and provides structural support (Evert, 2006; Romero, 2014; Hoffmann et al., 2012; Schafer et al., 2015; Figure 1). The inner bark is usually thicker in drier ecosystems possibly due to selection favouring its water storage capacity (Rosell, 2016; Rosell & Olson, 2014). Many of barksp' functions were probably in place from the very origin of bark and contributed to the ability of early woody species to colonize and diversify in a range of environments. The diversity of bark functions is also reflected in its diverse anatomy (Figure 1) and chemistry, as well as in the diversity of colours, textures, smells, densities and thicknesses (Pausas, 2017). Despite its importance and visibility, bark has been little studied from an ecological perspective in comparison with other plant parts such as leaves (Pausas, 2015).

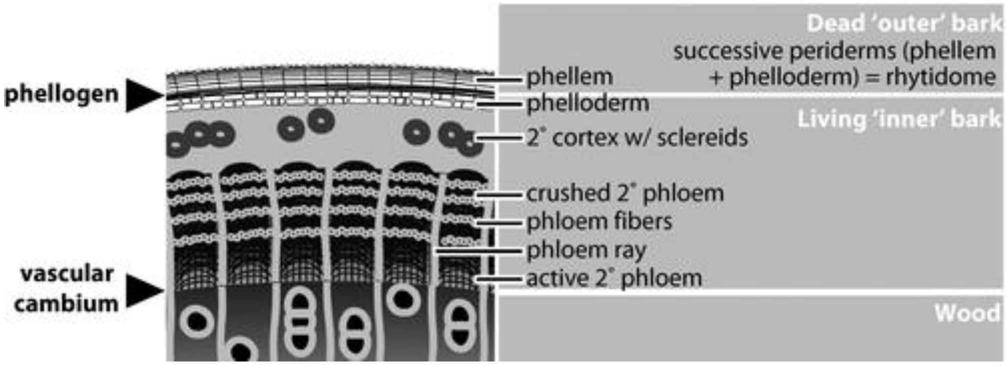

Figure 1. Bark structure in cross-section. From Rosell (2016).

While numerous evolutionary studies have investigated the protective role of bark thickness — most of them focusing on tropical ecosystems and examining a few to many species in natural populations and diverse ecosystems, often emphasizing species whose fire-adaptive strategy is adult survival (Keeley and Zedler, 1998; Paine et al., 2010; Dantas and Pausas, 2013; Poorter et al., 2014; Rosell et al., 2014; Rosell, 2016)— relatively few studies have explored intraspecific variation and plasticity in bark thickness (Climent et al., 2004; Tapias et al., 2004; Stephens and Libby, 2006; Matziris, 2000; Martín-Sanz et al., 2019). Furthermore, to our knowledge, the construction costs of bark thickness in terms of resource allocation have not been examined, nor have the comparative costs between bark and wood.

Bark and wood densities can be very similar in several species (Miles, 2009), but these two tissues have different physical properties, chemical composition and biological functions (Romero, 2014). Wood is mainly formed by lignin and cellulose, but bark lignin is much more complex in structure than wood lignin (Romero, 2014). Bark has higher reserves concentration and contains other large chain compounds that are costly to produce (Barbaroux et al., 2003; Larjavaara & Muller-Landau, 2010), making bark possibly more costly than wood. These presumably high costs suggest that the relative allocation of resources to bark is subject to strong evolutionary trade-offs between the fitness benefits of thicker bark and its associated costs at different ontogenetic stages (Schwilk and Ackerly, 2001). In other words, thick bark would not have evolved in ecosystems where variations in thickness did not confer significant fitness advantages. Consequently, understanding the construction costs of bark, as well as comparing the production costs of bark and wood, is crucial for elucidating patterns of resource allocation, making their determination essential. Estimating these construction costs could involve straightforward methods, such as performing a carbon-to-nitrogen (C/N) ratio analysis followed by ashing or nitrate determination, as carbon concentration is often a reliable indicator of construction costs (Poorter, 1994).

1.3. Study species: *Pinus halepensis*

1.3.1. Distribution and characteristics

In this study, we focused on *Pinus halepensis* Mill. (Aleppo pine), a Mediterranean pine species. This species originates from Syria, but there are populations alongside the western Mediterranean, Spain and the Maghreb countries (Figure 2). The current distribution results from a westward range expansion, which accounts for the reduced genetic diversity observed in both neutral markers and certain quantitative traits, particularly in Iberian populations (Bucci et al., 1998; Tapias et al., 2001; Gómez et al., 2005; Grivet et al., 2009).

Figure 2. Natural distribution of *Pinus halepensis*. From EUFORGEN (2022).

Its distribution is determined by a low resistance to cold (around -12ºC, their needles suffer damages; Calamassi et al., 2001; Climent et al., 2009), and by their drought tolerance. It is considered a pioneer species, which forms an extensive and highly branched root system, with a high capacity for initial soil colonization compared to other Mediterranean forest species (Domínguez-Lerena et al., 2001; Puértolas et al., 2009). It has low tolerance to shade, although it is able to grow well under intermediate shading (Fernández & Martín, 2005).

Pinus halepensis is a medium-sized tree that can grow up to 20-22 m high. The trunk has light grey bark (Figure 3). The crown is irregular and provides little dense shade. In early stages, it has a monopodic branching system with a strong apical dominance and a profusion of lateral branches from the base if it grows without light limitations. With age, or in very adverse seasonal conditions, this apical dominance is gradually lost until the crown type described above is formed. The needles of Aleppo pine are gathered in fascicles of two, are flexible and measure from 6 to 12 cm in length (Figure 3; Castroviejo, 2020; Puértolas et al., 2009).

Figure 3. Aleppo pine morphology and growth: a) female cones of different reproductive cycles, b) mature cones, c) serotinous cone in a young tree showing the thin light grey bark, d) male strobili (i.e. male cones), e) winter buds in a 3-year-old plant at the nursey, f) seeds, g) an example of a provenance trial in Central Spain, h) reforestation with *Pinus halepensis* in Sierra Espuña (Murcia), i) grey thicker bark at the base of the tree. From Puértolas et. al (2015), Salhi et. al (2020) and R. C. Martín-Sanz©.

Due to its high capacity for rapid colonization and its drought tolerance, this species is commonly used for afforestation in semiarid environments. Therefore, is found in extensive planted stands across Mediterranean dry zones (Gil, 1996), targeting ecological restoration, or less frequently, wood production. It is also frequently used in low demanding ornamental plantations under unsuitable conditions for other species (Chambel et al., 2013).

Moreover, *P. halepensis* is an excellent model species for studying past and future local adaptation processes (Lev-Yadun & Sederoff, 2000), as well as for investigating fire adaptive traits. This is due to several characteristics of its life cycle, such as early age of first reproduction (permitting an early measurement of reproductive traits) or the fact that female cones remain attached to the branch after opening and releasing of seeds (facilitating the retrospective record of female reproduction events even from its beginnings; Martín-Sanz, 2018a; Ne'eman et al., 2004; Ne'eman et al., 2011; Tapias et al., 2004).

1.3.2. Fire-adaptive traits: serotiny and bark thickness

Serotiny, i.e. the retention of seeds within close fruits or cones for several years after ripening until an environmental driver causes dehiscence and seeds release, is a common fire-adaptive trait in areas characterized by high diversity and diversification rates, as well as severe threats to natural ecosystems (Myers et al., 2000; Sauquet et al., 2009). Specifically, serotiny is more common in species from the south hemisphere, but also of plants from Mediterranean-climate, characterized by natural crown fire regimes (Keeley et al., 2012). Fire is the most known factor that causes the opening of serotinous fruits or cones (what is more properly called *pyriscence*, Lamont, 1991), but there are also other causes of cone or fruit opening such as death of the supporting plant or branch (see, Lamont, 1991 for all the different seed released causes). Another factor causing the opening of serotinous cones is dry atmospheric conditions, which is called *xeriscence* (Nathan et al., 1999). Serotiny implies higher costs per seed (carbon, water, and canopy growth constraints) in return for potentially decreased risk of seed predation or losses to pathogens and decay (Lamont & Enright, 2000; Martín-Sanz, 2018a) and seed release into an unsuitable environment due to plant senescence could lead to seed mortality (Bond, 1980; Lamont et al., 1991; Martín-Sanz, 2018b).

Pinus halepensis is considered a post-fire obligate-seeder, which does not survive fires but regenerates from seeds in the post-fire environment (Ne'eman et al., 2004). This means that their fire-adaptive traits should lead to population persistence and not adult survival. Therefore, this species shows a variable and intermediate level of serotiny, which has been extensively studied (Tapias et al., 2001; Ne’eman et al., 2004; Hernández-Serrano et al., 2014, Martín-Sanz et al., 2016; 2017). It shows xeriscent cones that eventually open even in the absence of fire after a variable number of years or under particularly dry and warm conditions (Nathan et al., 1999; Martín-Sanz et al. 2017), if fires occur after the cones open because of droughts, the survival of those populations would be in danger. Climate change and more frequent and severe dry periods can be a problem for the adaptation of the species or certain populations. Consequently, with this obligate-seeder strategy, *P. halepensis* is characterized by a low investment in a thick bark allowing adult endurance. However, some previous works reported variable survival of adult trees to low or moderate intensity fires (Ducrey et al., 1996; Trabaud and Valina, 1998; Rigolot, 2004; Fernandes et al., 2008; Martín-Sanz et al. 2019), and there is evidence of wide differences in bark thickness among populations and between breast height and the base of the tree (Martín-Sanz et al. 2019). Actually, these authors showed that some *P. halepensis* populations can survive low or moderately intense fires thank to a thick basal bark, allowing these populations to reach a sufficient aerial seedbank (usually achieved when trees are 15-20 years old; Moreira et al., 2011) under frequent fires.

Basal bark thickness is relevant since trees can die by the girdling of their basal stem in surface fires, even of low intensities (Jones et al., 2004). Nonetheless, several experiments have also shown that trees with less than 85% of basal girdling can survive after fire (Wagener, 1961; Ryan et al., 1988; Ryan, 1993; Ducrey et al., 1996). Bark thickness decreases along the bole of the tree from the base, so studying bark thickness at breast height (where it is usually measured; but see, Harmon, 1984; Stephens and Libby, 2006; and Rosell et al., 2015 for exceptions) or along the bole is also essential, considering the so-called chimney effect on trunks that occurs during fires. Therefore, the results found by Martín-Sanz et al. (2018a) for *P. halepensis* populations are key to understand better the fire-survival-strategy of this species. These authors found that some populations of this species have thicker bark at the tree base, but not at breast height, where all populations show less absolute bark thickness with little variation among them. This is consistent with a greater thermal insulation at the stem base and a steep bark tapering along the bole found in several Mediterranean pine species (Jackson et al., 1999; Martín-Sanz, 2018a) and can be seen as an adaptive solution to lower immaturity risk (Keeley & Zedler, 2000; Lamont et al., 1991). Moreover, bark thickness in *P. halepensis* showed high genetic differentiation among populations. Eastern Europe populations have higher growth and lower reproduction, and show thicker bark, while populations from North Africa and Southern Spain –with lower growth, and precocious and intense reproduction– display thinner absolute bark. Bark in this species also show strong allometric plasticity and high phenotypic plasticity linked to site effects, with environments displaying lower resource availability limiting both the relative allocation to the bark and the absolute bark thickness (Martín-Sanz, 2018a). However, bark thickness in this species did not show population differentiation for plasticity (that is, genotype by environment interaction did not affect this trait), which means that the ranking of populations and the differences of variance among them is extremely similar between environment with great ecological and climatic differences (Martín-Sanz et al. 2018). This could be attributed to multiple non-exclusive factors, such as a strong dependence of bark thickness on vegetative traits, the influence of environmental heterogeneity driving selection for plasticity, which varies among traits with different functions, or intense stabilizing selection acting on the plasticity of this trait across populations. The latter appears to be particularly relevant for basal bark thickness, where the absence of ecotypic patterns may suggest that all individuals, irrespective of population origin, must attain a minimum basal bark thickness to survive surface or moderately intense fires until they establish a sufficient aerial seed bank to ensure population persistence under wildfire conditions. Furthermore, the quantitative differentiation among populations for bark thickness at breast height consistently exceeds the neutral genetic differentiation, indicating a pronounced ecotypic pattern and providing strong evidence of local adaptation (Martín-Sanz et al., 2018). Actually, this trait has been demonstrated to be moderately heritable in *P. halepensis* ($h^2 = 0.15\text{-}0.24$; Matziris, 2000).

Even though bark thickness has a crucial impact on *P. halepensis* survival, the role of bark in plant ecological strategies and the causes of its variation remain poorly understood. The critical bark thickness value for *P. halepensis* survival, the possible differences in bark morphology, its internal structure (separating between inner and outer bark), and the rate of bark thickness tapering along the entire bole are aspects yet to be discovered and might be critical for Aleppo pine persistence (Harmon, 1984).

1.3.3. Resources allocation and construction costs of bark and wood

Plant resource allocation encompasses the distribution of molecular constituents and energy across organs and physiological functions. Through photosynthetic metabolism, plants transform light energy into chemical energy reserves that serve as fundamental building blocks for cellular processes (Madsen, 1991).

Although bark performs numerous ecological functions, most research has focused on single functional aspects (Rosell et al., 2014). These studies have provided valuable insights, particularly into bark's roles in fire resistance (Pinard & Huffman, 1997; Lawes et al., 2011b) and photosynthesis (Pfanz et al., 2002; Cernusak & Hutley, 2011). However, by examining functions in isolation, these studies have overlooked how variation in bark traits is influenced by interactions among its multiple functions. Since bark performs several roles simultaneously, resource allocation trade-offs may arise (Niklas, 2013), preventing the simultaneous optimization of multiple functions.

There are strong theoretical reasons to expect associations between wood and bark traits. Both tissues play roles in storing water and photosynthates (Srivastava, 1964) and contribute to the mechanical support of stems (Niklas, 1999; Rosell & Olson, 2007). Additionally, both wood and the inner bark layer originate from the vascular cambium (Evert & Eichhorn, 2006). This shared developmental origin may establish links between the characteristics of these tissues, resulting in covariation (Olson & Rosell, 2006).

Data from the literature on the construction costs of plant biomass —the amount of glucose required to produce one gram of biomass— indicate only minimal variation in these costs. Herbaceous species' leaves tend to have slightly higher construction costs compared to stems or roots. Environmental conditions show little or no influence on these costs, and differences between ecologically distinct species groups are minimal. However, most studies in this area have concentrated on leaves, seeds, and fruits, leaving other plant tissues, such as bark, less explored (Poorter, 1994).

The definition of these construction costs is as simple as performing a C/N analysis and an ashing/nitrate determination afterwards, because carbon concentration is generally a good indicator of construction costs (Poorter & Villar, 1997).

2. Objectives

The principal objective of this work is to determine the construction costs of bark versus wood in *P. halepensis* populations to understand the ecotypic patterns and phenotypic plasticity previously found in bark thickness of this species, as well as the patterns of resource allocation among populations. For this, we studied the construction costs of bark versus wood in these species populations along its natural distribution, considering bark at breast height and at the base of the tree. This information will help us determine if the different populations of this species allocate different amount of resources to this trait. Furthermore, this information is valuable for forest management, enabling the selection of the most suitable populations for current and future ecosystems affected by climate change.

The specific objetives are:

- To analyze the construction costs of bark and wood in different populations of *Pinus halepensis* grown in a common garden experiment, both at breast height and at the tree base.
- To compare the construction costs of bark and wood between the base of the tree and at breast height, as well as among the different populations.

We hypothesized that different populations of *P. halepensis* growing in a common garden experiment -under the same favourable climatic and soil conditions-, allocate more resources to bark at the base of the trunk than at breast height, showing different construction costs of bark among populations. For wood, differences between populations are not expected, but differences are anticipated between construction costs at breast height and at the base of the tree at the species level.

When comparing the construction costs of wood and bark, differences are also expected, primarily between costs at the base of the tree, with higher costs associated with populations exhibiting thicker bark thickness.

3. Relevance of the study

The significance of this study lies in the fact that construction costs have never been examined in tree bark, having only been studied in fruits, leaves, and other plant tissues. Even less, in a species like *Pinus halepensis*, which, as an obligate-seeder, is not expected to allocate resources to bark formation.

Additionally, it is important to highlight the absence of a standardized laboratory protocol for determining construction costs in hard tissues such as bark and wood. For this reason, existing protocols for other plant samples and soil, along with Poorter & Villar's (1997) equations, were used as a foundation to develop the final procedure employed in this study. The method proposed by Poorter & Villar's (1997) just required minor adaptations to the equipment and materials available in our laboratory. However, the second step of the procedure, aimed at determining nitrates content, proved more challenging. The initial procedure (Cataldo et al., 1975) was unsuitable for our samples. After extensive testing, bark pigmentation presented a significant issue for spectrophotometric measurement, and even the standards required for the calibration curve produced measurement errors. The next method tested was Yang et al.'s (1998), which showed greater success. With only a few trials, it was confirmed as suitable for our samples. However, the preparation of reagents was somewhat more complex. Despite this, even the most pigmented bark samples did not interfere with the analysis, allowing for valid and consistent results.

After extensive experimentation for laboratory procedure see Supplementary information 11.2 to 11.3; from page 39 to 41), we successfully established a valid protocol for determining the construction costs of wood and bark.

4. Materials and Methods

4.1. Plant material

Common garden experiments, a specific type of a forest genetic trial, involve the replication of individual genotypes (or, more broadly, genetic entries) across multiple environments. This design facilitates precise phenotyping by enabling the estimation and partitioning of environmentally induced variation differentiating it to the genetic induced variation (Ingvarsson and Street, 2011). Consequently, these experiments provide insights into phenotypic plasticity and genotype-by-environment interactions. While common garden studies are effective for evaluating genetic divergence across numerous populations, they do not directly test the hypothesis of local adaptation for all populations (Savolainen et al., 2007).

Common garden experiments can be categorized based on the genetic material used for their establishment into provenance, progeny, or clonal trials (Mesen, 1994). Provenance trials provide a broad overview of adaptive variation within a species but do not allow the estimation of quantitative genetic parameters, such as heritability, which describe the genetic architecture of phenotypic traits. In contrast, progeny and clonal trials enable the estimation of such genetic parameters. Combined trials, such as provenance-progeny or provenance-clonal trials, can achieve both objectives but are associated with the challenge of managing large-scale experimental designs.

It is crucial to distinguish between the concepts of provenances and test sites in these experiments. Provenances refer to the origins or source environments of the genetic materials tested, typically sampled from natural populations. Test sites, on the other hand, are the specific locations where these sampled provenances are planted and evaluated under common environmental conditions.

We used samples from a *P. halepensis* common garden experiment (Figure 4) that is part of a national genetic forest trials network, GENFORED (www.genfored.es; for more information see Climent et al. (2008)).

The provenance common garden trial used in this study was established in 1997 and replicated across six sites in eastern and central Spain. The trial encompasses 52 populations of *Pinus halepensis* originating from continental Spain, the Balearic Islands (Spain), France, Italy, Greece, and Tunisia, thereby representing a substantial portion of the species' natural range (Climent et al., 2008; Martín-Sanz, 2018). For more information about the provenances, see Supplementary information 11.1. (Page 38).

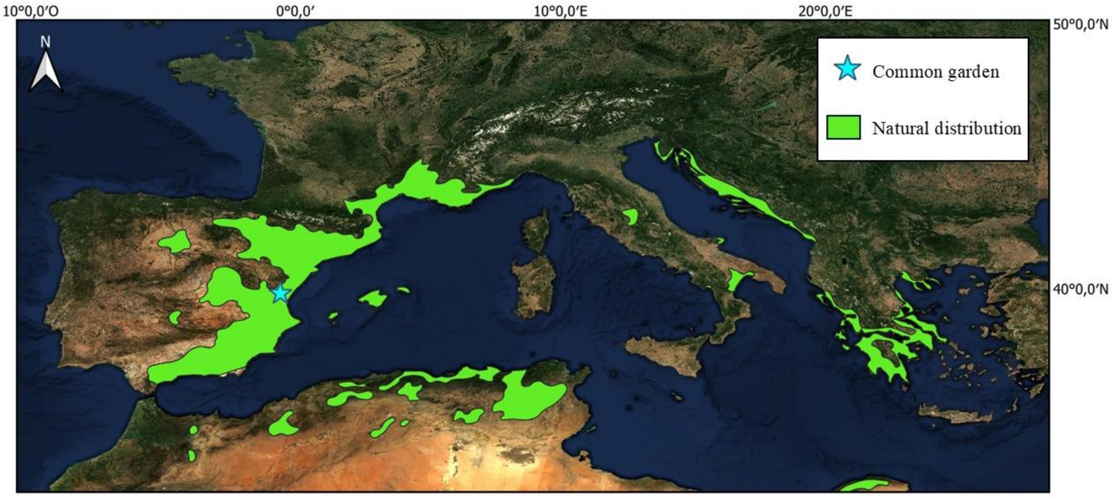

Figure 4. Map of the natural distribution of *Pinus halepensis* (green color) and the location of the study site in Spain (blue star).

In this common garden site, there are populations representing the natural distribution of the species. However, in our study we use only eight provenances due to the thinning done in the site and explained below (Table 1).

Table 1. Provenances used in this study.

Provenance code	Field code	Natural origin
P142MVA	142	Monovar (Alicante)
P152BEN	152	Benamaurel (Granada)
P154SAN	154	Santiago-Pontones (Jaén)
P157ESP	157	Alhama de Murcia (Murcia)
P172CAR	172	Carratraca (Málaga)
P211IST	211	Istaia-eyboia (Grecia)
P214KAS	214	Kassandra (Grecia)
P241THA	241	Thala (Tunisia)

The study site has Mediterranean humid climate with warm winters and moderate summer drought conditions, it is considered a high resource site for this species. The following table shows additional information of the trial site where the samples come from (Table 2).

Table 2. Description of the trial site from *Pinus halepensis* common garden experiment (PCG Site).

PCG Site	**Experimental site**
Latitude	39º49’29’’N
Longitude	0º34’22’’W
Altitude (m)	605
P (mm)	509
Ps (mm)	79
Psp (mm)	129
T (ºC)	14
MTWM (ºC)	22,9
MTCM (ºC)	7,8
A (months)	2,51
K	0,2
WS (m/s)	5,4
WSs (m/s)	3,9
Soil Type	Calcaric Cambisol (B)

Notes: P, annual precipitation; Ps, summer precipitation (June, July and August); Psp, spring precipitation (March, April and May); T, mean annual temperature; MTWM, mean temperature of the warmest month; MTCM, mean temperature of the coldest month; A, drought duration parameter; K, drought intensity parameter (all previous variables from Gonzalo-Jiménez, 2010); WS, mean annual wind speed at 80 m above surface and WSs, mean summer wind speed at 80 m above surface (from http://atlaseolico.idae.es/meteosim); soil type classification according to FAO (2015) guidelines, letter in parentheses indicate that the soil has a basic pH.

In Figure *5* is shown the average tree size present in the study site at age 18 (year 2016), highlighting also the tapering along the bole and the different bark thickness at different tree heights (Martín-Sanz et al., 2019).

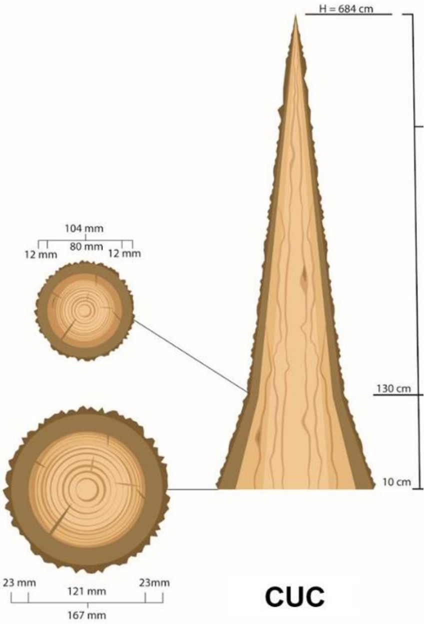

Figure 5. Scheme of the average tree at the test sites. H is the total height of the tree. Diameters and bark thicknesses of the circular cross-sections were measured at 10 and at 130 cm from the ground. From Martín-Sanz et al. (2019).

In this study, the samples come from a thinning from below done due to a high tree density in the common garden experiment. This high density began to affect the development of individuals and the measurement of vital traits for evolutionary ecology purposes. The thinning was made randomly in all the four blocks of the trial. As a result, we obtain logs from the ground to 30 cm and between 110 to 140 cm, when possible. These logs have been used for several purposes (not just the one in this work). For this study, we obtained small samples of wood and bark at the tree base (10 cm above ground) and at breast height (130 cm). Consequently, we obtained four sample types:

- Wood samples at breast height.
- Wood samples at tree base.
- Bark samples at breast height.
- Bark samples at tree base.

Construction cost analyses are invasive procedures, since they require the samples' destruction to obtain results, which makes such studies relatively uncommon, and mainly in forest genetic trials which are usually permanent plots.

4.2. Laboratory experiments: determination of construction costs of bark and wood in *P. halepensis*

Before moving on, we need to prepare the samples so that they are suitable for analysis. We cut down the logs into smaller pieces (Figure 6) to make them manageable for different purposes and research. Using the new pieces, we obtained sample dust to work with it in the laboratory. Various technics were used to obtain powder such as sandpapers (it was turned down because it left traces), rasp or wood file (it was useful for bark, but not for wood) and drill with wood bits, which was the best option. Sawdust was extracted from wood and bark, using bigger drills for wood than for bark.

Figure 6. Pieces used for wood and bark powder extraction. From R. C. Martín-Sanz©.

Afterwards, the wood sawdust was processed through a ball mill (Retsch MM301) to reduce the size of the sawdust to wood powder; this step was not necessary with the bark. This procedure took quite a long time. This material was then dried, weighed, and stored well identified in airtight jars for later cost analysis (Figure 7).

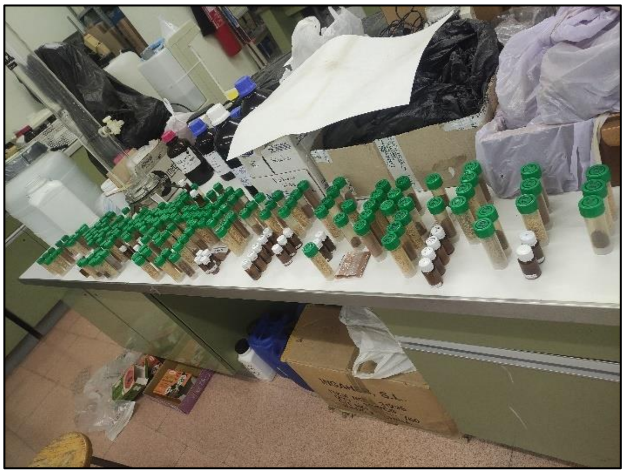

Figure 7. Samples in airtight jars for later cost analysis. From E. Herrero de San Luis©.

Tests carried out in the laboratory were designed to determine the construction costs of bark and wood, based on the determination of N and C content, ash, alkalinity and nitrate content, that are the parameters for the costs' equation from Poorter & Villar (1997).

Firstly, we estimated the water content of each tissue (wood and bark) to know if this parameter was similar in each sample. This part was already done by drying the samples in a heater to get rid of the water content and to know the weight differences before and after the treatment to determine the humidity of the samples (Figure 8). The water content of the samples was similar (3-7%) so it would not influence the rest of the analysis. All data is referred to as dry weight (DW).

Figure 8. Determination of water content in wood and bark samples. From R. C. Martín-Sanz©.

Following Poorter & Villar (1997) equations, firstly we must calculate the mineral concentration (Min; mg/g):

$$(1)\ Min = Ash - AA \times 30 + Nit$$

Where:

- Ash (mg/g dry weight) is determined by weighing 0,150 g of sample, followed by the elimination of the first smoke in a heater and the calcination of the sample in a crucible (550ºC, 2h) (Figure 9). The weight difference would be the ash content of each sample.

Figure 9. Determination of ash content in wood and bark samples. From E. Herrero de San Luis© and R. C. Martín-Sanz©.

- AA (mEq/g) or ash alkalinity is determined by indirect titration with NaOH 0,05N (Figure 10).

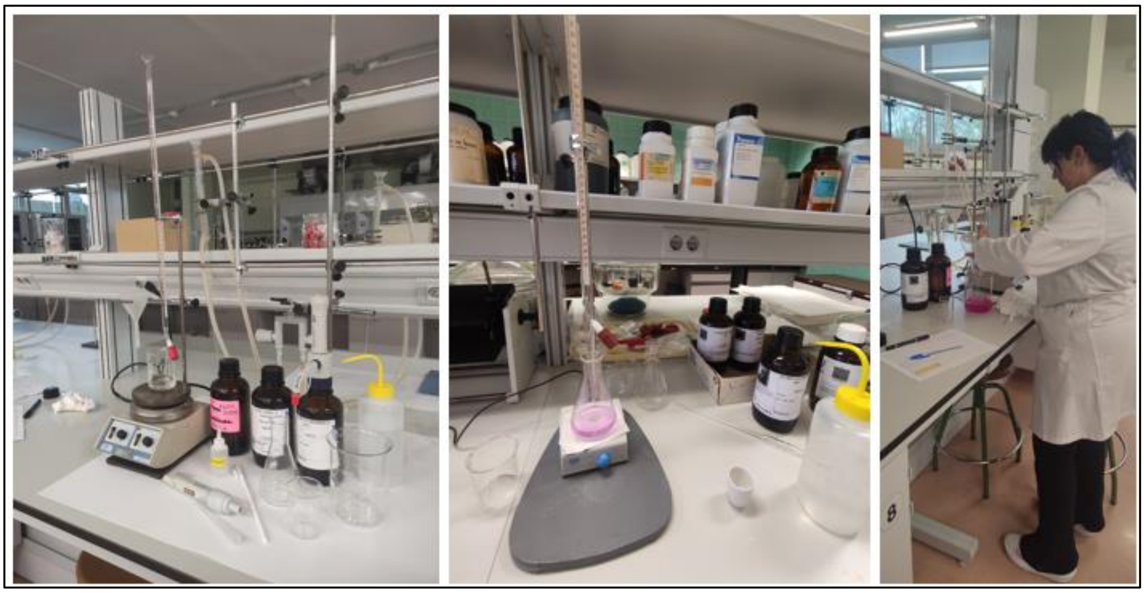

Figure 10. Laboratory equipment for determination of alkalinity by indirect titration with NaOH 0,05N. From E. Herrero de San Luis© and R. C. Martín-Sanz©.

- Number 30 is the weight of carbonate per equivalent of charge.
- Nit. or nitrate content (mg/g dry weight) has been determined by extracting the nitrates incubating the sample 1h and centrifuging the sample to purify the extract (Cataldo et al. 1975). Followed by the salicylate method that is based on the electrophilic aromatic substitution reaction, in which salicylate reacts with nitronium ions (NO_2^+) to form mostly the nitrobenzoic compound (Yang et al., 1998; Figure 11).

Figure 11. Determination of nitrates by spectrophotometry. From E. Herrero de San Luis©.

Secondly, we use the second equation of Poorter & Villar (1997) to calculate the construction costs (g glucose*g^{-1}) (2).

$$(2)\ CC = \left(-1.041 + 5.077 \times \frac{C}{1000-Min}\right) \times \frac{1000-Min}{1000} + \left(5.325 \times \frac{N_{org}}{1000}\right)$$

Where C is total carbon, Min is the mineral concentration (from Eq. 1) and N_{org} is organic nitrogen.

We determined the concentration of total C (C) and total N (N) in each type of sample by dry combustion using a Leco CSN 928® elemental analyser (LECO inc., St. Joseph, Michigan, USA). For this, we weight a representative portion (0,150 – 0,200g) of each type of sample already grounded (Figure 12). To determine the organic nitrogen (N_{org}) we multiply total N by ten and subtract the nitrate content (Nit; see Eq. 1).

Figure 12. Determination of total C and N by dry combustion using Leco CSN 928® elemental analyser. From R. C. Martín Sanz© and E. Herrero de San Luis©.

For more information on laboratory procedure and equations see Supplementary information (11.2. to 11.4, Page 39 to 41).

4.3. Data analysis

For the statistical analysis, we used a generalized linear mixed model with three fixed factors (provenance, sample type and sample height) in a complete factorial design and two covariates (the diameter and the absolute bark thickness, both nested within the sample height). These covariates were included to assess potential allometric effects on construction cost. The sample type and the sample height were considered as within-subjects factors and the provenance was considered as a between-subjects factor. The trees were the experimental units and four groups of the factors sample type and sample height were considered as a repeated measurements factor. An unstructured matrix with correlations was considered for the repeated measurements and different between-subjects variance parameters were considered for the bark and wood measurements. A Gamma distribution was considered for the dependent variable and the log function was used as the link function. If we denote $\mu i;jk$ the expected value for the dependent variable (construction costs) in a sample type j at the sample height k for the provenance i, the formulation of the model was as follows:

$$(3)\quad log(\mu i;jk)=\mu+\alpha i+\beta j+\gamma k+\alpha\beta ij+\alpha\gamma ik+\beta\gamma jk+\alpha\beta\gamma ijk+\lambda jDi;k+\delta jTi;k$$

with the following definitions of the subscripts: $i=1,\ldots,8$ for the provenances; $j=1$ (bark), 2 (wood) for the two sample types; $k=1$ (breast height), 2 (base height) for the two sample heights.

The independent variables and the parameters of the model were:

$\mu i;jk$=expected value of the cost for the sample type j at the sample height k in the provenance i.

μ=overall mean for the natural logarithm of the construction costs.

αi=main effect of the provenance i on the natural logarithm of the construction costs.

βj=main effect of the sample type j on the natural logarithm of the construction costs.

γk=main effect of the sample height k on the natural logarithm of the construction costs.

$Di;k$=diameter of the tree at the sample height k for the provenance i.

λj=linear effect of the diameter of the tree on the natural logarithm of the construction costs for the sample type j.

$Ti;k$=bark thickness at the sample height k for the provenance i.

δj=linear effect of the bark thickness on the natural logarithm of the construction costs for the sample type j.

The other terms of the equation were the second and third interactions between the three factors.

The probability distribution considered for the residuals was a normal distribution with an unstructured matrix with correlations for the within-subjects factors (four variances and six correlations) and two different variances (bark and wood) for the between-subject-factor (provenance).

Pairwise t-tests were used for the comparison of the means of the natural logarithm of the construction costs. The estimators $\hat{\mu}i;jk$ of the expected means $\mu i;jk$ and their confidence intervals were obtained with the inverse of the link function. A normal probability plot and the Kolmogorov-Smirnov test were used to test the normality of the studentized residuals of the model.

We used the PROC GLIMMIX procedure in SAS software (SAS Institute Inc., 2015).

5. Results

The general model conducted for all data indicates that sample type ($\chi^2 = 58.04$, $P < 0.001$), the sample height ($\chi^2 = 5.37$, $P = 0.048$) and the interaction between sample type and provenance ($\chi^2 = 14.46$, $P < 0.001$) showed a significant effect on construction costs. None of the covariates considered had a significant effect on construction costs.

As hypothesized, the construction costs (CC) of bark were significantly higher than those of wood at the species level (t-test = 8.71, $P < 0.001$; Table 3). In addition, the construction costs were higher at the base of the tree compared to breast height, without differentiating by sample type (Table 3; t-test = 2.18, $P = 0.041$).

Table 3. Mean, standard error (SE), minimum and maximum values of construction costs (g/glucose g) of bark and wood at the species level for each sample type and each sample height (BH = breast height)

Sample type	**Mean**	**SE**	**Min**	**Max**
Bark	1.56	0.023	1.48	1.63
Wood	1.29	0.024	1.22	1.35
Sample height				
BH	1.39	0.028	1.29	1.44
Base	1.46	0.025	1.38	1.53

Bark construction costs were higher than those of wood across all studied populations (Table 4, Figure 13). Variation in wood construction costs among provenances was relatively low, approximately 35% lower than the variation observed in bark construction costs. Provenance 211 (from Greece) exhibited the highest construction costs for both wood and bark, whereas provenances 241 (from North Africa) and 172 (from southern Spain) allocated fewer resources to bark and wood, respectively (Table 4). Regarding wood, construction costs were largely similar across provenances, with the exception of provenance 211, which showed significantly higher wood construction costs compared to the other provenances (Figure 13).

Table 4. Mean, standard error (SE), minimum and maximum values of construction costs (g/glucose g) of bark and wood for each studied provenance.

Sample type	PROV	Mean	SE	Min	Max
Bark	142	1.61	0.071	1.39	1.86
	152	1.58	0.063	1.39	1.79
	154	1.65	0.058	1.47	1.86
	157	1.59	0.064	1.39	1.81
	172	1.44	0.071	1.25	1.66
	211	1.72	0.077	1.47	2.01
	214	1.58	0.066	1.38	1.81
	241	1.35	0.062	1.19	1.54
Wood	142	1.22	0.069	1.06	1.41
	152	1.32	0.064	1.16	1.50
	154	1.31	0.063	1.16	1.49
	157	1.25	0.072	1.08	1.44
	172	1.16	0.067	1.01	1.33
	211	1.49	0.086	1.25	1.77
	214	1.30	0.069	1.13	1.49
	241	1.30	0.068	1.13	1.49

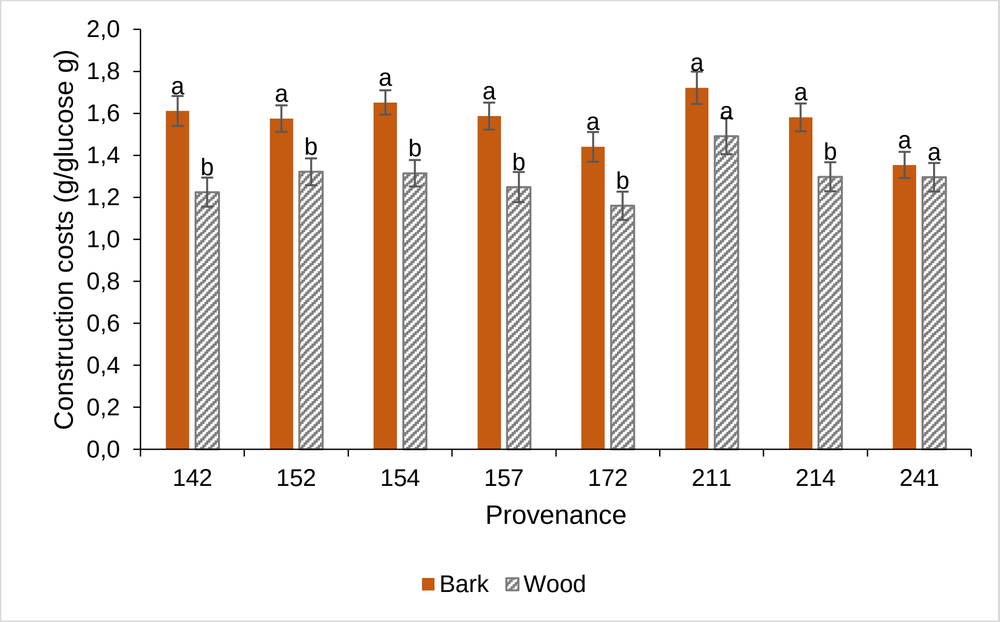

Figure 13. Construction costs of bark and wood for each provenance. Letters show significant differences among costs for each type of tissue in each provenance.

According to the model the interaction provenance*height does not show significant differences. However, the interaction sample type by sample height displayed significant differences. Construction costs of bark were consistently higher than those of wood at both sampling heights, with a slightly reduced difference observed at breast height compared to tree base (Table 5). At the base of the tree, bark costs were notably higher relative to wood costs. Both tissue types exhibited a variation of approximately 17% in their construction costs, with bark consistently maintaining higher values (Table 5, Figure 14). These findings indicate a differential resource allocation strategy between bark and wood that varies along the trunk of the tree.

Table 5. Mean, standard error (SE), minimum and maximum values of construction costs (g/glucose g) of bark and wood by height of the sample, at the base of the tree and at breast height (BH).

Sample type	Height	Mean	SE	Min	Max
Bark	Base	1.60	0.029	1.50	1.70
	BH	1.50	0.035	1.38	1.61
Wood	Base	1.32	0.031	1.23	1.41
	BH	1.24	0.028	1.16	1.31

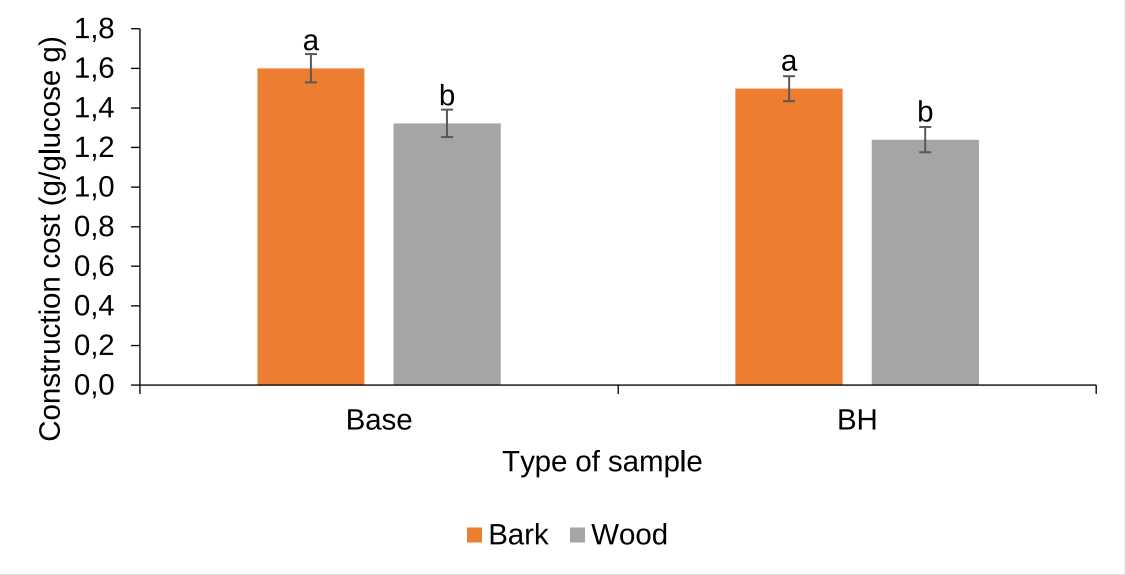

Figure 14. Construction costs of bark and wood by height of the sample, at the base of the tree and at breast height (BH). Letters show significant differences among costs for each type of tissue at each height.

6. Discussion

In this study, we provide the first experimental evidence of construction costs of bark and wood in a conifer, based on the analysis of samples from various populations across the species' natural distribution growing under the same environmental conditions in a common garden experiment.

Following our expectations, our results revealed differences in the construction costs of bark and wood at the species level, with bark exhibiting higher costs than wood. Significant differences in construction costs of both tissues, wood and bark, were also found among provenances, showing higher costs of the bark the populations from the Eastern part of the distribution, those with thicker bark, higher growth and lower reproduction (Martín-Sanz et al., 2019), while provenances from Southern Spain and North Africa showed lower constructions costs of the bark. This could be attributed to the differential resource allocation strategies exhibited by different populations. Moreover, this pattern shown by the Eastern populations is associated with the need to attain a minimum bark thickness to survive fires until achieving a sufficient aerial seed bank for population persistence, thus reducing the immaturity risk in those populations (Keeley & Zedler, 2000; Lamont et al., 1991). Therefore, the adaptive geographic variation in this species appears to result from differential resource allocation among key life-history traits, including also bark thickness as a fire-tolerance trait.

Regarding differences found at different tree heights, no significant differences were observed in wood, following expectations. Because we are comparing populations from the same species, wood properties should be highly genetically constrained, so we did not expected differences in constructions costs of this tissue among our populations. This result is in line with previous studies that indicated no significant variation in wood traits within species (Corriveau et al., 1991; González-Martínez et al., 2007; Zobel & Jett, 2012).

Our results contradict our hypothesis, and we did not find significant differences between heights among populations. However, previous studies found significant differences in the absolute bark thickness among populations of *P. halepensis*. This outcome could be explained by the small sample size used in this work, as only samples from eight provenances were available. This type of analysis requires four samples per individual —bark and wood at both heights, and this could be difficult to obtain for all trees. In fact, in our case, before the thinning was carried out in the common garden experiment, all the trees to be cut were marked, indicating perfectly the logs that we wanted to keep (from the base to 15 cm and from 120 to 140 cm on the trunk). However, once the thinning ended, the subcontracted company did not properly cut the logs in all the trees and therefore, we have missing data on some trees.

As expected, we found significant difference between samples from the tree base and breast height. Construction costs were higher at the base of the tree for both wood and bark. However, bark consistently exhibited greater construction costs compared to wood.

Despite *P. halepensis* is typically classified as an obligate-seeder, studies have shown that some populations can develop sufficient bark thickness to survive surface and moderately intense fires (Martín-Sanz et al., 2019). This suggests a more variable adaptive strategy for fire response than previously considered for this species (see Ducrey et al., 1996; Rigolot, 2004; Fernandes et al., 2008). Our results on constructions costs support these previous evidences.

To our knowledge, this is the first study providing experimental evidence on the construction costs of bark, a key fire-adaptive trait. Investigating construction costs is complex and challenging; however, it is crucial for understanding ecotypic patterns, phenotypic plasticity, and resource allocation within species. Knowing that some populations find it more difficult than others to build their bark in terms of resource allocation is vital to being able to offer sustainable management alternatives. Our findings can be highly valuable for the sustainable management of forest stands of *P. halepensis* in the current context of climate change and under the new fire regimes, by identifying populations better adapted to fire regimens and knowing that the scarcity of resources (mainly water) clearly affects the resource allocation on this species. Additionally, the increasing frequency of wildfires in Mediterranean regions must be considered, as it will inevitably influence future management strategies and the selection of populations for species propagation and reforestation in the short, medium, and long term.

7. Conclusions

1. Construction costs of bark were higher than those of wood across populations of *P. halepensis*.
2. Construction costs of bark were higher than wood costs also comparing between sample heights (basal vs. breast height).

3. Construction costs at the tree base were higher than at breast height. This appears to be particularly relevant considering that trees must attain a minimum basal bark thickness to survive surface or moderately intense fires until they establish a sufficient aerial seed bank to ensure population persistence under wildfire conditions.
4. Significant differences exist in resource allocation between bark and wood among provenances, with higher allocation observed in Eastern European populations compared to those from Southern Spain or North Africa. This is consistent with reducing the immaturity risk in Eastern European populations that allocate more resources to growth and thus, need more time to reach a sufficient aboveground seedbank.
5. No significant differences were found among provenances for the two sampling heights for any tissue studied, may be due to the limited sample size.
6. These types of studies are challenging and complex to conduct, yet they are essential for improving our understanding of ecotypic patterns, phenotypic plasticity, and resource allocation in forest species. Additionally, they provide valuable information for enhancing the management of forest stands.

8. Conclusiones

1. Los costes de construcción de la corteza fueron más altos que los de la madera en las distintas poblaciones de *P. halepensis*.
2. Los costes de construcción de la corteza fueron superiores a los de la madera, también al comparar las alturas de las muestras (altura basal vs. altura del pecho).
3. Los costes de construcción en la base del árbol fueron más altos que a la altura del pecho. Esto parece ser particularmente relevante considerando que los árboles deben alcanzar un espesor mínimo de corteza basal para sobrevivir a incendios superficiales o moderadamente intensos hasta que establezcan un banco de semillas aéreo suficiente para asegurar la persistencia de la población en condiciones de incendios forestales.
4. Existen diferencias significativas en la asignación de recursos entre corteza y madera entre procedencias, observándose una mayor asignación en las poblaciones de Europa del este en comparación con las del sur de España o el norte de África. Esto es consistente con la reducción del riesgo de inmadurez en las poblaciones de Europa del este que asignan más recursos al crecimiento y, por lo tanto, necesitan más tiempo para alcanzar un banco de semillas aéreo suficiente.
5. No se encontraron diferencias significativas entre procedencias para las dos alturas de muestreo para ningún tejido estudiado, lo que puede deberse al tamaño limitado de la muestra.
6. Estos tipos de estudios son difíciles y complejos de realizar, pero son esenciales para mejorar nuestra comprensión de los patrones ecotípicos, la plasticidad fenotípica y la asignación de recursos en las especies forestales. Además, brindan información valiosa para mejorar la gestión de las masas forestales.

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11. Supplementary information

11.1. Provenance from the common garden experiment

Table S 1. Provenances used in this study.

Country	Code	Ecological region	Provenance	Location	Longitude	Latitude	Altitude (m)	Mean temperature (ºC)	Annual temperatura range (ºC)	Precipitation (mm)	Summer precipitation (mm)
Spain	P142MVA	6	Bética Septentrional	Monovar	0º57'27''W	38º23'05''N	760	14.7	28	387	51
Spain	P152BEN	7	Bética Meridional	Benamaurel	2º44'19''W	37º42'05''N	914	14.3	31.8	394	44
Spain	P154SAN	7	Bética Meridional	Santiago de la Espada	2º28'03''W	38º13'35''N	761	13.6	32.6	608	66
Spain	P157ESP	7	Bética Meridional	Alhama de Murcia	3º01'19''W	37º45'10''N	818	15.6	32.6	433	29
Spain	P172CAR	8	South	Carratraca	4º50'01''W	36º50'32''N	643	14.85	28.6	699.5	33
Greece	P211IST	12	Greece	Istaia-eyboia	23º30'46''E	38º44'29''N	27	17.47	27.2	506	35
Greece	P214KAS	12	Greece	Kassandra	23º54'27E	40º05'13''N	173	14.37	27.7	510	61
Tunisia	P241THA	15	Tunisia	Thala	8º39'00''E	35º33'60''N	527	14.89	33.6	467	63

11.2. Procedure for determination of ash and ash alkalinity

To determine the ash content of the samples we use a calcination method and for the ash alkalinity a back titration of NaOH 0,05N.

MATERIAL

- Precision scale
- Crucible
- Stove
- Flask
- Automatic pipette
- Beakers
- Volumetric flasks 1L
- Magnetic stirrer and rod
- Mechanical rotator shaker
- 1 L beaker
- Adjustable dispenser
- Muffle furnace
- Wash bottle
- Distilled water
- Phenolphthalein

REAGENTS

- HCl 0,05N: Dissolve 4.14 ml of 37% HCl in one litre of distilled water in extractor hood.
- NaOH 0,05N: Dissolve 2 g of NaOH in one litre of distilled water.
- Oxalic acid: Dissolve 3.152 in one litre of distilled water.

PROCESS FOR ASH CONTENT

1. Weigh 0.150g of sample in a crucible.
2. Eliminate smoke in a stove in extractor hood before using muffle furnace.
3. Take to muffle furnace for 2h at 550º, and let it cool down.
4. Weigh the samples once they have cooled down.

PROCESS FOR ASH ALKALINITY

1. Make 2 blanks with 10 ml HCl in order to compare the titration data (to add the HCl use the dosing device on the bottle).

2. Transfer the ashes to an Erlenmeyer flask, add 10 ml 0,5N HCl (reacts with the ashes and consumes part of this HCl). For titration use magnetic stirrer and phenolphthalein as an indicator.
3. To find out what is left over, titrate back with 0.05N NaOH. This way you can calculate the moles of HCl that have reacted with the ashes. For the oxalic, do 3 repetitions, it is taken with an automatic pipette.

11.3. Procedure for determination of nitrate content

To determine nitrates, we extract them and use a colorimetric method.

MATERIAL

- Precision scale
- Crucible
- Stove
- Flask
- Automatic pipette
- Beakers
- Volumetric flasks 1L
- Wash bottle
- Distilled water

REAGENTS

- NO_3 standard: Dissolve 0.7218g of KNO3 (dried for 6h at 105°C) in 1 l of distilled water.
- Sulfuric acid.
- 1% Sodium salicylate: Dissolve 0.25g of NaOH in 50 ml of distilled water, after in that dilution dissolve 0.863g of salicylic acid (if it does not dissolve use an ultrasound bath) in a 100ml flask.
- 40% NaOH: Dissolve 40g of NaOH in 100ml of distilled water.

PROCESS FOR NITRATE EXTRACTION (Cataldo et al. 1975)

1. Take 50 mg of sample weighed to the highest precision (4 decimal places, if possible), add 5 ml of distilled water with dosing device, shake by tumbling to mix well.
2. Incubate at 45°C for 1h in the oven.
3. Centrifuge for 10 min at 10,000 rpm.

PROCESS FOR COLORIMETRIC METHOD (Yang et al., 1998)

1. Transfer 1 mL of sample containing 0 to 4 mg NO_3-N L"1 into a flat-bottom sample vial.
2. Add 0.5 mL of sodium salicylate.

3. Evaporate to dryness on a hot plate (or in an oven overnight) and cool the residue.
4. Wet the residue with 1 mL of concentrated H_2SO_4 and allow to stand for 5 min.
5. Add 5 mL of H_2O down the vial wall and swirl to mix. Allow the solution to cool.
6. Add 5 mL of 40% NaOH down the vial wall, swirl, and cool**.
7. Read absorbance or transmittance at 410 nm.
8. Calculate NO_3-N concentration from the standard calibration curve.

**Handle the samples with care, as crystallization begins if the time elapsed between the addition of the last reagent and the measurement in the spectrophotometer exceeds 30 minutes.

11.4. Example of calculations

We use Poorter & Villar's (1997) equations to determine construction costs in this study, which are the following:

$$(1)\quad Min\ = Ash - AA\ \times 30 + Nit$$

Where:

- Min: Mineral concentration in mg/g.
- Ash: ash content of the sample in mg/g dry weight.
- AA: ash alkalinity in mEq/g.
- Nit: nitrate content in mg/g dry weight.

$$(2)\quad CC\ = \left(-1.041 + 5.077\ \times \frac{C}{1000 - Min}\right) \times \frac{1000 - Min}{1000} + \left(5.325\ \times \frac{N_{org}}{1000}\right)$$

Where:

- CC: construction costs in g/g glucose.
- C: total carbon in g.
- N_{org}: organic nitrogen in g.

The next section provides a numerical example of calculating the construction cost for a bark sample.

To calculate the ash content (Ash), we use the data in Table S 2 and equation $Ash = W_{is} - (W_{PC+D} - W_{PC}) = 0{,}001\ g$(3).

Table S 2. Data to calculate ash content (Ash).

Sample	W_{PC} (g)	W_{IS} (g)	W_{PC+D} (g)
141BC	30.783	0.150	30.786

W_{PC}: Porcelain capsule weight. W_{IS}: initial sample weight. W_{PC+D}: Porcelain capsule + dry weight.

$$(3)\quad Ash = W_{is} - (W_{PC+D} - W_{PC}) = 0{,}001\ g$$

To calculate ash alkalinity (AA), we use the data in Table S 3 and equation (4).

Table S 3. Data to calculate ash alkalinity (AA).

Sample	W_{IS} (g)	NaOH Vol. (ml)	Oxalic Vol. (ml)	HCl Vol. (ml)
141BC	0.150	8.8	19.3	9.15

$$(4)\quad AA = \frac{\left(HCl\,Vol. \times \frac{20 \times 0.05}{Oxalic\,Vol.}\right) - \left(NaOH\,Vol. \times \frac{20 \times 0.05}{Oxalic\,Vol.}\right)}{W_{IS}} = 0{,}1208981\, mEq/g$$

To calculate nitrate content (Nit), we use the data in Table S 4, Figure S 1 and equation (5).

Table S 4. Data to calculate nitrate content (Nit).

Sample	W_s	ABS_s	ABS_b	CV	C
141BC	0,05	1,951	1,853	0,098	0,0072368

W_s: sample weight. ABS_s: Sample's absorbance. ABS_b: Blank sample's absorbance. CV: Sample's corrected value. C: Concentration of sample using the Standard curve.

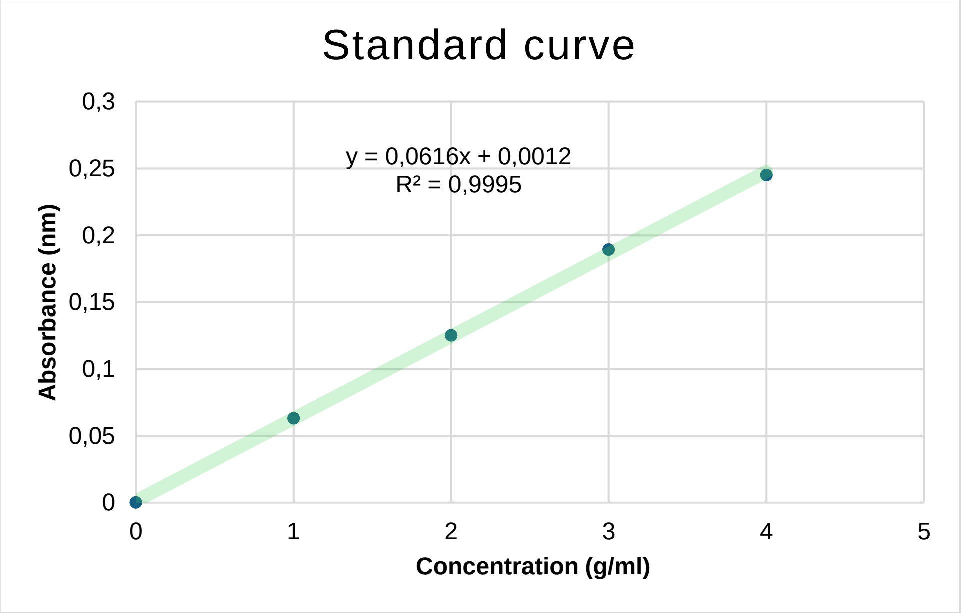

Figure S 1. Standard curve for sample 141BC.

$$(5)\quad Nit = \frac{C \times 0.05}{ABS_S} = 1{,}854E^{-05} mg/g\ dry\ weight$$

Considering the data from equations (3), (4) and (5), and using equation (1), we obtain a mineral concentration for sample 141BC of -7.771 mg/g.

To calculate organic nitrogen concentration (N_{org}) we use data from Table S 5 and equation (6).

Table S 5. Data to calculate organic nitrogen (N_{org}).

Sample	N LECO (%)	Nit. (mg/g)
141BC	0.1983	$1.8546E^{-05}$

$$(6) \quad N_{org} = \frac{N\ LECO \times 10}{Nit.} = 198.299\ g$$

Considering all the data above and equation (2) we obtain construction costs value for sample 141BC is 2.704 g/ glucose g.