Resin ducts as resistance traits in conifers: Linking dendrochronology and resin-based defences

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Abstract

Conifers have evolved oleoresin-based chemical and anatomical defences to protect them from a wide range of antagonists. Resin ducts produce, store and translocate oleoresin, a complex terpenoid mixture that acts as both a physical and a chemical defence. Although resin duct characteristics (e.g., number, density, area) have been positively related to biotic resistance in several conifer species, the literature reporting this association remains inconclusive. Understanding the sources of phenotypic variation in defences, such as genetic differentiation and environmental plasticity, is essential for assessing the adaptive potential of forest tree populations to resist pests under climate change. Axial resin ducts recorded in annual growth rings are an archive of annual defensive investment in trees. This whole-life record of defence investment can be analysed using standard dendrochronogical procedures, allowing us to assess interannual variability and the effect of understudied drivers of phenotypic variation on resin-based defences. After reviewing the evidence supporting the importance of resin ducts in conifer resistance, we summarised current knowledge about the sources of variation in resin duct production. We propose the application of a standardized methodology to measure resin duct production by means of dendrochronogical procedures. This approach will illuminate the roles of resin ducts in tree defence across species, while helping to fill pivotal knowledge gaps in plant defence theory, and leading to a better understanding of the patterns of variation in resin-based defences throughout the tree's lifespan.

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1. Introduction

Considerable effort has been made to elucidate the mechanisms that have given rise to the enormous array of defences among plant species. One of the ideas first embraced in plant defence theory is that plants and herbivores are engaged in an ‘arms race’ (Ehrlich and Raven 1964). Accordingly, adaptations in the phytophagous antagonists can shift the selective pressures acting on host plants and *vice versa*, leading to counter-adaptations. This theory provided an initial basis for understanding the relevance of host plant-herbivore interactions for the evolution of plant defences.

Among woody plant species, conifers have evolved specific defensive traits and strategies that have contributed to their evolutionary diversification and colonisation success (Franceschi et al. 2005). The defensive system of Pinaceae, a family that includes pines and spruces among other tree species, relies heavily on oleoresin (hereafter ‘resin’), a chemical mixture of diterpenes, sesquiterpenes and monoterpenes that are toxic to herbivores and pathogens (Celedon and Bohlmann 2019; Erbilgin 2019; Phillips and Croteau 1999). Resin is produced, stored and translocated in secretory structures of differing complexity, from single cells or blisters to interconnected resin ducts (Franceschi et al. 2005). Resin ducts are tube-like structures formed by secretory epithelial cells that produce and exudate resin into the lumen (Fahn 1979). These anatomical structures act as a mechanical and chemical barrier to prevent the entry of organisms by flushing out resin on wounding (Berryman 1972; Franceschi et al. 2005).

Resin ducts are common features in the stem, roots, needles and reproductive structures of many conifer genera, including *Picea, Pseudotsuga* and *Larix*, but reach their most sophisticated expression in *Pinus* (Bannan 1936; Wu and Hu 1997). On one hand, axial resin ducts are present in the xylem (Fig. 1a, b, d) and in the cortex of roots and stems (Fig. 1c). Cortical resin ducts are important resin reservoirs during the first developmental stages of conifers, but xylem resin ducts become the main source of resin in adult trees of some species (Franceschi et al. 2005). On the other hand, radial resin ducts are present in the bark and also in the xylem, and are usually connected to axial resin ducts, creating a complex three-dimensional network that ensures resin exudation when the tree is injured (Franceschi et al. 2005). Moreover, resin ducts can be induced in response to wounding, fungal infestation, or insects attack (Franceschi et al. 2005).

Several authors have suggested that resin ducts are the result of adaptive coevolutionary interactions between conifers, bark beetles (Curculionidae: Scolytinae) and their symbiotic pathogens. This hypothesis is supported by evidence indicating that conifer species commonly attacked by aggressive bark beetles – those that orchestrate a pheromone mediated mass attack to deplete tree defences – evolved a better developed and more complex network of constitutive resin ducts (Franceschi et al. 2005; Labandeira et al. 2001; Mason et al. 2019; Raffa et al. 2008). However, as a coevolutionary response to tree defence, aggressive bark beetles use resin compounds, primarily monoterpenes, as precursors of aggregation pheromones (Seybold et al. 2000). From an evolutionary perspective, resin-based defences are therefore critical in understanding the evolutionary history of conifers and insect herbivores (Celedon and Bohlmann 2019; Franceschi et al. 2005).

A previous meta-analysis revealed that anatomical defensive traits can often be more reliable than chemical traits for assessing biotic resistance across plant species (Carmona et al. 2011). Supporting this hypothesis, the abundance and size of constitutive and induced resin ducts were associated with resistance to many insects and pathogens in numerous conifer species (Alfaro et al. 1997; Ferrenberg et al. 2014; Kane and Kolb 2010; Krokene et al. 2008; Krokene et al. 2003; Mason et al. 2019; Zhao et al.

2019). Despite this evidence, the outcomes of various studies are inconsistent and show positive, negative or no relationships between resin duct characteristics and biotic resistance. A synthesis is therefore needed to elucidate the role of resin ducts in tree resistance to biotic stress.

One of the main research challenges in plant defence theory relies on the understanding of the patterns and drivers of variation in plant defences within species. Intraspecific variation in defence production is expected, because plants must optimise resource allocation between defence and other life functions in relation to the local environment (Coley et al. 1985; Herms and Mattson 1992; Stamp 2003). Evolutionary trade-offs between growth and defensive investment resulting from adaptation to the local environment have been specially relevant at explaining patterns of variation in defences at the interspecific level (Resource availability hypothesis - RAH, Coley et al. 1985; Endara and Coley 2011). However, it is not clear yet how local adaptation to environmental variation drives genetic differentiation in defensive allocation within species (Hahn and Maron 2016). In addition to genetic differentiation, environmental plasticity is known to contribute greatly to phenotypic variation in plant defences within species (Moreira et al. 2015; Rigling et al. 2003; Sampedro et al. 2011). Both intraspecific genetic variation and phenotypic plasticity have enormous implications for future acclimatisation, expansion and adaptation of forest tree populations (Aitken et al. 2008; Alberto et al. 2013; Valladares et al. 2014). Importantly, recent studies have also focused on the interplay between genotype and environment (i.e., G×E interactions) due to its relevance in predicting population responses to climate change (Li et al. 2017; Wang et al. 2010). Furthermore, epigenetic processes and transgenerational effects have also been identified as potential sources of variation in plant defensive investment (Holeski et al. 2012).

The ongoing rapid global change has generated an urgent need to understand the micro-evolutionary patterns driving intraspecific variation in defences, especially in long-lived forest tree species exposed to environmental changes throughout their lifespan. This requires a disentangling of the interactive effects of genetic and environmental factors on defensive traits. In this paper we provide a novel approach for exploring phenotypic variation in resin-based defences in conifers through application of dendrochronological methods. Axial resin ducts remain in the wood throughout the lifetime of the tree, making them an annually-resolved archive of defensive allocation for retrospective analysis by standard dendrochronological procedures (Fritts 1976). Wood cores for tree-ring analysis can be obtained by non-destructive sampling in natural and experimental stands and are a powerful source of easily replicable ecological information. Tree-ring analysis is an especially relevant technique for analysing interannual plasticity in anatomical traits as a function of climate variability (Fritts 1976). It also allows us to evaluate the adaptive and evolutionary significance of climate sensitivity in anatomical traits when applied to mature individuals in common garden experiments (Housset et al. 2018). Similarly, dendrochronological data gathered from genetic trials can be analysed to determine the existence of genetic trade-offs between growth and defences throughout the lifespan of trees (Hahn and Maron 2016). Because resin duct measurements from wood cores provide a whole-life record of defensive investment, age-associated effects on defence allocation can also be explored by tree-ring analysis (Barton and Koricheva 2010; Boege and Marquis 2005; Erbilgin and Colgan 2012).

Here we compiled results from studies reporting a comparison of resin duct characteristics in resistant and susceptible trees across different conifer species and conducted a meta-analysis to quantify the mean effect size of resin duct production on tree biotic resistance. We then reviewed and synthesised knowledge about genetic and environmental variation in conifer resin ducts and identified current knowledge gaps. Our review showed that higher resin duct production has a positive effect on biotic resistance. However, the limited number of studies and heterogeneous methodologies for evaluating resin ducts highlight the need for more research to assess the role of this anatomical defensive trait in conifer resistance. We provide a novel approach for studying resin-based defences in conifers through retrospective analysis of axial resin ducts in xylem tree rings. This approach will shed light on the micro-evolutionary patterns of resin-based defences in conifers and address unanswered questions in plant defence theory.

2. Association between resin duct characteristics and resistance in conifers

The network of resin ducts acts as an initial anatomical and physical barrier to protect the tree against insect herbivores and pathogens. After damage, resin exudation seals wounds and prevents the entry of organisms by forming a crystallised resin barrier (Berryman 1972). The amount of resin a tree produces could therefore determine the probability that the tree will resist a biotic challenge (Christiansen et al. 1987). According to Hagen-Poiseuille's law, resin flow is proportional to the fourth power of the resin duct diameter (Schopmeyer et al. 1954). Small increases in resin duct size or production can therefore lead to large increases in resin flow, as has been supported by a number of studies finding a positive association between resin flow and resin duct size and/or production (Baier et al. 2002; Blanche et al. 1992; Hood and Sala 2015; Lombardero et al. 2000; Rodriguez-Garcia et al. 2014). Despite wider resin ducts are expected to produce larger amounts of resin, the complexity of the resin duct system, arising from connections between radial and axial resin ducts (Fahn 1979; Franceschi et al. 2005), may also determine its efficiency as an anatomical barrier. For example, Boucher et al. (2001) found that *Picea* and *Pinus* trees with narrower but more frequent resin ducts in the cortex showed reduced performance of the white pine weevil (*Pissodes strobi*). These results suggest that a denser network of smaller resin ducts can constitute a better resistance mechanism than fewer but bigger resin ducts, since it might leave fewer undefended gaps reducing the chance of insect accessibility.

The relevance of resin ducts as proxies of defensive investment in conifers was reported in various studies linking resin duct characteristics (e.g., higher conducive area or abundance) to enhanced resistance against a number of insect herbivores and their symbiotic phytopathogenic fungi (Christiansen et al. 1999; Ferrenberg et al. 2014; Kane and Kolb 2010; Krokene et al. 2003). Trees that are more resistant to insects and pathogens are assumed to allocate proportionally more resources to defences and produce more or larger resin ducts. This hypothesis is supported by studies documenting greater allocation to resin ducts in trees that survived a pest outbreak. For instance, comparisons in lodgepole pine (*P. contorta*) and limber pine (*P. flexilis*) showed that trees surviving attacks during a mountain pine beetle (*Dendroctonus ponderosae*) outbreak had more or denser axial resin ducts in the xylem than susceptible trees that died (Ferrenberg et al. 2014). Likewise, *P. ponderosa* trees that either avoided (Kane and Kolb 2010) or survived bark beetle attacks (Hood et al. 2015) had a larger area or density of resin ducts in the xylem than trees that died. Pinyon pine (*P. edulis*) trees that were attacked by bark beetles and died after severe drought events had smaller resin ducts than trees that were not affected by drought or bark beetles (Gaylord et al. 2015; Gaylord et al. 2013). Beyond *Pinus* species, a better developed system of resin-based defences is also known to contribute significantly to enhanced resistance in *Picea* trees, particularly to insect herbivores such as *P. strobis* (Alfaro 1996; Alfaro et al. 1997; King et al. 2011; Tomlin and Borden 1994; Tomlin and Borden 1997). Differences have been also determined in resin duct characteristics between resistant and susceptible species to common pests, in particular in relation to potential hosts of the mountain pine beetle (Bentz et al. 2017). However, more studies are needed to determine any broad-scale patterns.

Other studies have documented correlational evidence between characteristics of bark resin ducts and reduced pest performance or attack success. O'Neill et al. (2002) found that the size of resin ducts in the bark of *P. sitchensis* was positively correlated with the percentage of bark free of *P. strobi* attacks. More recently, Moreira et al. (2012) reported that the density of resin ducts in this species was negatively correlated with weevil damage. Wainhouse et al. (2005; 2009) showed that the depth of bark feeding by the European weevil (*Hylobius abietis*) in *P. sitchensis,* European black pine (*P. nigra*) and Scots pine (*P. sylvestris*) was negatively correlated with the area of cortex occupied by resin ducts.

Contrary to the hypothesis of enhanced resistance associated with a better developed resin duct system, other studies found a negative or no relationship between resin duct characteristics and resistance. Aleppo pine (*P. halepensis*) trees that survived attacks by *Tomicus* or *Orthomicus* bark beetles had fewer or less dense resin ducts in their xylem than dead trees (Sangüesa-Barreda et al. 2015). However, surviving trees still had more resin ducts in the years before the attacks when expressed as a function of annual growth. The contrasting results among studies may indicate that the importance of resin ducts for resistance can be context dependent and/or specific to certain antagonist organisms. For instance, *Tomicus* and *Orthomicus* are secondary bark beetles that affect weakened trees, circumstances in which resin based defences might not be so relevant at determining resistance. Indeed, resistance to those insects in the later mentioned study was related to growth patterns associated with drought events (Sangüesa-Barreda et al.

2015). Moreover, differences in the metrics applied when reporting resin duct characteristics (e.g., absolute vs relative metrics) could also be responsible for the discrepancies between reported results (see supporting information, Table S1).

The effectiveness of the resin duct network may also depend on the nature of the biotic challenge. An obvious exception could be pathogens taking advantage of resin ducts as a pathway for preferential infection and expansion. The pine wood nematode (*Bursaphelenchus xylophilus*), a serious exotic threat for Eurasian pine forests, is known to migrate from branches to the main stem through the resin duct system in the cortex and xylem (Ichihara et al. 2000; Kuroda 2008a; Kuroda 2008b). Given this pathway of infection, larger or more numerous resin ducts can facilitate migration of the nematode from infected to healthy tissues, helping to spread the disease (da Silva et al. 2015; Kawaguchi 2006; Kuroda 2004; Zas et al. 2015). Pines susceptible to this nematode were reported to have more resin ducts than pines capable of restricting nematode migration (Son et al. 2015). In a similar example, the pathogen *Fusarium circinatum* is able to colonise resin ducts and expand through the xylem via axial resin ducts in Monterey pine (*P. radiata*), spreading pitch canker disease (Martin-Rodrigues et al. 2013).

Despite the particular cases in which resin ducts are colonised by specialised antagonist organisms that use them as a pathway for disease spread, the literature indicates that resin ducts tend to be positively related to increased biotic resistance. We tested this hypothesis by conducting a random effects meta-analysis (Box 1) to assess the mean effect size (standardised mean difference - SMD) of resin duct investment on resistance to bark beetles. While we found a positive mean effect size of resin duct production on resistance, the small number of studies and considerable heterogeneity among them, most likely due to variations in methodology and experimentation, precludes a definitive conclusion. When the data were grouped by variables associated with the area, number and density of resin ducts, we found that variables related to area were the most reliable for studying resistance. In light of these results, we conclude that most studies provide evidence of enhanced tree resistance as a result of greater resin duct production. However, further studies are needed to assess this relationship before more detailed outcomes can be assessed. It is also crucial to determine which resin duct characteristics are better related to resistance, and to develop a unified methodology for analysing anatomical resin-based defences. This will reduce the heterogeneity among results and improve our quantitative understanding of the overall effect of resin duct investment on tree resistance.

3. Induced resin duct production may enhance tree resistance

Resin duct formation can also be induced in response to biotic damage by production of traumatic resin ducts (TRD). Hudgins *et al.* (2004) reported the inducibility of resin duct formation in different conifer species, such as those in the Pinaceae and Cupressaceae families (Fig. 2). According to these authors, constitutive resin ducts (CRD) are produced in the bark, and as either constitutive or inducible structures in the xylem of *Pinus*, *Picea*, *Larix* and *Pseudotsuga* (Fig. 2). Other Pinaceae genera have CRD in their bark, but only TRD in their xylem. As described by Hudgins *et al.* (2004), some Cupressaceae species also produce CRD in the bark and TRD in the xylem, while some others in this family can only activate or produce new resin ducts in their bark (Fig. 2). The fact that species from two evolutionarily divergent families such as Pinaceae and Cupressaceae both produce TRD in their xylem highlights the importance of inducible resin-based defences for biotic resistance in conifers. The balance between constitutive and inducible resin ducts is thought to be shaped by interactions and coevolution with insects and fungal pathogens. Species that are highly threatened by aggressive bark beetles appear to rely more on a better developed network of CRD, while those that are not host to these beetles prioritise inducible resin ducts (Franceschi et al. 2005; Seybold et al. 2000).

Several external factors can induce TRD formation, including fungal pathogens, insects and wounding (Franceschi et al. 2000; Krokene et al. 2003; Nagy et al. 2000; Nagy et al. 2006). TRD formation in the xylem can also be induced by physical or thermal damage, such as debris impact or fire (Arbellay et al. 2014; Bollschweiler et al. 2008). *De novo* formation of resin ducts has been widely reported as inducible by external application of the hormone methyl jasmonate, probably via ethylene downstream signalling (Hudgins et al. 2004; Hudgins and Franceschi 2004; Martin et al. 2002; Moreira et al. 2015). Furthermore, studies suggest that inducibility of resin ducts involves systemic responses. For instance, TRD production in *Picea* and *Pinus* trees inoculated with different pathogens were reported to produce TRD at certain distance away from the inoculation site (Krekling et al. 2004; Luchi et al. 2005).

Induction of TRD might lead to reduced pest damage according to a number of studies. For instance, inoculations with *Endoconidiophora polonica* (formerly *Ceratocystis polonica*) and the application of methyl jasmonate induced resin duct development in *P. abies*, where higher TRD production negatively correlated with the damage caused by the fungus (Christiansen et al. 1999; Krokene et al. 2008; Krokene et al. 2003). Induced TRD production was also negatively correlated with the performance of the insect *P. strobis* in *P. abies* and *P. sitchensis* trees previously elicited by wounding (O'Neill et al. 2002; Poulin et al. 2006). Resistant trees were also shown to produce more TRD than susceptible trees in *Picea* species in response to a range of elicitors (Byun-McKay et al. 2006; Schmidt et al. 2011; Tomlin et al. 1998). These studies suggest that traumatic resin duct production might be a key mechanism involved in conifer resistance to both insects and pathogens.

Mechanistically, *de novo* formation of resin ducts (or a larger area of TRD) may result in increasing resin flow, helping to block wounding sites and expelling invading organisms. Moreover, while constitutive resin ducts have a scattered distribution in the xylem, TRD are distributed in dense tangential series (Franceschi et al. 2002; Krokene et al. 2003; Martin et al. 2002). This distribution of resin ducts may act as a powerful barrier, sealing the infected zone and limiting the spread of pathogens (Nagy et al. 2000). This phenomenon can be conceptualized under the framework of the model of 'compartmentalization of damage in trees' (i.e. CODIT) (Morris et al. 2019). Accordingly, the production of layers of induced resin ducts would favour the development of a reaction zone that prevents the spread of any infection or damage. TRD production involves differentiation of epithelial cells from mother cells in the cambium, a process that can take several weeks (Nagy et al. 2000). Considering bark beetles as an example, TRD production may therefore be too slow to deter or repel a mass attack in a short time frame (Berryman 1972). However, TRDs may be a successful defensive mechanism against future attacks or fungal infections introduced by bark beetles (Krokene et al. 2003).

4. Environmental factors affecting resin duct differentiation

During their long lifespan, conifers are exposed to variable environmental conditions. Phenotypic plasticity is therefore essential for their persistence and allows trees to acclimatise to the environment. The extended growth-defence balance hypothesis (GDBe, Herms and Mattson 1992), a longstanding hypothesis within the large body of plant defence theory, predicts plastic responses in plant defences, where carbon allocated to defensive traits is increased under scenarios with low resource availability. This hypothesis addresses the expression of physiological growth-defence trade-offs, stating that when the shortage of a resource limits growth but not photosynthesis, the excess of photoassimilates can be allocated to the secondary metabolism and, therefore, defence production in enhanced. Because resin duct production is a costly process involving cell production and differentiation (Bonello et al. 2006; Moreira et al. 2015), resource availability is expected to affect their production.

While evidence for growth-defence trade-offs predicted by plant defence theory is available for some species of conifers (Moreira et al. 2015; Sampedro et al. 2011), experimental tests of nutrient availability on resin duct production are relatively rare and have yielded conflicting results. For instance, *P. pinaster* trees exposed to nutrient limiting conditions grew less but showed higher resin duct density (Ferrenberg et al. 2015; Moreira et al. 2008; Moreira et al. 2015). Contrarily, other studies have reported that fertilisation and light availability increased absolute resin duct production (e.g. their number or size) in different conifer species (Kyto et al. 1999; Wainhouse et al. 2005; Wainhouse et al. 2009). These contrasting results highlight the importance of using different resin duct metrics, and how this can affect the outcomes when exploring environmental effects on resin duct differentiation (Hood and Sala 2015). While absolute resin duct metrics (e.g. number or size) tend to be positively correlated to growth, their relative production (e.g. resin duct density) usually shows negative correlations (Ferrenberg et al. 2014; Hood and Sala 2015; Kane and Kolb 2010; Klutsch and Erbilgin 2018; Mason et al. 2019; Moreira et al. 2015; Wainhouse et al. 2005). In overall, it seems that although favourable conditions might enhance both growth and absolute resin duct production, their production relative to growth, (e.g. resin duct density) decreases under such circumstances (Van Akker et al. 2004).

Water availability, in the form of variation in interannual precipitation, could be a key driver of the production of resin duct defences in conifers. According to the GDBe, drought might modulate growth-defence relationships by slowing tree growth but potentially enhancing resin duct production. Supporting this hypothesis, Rigling et al. (2003) demonstrated in a field experiment that irrigated *P. sylvestris* trees had lower resin duct density than control trees. On the other hand, various studies have shown that severe drought can also negatively impact resin duct production. Smaller axial resin ducts in the xylem of sugar pine (*P. lambertiana*) were associated with years when growth was reduced by higher water deficits (Slack et al. 2017). *Picea abies* trees in drier stands produced narrower radial resin ducts than trees in stands under higher precipitation (Rosner and Hannrup 2004). *Pinus edulis* trees growing under extreme drought treatments had a lower number, density and total area of axial resin ducts (Gaylord et al. 2013). According to the GDBe, it is likely that trade-offs emerge only at intermediate states of physiological stress (Gaylord et al. 2013; Herms and Mattson 1992; Sampedro et al. 2011). This could explain both negative and positive relationships between drought and defensive investment, depending on the extent to which drought limits growth rates more than differentiation processes. In environments where water stress exerts excessive physiological constraint, both growth and defence could be negatively affected.

Other climate factors can also affect resin duct production. High temperatures have been widely reported to enhance resin duct differentiation in various tissues in *Picea* (Wimmer and Grabner 1997; Wimmer and Grabner 2000) and *Pinus* species (Heijari et al. 2010; Hood et al. 2015; Kivimaenpaa et al. 2016; Rigling et al. 2003; Rodriguez-

Garcia et al. 2015; Saracino et al. 2017; Zamski 1972). High temperatures are known to enhance ethylene production, a phytohormone that causes a shift in cambial activity from tracheid production to production of resin duct epithelial cells (Franceschi et al. 2002; Hudgins and Franceschi 2004; Yamamoto and Kozlowski 1987). The positive relationship between temperature and increased resin duct differentiation may therefore be ultimately due to enhanced ethylene production.

Factors other than climate have been related to resin duct production. *Pinus ponderosa* trees that experienced a history of low-severity fire had more xylem resin ducts than trees growing in fire-supressed forests (Hood et al. 2015; Sparks et al. 2017). However, the opposite effect of fire on resin ducts was observed in *P. palustris* (Slack et al. 2016). Fires could affect tree defences not only by reducing tree density and thus competition for resources, but also by promoting TRD formation by direct injury (Arbellay et al. 2014). Low-severity fires therefore appear to have a positive effect on defences by inducing resin duct development, at least in environments where trees have evolved under both fire and herbivore pressure. In contrast, high-severity fires could negatively affect tree vigour, enhance tree mortality, and negatively affect tree defences. Finally, the extent to which positive biotic interactions determine defensive allocation and resin duct differentiation remains unclear. These effects could be direct, by modulating defensive signalling (Pozo and Azcón-Aguilar 2007), or indirect, such as those mediated by ectomycorrhizal associations affecting nutrient uptake and ultimately the growth-differentiation balance.

5. Within species genetic variation in resin duct characteristics

Resin duct characteristics display large intraspecific genetic variation among populations within species (Esteban et al. 2012; Martin et al. 2010; O'Neill et al. 2002; Vázquez-González et al. 2019; Zas et al. 2015). Population differentiation in defensive traits might occur from adaptation to both biotic and abiotic factors exerting selective pressures (Hahn et al. 2018; Hahn and Maron 2016; Maron et al. 2019). Particularly, resource availability is thought to drive variation in plant defences. The Resource Availability Hypothesis (RAH; Coley et al. 1985) states that under high resource availability, plants would tend to allocate more resource to growth than to defences. Conversely, in resource-poor environments, defensive production would be enhanced over growth, as the costs associated to replace the tissue lost by herbivory would be higher in such environments. This hypothesis is supported by studies addressing patterns of variation in defensive allocation among species (Endara and Coley 2011). It is not clear, however, how environmental factors might drive genetic differentiation in defensive investment among populations within species (Hahn and Maron 2016).

Genetic variation among populations in resin ducts characteristics has been associated with environmental gradients at the origin of those populations, specifically with climate conditions. For instance, a provenance study in British Columbia, Canada, revealed that the extant genetic variation in resin duct characteristics in *P. sitchensis* was explained by geographical and climate variables in the region of provenance (O'Neill et al. 2002). Similarly, genetic variation in allocation to resin ducts among populations of *P. pinaster* was found to be associated with climate gradients at the origin of populations (Vázquez-González et al. 2019). Both studies point to enhanced resin duct production in populations adapted to more limiting environments, supporting predictions of current hypothesis (e.g. GDBe, RAH) that predicts increased defensive investment under resource-poor conditions (Coley et al. 1985; Endara and Coley 2011;

Herms and Mattson 1992). Given the relevance of resin duct characteristics on tree resistance, previously summarized here (Box 1), genetic differentiation among populations in resin duct investment would be also expected to be associated with differential biotic pressures across a species distribution range. However, this hypothesis remains largely unexplored for this anatomical defensive trait.

The afore mentioned studies finding genetic clines associated with environmental gradients, suggest that adaptation to local environmental conditions might drive population differentiation in allocation to resin duct, highlighting its importance as defensive mechanism in conifers. However, among-population variation includes not only adaptive variation, but also neutral genetic variation caused by the specific demographic history of the species (González-Martínez et al. 2006; Grivet et al. 2010). Patterns of neutral variation within species can generate spurious correlations between defences and environmental gradients, resulting in misinterpretation of clinal patterns (López-Goldar et al. 2019; Vázquez-González et al. 2019). To reach a broad understanding on how adaptation drives intraspecific genetic variation in defensive traits, it is therefore necessary to accurately disentangle adaptive from neutral variation.

In addition to variation among populations within species, resin duct production has also been found to differ largely among families and genotypes in a number of conifer species (Alfaro et al. 2004; King et al. 2011; Moreira et al. 2012; Moreira et al. 2015; Rosner and Hannrup 2004). Furthermore, current evidence suggests that resin duct characteristics are heritable and subject to genetic control (Hannrup et al. 2004; Moreira et al. 2012; Rosner and Hannrup 2004; Westbrook et al. 2015). High intra-population genetic variation and moderate heritability values for resin duct characteristics pave the way for artificial selection and tree breeding aiming to enhance biotic resistance through increased production of resin-based defences. Moreover, Intra-population variation ultimately allows adaptation according to current selection pressures, being therefore especially relevant to forecast evolutionary responses of forest tree populations.

Genetic variation in plastic responses to environmental factors can also play a major role in modulating allocation to defences in plant species (Donaldson and Lindroth 2007; Wagner and Mitchell-Olds 2018). Genetic variation in plasticity is usually assessed as the effect of genotype by environment interactions (G×E) using replicated genetic trials, where the performance of different genotypes is compared across environments (Li et al. 2017). As far as we are aware, relatively few studies have assessed G×E effects on the expression of resin ducts, with contradictory results. For instance, G×E interactions were found to affect resin duct production in *P. taeda* (Westbrook et al. 2013), while the opposite was found in *P. abies* (Hannrup et al. 2004; Rosner and Hannrup 2004). Patterns of genetic variation in plastic traits conferring enhanced fitness will have immediate implications for forecasting the performance of forest tree populations exposed to changing environmental conditions (Valladares et al. 2014). Furthermore, G×E effects are especially relevant in the forestry industry, because they can lead to loss of heritability and genetic gain in functional traits (Li et al. 2017). Understanding this interactive source of variation is therefore crucial for developing breeding programmes to ensure durable, broad adaptation in the face of global change.

6. Dendrochronological analysis of resin ducts: Filling knowledge gaps in plant defence theory

Evidence suggests that resin duct traits are a valuable proxy of biotic resistance and useful metrics of defensive investment in conifers (Box 1). However, the literature on this topic is still narrow, both in coverage of tree species and number of studies.

Additionally, the wide variation in the methodology used to investigate resin duct metrics has led to high heterogeneity among results, making it difficult to reach clear conclusions. A unified methodology for adding resin duct metrics to dendrological analysis is needed to elucidate the relevance of resin-based defences in biotic resistance. Four main ways in which tree-ring analysis can provide a deeper understanding of resin-based defences in conifers are: (1) retrospective quantification of resin ducts and their association with resistance; (2) evaluation of time-varying trade-offs between growth and defence; (3) assessment of the influence of climate variation on inter-annual resin duct plasticity; and finally (4) assessment of genetic variation in the climatic modulation of resin-based defences production.

Firstly, adding resin duct metrics to tree-ring analysis would be very useful in retrospectively exploring the association between resin ducts and biotic resistance to insect herbivores and pathogens. Wood cores can be easily sampled in natural stands to explore the resin duct system of trees suffering pest outbreaks. Comparisons of dead and living trees are possible because wood cores can be crossdated (i.e., growth ring patterns can be matched among samples) and the exact year of tree death can be determined (Fritts 1976). Resin duct characteristics (e.g., area and abundance) can easily be measured year by year in the xylem tree rings developed before death and compared to those produced by living trees in the same time frame (Ferrenberg et al. 2014; Hood et al. 2015; Kane and Kolb 2010). This approach could be applied to wood cores that have been sampled for other purposes, representing an ample dataset and allowing comparison of results over wide spatial and temporal scales.

Secondly, linking dendrochronology and genetic trials (e.g., common garden trials) will provide an understanding of patterns of growth-defence strategies between and within species. The RAH posits that interspecific divergence in defensive traits may be explained by the trade-offs between growth and defences (Endara and Coley 2011). However it is still to know whether such compromises between both life functions also drive patterns of variation in defensive allocation within species (*i.e.,* across populations or families within populations) (Hahn et al. 2018; Hahn and Maron 2016). To date, studies exploring intraspecific variation of trade-offs between growth and defences have yielded contrasting results (Hahn and Maron 2016). In this sense, radial growth and resin duct measurements obtained from annual growth rings can provide relevant information about intraspecific growth-defence relationships. Correlations between annual investment in growth and defences can be tested to assess potential trade-offs and their variation across ontogenetic stages (Barton and Boege 2017; Boege and Marquis 2005; Ferrenberg et al. 2014; Hood and Sala 2015).

Thirdly, through tree-ring analysis, year-to-year variation in defence traits can be linked to climate (Fritts 1976). This is easily performed by computing response functions (Briffa and Cook 1990) for resin duct measurements, a method widely used in dendrochronology to quantify the effect of seasonal or monthly climate variables (e.g., temperature, precipitation, PET, etc.) on the yearly expression of dendrochronological traits (Box 2). Response functions can be used to establish the period of the growing season when certain climate variables limit the development of xylem anatomy. Several studies have documented the sensitivity of resin duct traits to temperature and precipitation variation using this approach. For instance, a number of studies showed that high summer temperature promoted annual resin duct differentiation in different *Picea* and *Pine* species (Rigling et al. 2003; Saracino et al. 2017; Wimmer and Grabner 1997). Similarly, interannual variation in the size of xylem resin duct of *Pinus lambertiana* showed a negative association with water deficit (Slack *et al.* 2017). Moreover, such approach allows to detect lagged effects (i.e., the effect of past climate conditions on current development rates). Particular climate conditions in the previous year (e.g. precipitation) can affect photosynthetic rates and/or resource availability and/or acquisition in the current growth year (Zweifel and Sterck 2018). Thus, lagged climate effect can significantly influence interannual variation in allocation priorities between defence investment and other life functions.

Fourthly, current research has shown that dendrochronological data gathered from common gardens can help to elucidate the patterns of intraspecific genetic variation in plasticity to climate (Housset et al. 2018). Genetic variation in climatic responses (i.e. response functions) can ultimately be considered a G×E effect, a little explored source of variation in tree defensive traits. G×E effects are usually assessed in replicated genetic trials that allow disentanglement of environmental plasticity from genetic variation, and therefore their interaction. However, well-replicated genetic trials in sites with contrasting environmental conditions are not always available since such trials are difficult and costly to establish and maintain. Retrospective analysis of resin ducts in tree-ring samples from individuals in long-term common gardens already exposed to climate change will make it possible to explore differences between genotypes in the climate sensitivity of resin-based defences.

In overall, the application of dendrochronological methods to quantify resin duct production in the xylem tree rings will enable to account for the temporal variation in the production of resin-based defences along the lifespan of trees. This is especially relevant since defence production is usually quantified as punctual snapshots, introducing a significant source of bias across studies, as investment in defences is known to vary during the lifespan of long-lived species (Barton and Koricheva 2010). Applying this methodological approach to mature trees in long-term genetic trials will therefore lead to a robust assessment of the interactive effects of genetic and plastic factors driving allocation to defences in conifer tree species.

7. Conclusions

The range and host expansion of native and invasive insect herbivores and pathogen species due to global climate change could pose a major threat to many forest ecosystems in the near future (Erbilgin 2019; Raffa et al. 2008). It is therefore especially important to understand patterns of variation in functional defensive traits that confer resistance to biotic threats. This knowledge will assist in forecasting adaptation and survival of forest tree populations. We reviewed here the evidences suggesting that higher resin duct production in conifer trees enhances biotic resistance. However, to understand the underlying cause-effect mechanisms, further studies are needed on the role and implications of resin ducts and their inducibility in conifer resistance. Retrospective tree-ring analysis of resin ducts is proposed as a promising methodology to investigate the relevance of resin based-defences in conifer resistance and resilience. Tree-ring analysis of resin duct production may help fill current knowledge gaps in plant defence research and shed light on the drivers of growth-defence strategies in long-lived conifer trees. This approach will therefore provide critical information for inferring the micro-evolutionary patterns of resin-based defences and anticipating long-term, large-scale tree responses and survival patterns in the face of global change pressures.

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List of figures & boxes

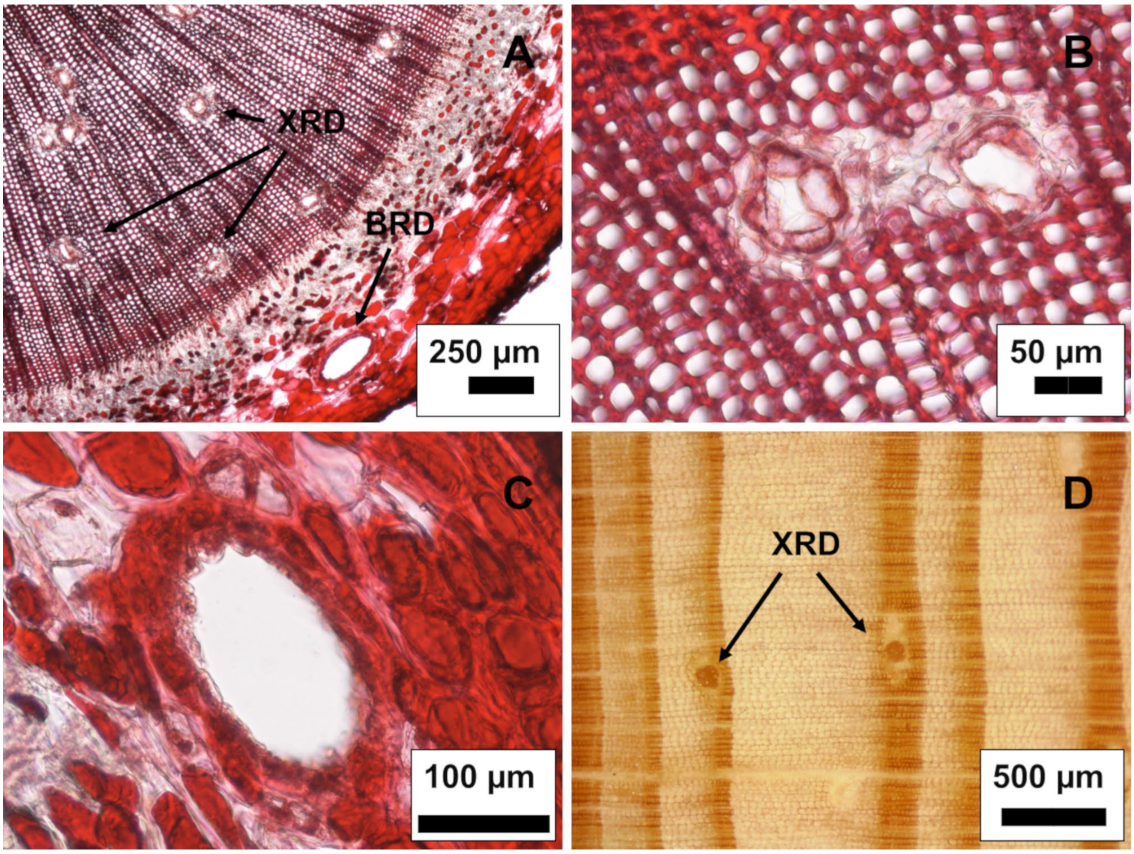
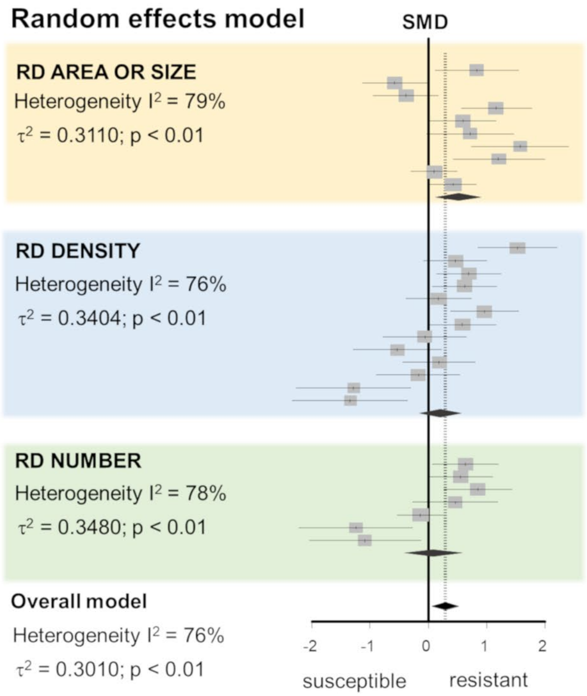


Figure 1 Transverse section of young *Pinus pinaster* stem **(A)** showing axial resin ducts in xylem (XRD) and cortex within the living bark (BRD). Xylem resin ducts **(B)** and cortex resin duct **(C)** with epithelial cells surrounding resin duct lumen. Xylem axial resin ducts (XRD) in annual growth rings of adult *Pinus ponderosa* tree **(D)**.



Methods: We included eight independent studies from a systematic review process reporting results for differences in resin duct characteristics between groups of resistant and susceptible trees to various beetle species. We applied a random effects model to compute the mean effect size (standardised mean difference between groups - SMD) and confidence intervals using the *meta* package in R (Schwarzer, 2007).

Main results: We found a positive and significant mean effect when considering all metrics together (SDM = 0.29; $p < 0.05$), indicating that resistant trees allocate more than susceptible trees to resin duct production. However, when the analysis was performed by categories of metrics applied, we found a significant mean effect only for metrics related to the area of resin ducts (SDM = 0.52; $p < 0.05$). The high heterogeneity between studies (I^2 = 76–79 %; τ^2 =0.30–0.35; $p < 0.01$) is probably due to wide variability in methodology and experimentation.

Conclusions: Despite the reported evidence of resin ducts as important traits in conifer resistance, the few studies and high heterogeneity among them limit our ability to perform a quantitative synthesis to assess the effect of this trait on tree resistance. The reported results suggest that metrics related to area are better resistance indicators than the number or density of resin ducts. However, more research and a unified methodology for reporting resin duct metrics is needed for accurate conclusions based on quantitative evidence.

Box 1 Random effects meta-analysis assessing the effect size (SMD – standardised mean difference) of resin duct characteristics on conifer resistance to species of phytophagous beetles. Random effects computed for each study case belonging to each individual study (± 95% confidence interval) are represented as grey squares with lines representing 95% confidence intervals. Black diamonds represent mean effects for groups of studies. The vertical dotted line represents the mean effect of resin duct production on biotic resistance computed from the overall model, considering all study cases together. Supplementary material (Table S1) gathers detailed information on the eight independent studies included in the analysis, which tested the differences in resin duct characteristics between resistant and susceptible trees.

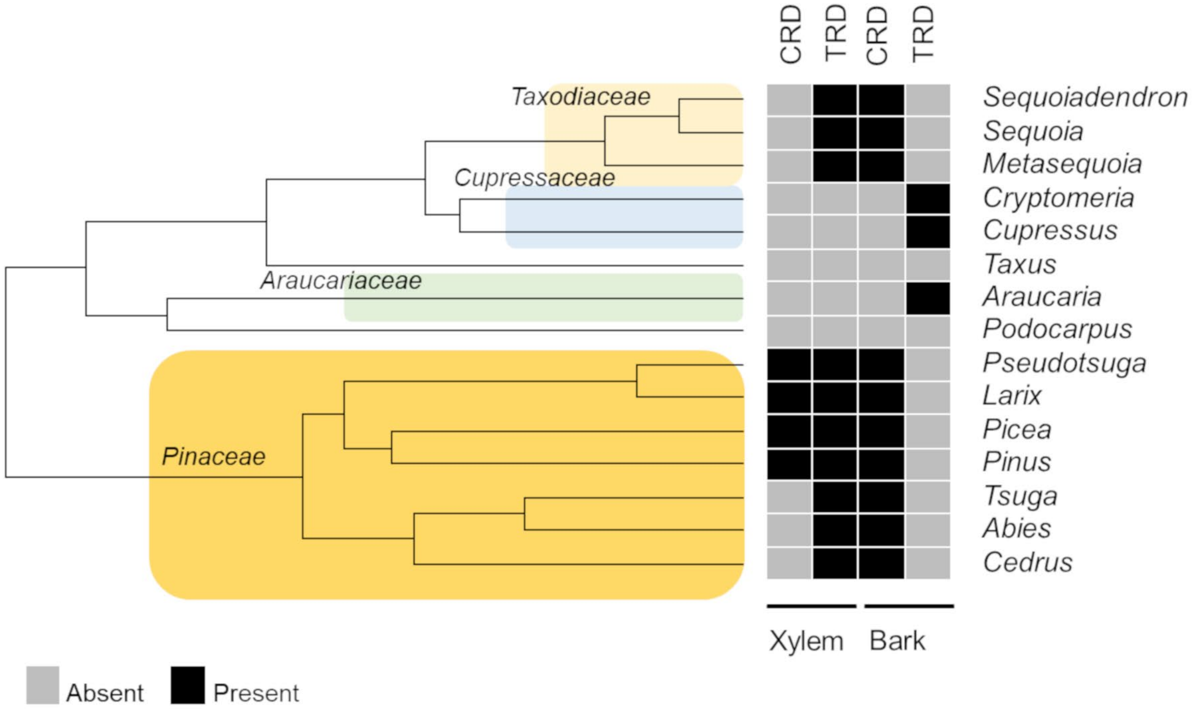
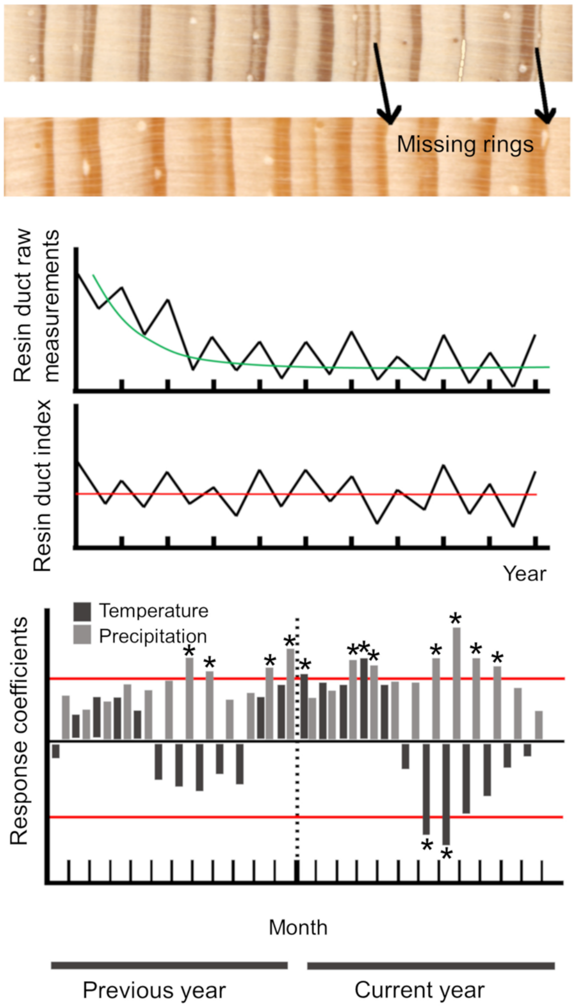


Figure 2 Occurrence of constitutive (CRD) and traumatic (TRD) resin ducts in the xylem and bark of conifer genera according to Hudgins *et al.* (2003, 2004) and the most recent phylogenetic tree from Leslie *et al.* (2018). CRD are those that are always present as a baseline level, while TRD refer to resin ducts produced only after biotic or mechanical damage.



1) *Crossdating*

Inter-annual climate variability is recorded in the pattern of wide and narrow rings (Fritts, 2012). This pattern allows us to synchronously match the ring widths of all samples within and across trees in the same stand to account for missing rings and accurately crossdate the samples.

2) *Standardisation and master chronology.*

To assess the influence of climate on any dendrochronological trait, it is usually necessary to remove variability due to other factors and the age-related trends that characterise tree ring features (Hughes et al., 2010; Meinzer et al., 2011). After detrending each series and removing the undesired variability, a standardised master chronology showing the annual index of the mean population value for a given trait can be used for further climate analysis.

3) *Response functions.*

Response functions are used in dendroclimatology to assess the influence of interannual climate variability on dendrochronological traits. They are bootstrapped regression models in which climate variables (precipitation, temperature, PET etc.) are the predictors and tree-ring variables are the response. The aim is typically to determine which climate variables and time of year are most likely limiting a process. For this reason, monthly climate variables from the growing season and the previous year are included in the analysis to assess the existence of lag effects. From this we obtain a set of regression coefficients showing the effect of each monthly climate variable on the relevant dendrological trait after accounting for collinearity between explanatory variables.

Box 2 Description of main standard dendrochronological procedures to assess climate signals in tree-ring variables, including sample crossdating, raw data standardisation, calculation of master chronologies, and assessment of climate response functions.