

INTRASPECIFIC GENETIC VARIATION IN DEFENSIVE INVESTMENT OF A MODEL MEDITERRANEAN PINE: A DENDROECOLOGICAL ANALYSIS OF RESIN DUCTS

Title presented at workshop:

INTRASPECIFIC GENETIC VARIATION IN DEFENSIVE INVESTMENT VARIES ALONG THE ONTOGENY OF A MODEL MEDITERRANEAN PINE: A DENDROECOLOGICAL ANALYSIS OF RESIN DUCTS

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Conifers, the largest and longest-lived organisms on the planet, have evolved a sophisticated defensive system which has contributed to their colonization of diverse habitats (Franceschi et al. 2005). The defensive system of conifer trees heavily relies on the production of oleoresin, a terpenoid-based viscous fluid which is toxic for insect herbivores and pathogens (Phillips and Croteau 1999). Oleoresin is produced and accumulated in structures of different specialization degree, from single cells to a complex network of resin ducts in some genera such as *Picea* or *Pinus* (Bannan 1936, Wu and Hu 1997). Resin ducts protect the tree against pests by flushing out resin upon wounding, but also by acting as a mechanical barrier that prevents invasion by tree enemies (Franceschi et al. 2005). Importantly, resin duct characteristics (e.g., abundance and size) have been reported to be strongly associated with tree resistance against insect pests and pathogens in several conifer species. For instance, previous studies in different *Pinus* species have shown trees that survived after bark beetle attacks had more and/or larger xylem resin ducts than trees that died (Ferrenberg et al. 2014, Hood et al. 2015, Kane and Kolb 2010).

As other plant defensive traits, genetic and environmental factors mediate resin duct production. On the one hand, genetic variation in resin duct production has been reported in several conifer species (Esteban et al. 2012, Martin et al. 2010, Zas et al. 2015). Such differences among populations are expected to be, at least partially,

the result of local adaptation. Supporting this idea, Esteban et al. (2012) found that geographic and climatic variables at population origin explained intraspecific genetic variation in resin duct characteristics in *P. nigra*. On the other hand, environmental conditions that limit plant growth may enhance resource allocation to defences in trees due to the existence of trade-offs between both plant functions (Endara and Coley 2011, Herms and Mattson 1992). In this sense, previous studies have shown that trees growing under low resource (e.g., nutrients, light, water) conditions tend to grow less and increase their resin duct production (Moreira et al. 2015, Moreira et al. 2008). In addition to the individual effects of plant genotype (G) and environment (E), there may also be interactive effects of these two major sources of variation (G×E interactions). An increasing number of studies have addressed G×E effects on resin duct production with contrasting results (Hannrup et al. 2004, Moreira et al. 2015, Rosner and Hannrup 2004, Westbrook et al. 2013). Such interactions may have important implications for tree breeding, such as unstable selection for resistance when considering global warming scenarios. More research is thus needed to derive general patterns.

Plant defence allocation can be also determined by plant ontogeny (Barton and Koricheva 2010, Boege and Marquis 2005). In particular, previous studies have reported both positive and negative trends in defensive investment with tree age (Boege and Marquis 2005). However, none of

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these previous studies have investigated the effect of plant ontogeny on resin duct production. Axial resin ducts are recorded in growth rings, representing thus an annually resolved archive of plant defences. This special feature allows their quantification by standard dendrochronological procedures (Fritts 2012) to retrospectively assess the existence of age-related effects on defensive investment.

In this study, we aimed at disentangling the independent and interactive effects of plant genotype and environment on annual resin duct production across the last 30 years of growth of a model Mediterranean pine (*P. pinaster*). Additionally, we explored the adaptive origin of this anatomical defensive trait by testing whether climatic gradients at the population origin explain intraspecific genetic variation in resin duct production.

We sampled *P. pinaster* trees from two replicated genetic trials established in 1967 located in two different sites (Cabañeros and Riofrío). Cabañeros was located on a plain top-hill while Riofrío was on a mid-hill with irregular topography and stonier soil. These contrasting abiotic conditions caused that tree growth was significantly higher in Cabañeros than in Riofrío. In each genetic trial, we sampled stem cores from trees belonging to nine different populations (N = 10 trees per population) covering most of the natural distribution of this species. Stem cores were extracted at breast height with a Pressler borer, obtaining four cores per tree, each from a cardinal point. Wood cores were progressively sanded and visually crossdated following standard dendrochronological procedures (Fritts 2012). We measured tree-ring width and statistically validated the crossdating applying a cross-correlation analysis in COFECHA software (Grissino-Mayer 2001). We counted axial resin ducts (RD) in each annual growth ring in the latewood (LW), the earlywood (EW) and the total ring (TR) for the last 30 years of growth. Annual RD density was calculated as the number of RD per squared millimetre of wood and year (RD mm⁻² yr⁻¹). We ran linear mixed models to test for the effects of site, population and their interaction (all fixed factors) on annual RD

density. To test for ontogenetic effects on resin duct production during three 10-year periods along the tree lifespan (10–20, 20–30, 30–40 years old), we also included the effect of year as a fixed categorical factor in the model. Block within site and population by block within site were included as random factors. Finally, to assess the existence of climatic clines explaining among population variation, we performed correlations between climatic variables at the population origin and annual RD density. The climatic data was obtained according to a climatic model detailed in Gonzalo (2008). All statistical procedures were performed in R software version 3.4.3 (RStudio Team 2016).

Our results showed no significant differences in mean annual RD density in the TR among pine populations and sites (table 1). However, when we separately analysed EW and LW, we found significant variation in annual RD density in the EW and LW among pine populations (table 1). A number of studies have also reported intraspecific genetic variation in RD production among populations in different *Pinus* species (Esteban et al. 2012, Martin et al. 2010, Zas et al. 2015). However, none of the previous studies have measured RD production separately in both ring compartments. Similarly, we found significant differences in RD density in the EW across sites (table 1), being such RD density markedly higher in the low quality site (Riofrío). These findings agree with a long-standing ecological paradigm which predicts that trees growing under poor-resource environments tend to invest less in growth and more in defence (Endara and Coley 2011, Herms and Mattson 1992).

Our results also showed that the G×E interaction did not significantly affect RD density (fig. 1, table 1), indicating that the relative performance of the populations regarding resin duct differentiation is stable across environments. Our results agree with those reported previously in *Picea abies* under drought conditions (Hannrup et al. 2004, Rosner and Hannrup 2004). Contrary to our findings, Moreira et al. (2015) found that *P. pinaster* genotypes markedly differed in the phenotypic plasticity of resin duct production in response to soil nutrient availability (i.e., a

Table 1—Effect of Population (Pop), site (S), year and their interactions on *Pinus pinaster* resin duct (RD) density (RD mm⁻² yr⁻¹) in total ring, the earlywood and the latewood, F-ratio and associated p-values are shown

	Total ring		Earlywood		Latewood	
	F	p-value	F	p-value	F	p-value
Pop	2.1	0.06	2.9	0.011	4.40	< 0.01
S	4.2	0.09	18.8	< 0.01	5.14	0.06
Year	39.1	< 0.01	74.2	< 0.01	68.49	< 0.01
Pop × S	0.7	0.72	0.7	0.71	0.55	0.81
Pop × Year	2.6	< 0.01	1.4	< 0.01	1.50	0.01
S × Year	10.7	< 0.01	12.6	< 0.01	10.93	< 0.01

Linear mixed model results. Significant p-values are indicated in bold.

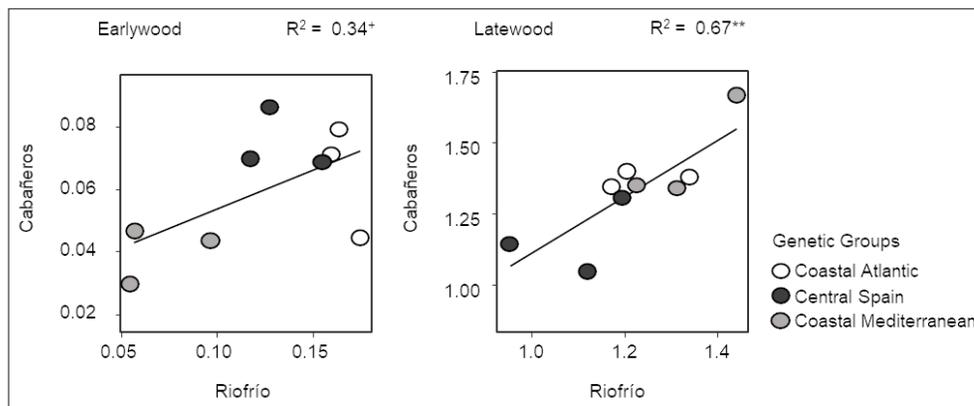


Figure 1—Relationship between annual RD density in earlywood and latewood in both common gardens (Cabañeros and Riofrío). Each dot represents a *Pinus pinaster* population (N = 9). R-squared coefficients (R²) are shown. Asterisks indicate significant (*P < 0.1, *P < 0.05, **P < 0.01) P-values.

significant G×E interaction). However, these contrasting findings could be explained by ontogenic effects. In particular, these authors measured resin duct production in juvenile trees (Moreira et al. 2015), whereas we focused our study in adult (reproductive) trees. Supporting this idea, we found that plant ontogeny significantly affected resin duct production (significant effect of year; table 1), where RD density in the EW was significantly greater at younger stages and RD density in LW was significantly greater at older stages (fig. 2). Previous studies have commonly reported increased resin production with tree age in other tree species (Bryant and Julkunen-Tiitto 1995), but to our best knowledge this is the first study reporting plant ontogenetic effects on resin duct production.

Finally, our results showed that local climate at the population origin significantly explained resin duct differences among populations. Specifically,

RD density in EW was negatively correlated with summer temperature, whereas RD density in LW was positively correlated with spring temperature ($r = -0.89$ and $r = 0.72$ respectively; $p < 0.05$). Accordingly, our results suggest that pine trees from populations at warmer regions produce more RD in the late growing season, while resin duct development takes place earlier in trees from populations at colder regions. Supporting our findings, Esteban et al. (2012) found that temperature at the population origin negatively affected resin duct investment among *P. nigra* populations.

Despite the implications of G×E interactions for tree breeding, to date most breeding programs have been developed based on genetic or environmental factors, disregarding the effect of their interaction. Our results indicate that, despite we observed significant effects of plant ontogeny and environment on resin duct production,

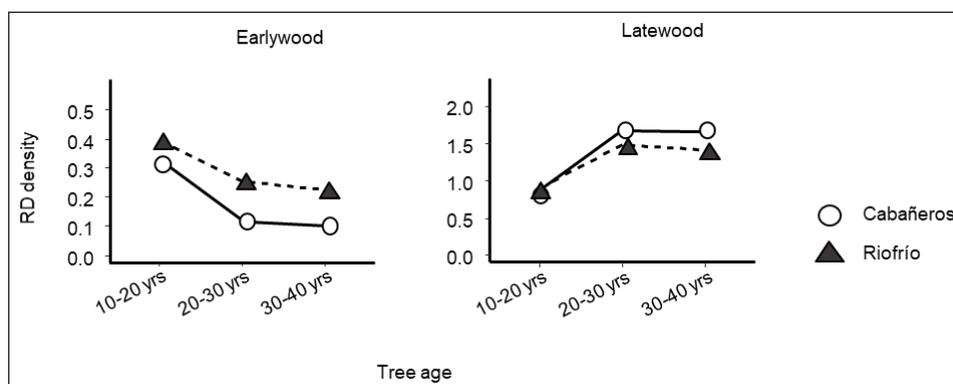


Figure 2—Mean annual RD density (RD mm² yr⁻¹) in the earlywood and latewood of *Pinus pinaster* trees in both common gardens (white dots for Cabañeros and black dots for Riofrío). Values represent mean annual RD density in three different 10-year periods along tree lifespan.

intraspecific genetic variation in this anatomical defence can be exploited in breeding programs for stable resistance, due to the absence of G×E effects. Moreover, the contribution of climatic clines explaining among population differentiation highlights the importance and putatively adaptive value of this trait. However, other processes that generate non-adaptive intraspecific genetic variation (e.g., genetic drift and demographic or migratory patterns) can also contribute to explain phenotypic differences among populations (Grivet et al. 2010). For this reason, in order to fully understand the adaptive value of this anatomical defensive trait, further work should also take into account the effect of neutral genetic variation.

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