Soil Fertility and the Emergence of Genetic Trade-Offs related to Defensive Strategies in Pine Trees
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Pine trees are big, apparent, long lived plants that have to cope against a vast array of biological enemies and lots of herbivory events during their lifespan. They have several defensive traits against insect herbivores, mainly carbon-based chemical defences such as oleoresin (terpenoids) and phenolic compounds. Plant genotypes can express preformed defensive phenotypes commonly called “constitutive defences”, however the defensive function is a plastic trait, and plants can increase the investments in defences when receiving a signal of attack by herbivores or pathogens. This newly formed or mobilized defences to the site of attack are called induced defences. Induced defences have been considered a “cheaper” strategy than constitutive defences since the cost is realized only when required. As other conifer species, pine species usually show large distribution ranges across heterogeneous soil fertility and climatic conditions, and they have to continuously readjust the allocation of the limited resources to their live functions according to the changing environmental circumstances. As resources are limited and growth and defences costly, it would be expected the existence of trade-offs among those functions, and even between defensive traits and strategies. They have been many times suggested in the literature and some times reported for angiosperms, but not yet in conifers. The aim of this paper is to explore the existence of these trade-offs in two southern pine tree species, *Pinus pinaster* (Pp) and *P. radiata* (Pr), and to explore whether nutrient availability can modify the emergence of those negative genetic correlations.

We performed three independent greenhouse experiments with 1-2 yrs old seedlings of both species belonging to open-pollinated (half-sib) families. In exp#1 (Pp) and exp#2 (Pr) we explored possible negative genetic correlations among growth, concentration of constitutive and methyl-jasmonate induced defences (resin content in the stem and total phenolics and condensed tannins in the needles), and effective antiherbivore resistance by means of in vivo bioassays with the large pine weevil *Hylobius abietis* and the pine processionary caterpillar *Thaumetopoea pityocampa*. In exp#3 we repeated the experiment for Pp using more families, a bigger sample size, and only chemical defences for confirming results, and we included to treatments of phosphorus availability (P-limited and complete fertilization).

We found (i) a negative genetic correlation ($R^2 = 0.17; P<0.05$) between growth rates and constitutive concentration of resin compounds but no with those of phenolics, and only under P-availability for Pp; (ii) no evidence of negative genetic correlations between chemical defences; (iii) significant negative genetic correlations between inducibility (the ability of increase the concentration of a given chemical defence for a given family) of phenolics and tannins (but not resin) and growth potential for Pp, but only under low nutrient availability; and, as a main result, strong negative genetic correlations between constitutive defences (or resistance against the insect in the in vivo bioassays) and the capability of express induced defences (or induced resistance); this significant constitutive-induced trade-offs were consistently found in both species for all the assayed traits with $R^2$ of about 0.60, 0.40, 0.30, 0.60 and 0.65 (all $P<0.01$) for resin, phenolics, tannins, damage by the weevil and damage by the caterpillar (all confirmed as non-spurious with MonteCarlo analyses). The trade-offs were context-dependent and did not emerge when plants were supplied with plenty of nutrients. These negative genetic correlations, within each kind of chemical defence, between the constitutive and inducible defences, at the physiological and functional level, constitute strong experimental evidences that this trade-off exists in pine trees.