

Pine tree chemical defensive strategies and the evolutionary trade-off between induced and constitutive defenses

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Induced defences have been considered less expensive than constitutive preformed defences since the cost is realized only when required. But induced chemical resistance has some associate costs due to possible losses in term of fitness derived from the time required for their synthesis or re-allocation.

In pine trees, resin and phenolic compounds are the major carbon-based chemical defences. As both are linked by common carbon sources, it could be expected that they would be not maximized at the time. Furthermore, preformed defences and plastic defensive responses differ in their benefits in terms of fitness for long-lived plants. Genotypes constitutively well-defended are expected to gain little boosting their defences after damage to be protected against subsequent attacks; conversely genotypes with low constitutive defences are likely to be under the pressure for expressing effective inducible responses. The existence of these evolutionary conflicts has been many times suggested in the literature and sometimes reported for angiosperms, but rarely in conifers and not yet in pine trees. The aim of this paper was to explore the existence of this trade-off in the maritime pine (*Pinus pinaster* Ait.).

We grew pinions from 18 open pollinated mother trees from Galicia (NW Spain) in a greenhouse under controlled conditions. After two years, half of the pine seedlings were sprayed with MJ (100 mM in 0.1% Tween-20) and the remaining acting as controls (0.1 % Tween-20). We analyzed the secondary chemistry (total polyphenolics in the needles and the gravimetric resin content in the stem) and we performed an *in vivo* feeding bioassay with a generalist insect herbivore (the pine weevil *Hylobius abietis* L., a phloem-feeder herbivore) to check how the expressed defences reflected the ability to avoid the attack.

We found negative genetic correlations between the constitutive and inducible defences, measurable in the physiological defensive traits and in the effectiveness of the defensive compounds against the herbivory insect, which constitute strong experimental evidences that this genetic trade-off exists in this pine tree. We explored that these negative correlations were not spurious using a Monte Carlo iteration procedure. We confirmed strong negative genetic correlations between induced and constitutive levels of resin content ($R^2 = 0.72$), and also for the realized damage by the weevil ($R^2 = 0.71$), but the relationship observed for total polyphenolics ($R^2 = 0.48$) appeared to be spurious. The analyzed genetic entries of *P. pinaster* showed a continuous range of defensive strategies from families with reduced expression of constitutive defences which showed the ability of dramatically increase their defences after induction signals, and families with strong expression of constitutive defences which are poorly capable of increase their defences after attack.