Experimental evidences of a genetic trade-off between induce and constitutive defenses in a pine species: secondary chemistry and effectiveness on the realized damage

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Secondary metabolism is costly for the plant, and presenting effective constitutive defense levels and the ability of expressing efficient inducible defenses by a plant are two resource related attributes that are not likely to be maximized at the time. Induced defenses have been considered "cheaper" than constitutive defenses since the cost is realized only when required. Moreover genotypes constitutively well-defended are expected to gain little boosting their defenses after damage to be protected against subsequent attacks; conversely genotypes with low constitutive defenses are likely to be under the pressure of being able to express effective inducible responses. A negative, non spurious, correlation between constitutive and inducible defenses illustrates this classical trade-off. It have been many times suggested in the literature and some times reported for angiosperms, but rarely in conifers and not yet in pine trees. The aim of this paper is to explore the existence of this trade-off in the pine tree *Pinus pinaster*. We analyzed the secondary chemistry of methyl jasmonate (MJ) induced and control pine seedlings belonging to 18 genetic entries and we performed a *in vivo* feeding bioassay with a generalist insect herbivore to check how the expressed defenses reflected the ability to avoid the attack. We found a negative genetic correlation between the constitutive and inducible defenses, at the physiological and functional level, which constitute strong experimental evidences that this genetic trade-off exists in this pine tree.

We grew pinions from 18 open pollinated mother trees from Galicia (NW Spain) in a greenhouse under controlled conditions. Sample size was 6 half-sibs for each genetic entry. After two years, half of the pine seedlings were sprayed with MJ (25 mM in 0.1% Tween 20) and the remaining acting as controls (0.1 % Tween 20). Statistical design was a split plot, with 3 blocks, 2 MJ treatments and 18 families. Sixty days after induction we performed a bioassay exposing a part of the stem to experimental attack by the pine weevil *Hylobius abietis*, a phloem herbivore. We labelled a control part without insects in a more basal position. After 48 h we harvest the pines for measuring the debarked area consumed by the weevil in the experimental section, and measuring total phenolics in the needles and the gravimetric resin content in the phloem and of the experimental and control section of each tree.

We explored the existence of trade-offs regressing the difference in mean resistance levels between experimentally MJ induced individuals and control individuals from a given family (induced - control), against the family means of control treatment. The first variable indicates the potential to express induced resistance in each family, and the second one the actual level of constitutive resistance. In order to avoid the common mathematical problems leading to spurious correlations when comparing a difference against one of the terms, we checked whether the regression of the induced response against the controls had a slope different from 1. Using this metric, we identified strong negative genetic correlations between induced and constitutive levels in total polyphenolics compounds (R^2 = 0.48), resin content (R^2 = 0.72), and also for the realized damage by the weevil (R^2 = 0.71). All relationships were significant at $P < 0.001$. Thus the results evidenced this classical trade-off in *P. pinaster*, measurable in the physiological defensive traits and in the effectiveness of the defensive compounds against the insect herbivore. We found a range of strategies within the genetic entries o *P. pinaster* from families with reduced expression of constitutive defenses which showed the possibility of dramatically increase their defenses after induction signals, and families with strong expression of constitutive defenses which are poorly capable of increase their defenses after attack.