Trade-offs between induced and constitutive resistance in two pine species: secondary chemistry, effective antiherbivore-resistance and effect of nutrient availability.

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During millions of years of co-existence with herbivores, conifer trees have evolved effective traits and strategies for defending themselves. Morphological and chemical defences present in trees have been traditionally classified as either constitutive, the preformed defensive levels always expressed in the plants, and inducible, plastic defences, synthesized or mobilized in response to plant injury or other cues or herbivory risk. Preformed defences and plastic defensive responses differ in their benefits in terms of fitness for long-lived plants. Induced defences are considered to be less expensive than constitutive preformed defenses since the cost is realized only when required. But induced chemical resistance also have disadvantages derived from the time required for their synthesis or re-allocation. Plant defence theory predicts that, as secondary metabolism is costly for the plant, presenting effective levels of constitutive defences and the ability of expressing efficient inducible defences by a plant are two resource-related attributes that are not likely to be maximized at the time. Furthermore, selective pressure favoring the expression of induced responses is likely to be lower in those lineages well defended constitutively, as they would be less subjected to herbivore attacks. 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, genetic correlation between constitutive and inducible defenses illustrates this classical trade-off. 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. However, the emergence of this genetic constrains could be hidden by environmental factors affecting growth potential, such as nutrient availability.

In this paper we present the results from three independent experiments aimed to explore the existence of this trade-off in two year old seedlings of Maritime pine (Pinus pinaster) and Monterey pine (P. radiata), native from similar climate regions in Europe and North America. We used 22 mM methyl-jasmonate (MJ), a fitohormone involved in the biosynthetic pathways of chemical defences, to induce pine defensive responses. In the first experiment we analyzed the secondary chemistry of constitutive (control) and MJ-induced pine seedlings belonging to 18 half-sib families of P. pinaster from the Atlantic population of Galicia (NW Spain). One month after induction, we performed an in vivo feeding bioassay with a generalist insect herbivore (the large pine weevil, Hyllobius abietis) to check how the expressed defenses reflected the ability to resist the attack. In the second experiment we reproduced the same design with 34 half-sib families belonging to P. radiata. And in the third experiment we analyzed this trade-off in a wider collection of P. pinaster genetic entries (33 half-sib families) grew either in low or in high phosphorus availability, to test if this trade-off could be dependent on environmental context. 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. Correlations were checked not to be spurious by means of Monte-Carlo analysis.

We identified, in both species, strong negative genetic correlations between induced and constitutive concentrations of total polyphenolics, resin content and also for the effective resistance against weevil damage, with $R^2$ ranging from 0.38 to 0.80 (all relationships significant at $P < 0.001$), which constitute strong experimental evidences that this genetic trade-off exists in this pine tree. All the three experiments confirmed the existence of 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. Besides, in the third experiment, Monte-Carlo only confirmed as non-spurious those negative genetic correlations observed under phosphorus limitation, while those under complete fertilization appeared to be likely spurious. Thus, in P. pinaster, this trade-off only emerged in circumstances of nutrient limitation, but it is not measurable when plants are plenty of nutrients. We found, within the studied populations, a range of strategies from families with reduced expression of constitutive defenses which exhibited the ability of increase dramatically their defenses after induction signals, and families with a strong expression of constitutive defenses which are poorly capable of increase their defenses after attack.