Physiological trade-offs in the complexity of pine tree defensive chemistry
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[Invited commentary on Villari et al. (2014) Testing phenotypic trade-offs in the chemical defence strategy of Scots pine under growth-limiting field conditions. Tree Physiol 34: 919-930]

Like all organisms on Earth, trees must finely tune the relative allocation of resources to their living functions (namely growth, maintenance, defence and reproduction), seeking to optimize the costs and benefits (Bazzaz et al. 1987). As resources are limited and those allocated to one trait cannot be allocated to another, conflicts in resource allocation may result in trade-offs among different functions or traits (Agrawal et al. 2010). Such trade-offs might emerge as negative phenotypic correlations between pairs of traits with a shared source (reviewed by Saeki et al. 2014). Thus, patterns of phenotypic covariation (i.e. individual-based correlations) between traits help reveal possible conflicts in resource allocation and shared regulatory processes. One example in tree ecology and physiology is the relative allocation of resources to chemical defences (e.g., Keinanen et al. 1999, Koricheva et al. 2004, Donaldson et al. 2006, Agrawal 2011). Because of their life-history characteristics (such as being long-lived, large and forming extensive and stable populations) trees usually support a particularly diverse, extensive and temporally variable community of herbivores and pathogens. The selective pressure imposed by their antagonists has led to the evolution of effective resistance mechanisms, which include both constitutive and inducible defences (Zangerl and Bazzaz 1992). Constitutive defences, which are permanently expressed irrespective of the incidence of herbivores and pathogens, represent the first line of resistance. By contrast, induced resistance traits are activated, synthesized or mobilized in response to biotic challenges or cues of biotic damage. Plant resistance based on inducible defences, although energy saving, is a risky strategy as its benefits are based on the reliable identification of biotic cues (Karban 2011). Moreover, a plant could remain vulnerable for a period of time while induced defences are not activated or synthesized (e.g., Gómez et al. 2010). Therefore, plants need to combine a defensive system based on constitutive defences (which are omnipresent but have high associated costs) with induced defences (where the delay in activation is compensated for by the reduced cost associated with its production). The overlap of both strategies in space and time is assumed to be crucial for an efficient defence strategy (Cipollini and Heil 2010). Although there is an increasing body of theoretical predictions about this framework, little is known about the phenotypic integration of defensive investment in long-lived plants, whose life history determinants (e.g. maintenance costs, delayed reproduction, multiple reproductive events and extent of life cycles) largely differ from those of herbaceous and annual plants examined to date.

In this issue Villari et al. (2014) present a case study of the defence allocation patterns in pine trees. Constitutive and inducible defensive chemistry in pine trees is based on high concentrations of a variable array of carbon-based secondary compounds of a diverse chemical nature, namely, terpenoids and phenolic compounds (Krokene et al. 2003, Mumm and Hilker 2006, Keeling and Bolhman 2006). Villari et al. challenged individuals of a mature Scots pine alpine population growing under extreme environmental stress with bark plugs inoculated with two fungi associated with pine bark beetles (see Villari et al., 2012). Three weeks after inoculation, samples of bark and phloem tissues of induced and control trees were subjected to metabolite profiling of terpenoids and phenolics. The authors looked for phenotypic, individual-based correlations that provide information about possible resource-derived trade-offs between investment in secondary chemistry and growth rates, between different chemical compounds and between constitutive and inducible variation in defensive chemistry.

Phenotypic correlation between growth rate, age and investment in defensive chemistry
A major finding of their study is that the tree ring growth rate over the past ten years was
a good predictor of constitutive investment in terpenoids (for both for total terpenoids and most of the individual terpenoids identified). However, tree ring growth was not related to the concentration of total phenolics or individual phenolic compounds. In addition, induced variation of most individual and total terpenoids and phenolics was not related to tree ring growth. In other words, Villari et al. (2014) found no evidence of allocation conflicts between primary and secondary metabolism, but rather, they uncovered a positive relationship between growth and defensive investment under that growth-limiting conditions, as predicted by the extended Growth-Differentiation Balance Hypothesis (GDBH) (Herms and Mattson 1992). From an evolutionary point of view, the theoretical background predicts that optimal relative investment in defences should be greater in slow-growing plant species or lineages adapted to stressful, growth-limiting environments due to greater construction costs (as posed by the Resource Availability Hypothesis by Coley, Bryant & Chapin (1985), reviewed by Endara & Coley 2011). However, from a physiological point of view, the GDBH predicts that optimal investment in defences at the individual level is not a linear function of carbon assimilation and growth rates across the entire growth rate range of a species (Herms and Mattson 1992).

Pine trees are a good model for studying these relationships, as they have differentiated secretory organs for production and storage of chemical defences. Terpenoids and phenolic compounds are secreted and stored in specialized tissues: the resin duct network of the xylem and cortex and the polyphenolic parenchyma cells in the phloem, respectively (Franceschi et al. 2005). Damage signalling triggers physiological processes and changes in the cambium, leading to swelling and/or differentiation of more or greater xylem resin ducts and polyphenolic parenchyma cells (Krokene et al. 2003, Hudgins and Franceschi, 2004). These tissues remain functional and active for a time, serving as a footprint of previous induced responses or past damage. This is advantageous for retrospective studies linking growth rates and net (current + past) defensive investment. In this sense, the results of Villari et al. agree with recent papers suggesting that there is no evidence of phenotypic resource-based trade-offs between growth and resin based defences in pine trees (Ferremberg et al. 2014, Rodríguez-García et al 2014).

On the other hand, studies characterizing the concentrations and profiles of both terpenoids and phenolics are scarce, making this paper particularly welcome. While numerous studies have focused on terpenoids and phenolic compounds in conifers, most of those studies have been devoted to examining the chemical ecology of a single type of compound (e.g., single groups of terpenes, phenolics or alkaloids). The relative lack of more comprehensive studies may be due to a lack of facilities needed for the analysis of chemicals of different natures, as well as the expertise required for data analysis and interpretation results. The complexity and diversity of defensive chemistry in trees is vast, and papers covering the entire array of chemicals (such as that from Villari et al.) are valuable for understanding the integration of tree defences and their environmental determinants.

An important point for fully understanding resource-derived trade-offs is that the limiting source of carbon constraining the allocation must be clearly identified (recently reviewed by Saeki et al. 2014). In the case of pine defensive chemistry, the energy source for defensive chemistry is likely a combination current assimilates and non-structural stored carbohydrates. Identifying the relative contribution of these two sources to pine defences may have important implications for plant physiology and ecology (Martinez-Vilalta 2014, Saffell et al. 2014). Although this topic has been studied at the cellular level (e.g., Schnitzler et al. 2004, Affek and Yakir 2003), more research is needed about the relative contribution of current carbon assimilation and stored carbohydrates to the synthesis of both constitutive and induced phenolics and terpenoids at the whole tree level (but see Goodsman et al. 2013). Advances in our understanding of this topic would help us to understand how environmental factors such as those leading to carbon starvation and altered carbon storage (e.g., water availability and winter temperature) will affect current and future defensive investment and capabilities in pine trees, subsequently impacting tree survival and performance in a changing climate.
Phenotypic correlations between constitutive and inducible variation

Villari et al. (2014) also explored the functional relationships between the constitutive concentration and inducible variation of pairs of compounds. Their analyses resulted in the classification of five response types, depending on the sign, shape (linear or quadratic) and slope of the response to their experimental treatments. Studying the pattern of phenotypic covariation between pairs of compounds is a valuable approach, as the functional linkage between resource-related traits is not necessarily linear, because gains in one trait could lead to multiplicative effects in the other. Thus, non-linear relationships are expected and must be explored to characterize the physiological trade-offs, as stressed by Saeki et al. (2014) in their recent review about concepts and methodology on this topic.

As constitutive and induced resistance cannot be maximized at the same time, and lineages expressing high levels of constitutive resistance would obtain limited fitness benefits from expressing induced resistance, an evolutionary trade-off between both strategies is predicted (e.g. Kempel et al. 2011, but see Morris et al. 2006 for a critical review of methodological approaches). This type of negative genetic correlation between traits may impose evolutionary constraints on the simultaneous improvement of both strategies and thus influence evolutionary trajectories within populations, and as well as at the macroevolutionary level (Sgro & Hoffmann 2004, but see Moreira et al. 2014). For this purpose, studies based on phenotypic correlations, although valuable, allow for only limited evolutionary inference compared to studies based on genotypic or family correlations, which provide information about heritable, genetic-based, trade-offs (reviewed by Agrawal et al. 2010). In long-lived trees, this type of research has mainly been performed using young individuals (e.g., Sampedro et al. 2012, Carillo-Gavilán et al. 2012, Moreira et al. 2013), while the results from Villari et al. (2014) should encourage further research on this topic at the adult stage.

Covariation between individual secondary compounds

Villari et al. (2014) found no phenotypic correlations between the concentration of total phenolics and total terpenoids, and they detected no significant correlations between individual chemical compounds. Importantly, both results suggest that there is no conflicts or substrate competition in the alternative allocation to the phenylpropanoid and isoprenoid defensive pathways at the individual level. Such an absence of negative correlations between defensive chemicals at the individual level, or even a positive covariation, is not uncommon in plants (Koricheva et al. 2004).

Due to the vast diversity in quality and concentration of pine chemical defences (i.e., Iason et al. 2011), and provided that defensive chemicals do not function in isolation, it seems that the next step is to take advantage of multivariate bioinformatic tools for the analysis of these types of large databases. Further efforts that combine the analytical approaches of evolutionary ecology, chemical ecology and tree physiology are required. The analysis of databases covering hundreds of compounds would greatly benefit from network analysis and whole plant metabolome approaches. A recent article by Harding et al. (2014) and some recent reviews on this topic (Kliebenstein 2014, Moore et al. 2014) illustrate this points, and they encourage new analytical approaches to understanding the complexity and common regulatory networks in constitutive and induced allocation of conifer defences. Advanced analysis of exhaustive chemical studies of adult trees in forest genetic trials with family or population structure would be crucial for understanding the multivariate structure of plant defences in long-lived plants, as well as the metabolic integration of costs and benefits of individual chemical species and their patterns of context dependency.

In summary, Villari et al. (2014) provide new ideas and a valuable model for new approaches that should motivate scientists to put more effort into the fine-scale characterization of the diversity of secondary metabolites in response to biotic challenges. These approaches would benefit from advanced multivariate analyses and experimental designs using plant material with a known genetic background. This would help advance our knowledge of the phenotypic integration and
physiological regulation of tree defensive chemistry, as well as the evolutionary ecology of defences in long-lived plants. This type of research has usually been performed on young individuals under controlled conditions, and it is clear that more effort is needed to examine adults in field conditions. Such research would be challenging, given the difficulties in establishing and maintaining mature field stands of known genetic backgrounds. Thus, we should encourage more intense interactions between forest tree breeders and tree physiologists, as future research in this field should utilize the long-term networks of progeny and provenance trials established by national and provincial forests services.

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References


