LETTER

Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences

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Abstract

There is increasing evidence that geographic and climatic clines drive the patterns of plant defence allocation and defensive strategies. We quantified early growth rate and both constitutive and inducible chemical defences of 18 Pinaceae species in a common greenhouse environment and assessed their defensive allocation with respect to each species' range across climatic gradients spanning 31° latitude and 2300 m elevation. Constitutive defences traded-off with induced defences, and these defensive strategies were associated with growth rate such that slow-growing species invested more in constitutive defence, whereas fast-growing species invested more in inducible defence. The position of each pine species along this trade-off axis was in turn associated with geography; moving poleward and to higher elevations, growth rate and inducible defences decreased, while constitutive defence increased. These geographic patterns in plant defence were most strongly associated with variation in temperature. Climatic and geographical clines thus act as drivers of defence profiles by mediating the constraints imposed by trade-offs, and this dynamic underlays global patterns of defence allocation.

Keywords

Altitude, growth rate, jasmonic acid, latitude, non-volatile resin, Pinus, precipitation, salicylic acid, temperature, total phenolics.

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INTRODUCTION

Ecologists have long been interested in understanding the extreme phenotypic variation in plant investment in defences against herbivores and pathogens. Several hypotheses have been put forward to provide a predictive framework explaining the patterns and mechanisms underlying this variation, mainly involving climate (e.g. Pearse & Hipp 2012), resource availability (e.g. Fine *et al.* 2006; Endara & Coley 2011), plant physiological constraints and resource allocation (e.g. McKey 1974; Karban 2011). Fundamental to these hypotheses is the prediction that interspecific variation in plant defences can be explained by the cost/benefit ratio of defences, to which resource availability, abiotic stress and the strength of herbivore pressure all contribute. For example, plants adapted to resource-poor and stressful habitats are predicted to be relatively slow growing, with high tissue value, leading to high cost of tissue removal by herbivores and thus to invest heavily in defences (Resource Availability Hypothesis, RAH, Coley *et al.* 1985; Endara & Coley 2011). At the same time, variation in herbivore pressure is predicted to lead to concomitant variation in defence (Feeny 1976), and herbivore pressure and the abiotic environment may interact to drive patterns of plant defence (Fine *et al.* 2004).

Because antiherbivore resistance is costly, there is a prediction (Coley *et al.* 1985) and empirical support (e.g. Mooney *et al.* 2010; Sampedro *et al.* 2011) for a trade-off between the allocation of resources to defences vs. other plant functions such as growth, competitive ability and reproduction (e.g. Fine *et al.* 2004) and among differing defensive traits or strategies (e.g. Bingham & Agrawal 2010; Rasmann *et al.* 2011). For example, the simultaneous evolution of both constitutive and induced defences could be constrained by such trade-offs (Bingham & Agrawal 2010; Kempel *et al.* 2011; Rasmann *et al.* 2011). When a plant defensive trait is constitutively expressed (i.e. always present in the plant irrespective of the biotic context) the allocation costs of defence are relatively high compared to when the same defence is inducible (i.e. produced or mobilised in response to chemical, physical or biotic external stimuli; Karban 2011). Thus, it can further be predicted that slow-growing plants should allocate more to constitutive defence, and fast-growing species to induced defence.

The study of environmental and geographic clines provides unique insight into the process by which herbivory and the abiotic environment interact to shape the evolution of plant defence over large geographic scales (e.g. Pearse & Hipp 2012). Besides abiotic environmental information, both latitude (e.g. Dobzhansky 1950; Janzen 1970; Pennings *et al.* 2009; Salazar & Marquis 2012) and altitude (Rodríguez-Castañeda *et al.* 2010; Garibaldi *et al.* 2011; Rasmann *et al.* 2014) have been recognised as predictors of fundamental biotic drivers of the evolution of plant defences such as herbivore pressure and diversity, although their connections with investment in plant defences is controversial, with examples of null, positive and negative relationships (Zehnder *et al.* 2009; Moles *et al.* 2011a, b; Woods *et al.* 2012; Pratt & Mooney 2013).

At least two factors may underlie the lack of consistency among latitudinal and altitudinal gradients in plant defence investment. First, herbivore pressure and plant resource availability may be shaped by climatic factors in opposing ways (Woods *et al.* 2012). For example, whereas herbivore pressure is predicted to increase at lower elevation and latitude, the RAH (Endara & Coley 2011) predicts that slow-growing species, generally occurring at low resource sites such as higher

latitudes, high elevation and other stressful environments, invest more in defences. Second, patterns of constitutive and inducible defences may respond differently to environmental variation, and the failure to explicitly consider their unique responses may obscure gradients in defence. Although both constitutive and inducible defences likely confer resistance against herbivores, they clearly differ in terms of costs and benefits (Karban 2011), they are differentially modulated by the abiotic environment (e.g. Sampedro *et al.* 2011) and they are assumed to evolve differentially depending on the intensity, frequency and specificity of the herbivore pressure (Bingham & Agrawal 2010; Karban 2011). These trade-offs, whose expression may be environmentally dependent (e.g. Sampedro *et al.* 2011), can therefore modulate the defensive investment of plant species. Accordingly, geographic and climatic clines could influence plant allocation to constitutive and induced defences differently, although this prediction has not been tested (but see Rasmann & Agrawal 2011).

Here, we examined whether allocation to constitutive and inducible plant chemical defences in pine trees is driven by the latitude, altitude and climatic factors defining each species' range, and to what extent resource allocation trade-offs between growth and defensive strategies could be driven those relationships. Specifically, we addressed the following questions: (1) Does interspecific variation in constitutive and induced defences trade-off among species? (2) Does species growth rate mediate relative investment in constitutive and inducible defences? and (3) How do such profiles of defence and growth relate to the latitude, elevation and climate within which a species occurs? To address these questions, we performed an experiment with 18 tree species in the family of Pinaceae whose combined ranges span a total of 31° latitude and 2300 m elevation, covering a broad range of environmental conditions in both Nearctic and Palearctic ranges. Pines present an excellent system to address these questions because there is little variation in growth forms (Richardson 1998); all species occupy similar habitats (Richardson 1998); and the chemical ecology for anti-herbivore defences is well established (Franceschi *et al.* 2005; Mumm & Hilker 2006). In addition, these species span a largely temperate gradient in latitude and elevation that avoids the variation in herbivore pressure common to tropical-temperate comparisons. Accordingly, this system provides opportunities for testing whether the macroevolutionary patterns of variation in pine defence are driven by the effects of climate on growth rate.

We grew seedlings of each species in a common environment to assess needle and stem tissues for the constitutive investment in the two most important chemical defences in conifers, phenolics and oleoresin (Mumm & Hilker 2006), as well as the potential inducibility of those defensive traits as driven by the jasmonic acid (JA) and the salicylic acid (SA) signalling pathways (Thaler *et al.* 2012). JA is a plant hormone involved in the defensive signalling against necrotrophic pathogens, chewing herbivores and (to a lesser extent) phloem-feeding insect. SA is involved in the defence against biotrophic pathogens and phloem-feeding insects. The JA and SA pathways are known to, at least in part, act antagonistically against one another (Thaler *et al.* 2012). By accounting for phylogenetic non-independence between species (Pagel

1999), we determined to what extent latitude, altitude and different aspects of the abiotic environment explain the patterns of variation in these contrasting modes of defences across pine species, as well as the relationships between constitutive and induced defences across species. This study thus elucidates the ecological factors and trade-offs driving multiple modes of plant defensive strategies in some of the Northern Hemisphere's most ecologically and economically important trees.

MATERIALS AND METHODS

Natural history

We studied 18 conifer species in the family Pinaceae that occupy and often dominate forested areas in the Northern Hemisphere. Seventeen of our study species belong to the genus *Pinus* (see Tables S1, S2 and Fig. S1 in Appendix S1); *Pinus canariensis*, *P. halepensis*, *P. nigra subsp. laricio*, *P. nigra subsp. salzmannii*, *P. roxburghii*, *P. pinea*, *P. pinaster* and *P. sylvestris* have a Paleartic range; while *P. banksiana*, *P. contorta*, *P. coulteri*, *P. muricata*, *P. patula*, *P. ponderosa*, *P. radiata*, *P. sabiniana* and *P. taeda* have a Nearctic range (Richardson 1998); and one to the genus *Pseudotsuga* (*Pseudotsuga menziesii*, Nearctic range). The later species, although not in the genus *Pinus*, was included in the experiment because it has similar defensive mechanisms and coexists in vast areas of mixed forests with most of the pine species in western North America (Richardson 1998).

Pines have well-defined defences (mostly based on the production of resin and phenolic compounds) against a broad range of ecologically important herbivores and pathogens (Mumm & Hilker 2006). Previous works using feeding trials with two widespread and harmful pine herbivores (the phloem feeder *Hylobius abietis* and the defoliator *Thaumetopoea pityocampa*) have shown that herbivore resistance in pine trees is mediated by the constitutive production and inducibility of oleoresins or phenolics (e.g. Carrillo-Gavilán *et al.* 2012; Moreira *et al.* 2012a, 2013; see Fig. S2, S3 in Appendix S2). Conifer oleoresin is composed of terpenoids, where about 50% are diterpenes (C_{20}) and diterpene acids (called resin acids). The remaining 50% is a variable mixture of volatile terpenes (monoterpenes – C_{10}– and sesquiterpenes – C_{15}–) that serve as a solvent for the transport of the heavier, nonvolatile, sticky and sealant diterpenoid fraction through the resin duct network system (Phillips & Croteau 1999). These chemical defences are present in large concentrations in all tissues along the plant (up to 100 mg g^{-1} tissue dried weight), imposing ecologically relevant physiological costs (Mumm & Hilker 2006; Sampedro *et al.* 2011). In addition, inducibility of the above-mentioned pine defences (i.e. the ability to increase the concentration of chemical defences in response to external challenges) has been shown to be predictive of plant survival and effective resistance against major insect herbivores in the field (e.g. Schiebe *et al.* 2012).

Experimental design

We carried out a factorial greenhouse experiment with the 18 conifer species and three treatments of plant defence induction: (1) control plants, for studying the constitutive allocation

to chemical defences, (2) jasmonic acid (JA)-induced plants and (3) salicylic acid (SA)-induced plants (treatments hereafter). The experiment followed a randomised split-plot design replicated in 10 blocks, with treatments as the whole-plot factor and pine species as the split-plot factor. Due to reduced availability of plant material of some pine species, only 15 species were included in the SA treatment. In total, there were 510 seedlings, corresponding to 18 species × 2 treatments (control and JA) × 10 blocks, plus 15 species × one treatment (SA) × 10 blocks.

Plant growth, greenhouse conditions and treatments

In October 2008, pine seeds were individually sown in 2-L pots filled with a mixture of perlite and peat (1 : 1 v : v), fertilised with 12 g of a slow-release fertiliser (N : P : K 15 : 15 : 15; Multicote®, Haifa Chemicals Ltd., Israel), and covered with a 1–2 cm layer of sterilised sand. To avoid interference from pathogens, seeds were treated with a fungicide before sowing (Fernide®, Syngenta Agro, Madrid, Spain). All the plants were grown in a research grade glasshouse (Forestry Research Center of Lourizan, Xunta de Galicia, Pontevedra, Spain), with controlled temperature (10 °C minimum temperature at night; 25 °C maximum temperature during daytime) and light (minimum 12 h per day), and automatic daily subirrigation (see Appendix S1 for more details about sowing and growing conditions).

During the first growing season, 13 months after emergence, we applied the treatments: (1) JA-plants were sprayed with a solution of 40 mM methyl jasmonate (MJ; #39270-7; Sigma-Aldrich, St. Louis, MO, USA) in deionised water with ethanol 2.5% (v : v), (2) SA-plants were sprayed with a solution of 5 mM benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH, Syngenta Bion® 50 WG) in deionised water with ethanol 2.5% (v:v) and (3) control plants were sprayed with the carrier solution, i.e. deionised water with ethanol 2.5% (v:v). Treatments were sprayed evenly over the foliage with a handheld sprayer until runoff occurred. MJ and BTH are derived from jasmonic acid and salicylic acid respectively. The appropriate concentrations of MJ and BTH were determined according to previous studies and literature search with pine seedlings (e.g. Sampedro et al. 2011; Moreira et al. 2012a). To avoid cross-contamination, treatments were applied in separate greenhouse chambers and plants remained in those separate spaces for 48 h to dry.

Sampling, measurements and chemical analyses

During the course of the greenhouse trial, and before treatment application, plants were measured for height (strongly associated with biomass; $R^2 = 0.88$, $P < 0.001$) as a proxy of early growth rate. Two weeks after treatment application plants were harvested, transported to the laboratory in ice coolers and immediately sampled for further chemical analyses. Immediately after harvesting, a fresh 5-cm-long segment of stem and a sample of needles (ca. 5 g fresh weight) were collected from the lowest part of the stem of each plant, weighed, and immediately frozen at −30 °C for analysis of non-volatile resin content and total phenolics. Our choice to assay these tissues and plant parts was based on our past work and experi-

ence (Moreira et al. 2012b). Concentration of total phenolics and non-volatile resin were measured in needles and stems of all experimental plants. We analysed these two chemical traits in needle and stem tissues as they largely vary in their allocation in constitutive and induced defences (Carrillo-Gavilán et al. 2012; Moreira et al. 2012b) as well as in the vegetative costs of their production (Sampedro et al. 2011).

Concentration of non-volatile resin in the stem (phloem + xylem) and needles was estimated gravimetrically (Moreira et al. 2012b). Briefly, plant material (ca. 5 g fresh weight) was transferred into pre-weighed test tubes, oleoresin was extracted with of hexane (15-min sonication and then 24 h at room temperature), the extract was quantitatively transferred into pre-weighed test tubes and the entire extraction step was then repeated again. The solvent in the tubes was evaporated to dryness under fume hood and the mass of the non-volatile resin residue was determined at the nearest 0.0001 g and expressed as mg of non-volatile resin g^{-1} stem d.w. This gravimetric determination of non-volatile resin was highly correlated with the concentration of the diterpenoid fraction ($r = 0.921$; $P < 0.001$) as quantified by gas chromatography (Sampedro et al. 2011). Total phenolics in the phloem and needles were extracted and analysed as described by Moreira et al. (2012b). Briefly, ca. 2 g of oven-dried plant material (45 °C to constant weight) was finely ground in liquid N and ca. 300 mg extracted with aqueous methanol (1 : 1 vol : vol) after sonication (15 min). Total phenolics in the extract were determined colorimetrically by the Folin-Ciocalteu method in a Biorad 650 microplate reader (Bio-Rad Laboratories Inc., Philadelphia, PA, USA) at 740 nm, using tannic acid as standard. Concentrations were expressed based on dry weights (d.w.). These analytical measures provide a good proxy of quantitative allocation to defensive chemistry and correlates with effective overall resistance against herbivores and pathogens (see Appendix S2).

Estimation of species distribution range and climatic variables

We acquired species range data from the Atlas of United States Trees (North American species; http://esp.cr.usgs.gov/data/little/), the European Forest Genetic Resources Programme (European and Mediterranean species; http://www.euforgen.org/distribution_maps.html) and the Banco de Datos de Biodiversidad de Canarias (P. canariensis; http://www.biodiversidadcanarias.es). Complete range data were not available for P. roxburghii, and thus we compiled coordinates from 22 georeferenced specimens in the Global Biodiversity Information Facility (http://data.gbif.org/welcome.htm) and the NOAA Tree Ring (http://www.ncdc.noaa.gov/paleo/treering.html) databases. Where necessary, range data were transformed to the WGS84 coordinate system (see Appendix S3, Table S3). Using these maps and literature about pine species distribution (Richardson 1998) we acquired the maximum and minimum latitude and altitude for each conifer species.

To characterise the mean climate within the distribution range of each species we used a subset of the BioClim climate variables (available at http://www.worldclim.org/), namely, BIO1 (annual mean temperature, °C), BIO4 (temperature seasonality, expressed as the standard deviation of temperature among months*100), BIO5 (maximum temperature of the

warmest month, °C), BIO6 (minimum temperature of the coldest month, °C), BIO12 (annual precipitation, mm), BIO13 (precipitation of the wettest month, mm), BIO14 (precipitation of the driest month, mm), BIO15 (precipitation seasonality, expressed as the coefficient of variation in precipitation across months). The procedures used to calculate these variables are fully described in Hijmans *et al.* (2005). Briefly, Hijmans *et al.* (2005) averaged records of weekly mean temperature (24 542 locations), temperature range (14 835) and precipitation (47 554) between the years 1950 and 2000 by location. Climate in unobserved locations was estimated by interpolating among the climate station locations, and the BioClim variables represent summary statistics across each month. Although there is substantial geographic bias in the location of climate monitoring stations at a global scale, the ranges of most species studied here were well covered. All species ranges and climate data were imported into ArcGIS 10.0 (ESRI, Redlands, CA, USA). For each species, the eight BioClim variables (see above) were averaged across all five arc min cells of the climate layer overlapped by the species range polygon(s) (or across all points for *P. roxburghii*) using the Zonal Statistics utility in the Spatial Analyst toolbox.

Statistical analyses

The analyses of plant growth and the concentration of chemical defences in stems and needles were carried out by fitting mixed models (Proc Mixed in SAS 9.2, Cary, NC, USA) with the main effects of treatments (T), pine species (SP) and T × SP interaction treated as fixed factors, and the block (B) and B × T interaction as random factors to test the whole-plot factor (T) with the appropriate error term (Littell *et al.* 2006). From this statistical model we obtained least square means ± SEM as descriptive statistics for each species, under both control and induced treatments, and in both stem and needle tissues.

To utilise all defence and climate information without inflating Type I error through multiple testing, variation in the four pine defensive traits analysed (i.e. non-volatile resin and phenolics in needle and stem tissues) was summarised by three independent principal component analyses (PCA) using Proc FACTOR (rotation = varimax) in SAS 9.2, one for constitutive defences, one for inducibility of defences by JA and one for inducibility of defences by SA. In each case, the four defensive traits were summarised into the two-first principal components. Similarly, the eight climatic variables were summarised into two principal components (PC) extracted by the PCA.

To investigate whether the latitude, altitude and climatic variables act as predictors of constitutive defences and their inducibility and whether the relative investment in constitutive and induced defences relate to species growth rate, we tested for across-species correlations among traits (defensive and climatic indices from the PCAs), accounting for phylogenetic non-independence in a maximum likelihood phylogenetic generalised least squares (PGLS) framework (Pagel 1999). We used the phylogenetic tree of Eckert & Hall (2006), with branch lengths estimated as the number of expected substitutions. Using PGLS, models of trait evolution can be compared using a likelihood ratio test (LR), in which LR = 2 [log-likelihood of the better-fitting model minus the log-likelihood of the worse-fitting

model]. Under the assumption of model equivalence, the LR statistic follows a chi-square distribution with degrees of freedom given by the number of parameters differing between the models compared. For the phylogenetic independent correlation analyses, the LR parameter was estimated from a random-walk model using Pagel's estimated lambda (λ) with and without an estimated covariance (Pagel 1999).

To test for trade-offs between constitutive and induced defences, we regressed the inducibility (i.e. the difference in mean phenotype values for each species between control and induced plants) of each individual defensive trait (not PC axes) against the species mean of that trait in the control treatment (i.e. the constitutive level). As we regressed a variable against a difference that includes the same variable (i.e. induced defence = constitutive component + induced component), the errors in the two axes are not independent, and thus there is a possibility of obtaining spurious correlations from these analyses (Morris *et al.* 2006). Therefore, to evaluate the significance of these correlations, we employed the Monte Carlo simulation procedure proposed by Morris *et al.* (2006), translating the MATLAB code into SAS. Specifically, we performed 9999 simulated correlations upon randomised data, and calculated *P* by comparing the observed correlation with this probability distribution. In addition, we also performed phylogenetically controlled analyses (PGLS) to test for correlations between constitutive defences and their inducibility.

RESULTS

Factor analysis of constitutive defences and their inducibility and climatic variables

Of the three PCAs performed on constitutive, JA- and SA-induced defences, in all cases > 62% of the variation in the four defensive traits analysed was explained by the two-first PCs. In the case of the constitutive defensive traits, two axes explained 72% of the variance across species (Table S4). PC1 was positively associated with constitutive non-volatile resin concentration in both tissues (hereafter called 'constitutive resin'), whereas PC2 was associated with constitutive concentration of total phenolics in the stem (hereafter 'constitutive stem phenolics') (Table S4). Two axes explained 67% of the variance in the inducibility by JA of the four defensive traits across species (Table S4). PC1 was a good indicator of inducibility of total phenolics in both tissues (hereafter 'phenolics induced by JA'), whereas PC2 was a good indicator of inducibility of non-volatile resin in the stem (hereafter 'stem resin induced by JA') (Table S4). Two axes explained 62% of the variance in the inducibility by SA of four constitutive defensive traits (Table S4). PC1 was a good indicator of inducibility of non-volatile resin and total phenolics in the needles (hereafter 'needle resin and phenolics induced by SA'), whereas PC2 was positively related to the inducibility of non-volatile resin in the stem (Table S4). However, concentration of resin in the stem decreased in 11 of 15 pine species in response to SA application (see Fig. S4 and S5). As SA application thereby promoted an induced susceptibility effect, we will refer to this axis hereafter as 'stem resin depletion by SA').

Two axes explained 76% of the variance in the eight climatic variables across species distribution ranges (Table S5). PC1 (hereafter 'temperature') was positively related to temperature, with high positive loading with annual mean temperature and minimum temperature of the coldest month, and negatively related to temperature seasonality (Table S5). PC2 (hereafter 'precipitation') was an indicator of precipitation with positive loading of precipitation in the driest month and negative loading of precipitation seasonality (Table S5).

Latitude and altitude as predictors of constitutive and induced pine defences

We found contrasting latitudinal clines in allocation to constitutive and inducible chemical defences across the 18 species. Whereas constitutive resin defence in stems and needles increased at higher latitudes (Fig. 1a), inducibility of stem resin by JA decreased (Fig. 1d). Inducibility of chemical defences by SA also tended to increase at lower latitudes, although this result was not significant (Fig. 1e, 1f). Consistently, constitutive resin defence in stems and needles was greater at higher altitudes (Fig. 2a). However, altitude did not explain the inducibility of pine defences by either hormone (Fig. 2c–f). Analyses of the single defensive traits were consistent with the results of the PC axis (Fig. S4, S5).
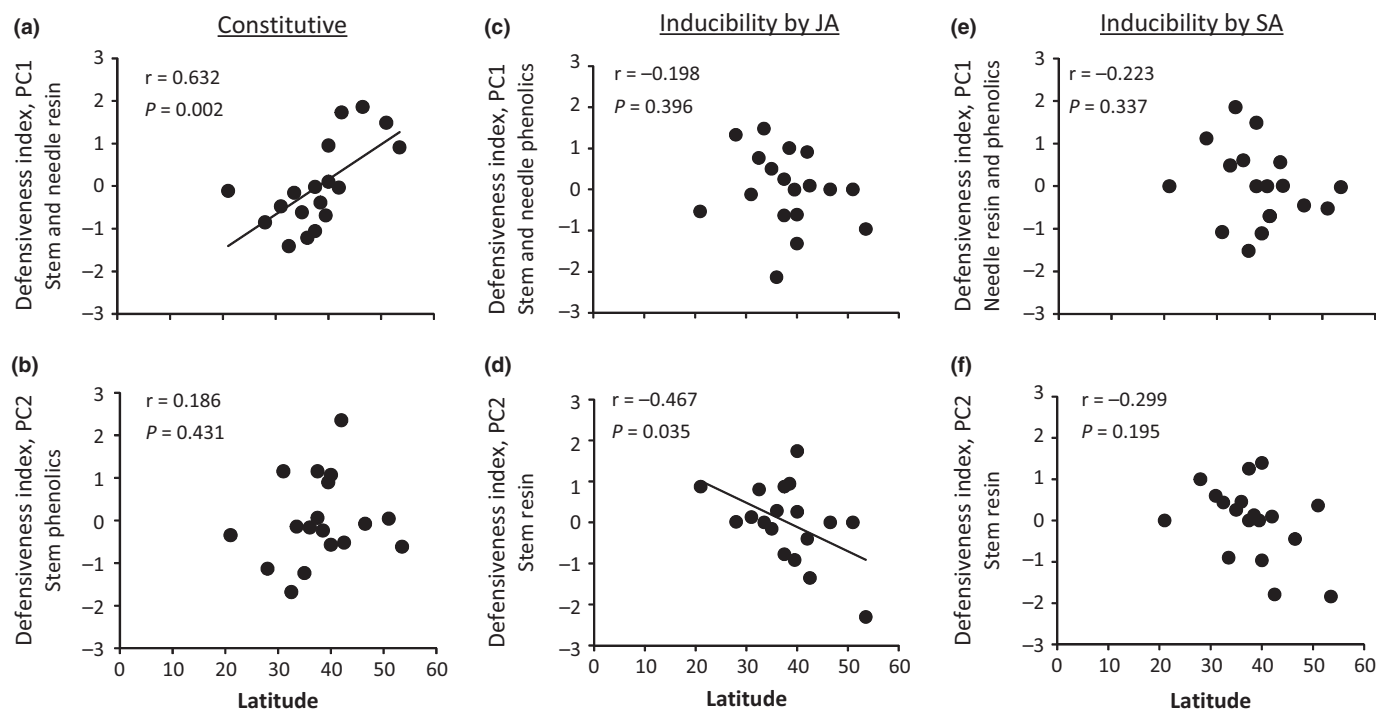
Climate as a predictor of constitutive and induced pine defences

Temperature predicted the allocation to constitutive chemical defences across species (Fig. 3a), where species from colder regions (lower mean annual temperature, lower minimum temperature of the coldest month and more temperature seasonality according to the climatic PCA) invested more in constitutive non-volatile resin in stems and needles (constitutive resins, Fig. 3a). Precipitation predicted the response of chemical defences to SA across species (stem resin depletion by SA, Fig. 4f), where species from the driest regions (less precipitation of the driest month and more precipitation seasonality) showed a lower depletion of non-volatile resin associated with SA application (i.e. induced susceptibility, negative values of resin inducibility). Analyses of the single climatic traits were consistent with the results of the PC axis (Table S6).

Trade-offs between constitutive defences and their inducibility

We found a significant negative relationship across species between the expression of constitutive non-volatile resin in the stem and its inducibility by both JA and SA, demonstrating a trade-off at the species level (Monte Carlo $r = -0.42$, $P_{MonteCarlo} = 0.081$ and PGLS $r = -0.46$, $P = 0.039$ for the JA pathway; Monte Carlo $r = -0.71$, $P_{MonteCarlo} = 0.003$ and PGLS $r = -0.68$, $P = 0.002$ for the SA pathway, Fig. 5a,c). Similarly, we also found a significant negative relationship between the expression of constitutive total phenolics in the needles and its inducibility by both JA and SA (Monte Carlo $r = -0.58$, $P_{MonteCarlo} = 0.012$ and PGLS $r = -0.60$, $P = 0.004$ for JA pathway; Monte Carlo $r = -0.49$, $P_{MonteCarlo} = 0.064$ and PGLS $r = -0.52$, $P = 0.030$ for SA pathway Fig. 5b, 5d). We did not detect significant relationships between the constitutive concentration and the induc-

Figure 1 Latitude as a predictor of constitutive defences (a, b), jasmonic acid-associated inducibility (JA) (c, d) and salicylic acid-associated inducibility of those defences (SA) (e, f) in 18 Pinaceae species. The r and P values shown were obtained with a phylogenetic independent model (PGLS). Each point represents a plant species mean ($N = 10$ replicate individuals).
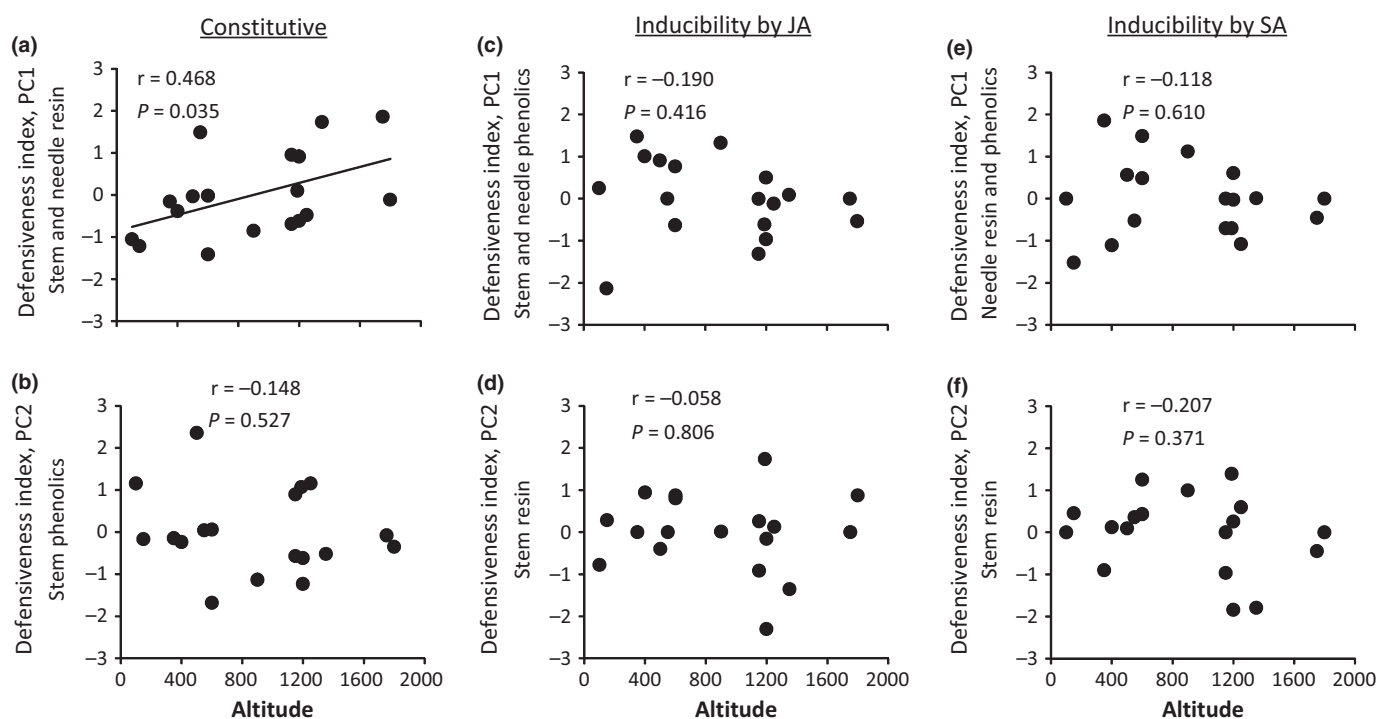
Figure 2 Altitude as a predictor of constitutive defences (a, b), jasmonic acid-associated inducibility (JA) (c, d) and salicylic acid-associated inducibility of those defences (SA) (e, f) in 18 Pinaceae species. The *r* and *P* values shown were obtained with a phylogenetic independent model (PGLS). Each point represents a plant species mean ($N = 10$ replicate individuals).

ibility (by JA either SA) of non-volatile resin in the needles and total phenolics in the stem (Fig. 5a–d).

Relative investment in constitutive and induced defences relate to species growth rate

We found a significant negative relationship across species between the species early growth rate and constitutive resin in stems and needles (Fig. 6a), indicating that slow-growing species invest more in constitutive resin. While there was a pattern for a positive association between species growth rate and inducibility of stem resin by JA (Fig. 6b), this was not statistically significant. Similarly, we did not find significant relationships between growth rate and the other defensive traits (data not shown). Growth rate was also associated with geography, decreasing with both latitude ($r = -0.639$; $P = 0.002$) and altitude ($r = -0.493$; $P = 0.017$). With respect to climatic factors, growth rate increased with temperature ($r = 0.429$; $P = 0.047$) but was not associated with variation in precipitation ($r = -0.364$; $P = 0.109$).

DISCUSSION

In accordance with predictions, our results indicate that climate is an important driver of pine defensive allocation, but constraints imposed by trade-offs between defensive strategies and growth rate are key for understanding the global patterns of pine defence allocation. While growth rate decreased and constitutive pine defences increased at higher latitudes and elevation and with the coldest temperatures, inducibility

of defences increased towards the equator. Constitutive defences thus traded-off with both inducible defences and growth rate, such that slow- and fast-growing species invested more in constitutive vs. inducibility of defences, respectively. The opposing patterns of geographic variation between the two modes of defence (constitutive and inducibility) may thus underlie the conflicting evidence for latitudinal and elevational gradients in plant defences (Zehnder *et al.* 2009; Moles *et al.* 2011a,b).

The climatic and biotic correlates of latitude and altitude are the main geographical drivers of species distributions, and can contribute to determine defensive phenotypes as well as functional traits related to plant defence. Theory has predicted that the strength of plant–herbivore interactions increases towards the equator (Dobzhansky 1950; Janzen 1970; Pennings *et al.* 2009; Salazar & Marquis 2012) and towards sea level (Rodríguez-Castañeda *et al.* 2010; Pellissier *et al.* 2012), leading to evolution of greater investment in chemical defences at lower latitudes and altitudes because of increased herbivore consumption rates (e.g. Rasmann & Agrawal 2011; Pearse & Hipp 2012; Raffa *et al.* 2013).

We found that the investment in some plant defences of pine seedlings was driven by latitudinal and altitudinal gradients, but these responses varied depending on the plant defensive strategy. On one hand, constitutive resin in pine species increased at higher latitudes and altitudes (i.e. more stressful environmental conditions). On the other hand, JA-associated inducibility of stem resin was greater at lower latitudes (i.e. favourable environmental conditions for primary
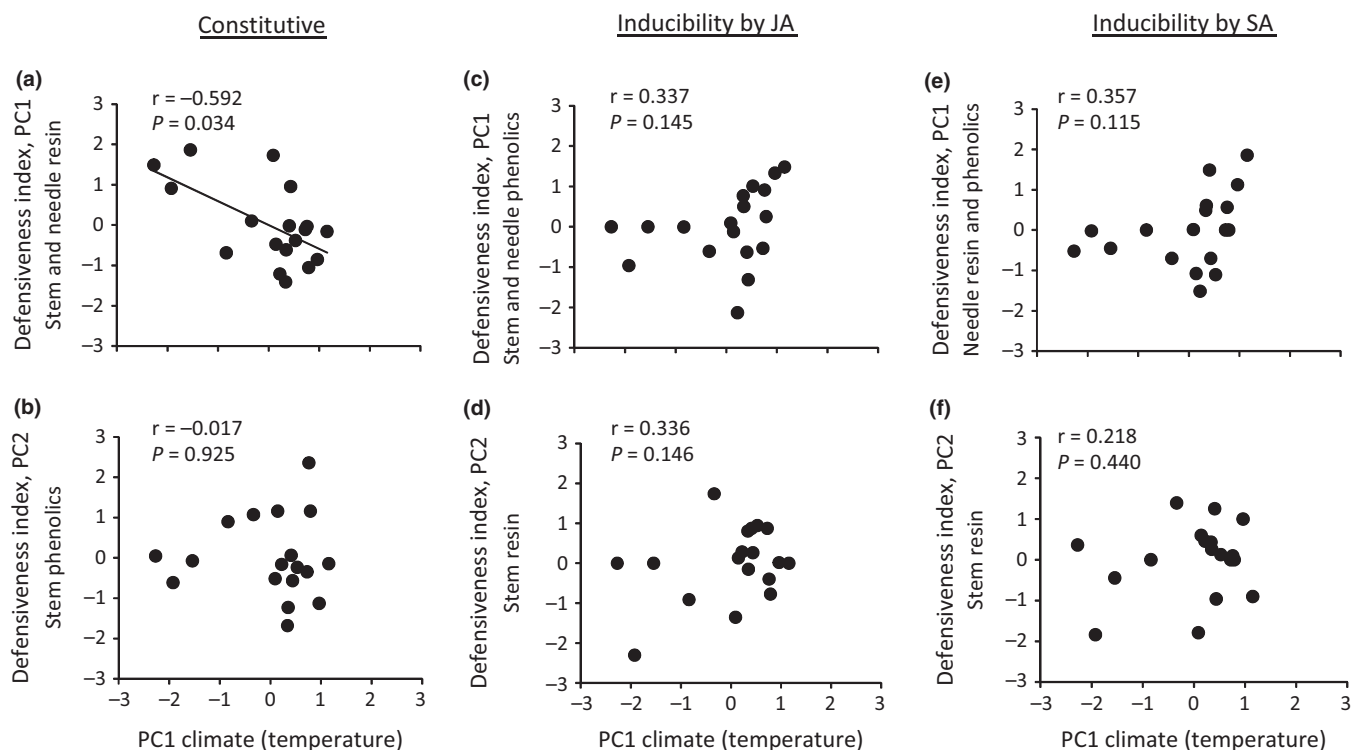
Figure 3 Temperature as a predictor of constitutive defences (a, b), jasmonic acid-associated inducibility (JA) (c, d) and salicylic acid-associated inducibility of those defences (SA) (e, f) in 18 Pinaceae species. The r and P values shown were obtained with a phylogenetic independent model (PGLS). Each point represents a plant species mean ($N = 10$ replicate individuals).
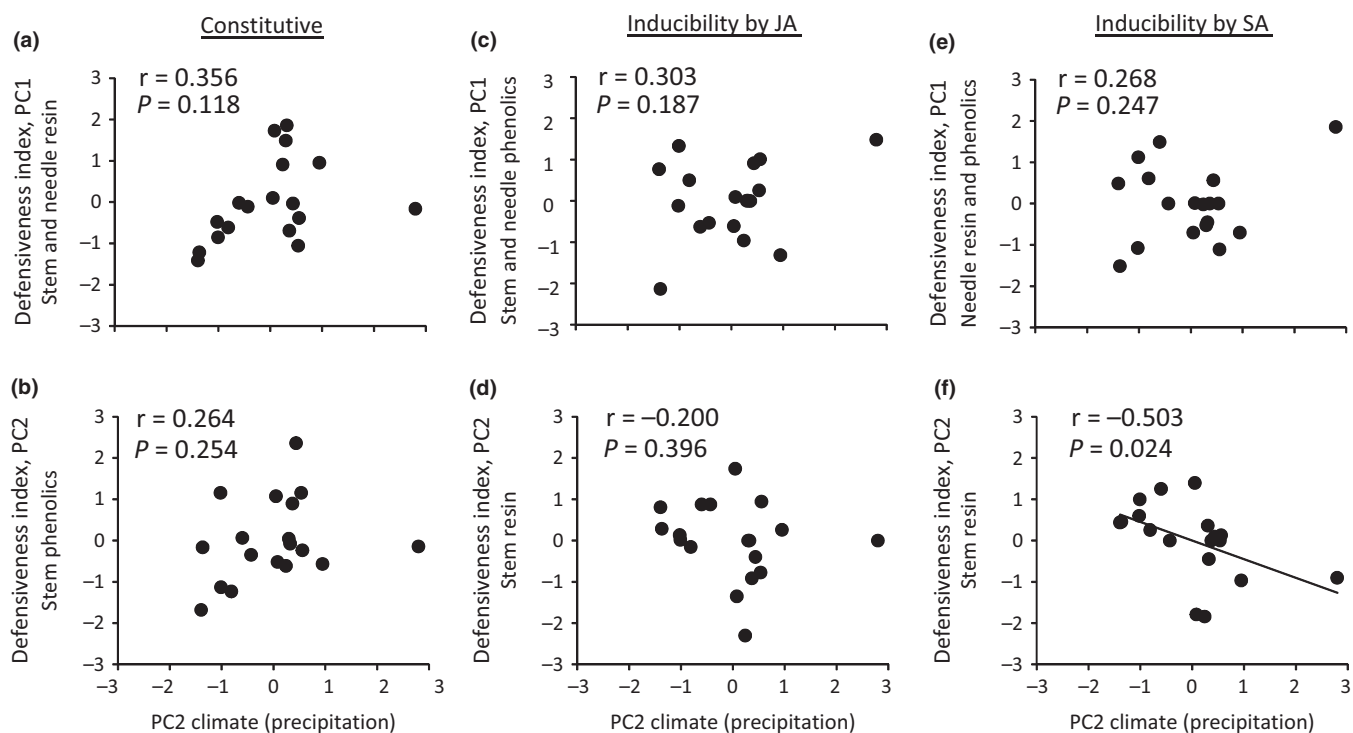
Figure 4 Precipitation as a predictor of constitutive defences (a, b), jasmonic acid-associated inducibility (JA) (c, d) and salicylic acid-associated inducibility of those defences (SA) (e, f) in 18 Pinaceae species. The r and P values shown were obtained with a phylogenetic independent model (PGLS). Each point represents a plant species mean ($N = 10$ replicate individuals).
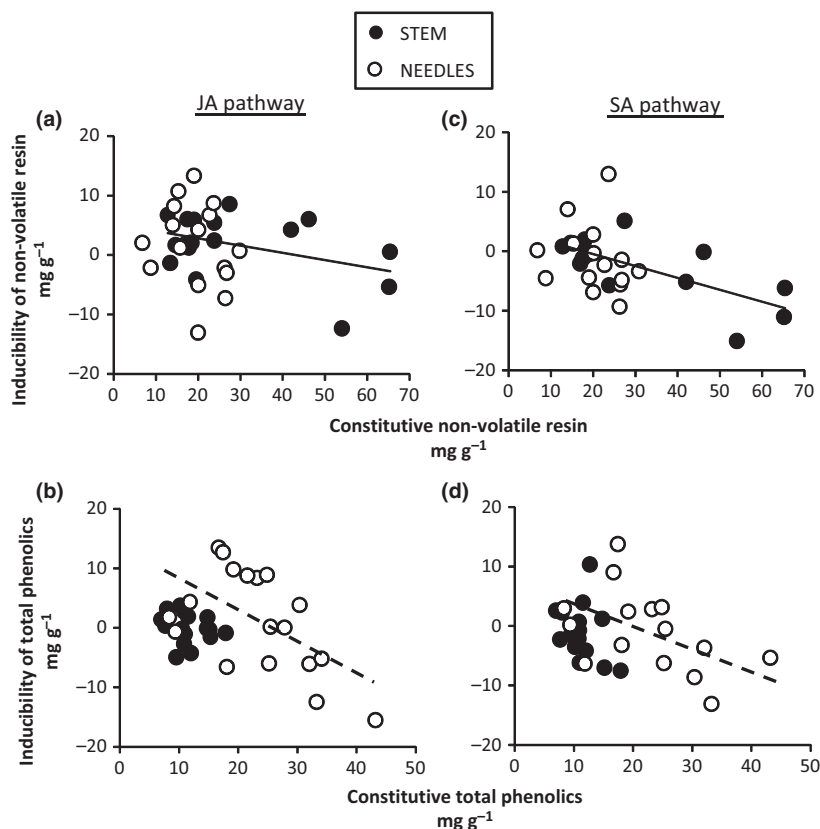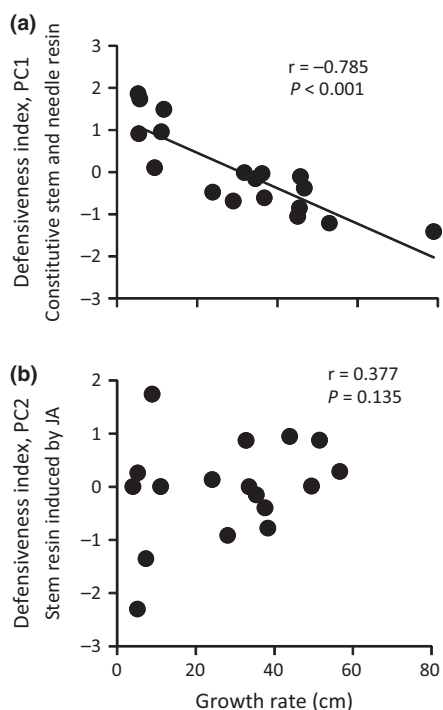
Figure 5 Relationships between constitutive levels and inducibility of (a, c) non-volatile resin and (b, d) total phenolics in the stem (black dots and solid lines) and needles (white dots and dashed lines). Panels in the left (a, b) represent those plants induced by jasmonic acid (JA) and panels in the right (c, d) represent those plants induced by salicylic acid (SA). Inducibility was measured as the species mean values in chemical-induced minus control plants. We independently performed Monte Carlo simulations and phylogenetically controlled analyses to test for correlations between constitutive defences and their inducibility. Significant negative relationships ($P < 0.05$; solid and dashed lines) denote a trade-off between constitutive and induced defences (see Pearson *r* and *P* values in the result section). Each point represents a plant species; $N = 10$ replicate individuals.

production). In parallel, we found a trade-off between constitutive defences (i.e. stem resin and needle phenolics) and their inducibility across pine species. These results suggest that trade-offs between constitutive defences and their inducibility may explain geographical patterns observed in our defensive traits. Specifically, we argue that constitutive defences could evolve as proposed by the Resource Availability Hypothesis (RAH). Colder environments (high latitude, altitude) are associated with slow-growing species with high tissue value (Coley *et al.* 1985; Fine *et al.* 2004). Consequently, plant species adapted to more stressful environments showed increased allocation to relatively costly constitutive defences (Karban 2011) as they cannot easily replace damaged tissues (Coley *et al.* 1985; Endara & Coley 2011). Inducibility showed opposing patterns to constitutive defence, increasing in fast-growing species from warmer climates (low latitudes and elevations), consistent with greater resource availability and with the constitutive-induced defences trade-off. Notably, these findings are contrary to those of Rasmann & Agrawal (2011), which found a positive correlation between constitutive and induced defences (cardenolides) in milkweeds (*Asclepias* spp.), and an increase in induced defence at lower latitudes. We speculate that long-lived woody pine trees and herbaceous milkweeds

may not be readily comparable due to their different modes of defence (carbon- and nitrogen-based defences respectively) and different growth habits.

Our results also showed that pine species from dry regions (associated with less precipitation in the driest month and more precipitation seasonality) showed the lower depression of chemical defences associated with SA application. This result contrasts with the predictions posed by the defence constraint (DC) hypothesis which proposes that, because of antagonistic crosstalk between signalling pathways for biotic and abiotic stress, the allocation to chemical defences is in conflict with the evolution of tolerance to abiotic stress (Siemens *et al.* 2009). Previous studies with herbaceous plants supported the major predictions of DC hypothesis, demonstrating that signalling pathway to drought stress by abscisic acid is antagonistic to JA/SA/ethylene signalling of herbivore damage (Fujita *et al.* 2006; Ton *et al.* 2009). From a physiological point of view, water deficiency for isohydric plant species, such as pines, is intrinsic to stomatal closure (Fujita *et al.* 2006; Ton *et al.* 2009). This fact would lead to carbon starvation in plants subjected to drought stress, thus compromising resource availability for carbon-based defences (but see Erb *et al.* 2009 as counter-example).

Figure 6 Relationships between growth rate and (a) constitutive level of non-volatile resin in the stem and needles, (b) jasmonic acid-associated inducibility of those defences (JA) in 18 Pinaceae species. The *r* and *P* values shown were obtained with a phylogenetic independent model (PGLS). Each point represents a plant species mean (*N* = 10 replicate individuals).

Non-volatile resin, especially in the stem, was the defensive trait most constrained by geographical and climatic factors. This result may be explained by the differential costs of synthesis and mobilisation between resin and phenolics. While the former herbivore-induced defence is based on changes involving cell division and differentiation, the latter only involves local changes in cell metabolism (Bonello *et al*. 2006). Resin compounds are produced by epithelial cells surrounding the resin ducts specialised in oleoresin storage (Franceschi *et al*. 2005). After herbivore damage to stems, most coniferous species produce numerous tangential rows of wound-induced resin canals in the early wood (i.e. traumatic resin ducts, Franceschi *et al*. 2005). The production of these wound-induced resin ducts is thus a slower (taking days to months) and more energetically expensive (depleting carbohydrate reserves) process compared to phenolic compound synthesis (Bonello *et al*. 2006). In addition, resources allocated to oleoresin defences are no longer available for other functions after damage, as resin cannot be further metabolised and reassimilated (Franceschi *et al*. 2005). On the other hand, it is well known that large quantities of phenolic compounds are quickly synthesised *de novo* in most plant tissues as a response to herbivore damage (Salminen & Karonen 2011; Carrillo-Gavilán *et al*. 2012). Because most phenolic compounds are water soluble, they can be rapidly synthesised and easily mobilised to damage zones, but also reassimilated after damage (Salminen & Karonen 2011).

In conclusion, we found that geographical and climatic factors act as global predictors of pine tree defensive strategies and functional defensive traits. Moving poleward, and into regions of colder temperature, constitutive defences increased and inducible defences decreased. In addition to this, we found trade-offs between constitutive and inducible defences across species. Climate thus acts as a key driver of defence profiles by mediating the constraints imposed by trade-offs. We propose that a consideration of this dynamic between climatic and evolutionary constraints may help to resolve the long-standing debate surrounding purported clines in plant defence.

AUTHORSHIP

XM, RZ, LS conceived and designed the experiment. KAM, SR provided new ideas and perspectives. RZ, LS contributed reagents and materials. XM, ACG, RZ, LS performed the experiment. XM, ACG performed the chemical analyses and collected plant defence data. WKP conducted spatial analyses for compiling climate data. XM, SR analysed data. XM wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

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