



# Ecogeographical determinants of investment in chemical defences in pines

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## Abstract

**Key message** Pine tree species exhibit significant levels of phenotypic variation in the investment in defences, which can be correlated with life-history traits, geographical affiliations and climate.

**Abstract** Understanding the ecological and environmental correlates of tree defences has value for understanding forest susceptibility to pests and pathogens in a time of global change. In the present work, we assessed how life-history attributes and biogeography are related to chemical defences of pine trees in Palaearctic and Nearctic forests. We studied adult trees of ten pine species in forests of Portugal and Eastern North America. We measured total phenols (using gallic acid as a standard) and condensed tannins (as catechin hydrate equivalents) in the phloem of pine branches. Pine trees in forests of Eastern North America presented higher levels of total phenolic content in their phloem tissues than pine trees in forests in Portugal. Higher values of precipitation were correlated with higher phenolic content and higher temperatures were associated with higher levels of condensed tannins. A few life-history traits—the maximum height reached by each pine species, the age at which they start reproducing, and the size of seeds—were positively related with defences. The present work points to interactions between life-history attributes, climate, and geographic location as predictors of defensive investment in pines. The results are useful for understanding differences within and among pine forests in susceptibility to pests and pathogens.

**Keywords** Condensed tannins · East North America · Phloem, Portugal · Secondary metabolites · Total phenols

## Introduction

It is well established that plants exhibit extreme phenotypic variation in the investment in defences against herbivores and pathogens, which often can be correlated with life-history traits and/or habitat properties. Accordingly, over decades, several hypotheses have been put forward to understand the patterns and mechanisms underlying this variation (described in Stamp 2003). Early theories postulated that plants invest more resources into defences when their fitness costs are outweighed by the benefits of reduced herbivory and disease load (Feeny 1976; Stamp 2003). However, besides the strength of herbivore/pathogen pressure, resource availability and abiotic stress were also found to govern the plant's investments in defences. The “resource availability hypothesis” (Coley et al. 1985; Endara and Coley 2011) is a prominent theory explaining the effects of abiotic selection pressures on shaping plant defences. It predicts that, since defences are costly and plants cannot produce unlimited amounts of resistance components, species

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adapted to a high-resource environment show high growth rates but low resistance to stress and herbivore/pathogen attacks. Another hypothesis considers growth rates as a response to other life-history attributes like longevity, shade tolerance, age of reproduction, seed dispersal, and competitive strength (Loehle 1988, 2000; Kempel et al. 2011). Thus, the different life-history attributes developed by plant species and consequent trade-offs that determine the strategies and defensive armaments of a plant, may predict levels of susceptibility to herbivores and pathogen.

Geographic location in itself has also been generally correlated with plant investment in defences. The greater diversity, greater specialization and higher numbers of herbivores in tropical environments are thought to lead to higher levels of herbivory at lower latitudes and the evolution of higher levels of defence in the tropics compared to temperate regions—the “low latitude/high defence hypothesis” (LHDH, Coley and Aide 1991; Marquis et al. 2012). Later studies extended LHDH to interpret differences between other biomes or even latitudinal gradients within biomes, diverting from the initial focus on the tropics (Anstett et al. 2016). Moreover, climate changes since the Pleistocene in combination with the different orientation of the large mountain systems in Eurasia and the Americas have given rise to different floristic and faunal realms and biotic interactions at the same geographical latitudes (Adams and Woodward 1989). This has led to marked differences in plant biodiversity and strength of biotic interaction between regions, resulting in regional differences in plant defensive traits (Huntley 1993; Liebhold et al. 2016).

Understanding responses to ecological and biological disturbances has become increasingly important in view of the unprecedented extent of global trade and traffic during the past decades. Forests have been exposed to an ever-increasing pressure by exotic invasive pests and pathogens (Aukema et al. 2010; Santini et al. 2013). High-impact exotic pest species have the potential to completely restructure forests even at the continental scale, as e.g. chestnut blight, Dutch elm disease, and beech bark disease (Brasier 1991; Milgroom et al. 1996; Garnas et al. 2012; Lovett et al. 2016; Schulz et al. 2020). Several hypotheses have been put forward to explain the locally differing susceptibility of forests to such biological disturbances. For example, diversity of tree species was positively related to the number of established pests due to the higher number of potential hosts (Liebhold et al. 2013; Santini et al. 2013). Historical lack of co-evolution between host and pest can result in a “defence-free space”, which favours the establishment, spread, and impacts of exotic pests (Gandhi and Herms 2010; Liebhold et al. 2016).

Pines are an economically and culturally significant group of tree species worldwide, due to their geographic distribution, high growth rates, production of quality wood, and partly edible seeds (Richardson and Rundel

1998). They inhabit regions with high climatic and edaphic variability in both the Palearctic and Nearctic, with life-history traits, that vary greatly among species, populations and stands (Tapias et al. 2004; Keeley 2012). This variation has been related to the capacity for adaptation to sites with different resources and the resulting differences in colonization dynamics (Proches et al. 2012). Moreover, there is growing evidence that natural selection by abiotic and biotic conditions and resulting different life-history traits generate marked differences in chemical defences and defensive strategies against herbivores and pathogens between related tree species (Zas et al. 2011; Moreira et al. 2014; Carrillo-Gavilán et al. 2015; Pimentel et al. 2017). However, this evidence originated mostly from common-garden experiments under controlled conditions. There is not much understanding of how differences in investment in defences between pine species translate into differences under natural conditions in corresponding biogeographical areas.

The first question pursued in the present work was whether affiliation with Nearctic or Palearctic realms is related to the investment in defences by adult pine trees in forest settings. The answer is relevant for understanding differences between Eurasia and North America in the performance of high-impact pests, including invasive species. Alternatively, species-specific life-history attributes and adaptation to climate may play a more crucial role within each realm, in accordance with the resource availability hypothesis and latitudinal herbivory-defence hypothesis. To pursue these questions we studied pine species with different life-history attributes and adapted to distinct climate regions in forests in Eastern North America (Nearctic) and Portugal (Palearctic). Phloem tissues are rich in nutrients and a resource for many heterotrophic organisms, including insects, vertebrates, fungi and bacteria, and thus, generally can be expected to contain robust armours of chemical defences. Distinct levels of defences in these concealed tissues have been found in different pine species, which could be associated with susceptibility to major pests affecting differently the Nearctic and Palearctic, such as bark beetles and the pinewood nematode *Bursaphelenchus xylophilus* (Pimentel et al. 2017; Raffa et al., 2017). This renders the phloem ideal for a comparative study. As in most conifers, chemical defence systems of pine trees are based on high concentrations of terpenoid oleoresins and phenolics (Franceschi et al. 2005). Thus, as a measurement of investment in defence by pine trees, we determined the concentration of phenolics and condensed tannins in phloem tissues. These analytics capture an aggregate of secondary metabolites, that are structurally diverse and can have multiple functions in plant ecology. They are all products of the shikimate pathway and are generally regarded as important components of constitutive plant defence systems (Franceschi et al. 2005;

Barbehenn and Constabel 2011; Karban 2011; Raffa et al., 2017; Holopainen et al. 2018).

## Materials and methods

### Pinus species and sampling

During summer 2010, samples of six pine species were obtained from subtropical and continental pine forests in three locations of eastern USA. Four of these species are native to East North America—longleaf pine, *P. palustris* Mill., loblolly pine *P. taeda* L., white pine *P. strobus* L., red pine *P. resinosa* Aiton—and two species were introduced from Europe—Austrian pine *P. nigra* Arnold and scots pine *P. sylvestris* L. During spring 2013 another set of samples of six pine species was collected from coastal and montane pine forests in three locations in Portugal: the European species Aleppo pine *P. halepensis* Mill., stone pine *P. pinea* L., maritime pine *P. pinaster* Aiton, *P. nigra* and *P. sylvestris*, and the introduced North American Monterey pine, *P. radiata* D. Don (Table 1).

Samples were taken from healthy trees (~3–5 m in height) with accessible canopies. Five to fifteen individuals were selected for each combination of species and location. From each of the selected trees, a large branch was collected, cut into pieces of ~20 cm length, and transported to the laboratory in ice coolers. From the subsamples the bark was removed and the phloem and cambium was separated from the xylem and freeze-dried at –80 °C for 48 h.

### Analytical procedures for secondary metabolites

The freeze-dried material was powdered with an MM2000 ball mill (Retsch, Haan) and aliquots of 0.1 g were extracted with 10 mL of boiling purified water. The solution was filtered and stored at –4 °C until analysis, which was performed within 24 h. The total phenolic content (TPC) was determined using the Folin–Ciocalteu method, with gallic acid as standard (Singleton and Rossi 1965). For analysis, 0.5 mL of the extract was mixed with 2.5 mL Folin–Ciocalteu reagent (10% v/v, Sigma-Aldrich, St. Louis) and 2 mL sodium carbonate (7.5% w/v, Fluka, Buchs) and heated for 5 min at 60 °C. After cooling to room temperature the absorbance was determined at 760 nm with an U-2000 spectrophotometer (Hitachi, Tokyo). The total phenolic content was calculated as gallic acid equivalent from the calibration curve (0.02–0.150 g gallic acid/L). Quantification of total proanthocyanidins (PAC) was performed according to Heil et al. (2002), using the DMCA (3, 4-dimethoxycinnamic acid) method. Plant material (0.5 g) was extracted

with 20 mL methanol at 25 °C during 48 h. After filtration, extraction was repeated twice with 10 mL MeOH each, and the three extracts were combined. The extracts were diluted (1 + 9) with ethanol, and 100 mL of the diluted extract were mixed with 1 mL of a DMCA solution (0.1% DMCA (Sigma-Aldrich, St. Louis) in methanol/conc.HCl (9:1 v/v)). The absorbance was read at 640 nm, and the concentrations were determined using a calibration curve with catechin hydrate (15–250 mg/L). Contents of condensed tannins were expressed as catechin hydrate equivalents per gram of dry plant material (g CE/g DW).

### Life-history, geographical and environmental variables

We gathered data for each of the pine species describing life-history traits and their climatic envelope. Data on life-history traits were obtained from the literature including online databases (Table S1 and references therein). The following traits were recorded: (1) Maximum height (m)—the height of the oldest and largest individuals reported in the literature; (2) h50—a common site index used in forestry, indicating the height (m) of trees at an age of 50 years; (3) Maximum DAP—maximum diameter at breast height (m) in the oldest and largest individuals of each species; (4) Tree ring width (mm)—the average width of tree rings formed during the initial 30 years of tree growth (these data were obtained from 20 trees each from the centre of each species distribution area, randomly selected from NOAA (<https://www.ncdc.noaa.gov/paleo-search/?dataTypeId=18>); for *P. radiata* see extra legend in Table S1); (5) Longevity—age of the oldest individual of each species; (6) Age of seed production—age at which each species starts producing viable seeds; (7) Seed mass—average seed weight. Each variable was calculated as the average from all sources (Table S1).

For the native geographic range of the investigated pine species and the corresponding climatic envelopes, distribution maps were acquired online in shape-file format from two sources: Data Basin (<https://databasin.org/search/#query=pinus>); and Euforgen—European Forest Genetic Resources Programme (<http://www.euforgen.org/species/>). *P. radiata*, is the world's most extensively planted softwood, mainly grown in forest plantations outside its native range where it is rare and sparsely distributed (Lavery and Mead 1998). In this case, data were used from the geographical ranges considered optimal for its growth (<http://www.fao.org/3/a-i3274e.pdf>). Climate data were obtained from WorldClim-Global Climate Data (<http://www.worldclim.org>; Hijmans et al. 2005). The following climate data were considered: BIO1: Mean annual temperature (°C), BIO4: seasonality of temperature, expressed as the standard deviation of temperature of months multiplied by 100, BIO5: maximum

**Table 1** Location of the sampling sites of pine species in Eastern USA and Portugal with the respective climate classifications according to Köppen and climatic variables obtained from World-Clim-Global Climate Data (<http://www.worldclim.org>, averages for the period 1950–2000)

Country	Location	Altitude (m)	Climate	BIO1 (°C)	BIO4	BIO5 (°C)	BIO6 (°C)	BIO12 (mm)	BIO13 (mm)	BIO14 (mm)	BIO15	<i>Pinus</i> species
USA	Wilmingtong, North Caro- lina	0–50	Humid subtropical köppen Cfa	15.7	6865	31.7	1.2	1266	192	75	32	<i>P. palustris</i> , <i>P. taeda</i>
	Upper valley, new hamp- shire/vermont	150–300	Humid conti- nental köppen Dfb	7.7	9857	27.1	– 14.3	1018	90	58	12	<i>P. strobus</i> , <i>P. resinosa</i> , <i>P. nigra</i>
	Finger lakes national for- est, New York	300–600	Humid conti- nental köppen Dfb	7.5	9173	27.9	– 8.7	949	88	42	22	<i>P. resinosa</i> , <i>P. sylvestris</i>
Portugal	Melides	0–50	Mediterranean köppen Csa	17.2	4243	29.3	7.7	444	90	2	63	<i>P. pinea</i> , <i>P. pinaster</i>
	Setúbal Peni- sula	0–50	Mediterranean Köppen Csa	16.3	4029	28.3	8.4	796	103	3	64	<i>P. pinea</i> , <i>P. pinaster</i> , <i>P. halepensis</i>
	Montesinho national Park	400–1200	Mediterranean köppen Csb	11.0	5676	28.4	1.2	1220	105	14	47	<i>P. pinaster</i> , <i>P. radiata</i> , <i>P. nigra</i> , <i>P. syl- vestris</i>

(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

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. In ArcGIS, for each species, a raster for each variable was extracted using the shape-files of the distribution range, and the mean value of each parameter was calculated. The average values of latitude and longitude describe the centre of the distribution range of each pine species (Table 2). The same climatic variables were extracted for the sampling site's location.

## Statistical analysis

We tested for differences in total phenolic content (TPC) and proanthocyanin content (PAC) considering the averages of each pine species/location combination. Differences among locations and co-existing species were tested with *t*-tests. We tested whether each life-history trait, geographical site and climatic variable could explain the variation in TPC and PAC starting with simple univariate general linear models. We tested geo-climatic variables obtained from each species distribution maps (Table 2), as an indication of the conditions leading to species-specific characteristics; and from the sampling locations for each species/location combination (Table 1), as an indication of the effect of local conditions on sampled trees. The explanatory variable that made significant contributions to the model were then combined following the hierarchical partitioning model of Quinn and Keough (2002). Models were compared with respect to the Akaike information criterion, corrected for small sample sizes (AICc). If the inclusion of a new variable reduced AICc, it was retained in the model. This procedure ended when the next hierarchical level failed to include new significant variables or reduce AICc. To reduce the effects of collinearity, intercorrelated variables were not included in the same model (Table S2). These analyses were performed using the software GraphPad Prism 8.0.2 (GraphPad Software Inc.).

## Results

Generally, pines in Eastern North American trees contained higher concentrations of TPC than pines in Portugal ( $t=4.04$ ,  $df=14$ ,  $P=0.001$ ; Fig. 1), but did not differ with respect to PAC ( $t=1.39$ ,  $df=14$ ,  $P=0.19$ ; Fig. 2). Comparing the two pine species for which we have data from both continents: *P. nigra* from the norther USA showed higher concentrations of TPC than *P. nigra* from PT ( $t=3.45$ ,  $df=8$ ,  $P=0.009$ ), while no differences were found in level of PAC for this species ( $t=1.57$ ,  $df=8$ ,  $P=0.15$ ). In contrast

the concentrations of TPC in *P. sylvestris* did not differ between countries ( $t=1.12$ ,  $df=8$ ,  $P=0.30$ ), but PAC levels were higher in the trees from Portugal ( $t=5.96$ ,  $df=8$ ,  $P<0.001$ ) (Figs. 1 and 2, 3).

There were some clear differences among co-existing species. In North America: (1) *P. palustris* presented higher concentrations of both TPC and PAC than *P. taeda*; (2) *P. resinosa* presented significantly higher concentrations of TPC than co-occurring *P. sylvestris* and *P. strobus*. In Portugal, *P. pinea* tended to be the species with higher concentrations of both TPC and PAC, with significantly higher concentrations than in *P. pinaster* and *P. halepensis* (Fig. 1 and 2).

Two life-history traits and 10 geo-climatic variables were significantly related with the concentrations of TPCs in the phloem (Table 3, Fig.3). Precipitation of the driest month (BIO14) of both distribution areas and sampling locations, were the variables that rendered the best univariate models (Models 5 and 10, Table 3). The only model with several predictor variables providing more information than the best univariate model was that which included the precipitation of the wettest month and the geographical longitude of the sampling location (Model 13, Table 3). For the PAC content of the phloem, one life-history and six geo-climatic variables showed significance in univariate models (Table 4, Fig.3). The best single predictor was average annual temperature of the sampling location (Model 4, Table 4). Only one model with more than one predictor variable had greater information content than the best univariate model, including seed mass and the maximum temperature of the warmest month of the sampling location (Model 8, Table 4).

## Discussion

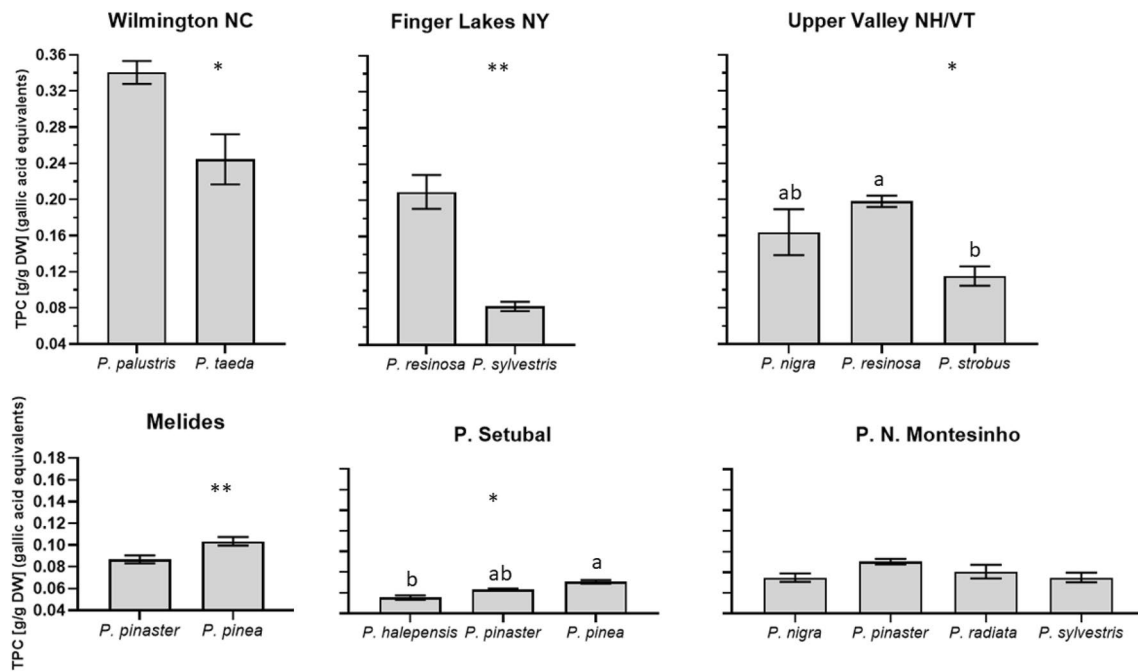
Trees of several pine species native to the north-eastern United States showed higher concentrations of phenolics in their phloem than those native to forests of Portugal, suggesting a higher level of chemical defence in the Nearctic than in the Palearctic. In contrast, tannins, another component of chemical defence against pests and pathogens proved to be similar in pines of both realms. Carrillo-Gavilán et al. (2015) also reported that Nearctic pine species invested up to 70% more in constitutive phenolics in their needles than Palearctic species, whereas inducible phenolics showed no differences in pine trees of both regions. These results were obtained in a common-garden experiment with pine seedlings, suggesting that biogeographical differences in phylogeny could have played an important role in the evolution of a constitutive chemical defence in pines. The results of the present work were obtained from adult trees in local forests, thus allowing neither differentiation between constitutive and induced chemical defences, nor a control of

**Table 2** Geographical coordinates and climate variables of the native habitat areas of the investigated pine species (extracted from <https://databasin.org/search/#query=pinus> and <http://www.eurofung.org/species/>)

Species	BIO1 (°C)	BIO4	BIO5 (°C)	BIO6 (°C)	BIO12 (mm)	BIO13 (mm)	BIO14 (mm)	BIO15	Altitude (m)	Latitude	Longitude
<i>P. palustris</i>	17.9	6477	33	2	1346	161	70	25	73	31°44'55.547"N	84°13'40.717"W
<i>P. taeda</i>	16.9	6977	33	1	1321	150	74	20	98	32°45'47.275"N	85°39'48.517"W
<i>P. resinosa</i>	3.6	10,764	25	-18	914	105	49	25	313	46°12'5.202"N	79°22'30.263"W
<i>P. strobus</i>	5.1	10,051	25	-15	979	108	56	22	321	44°27'52.596"N	78°30'54.662"W
<i>P. radiata*</i>	12.5	6288	32	-1	1225	98	4	70	870	37°42'27.232"N	119°37'18.491"W
<i>P. pinaster</i>	12.6	5361	27	2	832	113	23	39	528	41°4'28.52"N	4°22'42.514"W
<i>P. pinea</i>	14.2	5785	29	3	642	94	16	45	387	41°52'40.411"N	3°10'2.831"E
<i>P. halepensis</i>	13.8	6014	30	2	549	76	15	41	622	39°0'58.45"N	1°18'16.787"E
<i>P. nigra</i>	9.4	6732	26	-3	754	99	33	34	1023	41°52'24.99"N	20°56'55.93"E
<i>P. sylvestris</i>	-0.7	12,015	22	-22	656	80	23	41	303	59°24'33.718"N	56°5'27.434"E

Latitude and longitude refer to the central point of the distribution ranges and altitude was calculated as the mean elevation of the respective ranges. Climatic variables were obtained from WorldClim-Global Climate Data (<http://www.worldclim.org>, averages for the period 1950–2000) and means were calculated for each range; BIO1) annual mean temperature, °C; BIO4) temperature seasonality, expressed as the standard deviation of temperature between months  $\times 100$ ; 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 of precipitation between months

\**P. radiata*, is different from the other pine species studied in that it mainly occurs in forest plantations outside its native range. Thus, in this case, data was obtained from the geographical ranges considered optimal for its growth (<http://www.fao.org/3/a-i3274e.pdf>)



**Fig. 1** Total Phenolic Contents (TPC, means and SE) in the phloem of adult pines, measured as gallic acid equivalents (g/g DW). The samples were collected in three locations in eastern U.S.A. (upper panel) and in three locations in Portugal (lower panel). Note the different scaling of the y-axes for the two regions. Asterisks indicate statistically significant differences in the TPC contents between

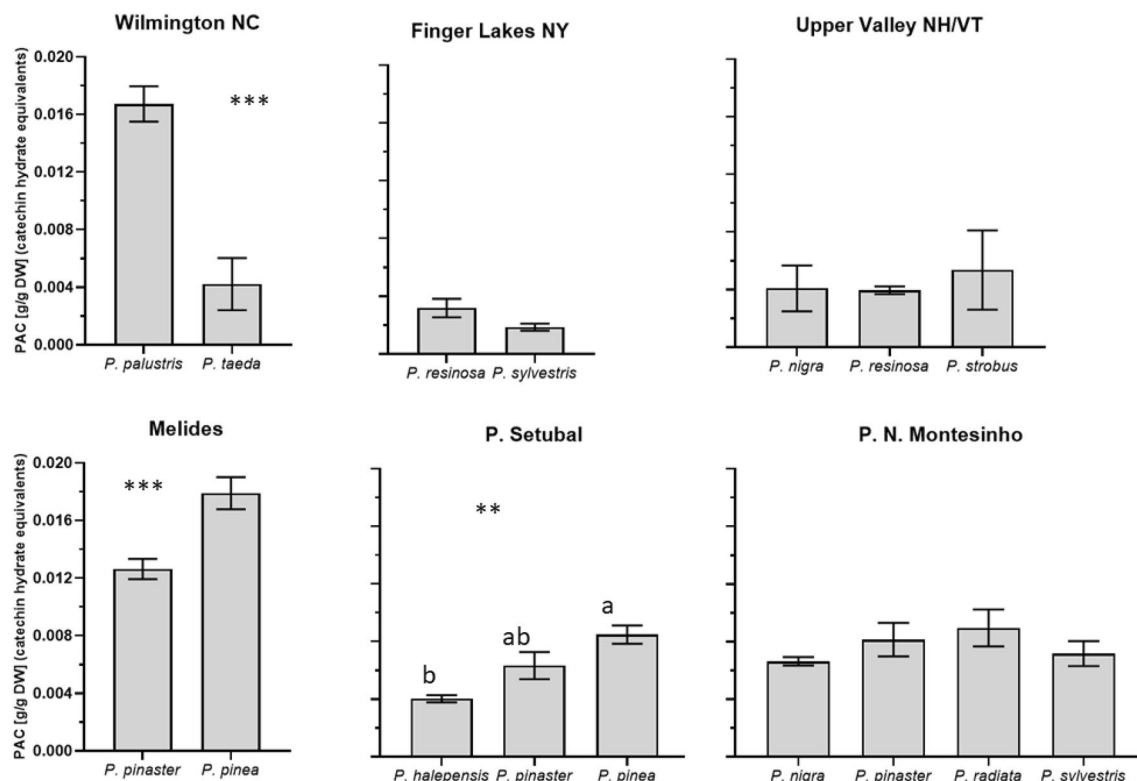
pine species from different provenances (Wilmington NC:  $t_8=2.79$ ,  $P=0.034$ ; Finger Lakes NY:  $t_8=6.53$ ,  $P=0.002$ ; Upper Valley NH/VT:  $F_{2,12}=6.42$ ,  $P=0.013$ ; Melides:  $t_{23}=-3.03$ ,  $P=0.005$ ; P. Setubal:  $F_{2,12}=3.93$ ,  $P=0.049$ ; P. N. Monteseinho:  $F_{3,16}=2.44$ ,  $P=0.103$ ; \* significantly different according to Tukey HSD  $P<0.05$  and \*\*  $P<0.01$ ; different letters indicate significantly different means)

environmental variables. High levels of defensive traits in the Nearctic pines could represent the response to ongoing or earlier biological pressure (Lev-Yadun and Holopainen 2009; He et al. 2012; Carrillo-Gavilán et al. 2015). More European phytophagous insects have successfully established in North American than in European forests (Liebhold et al. 2016). This was attributed to a higher adaptability and competitive strength of these pests, but also implies that European pines have a more effective chemical armament against these superior antagonists.

Considering the two species for which we have data from individuals in both continents, *P. nigra* also showed higher concentrations of total phenolics in North American forests, where it was introduced, compared to individuals of its native European regions, pointing to a higher biological pressure in the Nearctic. On the other hand, *P. sylvestris* presented only very low concentrations of phenolics in the phloem and did not show a clear distribution pattern between the two realms. Nevertheless, there is some evidence of pressure possibly inducing chemical defence in pines of North America. The pine wood nematode *Bursaphelenchus xylophilus* is native to North America where it is ubiquitous and abundant but does not cause significant damage due to a superior chemical defence potential of the pines, while as an invasive species it is one of the major threats to native pines

in Eurasia (Pimentel et al. 2014, 2017). Another important disturbance from which pine forests are periodically suffering is by bark beetle (subfamily Scolytinae) which are more diverse and more aggressive in North America than in Europe (Hicke et al. 2012; Schafstall et al. 2020).

Within each biogeographical region, adaptation to the local climate also appeared to affect the evolution of pine secondary metabolites. Secondary metabolites could be related with the climate of the area where the pine species originated, but more strongly from the actual location of the sampled trees. One pattern suggested that adaptation to higher precipitation leads to higher phenolic content in phloem tissues. Another pattern suggested that adaptation to higher temperature leads to higher levels of condensed tannins and that pines from lower latitudes tend to express more condensed tannins. Adaptation to the Nearctic realm in itself also leads to higher phenolic content, as is exemplified by the case of *P. nigra* that was sampled in both realms (Fig. 1). Moreira et al. (2014), also working with pine seedlings in a common-garden experiment, found that moving poleward and into regions of colder temperature was associated with higher levels of constitutive chemical defence but lower levels of inducible defence. The authors explained these patterns with the resource availability hypothesis: Colder climates are associated with slow-growing species for which



**Fig. 2** Condensed tannins or total proanthocyanidins content (PAC) in the phloem of adult pine trees, measured as catechin hydrate equivalents (g/g DW). The samples were collected in three locations in eastern U.S.A. (upper panel) and in three locations in Portugal (lower panel). Asterisks indicate statistically significant differences in the TPC contents between pine species from different provenances

(Wilmington NC:  $t_8 = -5.72$ ,  $P < 0.001$ ; Finger Lakes NY:  $t_8 = 1.93$ ,  $P = 0.090$ ; Upper Valley NH/VT:  $F_{2,12} = 0.18$ ,  $P = 0.84$ ; Melides:  $t_{23} = 4.21$ ,  $P < 0.001$ ; P. Setubal:  $F_{2,12} = 10.88$ ,  $P = 0.002$ ; P. N. Montesinho:  $F_{3,16} = 1.11$ ,  $P = 0.37$ ; \*\*significantly different according to Tukey HSD  $P < 0.01$  and \*\*\*  $P < 0.001$ ; different letters indicate significantly different means)

formation of new tissue is slow and costly, thus fostering a better protection of the existing tissue by a higher level of constitutive chemical compounds (Coley et al. 1985; Endara and Coley 2011; Karban 2011). However, in our study a tendency for increasing levels of chemical defence in warmer and wetter climates was observed, which is consistent with the low latitude/high defence hypothesis and implies higher resource investments in defence due to an increased herbivore pressure (Coley and Aide 1991; Marquis et al. 2012). Due to more favourable climatic conditions and a longer flight period of the pests, pine forests in subtropical and Mediterranean regions are subjected to a heavier attack by woodborers and bark beetles (Pimentel et al. 2014; Firmino et al. 2017). On the other hand, drought or dry weather negatively impact the vitality and infectiveness of fungal pathogens of trees (Sturrock et al. 2011). Thus, differences in environmental conditions, which favour either pressure by insects or by pathogenic fungi could explain the differences between our results and those of Moreira et al. (2014).

In addition to the geographical and climate-related differences in the production of secondary metabolites, we also found significant species-specific differences between

co-existing pine trees. We were able to relate a few life-history traits with the contents of phenolics in the phloem: the maximum height reached by each pine species, the age at which they start reproducing, and the size of its seeds. However, we could not correlate the levels of the chemical defences of the adult pines in any sense with their growth rates (as indicated by the parameters h50 and tree ring width; Table S2), as did Moreira et al. (2014) with pine seedlings.

Functional traits are, however, often involved in correlated syndromes (Reich et al. 2003). Thus, the life-history traits for which we found a correlation with secondary metabolites in the phloem might be indicative of special strategies in life history and chemical defence through their correlation with other traits. Generally, the evolution of larger seeds in plants is favoured by high-competition and low-resource environments that limit growth and development (Moles and Westoby 2006), conditions that favour other life-history attributes such as shade tolerance, slow growth rates and long-life spans (Coley et al. 1985; Endara and Coley 2011). Seed size in pines is a trait that sets apart evolutionary strategies. Pine species adapted to more stressful environments produce large seeds which are dispersed



**Table 3** Correlation data and model fits of the total phenol content (TPC in g/g DW) of the phloem of the investigated pines with climate variables (Models including BIO 12 – 15), geographical coordinates and two life-history traits

Model	Variable	$\beta \pm SE$	<i>F</i>	<i>P</i>	<i>R</i> <sup>2</sup>	AICc
1	Maximum height	0.006 ± 0.003	6.18	0.026	0.31	– 37.86
2	Age at seed production	0.010 ± 0.002	24.50	<0.001	0.64	– 82.10
3	BIO12 distribution	2.28e <sup>-4</sup> ± 0.59e <sup>-4</sup>	14.88	0.002	0.51	– 77.78
4	BIO13 distribution	0.003 ± 0.0006	24.19	<0.001	0.63	– 81.97
5	BIO14 distribution	0.003 ± 0.0006	39.37	<0.001	0.74	– 86.99
6	BIO15 distribution	– 0.004 ± 0.0014	0.21	0.009	0.40	– 74.50
7	Altitude distribution	– 1.43e <sup>-4</sup> ± 0.66e <sup>-4</sup>	4.75	0.047	0.25	– 71.30
8	Longitude distribution	– 8.57e <sup>-4</sup> ± 3.23e <sup>-4</sup>	0.07	0.019	0.08	– 73.05
9	BIO13 location	0.002 ± 0.0005	13.83	0.002	0.50	– 77.23
10	BIO14 location	0.003 ± 0.0004	33.25	<0.001	0.70	– 85.16
11	BIO15 location	– 0.002 ± 0.0009	5.94	0.029	0.30	– 72.22
12	Longitude location	– 0.002 ± 0.0004	17.77	<0.001	0.56	– 79.21
13	Bio13 location longitude location	0.001 ± 0.0003 – 0.001 ± 0.0003	28.29	<0.001	0.81	– 88.27*

Model fits were determined using the Akaike information criterion corrected for small sample sizes (AICc). Models Nr. 1–12 refer to univariate analyses; model 13 refers to a model using multiple variables

\*Best model fit. Climate variables BIO12-15 refer to precipitation data, and when specified by “Distribution” were obtained from pine species distribution maps, while “Location” refers to data of the sampling sites, determined for each species/location combination

**Table 4** Correlation data and model fits of the condensed tannins or total proanthocyanidins content (PAC in g/g DW) of the phloem of the investigated pines with climate variables (Models including BIO1, 5–6), geographical coordinates and one life-history trait

Model	Variable	$\beta \pm SE$	<i>F</i>	<i>P</i>	<i>R</i> <sup>2</sup>	AICc
1	Seed mass	1.04e <sup>-5</sup> ± 0.32e <sup>-5</sup>	5.74	0.031	0.29	– 158.58
2	BIO1 distribution	4.09e <sup>-4</sup> ± 1.76e <sup>-4</sup>	5.38	0.036	0.28	– 158.30
3	BIO6 distribution	2.37e <sup>-4</sup> ± 1.07e <sup>-4</sup>	4.90	0.044	0.26	– 157.92
4	BIO1 location	6.95e <sup>-4</sup> ± 2.47e <sup>-4</sup>	7.94	0.014	0.36	– 160.16
5	BIO5 location	0.002 ± 0.0008	5.43	0.035	0.28	– 158.34
6	BIO6 location	2.77e <sup>-4</sup> ± 1.23e <sup>-4</sup>	5.12	0.040	0.27	– 153.42
7	Latitude location	–2.335 ± 0.035	5.45	0.035	0.28	– 158.36
8	Seed mass BIO5 location	9.36e <sup>-6</sup> ± 3.73e <sup>-6</sup> 0.002 ± 0.0007	6.88	0.009	0.514	– 160.44*

Model fits were determined using the Akaike information criterion corrected for small sample sizes (AICc). Models Nr. 1–7 refer to univariate analyses; model 8 refers to a model using multiple variables

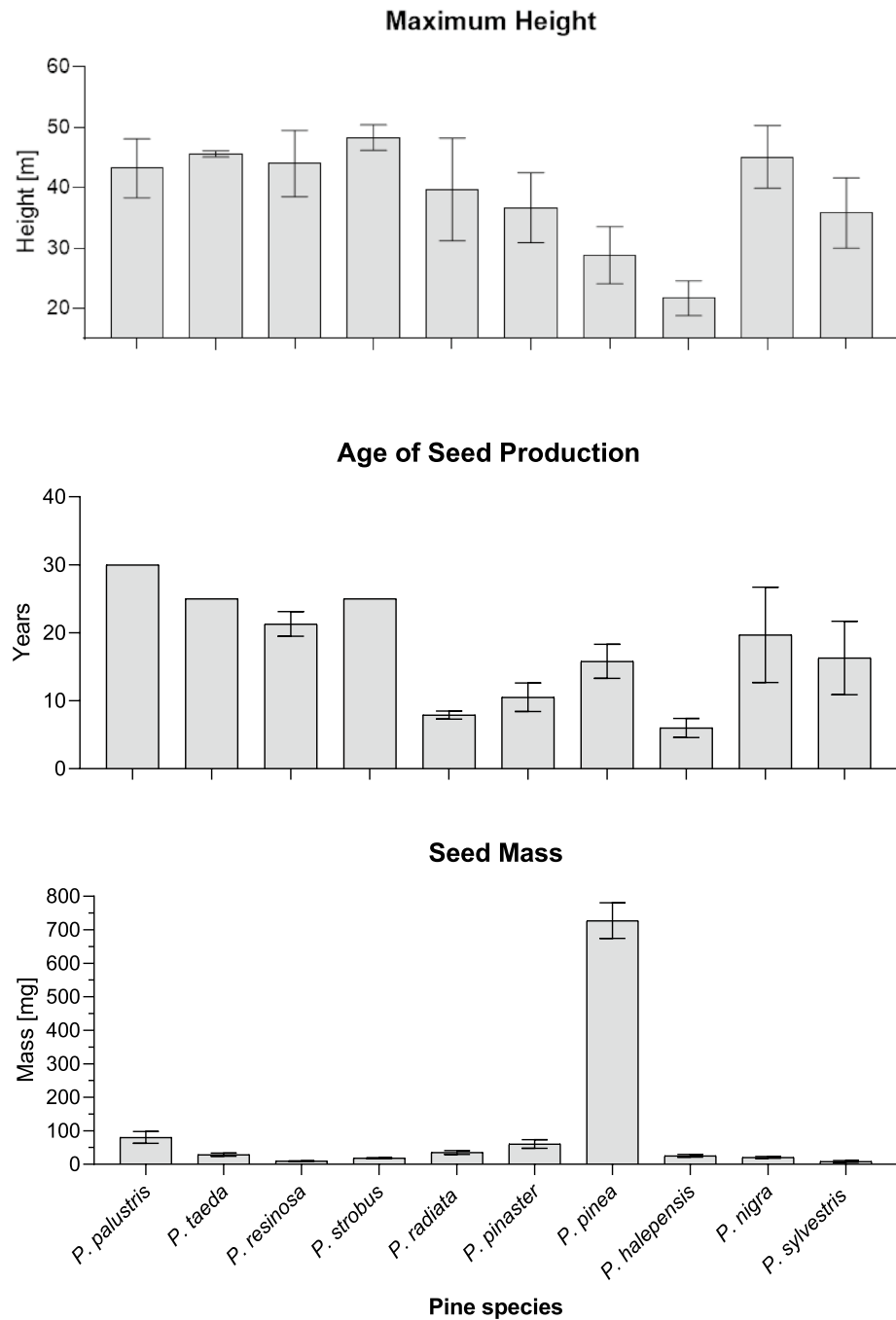
\*Best model fit. Climate variables BIO1-6 refer to temperature data and when specified by “Distribution” were obtained from pine species distribution maps, while “Location” refers to the climate data of the sampling sites, determined for each species/location combination

by animals, while others, growing in richer, but disturbance prone environments, propagate by small and wind-dispersed seeds (Tomback and Linhart 1990; Keeley and Zedler 1998). In the present study, seed mass correlated positively with the content of condensed tannins and with the maximum potential stem diameter (DAP,  $R^2 = 0.60$ ,  $P < 0.05$ ; Table S2 of supplementary material), but negatively with the maximum height ( $R^2 = - 0.52$ ,  $P < 0.05$ ). *Pinus pinea*, a xerophilic and drought-resistant species, native to fire-prone Mediterranean regions with poor sandy soils, invests its biomass in stem diameter growth rather than in tree height. It forms the largest pine seeds and produces higher levels of chemical defences than the less drought-resistant *P. pinaster* and *P.*

*halepensis* which, however, can be found in co-existence with it (Awada et al. 2003; De Luis et al. 2009).

The North American *P. palustris* also produces relatively large seeds (although much smaller than those of in *P. pinea*) and was the Nearctic pine with the highest levels of defences, much higher than the sympatric *P. taeda*. Compared to the other pines native to southeastern USA, *P. palustris* has a complex life cycle, passing through several distinct developmental stages, starting with an initial “grass stage” that lasts for some years, during which height growth is extremely slow, and the needles appear like clumps of grass. This long-lived species is also known for its drought resistance (Platt et al. 1988; Samuelson et al. 2014).

**Fig. 3** Averages  $\pm$  SD of three life-history traits of the investigated pine species, which significantly correlated with the levels of secondary metabolites in the phloem (Tables 3–4)



Age at seed production correlated positively with maximum tree height ( $R^2 = 0.69$ ,  $P < 0.01$ ) and longevity ( $R^2 = 0.59$ ,  $P < 0.05$ ), and the TPC content of the phloem was positively correlated with longevity and age at seed production (Table 3). These findings could imply that tree species with the potential to reach larger size and live longer, start to reproduce later and invest more resources in defences. This interpretation is compatible with the “resource availability hypothesis” which explains variation

of investment in defences. The differences in the content of chemical defences in the phloem might contribute to explain differences in the susceptibility to some high-pressure forest pests. For example, *P. pinea* has been proved to be much more resistant to *Bursaphelenchus xylophilus* than *P. pinaster* after the introduction of this pathogen in Portugal (Pimentel et al. 2017), while *P. palustris* is less susceptible to the bark beetle *Dendroctonus frontalis* than *P. taeda* in North America (Friedenberg et al. 2007).

## Conclusion

The present work shows that pines in the Nearctic tend to produce higher levels of chemical defences than those native to the Palearctic, thus corroborating results obtained with seedlings in common-garden experiments. This conclusion might indicate that Nearctic pines have been under a stronger evolutionary pressure to better defend their tissues. However, our results also point to interactions between chemical defences, species-specific life-history traits, and climatic adaptation.

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**Author contribution** CSP and MPA conceptualized the ideas behind the work; CSP, EVG and MPA collected the samples in the Field; CM, OC and EVG conceptualized the work on the secondary metabolites, while EVG did all the analytical work; JC and TC collected and analysed the data on life-history, geographical and environmental variables of the different pine species; CSP analysed the data and wrote the manuscript; All authors read and approved the final version of the manuscript.

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**Data availability** Data and code will be available upon request.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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