Photosynthetic capacity is negatively correlated with the concentration of leaf phenolic compounds across a range of different species

Sally Sumbele1, Mariangela N. Fotelli1, Dimosthenis Nikolopoulos1, Georgia Toulakou1, Vally Liakoura1, Georgios Liakopoulos1, Panagiota Bresta1, Elissavet Dotsika2, Mark A. Adams3,4 and George Karabourniotis1*

1 Laboratory of Plant Physiology, Department of Agricultural Biotechnology, Agricultural University of Athens, Iera Odos 75, Votanikos, 11855 Athens, Greece
2 Stable Isotope Unit, Institute of Material Science, National Center for Scientific Research ‘Demokritos’ 153 10 Aghia Paraskevi, Attiki, Greece
3 School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney NSW 2052, Australia
4 Present address: Faculty of Agriculture, Food and Natural Resources, Institute for Sustainable Solutions, The University of Sydney, NSW 2006, Australia

Received: 11 June 2012; Returned for revision: 22 June 2012; Accepted: 14 August 2012; Published: 30 August 2012

Citation details: Sumbele S, Fotelli MN, Nikolopoulos D, Toulakou G, Liakoura V, Liakopoulos G, Bresta P, Dotsika E, Adams MA, Karabourniotis G. 2012. Photosynthetic capacity is negatively correlated with the concentration of leaf phenolic compounds across a range of different species. AoB PLANTS 2012: pls025; doi:10.1093/aobpla/pls025

Abstract

Background and aims Phenolic compounds are the most commonly studied of all secondary metabolites because of their significant protective–defensive roles and their significant concentration in plant tissues. However, there has been little study on relationships between gas exchange parameters and the concentration of leaf phenolic compounds (total phenolics (TP) and condensed tannins (CT)) across a range of species. Therefore, we addressed the question: is there any correlation between photosynthetic capacity ($A_{max}$) and TP and CT across species from different ecosystems in different continents?

Methodology A plethora of functional and structural parameters were measured in 49 plant species following different growth strategies from five sampling sites located in Greece and Australia. The relationships between several leaf traits were analysed by means of regression and principal component analysis.

Principal results The results revealed a negative relationship between TP and CT and $A_{max}$ among the different plant species, growth strategies and sampling sites, irrespective of expression (with respect to mass, area or nitrogen content). Principal component analysis showed that high concentrations of TP and CT are associated with thick, dense leaves with low nitrogen. This leaf type is characterized by low growth, $A_{max}$ and transpiration rates, and is common in environments with low water and nutrient availability, high temperatures and high light intensities. Therefore, the high TP and CT in such leaves are compatible with the protective and defensive functions ascribed to them.

Conclusions Our results indicate a functional integration between carbon gain and the concentration of leaf phenolic compounds that reflects the trade-off between growth and defence/protection demands, depending on the growth strategy adopted by each species.

* Corresponding author’s e-mail address: karab@aua.gr

Published by Oxford University Press. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/uk/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
Introduction

Data for various leaf traits encompassing many species are important in order to understand the different plant strategies and the adaptation of each species in a particular environment. The correlations between leaf traits provide insights into the selective pressures that have shaped the evolution of vegetation, and can help with the calibration of models predicting vegetation and productivity dynamics with respect to climate and land-use change (Reich et al. 1997; Wright et al. 2004; Shipley et al. 2006; Westoby and Wright 2006; Kattge et al. 2011).

A great variation in photosynthetic capacity ($A_{\text{max}}$) is evident both within and between species. Within-species variation in $A_{\text{max}}$ has been ascribed to variations in leaf nitrogen concentration (Field and Mooney 1986; Evans 1989), due to the large fraction of leaf nitrogen that is invested in the photosynthetic apparatus. This is the reason that generally a strong positive correlation between $A_{\text{max}}$ and leaf nitrogen concentration within species has been observed. Between-species variation in $A_{\text{max}}$ occurs even among C$_3$ species, although they share the same photosynthetic metabolism. A global survey dealing with 1 % of vascular plant species on Earth revealed that $A_{\text{max}}$ varied by 120- and 40-fold when expressed on a dry mass and a leaf area basis, respectively (Glopnet; Wright et al. 2004). Such a large variation is believed to be related to the growth strategy and/or the niche of a specific species. Regarding growth strategies, higher $A_{\text{max}}$ is found in fast-growing species and in species with a shorter leaf lifespan than in slow-growing ones and species with a long lifespan. Regarding niches, higher $A_{\text{max}}$ is found in sun than in shade species, and in early successional than in late successional species (Chabot and Hicks 1982; Gulmon and Mooney 1986; Poorter et al. 1990; Lambers and Poorter 1992; Reich et al. 1992, 1997; Wright et al. 2004; Hikosaka 2010). The magnitude of $A_{\text{max}}$ of a species is associated with other functional and structural characteristics of the leaves. Therefore, strong relationships between $A_{\text{max}}$ and other key leaf parameters among species are observed globally. For example, net photosynthetic capacity with respect to mass ($A_{\text{max},n}$) is positively correlated with nitrogen content with respect to mass ($N_{\text{m}}$) and negatively correlated with leaf mass per area (LMA). Probably, these relationships are a consequence of the growth strategy of each species, e.g. rapid- versus slow-growing species (Coley et al. 1985; Coley 1988; Lambers and Poorter 1992; Reich et al. 1992).

Phenolic compounds are the most commonly studied of all secondary metabolites because of their significant concentration and their significant roles in plant tissues (Waterman and Mole 1994; Harborne 1997). The term ‘phenolic’ is used to define substances that possess one or more hydroxyl (OH) substituents bonded onto an aromatic ring. This highly diverse group of secondary metabolites includes mainly simple phenols, lignans, coumarins, flavonoids, tannins and quinines (Waterman and Mole 1994). These compounds fulfil at least three functions: (i) as defensive compounds—they inhibit the activity of herbivores or pathogens (Bennett and Walls 1994; Roberts and Paul 2006); (ii) as sunscreens—they reduce UV and visible-light penetration to sensitive tissues (Caldwell et al. 1983; Middleton and Teramura 1993); and (iii) as antioxidants—they are involved in reducing damage by reactive oxygen species (Rice-Evans et al. 1996; Close and McArthur 2002; Heim et al. 2002; Sakihama et al. 2002; Jaleel et al. 2009). The biosynthesis of phenolic compounds requires energy, carbon skeletons and investment of additional nutrients such as nitrogen, which are diverted from primary metabolism. Therefore, allocation of photosynthetic products and nutrients must be balanced between normal growth processes and defense/protection demands (Herms and Mattson 1992).

Since there have been relatively few studies of the associations between photosynthesis and the concentration of total phenolic (TP) compounds and condensed tannins (CT) across species (see Kattge et al. 2011), it would be interesting to examine whether there is any correlation between $A_{\text{max}}$ (and probably other gas exchange parameters) and TP and CT among species. In order to test such a general concept it is important to include common plant species thriving in different climate zones and ecosystems. In the present study, we tested this hypothesis using 49 common plant species of different life forms from different sampling sites located in the east Mediterranean basin (Greece) and Australia.

Materials and methods

Plant material and study sites

Our data set comprises common vascular plant species from two different countries located in two different continents differing in climate, biogeography and soil conditions: two Greek and three Australian ecosystems (see Table 1). The general criteria for species selection included: (i) a species had to be common, because common species are probably better adapted to the local conditions and, also, have a reasonably good chance of impacting upon major ecosystem processes (Grime 1998; Diaz et al. 2004) and (ii) the collection had to cover a wide range of growth forms, families and habitats. Our species selection was mainly local assembly based and restricted for practical reasons to common
species possessing sufficient leaf size for gas exchange measurements. In Greece, samplings and field measurements were conducted during late spring–early summer (May–June) on Mount Parnitha, Attica, Central Greece, in a typical maquis and phrygana (garigue) formation from 2006 up to 2008, and at Domnista, Eurytania, Central Greece, in a typical temperate forest of southern Europe in 2007. In Australia, corresponding samplings and field measurements were conducted during late spring–early summer (November–December) of 2006 at the following sites: Snowy Plains (New South Wales, Eastern Australia), at a subalpine ecosystem, Britannia Creek—Yara Valley (Victoria, Southern Australia), in a typical temperate ecosystem representing the wettest–coldest edge of the Mediterranean-type ecosystems and Perth (Western Australia, South-western Australia), in a typical Mediterranean-type ecosystem with climatic conditions similar to those in Parnitha, Greece (Table 1). A total of 49 plant species, 32 native in Greece and 17 native in Australia, were studied (Table 2).

For each species, sampling and measurements were conducted on three adult individuals and two fully expanded, current growth season’s leaves per individual. All three individuals were within an area of 100-m radius and had a similar age with leaves accessible for in planta measurements (regarding the limitations of the instruments). Measurements were conducted on fully expanded and sun-morphotype leaves: south-east facing (in the Northern Hemisphere) and north-east facing (in the Southern Hemisphere). There were no indications of temporary shading during their expansion. Leaves with obvious symptoms of herbivore or pathogen attack and senescent leaves of the previous growth period were excluded. Laboratory measurements were conducted on the same six leaves that were used for gas exchange measurements. The leaves were collected after measurements of gas exchange had been completed (see below), wrapped in sealed plastic bags and immediately transported to the laboratory in a portable coolbox.

**Morpho-anatomical measurements**

For calculation of the LMA (g m⁻²), leaves were oven-dried at 70 °C for 48 h. Leaf lamina area was determined from photographs of the leaves by image analysis using Image-Pro Plus (version 3.01, Media Cybernetics, Silver Spring, MD, USA). Image spatial calibration was ensured by the incorporation of a ruler and samples were photographed from a position perpendicular to the sample plane to avoid geometric distortion of the images. Leaf mass per area was estimated as the ratio of leaf dry mass to leaf area (g m⁻²). For total leaf thickness (LT) measurements, hand-cut cross-sections were made on fresh leaves (replicates as above) of all samples. Leaf density (LD; g cm⁻³) was calculated.

### Table 1 Study site coordinates and climatic data.

| Site             | Description                                           | Coordinates of meteorological station (lat.; lon.) | Altitude of meteorological station (m.a.s.l.) | Altitude of study site (m.a.s.l.) | T_min (°C) | T_max (°C) | Precipitation (mm) |
|------------------|-------------------------------------------------------|--------------------------------------------------|---------------------------------------------|---------------------------------|------------|------------|-------------------|
| Snowy plains     | Subalpine Eucalyptus *pauciflora* woodlands          | S36°17′38″; E148°58′21″                            | 930                                         | 1400–1500                      | 3.9        | 18.1       | 502.2              |
| Britannia creek  | Open messmate forests                                 | S37°51′36″; E145°44′24″                            | 189                                         | 400–600                        | 7.0        | 18.5       | 1445.7             |
| Perth            | Botanic garden (King’s Park & Botanic Garden)        | S31°55′39″; E115°58′35″                            | 15.4                                        | 50                             | 12.1       | 24.3       | 781.9              |
| Domnista         | Deciduous broadleaf *Quercus frainetto* and *Castanea sativa* forests | N38°54′00″; E21°48′00″                            | 690                                         | 1000                           | 4.5        | 18         | 1255               |
| Parnitha         | Deciduous (*Quercus macrolepis*) and evergreen (*Quercus coccifera* and *Pistacia lentiscus*) open woodlands | N38°06′05″; E23°46′48″                            | 235                                         | 200–400                        | 6.5        | 27.8       | 446                |

* AoB PLANTS 2012: pls025; doi:10.1093/aobpla/pls025, available online at www.aobplants.oxfordjournals.org © The Authors 2012

**Downloaded from** https://academic.oup.com/aobpla/article-abstract/doi/10.1093/aobpla/pls025/176906 by guest on 29 July 2018
Table 2 Studied plant species (per year and study site). The life form of the species is also presented.

| No. | Species                  | Year | Site              | Life form | Family               |
|-----|--------------------------|------|-------------------|-----------|----------------------|
| 1   | Derwentia derwentiana    | 2006 | Snowy Plains      | Herb      | Scrophulariaceae     |
| 2   | Eucalyptus pauciflora    | 2006 | Tree              | Myrtaceae |
| 3   | Acacia obliquinervia     | 2006 | Shrub             | Mimosaceae|
| 4   | Tasmannia xerophila      | 2006 | Shrub             | Winteraceae|
| 5   | Olearia megalophylla     | 2006 | Shrub             | Asteraceae|
| 6   | Daviesia mimosoides      | 2006 | Shrub             | Fabaceae  |
| 7   | Eucalyptus sieberi       | 2006 | Britannia Creek   | Tree      | Myrtaceae |
| 8   | Rubus sp.                | 2006 | Climber           | Rosaceae  |
| 9   | Eucalyptus radiata       | 2006 | Tree              | Myrtaceae |
| 10  | Correa reflexa           | 2006 | Shrub             | Rutaceae  |
| 11  | Correa lawrenciana       | 2006 | Shrub             | Rutaceae  |
| 12  | Olearia lirata           | 2006 | Shrub             | Asteraceae|
| 13  | Pomaderris aspera        | 2006 | Shrub             | Rhamnaceae|
| 14  | Platyllobium formosum    | 2006 | Shrub             | Fabaceae  |
| 15  | Banksia menziesii        | 2006 | Perth             | Proteaceae|
| 16  | Corymbia calophylla      | 2006 | Tree              | Myrtaceae |
| 17  | Eucalyptus marginata     | 2006 | Tree              | Myrtaceae |
| 18  | Pistacia terebinthus     | 2006 | Tree              | Anacardiaceae|
| 19  | Quercus ithaburensis     | 2006 | Tree              | Fagaceae  |
| 20  | Pistacia lentiscus       | 2006 | Shrub             | Anacardiaceae|
| 21  | Platanus orientalis      | 2006 | Tree              | Platanaceae|
| 22  | Rubus fruticosus         | 2006 | Shrub             | Rosaceae  |
| 23  | Olea europaea            | 2006 | Tree              | Oleaceae  |
| 24  | Styrax officinalis       | 2006 | Shrub             | Styraceae |
| 25  | Rosa canina              | 2006 | Shrub             | Rosaceae  |
| 26  | Pyrus amygdaliformis     | 2006 | Tree              | Rosaceae  |
| 27  | Smilax aspera            | 2006 | Climber           | Smilaceae |
| 28  | Phlomis fruticosa        | 2006 | Shrub             | Lamiaceae |
| 29  | Quercus cocifera         | 2006 | Shrub             | Fagaceae  |
| 30  | Malva sylvestris         | 2007 | Herb              | Malvaceae |
| 31  | Thapsia garganica        | 2007 | Herb              | Umbellifera|
| 32  | Echinops viscous         | 2007 | Herb              | Asteraceae|
| 33  | Securigera securidaca    | 2007 | Herb              | Fabaceae  |
| 34  | Bituminaria bituminosa   | 2007 | Herb              | Fabaceae  |
| 35  | Lotus ornithopodoides    | 2007 | Herb              | Fabaceae  |
| 36  | Castanea sativa          | 2007 | Domnista          | Tree      | Fagaceae  |
| 37  | Clematis vitalba         | 2007 | Climber           | Ranunculaceae|
| 38  | Quercus frainetto        | 2007 | Tree              | Fagaceae  |
| 39  | Juglans regia            | 2007 | Tree              | Juglandanceae|

Continued
according to Witkowski and Lamont (1991) as the ratio between LMA and LT.

**Gas exchange parameters**

Measurements of gas exchange were conducted between 0900 and 0012 h. Gas exchange parameters were: photosynthetic capacity ($A_{\text{max}}$), transpiration ($E$) and stomatal conductance ($g_s$) were measured in two leaves per individual; three individuals (six samples) using a portable photosynthesis system LI-6400 (Li-Cor Inc., Lincoln, NE, USA). $A_{\text{max,a}}$ was measured at ambient CO$_2$ atmospheric concentration under saturating photosynthetic photon flux density ($\approx 1850$ μmol m$^{-2}$ s$^{-1}$ PPFD). Leaves were acclimated to the saturating light intensity until rates of $A_{\text{max,a}}$ stabilized. $A_{\text{max,m}}$ was calculated as the ratio of $A_{\text{max,a}}$ to LMA. $E$ was also expressed with respect to mass ($E_m$).

**Total nitrogen concentration**

After weighing, dried plant material of two leaves per individual for three individuals (six samples) was ground to a fine powder with a ball mill. Nitrogen and carbon concentrations were determined by Dumas combustion. Aliquots of 50 mg of the finely ground foliage samples were combusted to N$_2$ and CO$_2$ in the presence of O$_2$, and quantified by means of thermo-conductivity (LECO CHN2000, St Joseph, MI, USA). The total nitrogen concentration of samples from Greek species was measured by the micro-Kjeldahl digestion method, properly modified for accurate measurements of small amounts of leaf samples and analysed colorimetrically (Mills and Jones 1996).

To assess the variability due to analysis with two different methods, comparisons were made between selected samples from Greece and Australia. The difference between the two analytical methods was constant ($\approx$1.5 %) and thus values were adjusted accordingly. Total nitrogen concentration was expressed per total leaf area ($N_a$) and per dry mass ($N_m$).

**Total phenolic compounds and CT determination**

Total phenolic compounds were measured in two leaves per individual for three individuals (six samples) according to the Folin–Ciocalteu method as described by Waterman and Mole (1994). Tannic acid (Sigma, USA) was used for a reference curve. Although the reagent also reacts with substances other than phenolic compounds, we used this method because it is recommended for corresponding field studies, it is the most popular and therefore, the results are comparable to the majority of studies (see Harborne 1989; Waterman and Mole 1994). Condensed tannins were determined according to the proanthocyanidin method as described by Waterman and Mole (1994). Delphinidin (Extrasynthese S.A., Genay, France) was used for the reference curve. The concentrations of TP compounds, CT and their sum ($TP + CT$) were expressed per total leaf area, per dry mass and per nitrogen content ($TP_N, CT_N$).

**Stable carbon and nitrogen isotope signatures**

All samples from two leaves per individual for three individuals (six samples) were analysed with a ThermoScientific Delta V Plus mass spectrometer. The samples were introduced into a Thermo-Flash EA elemental analyser where CO$_2$ and N$_2$ gas were produced by combustion at 1020 °C. The gases, moved along in a continuous flow of helium, were separated by a GC column, and then introduced into a continuous flow gas source mass spectrometer for carbon and nitrogen isotopic ratio determination. The isotopic ratios are expressed

| No. | Species               | Year | Site     | Life form | Family    |
|-----|-----------------------|------|----------|-----------|-----------|
| 40  | Ostrya carpinifolia   | 2007 |          | Tree      | Betulaceae|
| 41  | Rubus sp.             | 2007 |          | Climber   | Rosaceae  |
| 42  | Tussilago farfara     | 2007 |          | Herb      | Asteraceae|
| 43  | Fragaria vesca        | 2007 |          | Herb      | Rosaceae  |
| 44  | Platanus orientalis   | 2007 |          | Tree      | Platanaceae|
| 45  | Ballota acetabulosa   | 2008 | Parnitha | Herb      | Lamiaceae |
| 46  | Ceris siliquastrum    | 2008 |          | Tree      | Fabaceae  |
| 47  | Cionura erecta        | 2008 |          | Shrub     | Apocynaceae|
| 48  | Anchusa sp.           | 2008 |          | Herb      | Boraginaceae|
| 49  | Arbutus unedo         | 2008 |          | Tree      | Ericaceae |
for carbon as $\delta^{13}C$ versus Pee Dee Belemnite (a marine carbonate), and for $\delta^{15}N$ versus $N_2$ (atmospheric $N_2$):

$$X = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \times 1000$$

where $X$ is the $\delta^{13}C$ or $\delta^{15}N$ value and $R = ^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively.

The isotopic analyses were performed in the Stable Isotope Unit of the Institute of Materials Science (NCSR Demokritos), accredited according to EN ISO/IEC 17025:2005. Repeated measurements were made for each of the samples. Analytical precision was 0.1 ‰ for $\delta^{13}C$ and 0.2 ‰ for $\delta^{15}N$ values.

**Data analysis**

Spearman bivariate correlations among the pairs of all 20 initial parameters [see Additional Information Table S1] were performed with SPSS Statistics (version 17.0, IBM® SPSS® Statistics, New York, NY, USA) at a 95 % level of significance and correlation coefficients were recorded [see Additional Information Table S2]. Only eight parameters were selected for further analysis (Table 3) after elimination of derivatives and parameters that were different expressions of the same trait and the bivariate correlations between them had no physiological meaning.

Regression analyses were performed to determine the type of relationship that exists between pairs of defined parameters, the strength of the curve and coefficients of determination ($r$), and the statistical significance of correlation coefficients was recorded. Regression analysis was performed using Statgraphics Plus v. 4, (StatPoint Technologies, Inc., Warrenton, VA, USA) at a 95 % level of significance on the means of six samples per species. Correlations were displayed graphically as scatter graphs using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA, USA).

Results

Significant negative relationships of $A_{\text{max,m}}$ with the concentration of TP compounds, the concentration of CT, and the sum of TP and CT were found irrespective of how these concentrations were expressed (with respect to mass, area or nitrogen content [see Additional Information Table S2]). However, these relationships became stronger when the concentration of phenolic compounds was expressed per unit of nitrogen [see Additional Information Table S2]. Although the strength of the correlations was trait specific, in general, the concentrations of phenolic compounds were negatively correlated to gas exchange parameters and positively correlated to leaf structural parameters (Table 4). Regression analysis showed that the best-fitting model to the prediction of the relationships between $A_{\text{max,m}}$ and $TP_m$ (Fig. 1A, $r = 0.52$) and $CT_m$ (Fig. 1B, $r = 0.59$ at $P < 0.01$) was the reciprocal one (type of equation: $Y=1/(a + bx)$ [1]). Moreover, all the relationships between TP, CT and gas exchange traits [$A_{\text{max,m}}$, transpiration rate ($E$), stomatal conductance and intrinsic water-use efficiency] [see Additional Information Table S2] were described by the same model [though correlation and regression (data not shown) coefficients were lower].

The matrix of rank correlation and coefficients of determination ($r_s$) among all possible pairings of the eight examined traits (Table 4) confirmed some already known positive correlations, such as the ones between $A_{\text{max,m}}$ and $E$, between $A_{\text{max,m}}$ and $N_m$, and the negative correlation between $A_{\text{max,m}}$ and LMA (Table 4, Fig. 1C). The results from the regression analyses showed that

### Table 3

| Leaf trait                                      | Abbreviation | Units             |
|------------------------------------------------|--------------|------------------|
| Leaf mass per area                              | LMA          | g m$^{-2}$       |
| Leaf density                                    | LD           | kg m$^{-3}$      |
| Net photosynthetic capacity with respect to mass| $A_{\text{max,m}}$ | mmol CO$_2$ g$^{-1}$s$^{-1}$ |
| Transpiration rate                              | $E$          | mmol H$_2$O m$^{-2}$s$^{-1}$ |
| Nitrogen isotopic composition                   | $\delta^{15}N$ | %                |
| Nitrogen content with respect to mass           | $N_m$        | mg N g$^{-1}$    |
| Concentration of total phenolic compounds with respect to mass | $TP_m$ | mg tannic acid mg$^{-1}$ d.w. |
| Concentration of condensed tannins with respect to mass | $CT_m$ | mg delphinidin mg$^{-1}$ d.w. |
the relationship between LMA and $A_{\text{max,m}}$, ($r = 0.57$, at $P < 0.01$) was also reciprocal (Fig. 1C). Nitrogen isotopic composition ($\delta^{15}$N) was negatively correlated with leaf structural traits (LMA, LD) and positively correlated with $A_{\text{max,m}}$ (Table 4).

In the PCA (Fig. 2), the first two axes accounted for 77.9% of the total variation. Axis 1 (first PC), which explained 59% of the total variation, was well associated with traits related to growth (C, N gain and water losses—negative side of the axis) and protection (water saving and defense/protection—positive side). According to the eigenvector values of the traits on the first PC (Table 5), $A_{\text{max,m}}$, $E$, LMA and CT$m$ had the highest scores. Axis 2 (second PC: 18.9% of the total variation) was associated with TP$m$ and CT$m$, the traits that had higher eigenvector values on this axis (Table 5).

Increasing values on the first PC indicated a trend for higher water saving and defensive/protective demands (high LMA, LD, TP$m$ and CT$m$). Therefore, Australian plants that have higher LMA values are clearly separated from Greek plants (Fig. 2).

### Discussion

The most important findings of the present study concern the negative correlations between $A_{\text{max,m}}$ and TP and/or CT, irrespective of expression. Although the two curves $A_{\text{max,m}}$–TP and $A_{\text{max,m}}$–CT are described by the same model, the different strengths of these correlations may indicate a differentiation in the functional roles of TP and CT within plant tissues. The direct relationship between $A_{\text{max}}$ and TP has not been detected earlier, probably since ecological studies using a large number of species did not include the measurement of phenolic compounds, whereas studies in which TP or CT was measured did not include an efficient number of species or life forms. In a recent study, Ishida et al. (2008) found that leaf TP N or CT N was positively correlated to LMA and negatively correlated to $A_{\text{max,m}}$ and $A_{\text{max,p}}$. However, the correlations were weak ($r < 0.5$), probably because all the plants examined were drought tolerant and no herbs were included. Our results confirmed these correlations.

The results of the PCA indicate an interaction between growth (parameters associated with C and N gain, such as $A_{\text{max}}$, $N_m$, $E$ and $\delta^{15}$N, which is an indicator of soil N availability; see Schmidt and Stewart 2003; Craine et al. 2009) and defense/protection demands (parameters associated with mechanical and chemical reinforcement, such as LMA, LD and phenolic compounds), which is in accordance with the hypothesis of Herms and Mattson (1992). Additionally, the implication of growth strategy limitations in the interaction between structure and the concentration of phenolic compounds is also indicated since the analysis showed that high TP and CT are associated with thick, dense leaves with low N. Species with this leaf type are slow growing and are common in environments with low water and nutrient availability, and high temperatures and light intensities (Wright et al. 2002, 2004; Poorter et al. 2008), conditions that may also increase both the risk of photodamage and herbivory, and subsequently justify high TP and CT. This particular leaf type represents an indicator of the growth strategy of each species, and this is of use from the curves between $A_{\text{max,m}}$ and TP and CT. Indeed, the majority of herbs are positioned on the left part of the curve TP$m$–$A_{\text{max,m}}$ (Fig. 1A). On the other hand, species that are characterized by low $A_{\text{max,m}}$ and high TP and CT (mainly evergreen trees and shrubs) are positioned on the right part of the curve. This is in accordance with the already known trend that

| Table 4 | Spearman rank correlations for each pair of the eight traits examined. |
|----------|-------------------------|----------------|-------------|-----------------|-----------------|-----------------|-----------------|
|          | LMA                     | LD            | $A_{\text{max,m}}$ | $E$            | $\delta^{15}$N  | $N_m$           | TP$m$          |
| LD       | 0.595**                 |               |               |                |                |                 |                |
| $A_{\text{max,m}}$ | $-0.657^{**}$         | $-0.588^{**}$ |               |                |                |                 |                |
| $E$      | $-0.288^{*}$            | $-0.409^{**}$ |               |                | $0.837^{**}$   |                 |                |
| $\delta^{15}$N | $-0.601^{**}$         | $-0.508^{**}$ |               |                | $0.461^{**}$   | n.s.            |                |
| $N_m$    | $-0.323^{*}$            | n.s.          |               |                | $0.362^{**}$   | n.s.            | n.s.           |
| TP$m$    | n.s.                    | 0.351*        |               | $-0.415^{**}$  | $-0.346^{*}$   | n.s.            | n.s.           |
| CT$m$    | 0.336*                  | 0.350*        | $-0.521^{*}$  | $-0.455$       | $-0.330^{*}$   | n.s.            | 0.616**        |

Correlations with coefficient $r_s > 0.5$ are in bold.

$^{*}P < 0.05.$

$^{**}P < 0.01.$

---

AoB PLANTS 2012: pls025; doi:10.1093/aobpla/pls025, available online at www.aobplants.oxfordjournals.org © The Authors 2012

Downloaded from https://academic.oup.com/aobpla/article-abstract/doi/10.1093/aobpla/pls025/176906
by guest
on 29 July 2018
fast-growing species (mainly herbs) possess low levels of leaf defensive compounds, including TP, whereas slow-growing species show high levels of TP (Coley 1983, 1988; Coley et al. 1985; Bryant et al. 1989; Herms and Mattson 1992; Endara and Coley 2011).

Conclusions and forward look

Our results indicate a functional integration between carbon gain and the concentration of phenolic compounds that reflects the trade-off between growth and defence/protection demands, depending on the growth strategy adopted by each species. Further investigation is needed in terms of sample size and/or meta-analysis in order to obtain a more integrated picture of the relationship between the concentration of phenolic compounds and carbon gain. It would also be interesting to investigate whether this relationship is

---

**Table 5** Eigenvector values of eight leaf traits on the first two PCA axes in Fig. 2.

| Trait | 1st PC | 2nd PC |
|-------|--------|--------|
| $A_{\text{max},m}$ | -0.937 | 0.317  |
| $E$ | -0.799 | -0.153 |
| $\text{CT}_m$ | 0.703 | 0.610 |
| LMA | 0.664 | -0.296 |
| LD | 0.642 | -0.044 |
| TP$_m$ | 0.558 | 0.675 |
| N$_m$ | -0.406 | -0.213 |
| $\delta^{15}$N | -0.364 | -0.045 |

Values are ranked in the order of absolute magnitude along the first PC. The higher value for each parameter between the two axes is in bold.
strengthened in the case of species of the same genus. This remains to be answered in a future study.

Additional information
The following additional information is available in the online version of this article:
File 1. Table 1. List of abbreviations for all 20 leaf traits initially examined.
File 2. Table 2. Spearman rank correlations for each pair of the 20 traits initially examined.

Sources of funding
This work was conducted within a Greek–Australian bilateral co-operation project, funded by the Greek Ministry of Development, General Secretariat of Research and Technology. Financial support from the Greek Scholarship Foundation to S.S. as a post-graduate student is gratefully acknowledged.

Contributions by the authors
The project was conceived and planned by G.K. in collaboration with all co-authors. In Australia, the main experimental part of the study was carried out by M.N.F., G.T., V.L. and M.A.A. In Greece, the main experimental part of the study was carried out by S.S., as part of her PhD thesis, D.N., G.L. and E.D. The final data analysis was done by P.B. G.K. was the supervisor of S.S.

Acknowledgements
The authors thank Dr Tyrtron Turnball and Dr Tina Bell for their assistance during field and lab measurements in Australian sites, and Professor Diamantopoulos for the use of CANOCO software.

Conflict of interest statement
None declared.

References
Bennett RN, Wallsgrove RM. 1994. Secondary metabolites in plant defence mechanisms. New Phytologist 127: 617–633.
Bryant JP, Kuropat PJ, Frisby K, Cooper SM, Owen-Smith N. 1989. Resource availability hypothesis of plant antiherbivore defenses tested in a South African savannah ecosystem. Nature 340: 227–229.
Caldwell MM, Robberecht R, Flint SD. 1983. Internal filters: prospects for UV-acclimation in higher plants. Physiologia Plantarum 92: 207–218.
Chabot BF, Hicks DJ. 1982. The ecology of leaf life spans. Annual Review of Ecology and Systematics 13: 229–259.
Close DC, McArthur C. 2002. Rethinking the role of many plant phenolics—protection from photodamage not herbivores? Oikos 99: 166–172.
Coley PD. 1983. Intra-specific variation in herbivory on two tropical tree species. Ecology 64: 426–433.
Coley PD. 1998. Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. Oecologia 74: 531–536.
Coley PD, Bryant JP, Chapin FS III. 1985. Resource availability and plant antiherbivore defense. Science 230: 895–899.
Craine JM, Elmore AJ, Aidar MPM, Bustamante M, Dawson TE, Hobbie EA, Kahmen A, Mack MC, McLauchlan KK, Michelsen A, Nordugo GB, Pardo LH, Peñuelas J, Reich PB, Schuur EAG, Stock WD, Templer PH, Virginia RA, Welker JM, Wright IJ. 2009. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytologist 183: 980–992.
Diaz S, Hodgson JG, Thompson K, Cabido M, Cornelissen JHC, Jollil A, Montserrat-Martí G, Grime JP, Zarrinkamar F, Asri Y et al. 2004. The plant traits that drive ecosystems: evidence from three continents. Journal of Vegetation Science 15: 295–304.
Endara M-J, Coley PD. 2011. The Resource Availability Hypothesis revisited: a meta-analysis. Functional Ecology 25: 389–398.
Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78: 9–19.
Field CB, Mooney HA. 1986. The photosynthesis–nitrogen relationship in wild plants. In: Givnish TJ, ed. On the economy of plant form and function. Cambridge: Cambridge University Press, 25–55.
Grime JP. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology 86: 902–910.
Gulmon SL, Mooney HA. 1986. Costs of defense and their effects on plant productivity. In: Givnish TJ, ed. The economy of plant form and function. Cambridge: University of Cambridge Press.
Harborne JB. 1989. General procedures and measurement of total phenolics. In: Methods in plant biochemistry: Vol. 1 Plant phenolics. London, UK: Academic Press.
Harborne JB. 1997. Ecological biochemistry. London, UK: Academic Press.
Heim KE, Tagliaferro AR, Bobilsky DJ. 2002. Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. Journal of Nutritional Biochemistry 13: 572–584.
Herms DA, Mattson WJ. 1992. The dilemma of plants: to grow or defend. Quarterly Review of Biology 67: 283–335.
Hikosaka K. 2010. Mechanisms underlying interspecific variation in photosynthetic capacity across wild plant species. Plant Biotechnology 27: 223–229.
Ishida A, Nakano T, Yazaki K, Matsuki S, Koike N, Lauenstein DL, Shimizu M, Yamashita N. 2008. Coordination between leaf and stem traits related to leaf carbon gain and hydraulic across 32 drought-tolerant angiosperms. Oecologia 156: 193–202.
Jaleel CA, Riadh K, Gopi R, Manivannan P, Inês J, Al-Juburi HJ, Zhao CX, Shao HB, Rajaram P. 2009. Antioxidant defence responses: physiological plasticity in higher plants under abiotic constraints. Acta Physiologia Plantarum 31: 427–436.
Kattge J, Díaz S, Lavorel S, Prentice IC, Leadley P, Bönisch G, Garnier E, Westoby M, Reich PB, Wright IJ et al. 2011. TRY – a global database of plant traits. Global Change Biology 17: 2905–2935.
10

AoB PLANTS 2012: pls025; doi:10.1093/aobpla/pls025, available online at www.aobplants.oxfordjournals.org © The Authors 2012

Downloaded from https://academic.oup.com/aobpla/article-abstract/doi/10.1093/aobpla/pls025/176906
by guest
on 29 July 2018

Appendix

The complete references with the full list of authors for Diaz et al. (2004), Kattege et al. (2011) and Wright et al. (2004) are as follows:

Lambers H, Poorter H. 1992. Inherent variation in growth rate and between higher plants: a search for physiological causes and ecological consequences. Advances in Ecological Research 23: 187–261.

Middleton EM, Teramura AH. 1993. The role of flavonol glycosides and carotenoids in protecting soyabean from ultraviolet-B damage. Plant Physiology 103: 741–752.

Mills HA, Jones JB. 1996. Plant analysis handbook II. Athens, GA: Micro Macro Publishing.

Poorter H, Remkes C, Lambers H. 1990. Carbon and nitrogen economy of 24 wild species differing in relative growth rate. Oecologia 94: 434–440.

Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R. 2008. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytologist 182: 565–588.

Reich PB, Kloeppel BD, Ellsworth DS. 1992. Leaf lifespan in relation to leaf, plant and stand characteristic among diverse ecosystems. Ecological Monographs 62: 365–392.

Reich PB, Walters MB, Ellsworth DS. 1997. From tropics to tundra: global convergence in plant functioning. Proceedings of the National Academy of Sciences of the USA 94: 13730–13734.

Rice-Evans CA, Miller NJ, Paganga G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 20: 933–956.

Roberts MR, Paul ND. 2006. Seduced by the dark side: interacting molecular and ecological respectives on the influence of light on plant defence against pests and pathogens. New Phytologist 170: 677–699.

Sakihama Y, Cohen MF, Grace SC, Yamasaki H. 2002. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. Toxicology 177: 67–80.

Schmidt S, Stewart GR. 2003. δ15N values of tropical savanna and monsoon forest species reflect root specializations and soil nitrogen status. Oecologia 134: 569–577.

Shipley B, Lechowicz MJ, Wright I, Reich PB. 2006. Fundamental trade-offs generating the worldwide leaf economics spectrum. Ecology 87: 535–541.

Waterman PG, Mole S. 1994. Analysis of phenolic plant metabolites. Oxford, UK: Blackwell Science.

Westoby M, Wright IJ. 2006. Land-plant ecology on the basis of functional traits. Trends in Ecology and Evolution 21: 261–268.

Witkowski ETF, Lamont BB. 1991. Leaf specific mass confounds leaf density and thickness. Oecologia 88: 486–493.

Wright IJ, Westoby M, Reich PB. 2002. Convergence towards higher leaf mass per area in dry and nutrient-poor habitats has different consequences for leaf life span. Journal of Ecology 90: 534–543.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin FS, Cornelissen JHC, Diemer M et al. 2004. The worldwide leaf economics spectrum. Nature 428: 821–827.

Diaz S, Hodgson JG, Thompson K, Cabido M, Cornelissen JHC, Jalili A, Montserrat-Martí G, Grime JP, Zarrinkamar F, Asri Y, Band SR, Basconcelo S, Castro-Diez P, Funes G, Hamzehee B, Khoshnevi M, Pérez-Harguindeguy N, Pérez-Rontomé MC, Shirvany FA, Vendramini F, Yazdani S, Abbas-Azimi R, Bogaard A, Boustanl S, Charles M, Dehghan M, de Torres-Esparlony L, Falcuzuk V, Guerrero-Campo J, Nyd A, Jones G, Kowsary E, Kazemi-Saeed F, Maestro-Martinez M, Romo-Diez A, Shaw S, Siavash B, Villar-Salvador P, Zak MR. 2004. The plant traits that drive ecosystems: evidence from three continents. Journal of Vegetation Science 15: 295–304.

Kattege J, Diaz S, Lavelle P, Prentice IC, Leadley P, Bönisch G, Garnier E, Westoby M, Reich PB, Wright IJ, Cornelissen JHC, Violle C, Harrison SP, v. Bodegom PM, Reichstein M, Enquist BJ, Soudzilovskaia NA, Ackerly DD, Anand M, Atkin O, Bahn M, Baker TR, Baldocchi D, Bekker R, Blanco C, Blonder B, Bond WJ, Bradstock R, Bunker DE, Casanovas F, Cavender-Bares J, Chambers JQ, Chapin FS, Chave J, Coomes D, Cornwell WK, Crane J, Dobrin BH, Duarte L, Durka W, Elser J, Esser G, Estiarte M, Fagan WF, Fang J, Fernández-Méndez F, Fidelis A, Finegan B, Flores O, Ford H, Frank D, Freschet GT, Fyllas NM, Gallagher RV, Green WA, Guiterrez AG, Hickler T, Higgs S, Hodgson JG, Jalili A, Jansen S, Joly C, Kerkhoff AJ, Kirkup D, Kitajima K, Kleyer M, Klotz S, Knops JMH, Kramer K, Kühn I, Kurokawa H, Laughlin D, Lee TD, Leishman M, Lens F, Lenz T, Lewis SL, Lloyd J, Llusia’ J, Louault F, Ma S, Mahecha MD, Manning P, Massad T, Medlyn B, Messier J, Moles AT, Müller SC, Nadrowski K, Naeem S, Niinemets Ü, Nöllert S, Nöske A, Ogaya R, Oleksyn J, Onipchenko VG, Onoda Y, Ordoñez J, Overbeck G, Ozinga WA, Patino S, Paula S, Pausas JG, Penuelas J, Phillips OL, Pillar V, Poorter H, Poorter L, Poschlod P, Prinzing A, Proulx R, Ramig J, Reinsch S, Reu B, Sack L, Salgado-Negret B, Sardans J, Shiddea S, Shipley B, Sievert A, Sosinski E, Soussana J-F, Swaine E, Swenson N, Thompson K, Thornton P, Waldram M, Weihe E, White M, White S, Wright SJ, Yguel B, Zaehle S, Zanne AE, Wirth C. 2011. TRY – a global database of plant traits. Global Change Biology 17: 2905–2935.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin FS, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas E, Villar R. 2004. The worldwide leaf economics spectrum. Nature 428: 821–827.