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OIKOS

Research

Structural defence is coupled with the leaf economic spectrum across saplings of spiny species

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Given that the rate of resource capture constrains plant growth and defence, understanding the linkage between the leaf economic spectrum (LES) and defence and how it contributes to growth is central to predicting species performance. In spite of the prevalence of spiny plants in many plant communities, little is known about how the LES relates to defence and growth rate across these species. We grew 42 spiny species, from diverse environments, under common garden conditions for 15 weeks and measured LES (leaf N, SLA and assimilation rate), defence and growth traits. We assessed general relationships between LES and growth rate and tested whether structural defences (spines, leaf fibre and lignin content) and quantitative chemical defences (condensed tannins) are linked to the LES and growth and if different spine types (i.e. leaf spines, stipular spines, prickles and thorns), with distinct anatomical origins, partition out across the LES. We observed two independent trait axes that together explained ~68% of trait variation across species. The first axis showed that structural defences (spines, leaf fibre and lignin content) trade off with leaf productivity along the LES. Axis 2 revealed that condensed tannins is orthogonal and less integrated with the LES-structural defence axis. Bivariate trait analyses disclosed positive covariations between LES traits and sapling growth rate. All structural defence traits were negatively related to sapling growth. Across spine types, species with leaf spines were associated with the conservative end of the LES, characterized by high structural defences and lower leaf productivity relative to other spine types.

Synthesis: Our study shows that the LES and structural defences are coupled in spiny species such that constitutive growth – defence strategies range from fast-growing species with low allocation to defences to slow-growing species that invest heavily in structural defences (dominated by leaf spiny species).

Keywords: defence, growth–defence tradeoffs, growth rate, investment in spines, leaf economic spectrum, leaf productivity, spiny plant



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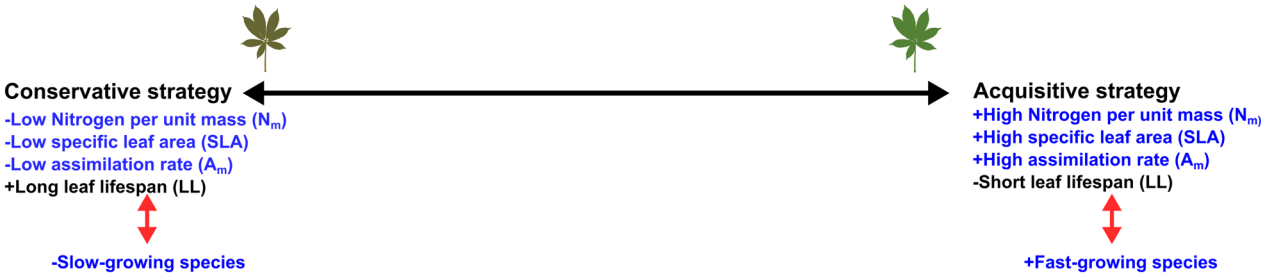
0 **Introduction**

Plant growth rate and defence are fundamental determinants of species ecological performance (Herms and Mattson 1992, Salguero-Gómez 2017). Because plant growth rate and defence are strongly dependent on resource acquisition (Zuest and Agrawal 2017), there is a renewed interest in understanding how the leaf economic spectrum (LES) (Wright et al. 2004, Shipley et al. 2006) relates to the growth–defence axis (Mason and Donovan 2015). The LES framework (Fig. 1a) identifies a trade-off in traits conferring higher leaf productivity (i.e. high leaf N, high specific leaf area and assimilation rates; acquisitive strategy) against those enhancing leaf durability (e.g. low SLA and leaf N; conservative strategy). Generally, acquisitive leaves are associated with fast-growing species (Fig. 1a) whereas slow-growing species tend to possess conservative leaves (Lambers and Poorter 1992, Shipley 2006, Reich 2014). The LES is also potentially linked to anti-herbivore defence given that fast-growing species are thought

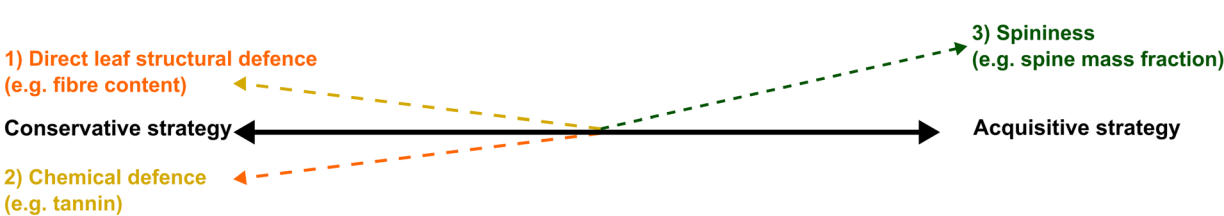
to have low constitutive defences (Coley et al. 1985, Herms and Mattson 1992, Reich 2014). However, the extent to which the underlying traits are related, across species, remains poorly understood.

Generally, leaf structural defences (e.g. fibre content) and quantitative chemical defences (e.g. tannins) are expected to be linked to the LES (Fig. 1b; Coley et al. 1985, Onoda et al. 2017) due to their shared dependence on C and N investment (Mason and Donovan 2015). Leaf durability, a key aspect of the conservative strategy, is largely driven by high investment in structural C (e.g. cellulose) resulting in high leaf toughness or low SLA (Poorter et al. 2009, Kitajima and Poorter 2010, Kitajima et al. 2012, Onoda et al. 2017). Investment in leaf structural components also enhances leaf defences by increasing leaf toughness as well as reducing palatability and digestibility (Bryant et al. 1983, Coley et al. 1985, Hartley and Jones 1997, Moles et al. 2013). Consistent with this expectation, Mason and Donovan (2015) and Chauvin et al. (2018) have recently shown that structural defence traits, such as leaf

(a) Traits covariation along the leaf economic spectrum (LES)



(b) Hypothesized relationships between different defence traits and the LES



(c) Expected distribution of different spine types across the LES

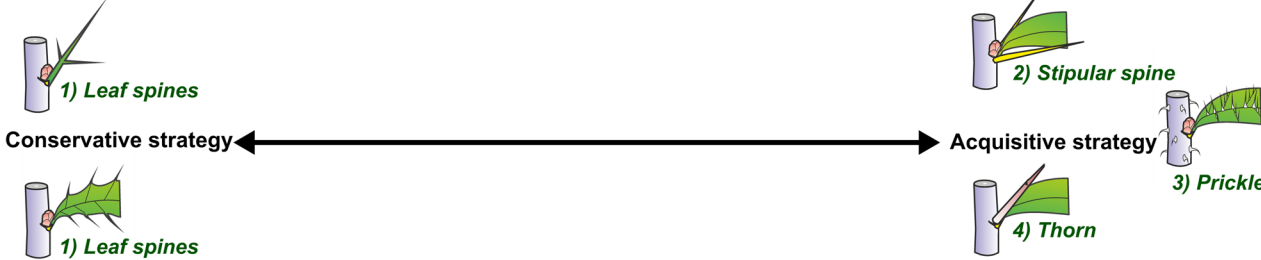


Figure 1. Conceptual framework of the relationships between different defence traits, spine types and the leaf economic spectrum (LES) traits. (a) Acquisitive leaf traits are generally associated with fast-growing species whereas conservative leaf traits are characteristic of slow-growing species. (b) Leaf structural and quantitative chemical defence traits are predicted to be linked to the LES but have been assessed by only a few studies. Non-direct leaf defences, such as spines, may also be linked to the LES but so far have not been considered by studies that seeks to integrate the LES-defence-growth axes. (c) Species with different spine types are likely associated with different ends of the LES given the divergent implications of spine construction on leaf morphology and physiology.

0 toughness and dry matter content, covary with the LES traits,
being negatively correlated with leaf productivity (Fig. 1b-1).

Variations in leaf chemical defences, particularly quan-
titative defences (e.g. tannins), are predicted to be coupled
5 with leaf lifespan such that long-lived leaves should be well-
defended (Coley 1988). Given that longer leaf lifespan is
associated with the conservative strategy, this suggests that
greater investment in quantitative chemical defences should
be associated with the conservative end of the LES spec-
10 trum (Fig. 1b-2). However, evidence for correlation between
LES and quantitative chemical defence traits is equivocal.
For instance, Mason and Donovan (2015) observed signifi-
cant correlations between tannin activity and the LES axis
in three *Helianthus* species across whole-plant ontogeny. In
15 contrast, Chauvin et al. (2018) observed that tannin content
was decoupled from the LES axis in 83 species in a tropi-
cal moist forest in Panama. Thus, despite its importance to
understanding carbon economy at the leaf-level, it remains
unknown whether there is a consistent covariation between
20 the LES traits and quantitative chemical defences. Here, we
provide insights into the link between the LES, chemical and
structural defences and sapling growth rate across a diverse set
of spiny species. Spines are plant structural traits that func-
tion mainly as defences against large mammalian herbivores
25 (Cooper and Owen-smith 1986, Hanley et al. 2007, but
see: Kariyat et al. 2017). Spines differ from direct leaf struc-
tural and chemical defences (such as toughness or high fibre
content) because they can function at either the individual
leaf-level (e.g. leaf spines and prickles) or whole plant level
30 (e.g. thorns and stipular spines), especially in combination
with ‘cagey’ branching (Archibald and Bond 2003, Charles-
Dominique et al. 2017). Thus, studying covariations between
LES and defence traits in this group of species has the added
35 advantage of providing further insights on whether and how
the LES is integrated with defence at the whole-plant scale.
Spiny species are widespread across terrestrial plant commu-
nities (Grubb 1992) and their evolution in Africa has been
shown to be tightly linked to evolution and diversification of
40 mammalian herbivores (Charles-Dominique et al. 2016). If
defence is coupled with the LES and constrain leaf productiv-
ity in spiny plants, then ongoing changes in large mammalian
herbivory pressure (Ripple et al. 2015, Hempson et al. 2017)
45 may have major consequence for systems dominated by spiny
plants given that mammalian herbivores impose significant
constraints on sapling recruitment in these systems (Prins
and van der Jeugd 1993, Augustine and McNaughton 1998,
Staver and Bond 2014).

In contrast to direct leaf defences, spines seem to be
associated with resource acquisitive life-history strategies
50 (Fig. 1b-3; Grubb 1992, Tomlinson et al. 2016, Wigley et al.
2018). Within biomes, spiny species tend to possess nutri-
tious and productive leaves (Grubb 1992), and are associated
with greater resource sites (Milton 1991, Grubb 1992). For
instance, Grubb (1992) noted that spiny species in closed for-
55 ests are either predominantly pioneer gap-loving species that
dominate tree-fall gaps (where both light and soil fertility are
likely high) or associated with permanently open sites (e.g.
61 rocky slopes or forest edges). Similarly, in African savan-
nas, spiny species tend to possess high-quality fine-leaves
often dominating nutrient rich soils (Scholes and Walker
65 2004, Charles-Dominique et al. 2016, Osborne et al. 2018)
where large herbivore biomass is high (Hempson et al. 2015,
Charles-Dominique et al. 2016). Recent studies have shown
that the defence syndrome of spiny savanna species involves
70 combining high leaf N with greater investment in spines
with high/low chemical defences (Tomlinson et al. 2016,
Wigley et al. 2018). Further, Rafferty and Lamont (2007)
also found weak positive correlations between an index of
‘spiness’, leaf N and specific leaf area (SLA) for saplings
75 of 19 species. Although these studies suggest that spines are
associated with resource acquisitive life-history strategies
(Fig. 1b-3), all the above analyses included both spiny and
non-spiny species (i.e. species that never produce spines),
potentially masking more subtle variation among different
80 spiny plants in their productivity–defence trait relationships.

Different spine types are derived from different plant
organs (Bell and Bryan 2008) and it is likely that produc-
ing these divergent spine types have differential effects on
leaf morphology and physiology and hence their relationship
85 with the LES (Fig. 1c). Spines can be derived from modi-
fied leaves (Fig. 1c-1; leaf spines), stipules (Fig. 1c-2; stipular
spines), as outgrowths of the epidermis or cortex (Fig. 1c-3;
prickles) or modified branches (Fig. 1c-4; thorns) (Grubb
1992, Gutschick 1999). Leaf spines may negatively affect
90 leaf productivity, because greater fibre and lignin are required
to modify leaf blades or margins into spines, and may be
associated with resource conservative life-history strategies
(Campbell 1986). Prickles and stipular spines generally incur
95 low biomass allocation cost (Bazely et al. 1991, Armani et al.
2019) and although prickles can be produced on leaf surfaces,
they have limited impact on leaf morphology and physiology
(Björkman and Anderson 1990) and thus can be compat-
ible with acquisitive leaf strategies. Similarly, although thorns
100 incur significant biomass allocation cost relative to stipu-
lar spines or prickles and thus likely constrain growth rate
(Armani et al. 2019), thorns are not directly linked to leaves
and should be compatible with acquisitive leaf strategies.
Therefore, different spine types likely partition out along the
105 LES (Fig. 1c) but this notion has never been experimentally
tested.

The aim of this study was to test whether different catego-
ries of defence traits (leaf structural defence, chemical defence
110 and spines) are related to the LES and how these relationships
are linked to sapling growth rate in spiny plants. In addition,
we were interested in exploring whether species with distinct
spine types partition out across the LES. Our approach was
to select diverse spiny species (i.e. different spine types, plant
115 families and contrasted environments; supplementary mate-
rial Table 1) and grow them under common garden condi-
tions for trait measurement. We measured eight traits that
have been demonstrated to be important for plant growth
and defence (supplementary material Table 2). Relative
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4

For each bivariate correlation, we included only species for which we had data for both traits.

We sampled seeds from the living collections of Xishuangbanna Tropical Botanical Garden of the Chinese Academy of Science (XTBG-CAS), located in Menglun, Yunnan, China (21°55'38"N, 101°15'6"E); the XTBG-CAS savanna field station in Yuanjiang Valley, Yunnan

Table 2. Results of phylogenetic least squares (PGLS) models testing for differences in the relationships between defence, leaf productivity and growth rate for different spine types.*

Trait–trait relationship	Model summary					Spine type replicates			
	Y-variable	X×Spine type	F-values			Leaf	Prickle	Stipular	Thorn
			X	Spine type	X×Spine type				
LES ~ growth	RGR	Am	5.75*	7.36**	0.70	0	6	5	8
	RGR	SLA	5.35*	2.313	0.31	0	8	6	15
	RGR	Nm	5.44*	3.92*	0.21	0	8	6	14
Defence ~ growth	SPMF	RGR	5.03*	1.99	0.90	0	6	6	4
	ADF	RGR	0.57	0.14	0.15	0	8	6	14
	ADL	RGR	0.03	0.74	0.06	0	8	6	14
Defence ~ LES	CT	RGR	1.34	1.59	0.44	0	8	6	14
	SPMF	Nm	16.15***	1.9634	1.43	4	8	6	6
	SPMF	SLA	13.24**	1.04	1.74	4	8	6	8
	SPMF	Am	0.46	21.42**	2.64	0	5	5	2
	ADF	Nm	6.74*	1.71	1.473	5	10	6	18
	ADF	SLA	3.07	1.2	0.50	5	10	6	18
	ADF	Am	0.00	0.25	0.02	0	6	5	8
	ADL	Nm	4.79*	1.62	0.62	5	10	6	18
	ADL	SLA	2.14	1.2	0.31	5	10	6	18
	ADL	Am	0.05	0.65	0.41	0	6	5	8
	CT	Nm	1.07	3.10*	1.69	5	10	6	18
	CT	SLA	0.90	3.42*	1.18	5	10	6	18
	CT	Am	0.93	0.48	2.25	0	6	5	8
Spine ~ chemical defence	SPMF	ADF	3.75	1.81	1.24	4	8	6	6
	SPMF	ADL	5.22*	1.37	1.00	4	8	6	6
	SPMF	CT	0.92	3.10	1.77	4	8	6	6

Traits are measured on saplings of spiny species grown under greenhouse conditions for 15 weeks. SPMF, spine mass fraction; ADF, acid detergent fibre; ADL, acid detergent lignin; CT, condensed tannins; Nm, nitrogen per unit mass; SLA, specific leaf area; Am, assimilation rate; RGR, relative growth rate.

Model summary values are F-values derived from phylogenetically – adjusted analysis of variance (ANOVA). Significant relationships are shown as $p < 0.0001 = ***$, $p < 0.001 = **$, $p < 0.05 = *$.

Spine types with missing data (e.g. leaf spines) or insufficient replicates (i.e. $n < 4$; e.g. thorns) were excluded from these analyses.

(23°28'15"N, 103°10'37"E); the Germplasm Bank of Wild Species, Kunming Inst. of Botany (KIB-CAS); and from South Africa from an earlier experiment (Tomlinson et al. 2012). In total we sampled 42 species, most of which are native to tropical China and Southeast Asia (24 species) and Africa (13 species), and that naturally grow in 'open' (e.g. savanna) or 'closed' (e.g. forest) from dry–wet environments (see Supplementary material Appendix 1 Table A1 for details). Of the 42 species (belonging to 17 plant families), 11 possessed prickles, 20 possessed thorns, five had leaf spines (all are *Berberis species*) and six had stipular spines.

Greenhouse experiment

Seeds were germinated either on agar or river sand, and ten (10) days after germination, they were transplanted into a greenhouse located in XTBG-CAS. The greenhouse was covered with shade netting from March to October to reduce irradiance levels to 40–50% of full sunlight to reduce desiccation and mortality of the young seedlings, particularly of the forest species. Temperature and relative humidity in the greenhouse ranged from 19.7 to 33.3°C and 42 to 100%, respectively. After germination, each individual seedling was transplanted into a plastic tube of 10 cm diameter and 80 cm length. We chose deep pots to allow space for taproot growth (Tomlinson et al. 2012). Tubes were filled with river sand mixed with 18–6–12 N–P–K fertilizer (8–9 month mixture) at a concentration of 5 kg of fertilizer per m⁻³ of river sand. Each tube was irrigated two times daily with each irrigation event lasting one minute using an automated irrigation system.

Transplanted seedlings were allowed to grow for a minimum of 15 weeks (hereafter week 15, but ranged between 15 and 19 weeks) before trait analysis and harvest ($n = 357$ plants total). Previous research on a subset of the species indicated that size-related changes in traits stabilised by 15 weeks. For a subset of species, we also obtained biomass at five weeks of age (hereafter week 5, but ranged between 5 and 8 weeks) to determine relative growth rate. For these 30 species (for which we had > 10 individuals; Supplementary material Appendix 1 Table A3), we randomly harvested five or more individuals (but three individuals for two species, a total of 225 individuals; Supplementary material Appendix 1 Table A3) at five weeks. For twelve species (including all species with leaf spines), we did not have enough individuals and therefore harvested all individuals of these species only during the final harvesting (Supplementary material Appendix 1 Table A3). The greenhouse experiment was conducted in two temporal blocks from July to December in both 2015 and 2016. Twelve species (two prickles, five stipular spines and five thorns) were grown in 2015, whereas 30 species (five

0 leaf spines, nine prickles, one stipular spines and 15 thorns) were grown in 2016 (Supplementary material Appendix 1 Table A1). Greenhouse conditions (minimum, mean and maximum temperature and relative humidity) did not differ between 2015 and 2016 ($p > 0.05$) and thus temporal block was not considered in the analyses.

5 **Trait measurements**

10 Prior to harvesting at week 15, we checked whether each plant bore spines or not (yes = 1, no = 0). For spine bearing individuals, we removed all spines on all plant organs. In all cases, we detached spines from the plant organ using either sharp razor blades or utility knives. Species with leaf spines often produced whole-leaf modified spines, or spines were clearly visible as modified leaf-tips. For thorny plants, we included only modified branches with pointed and lignified tips (most species that produced thorns had only thorns emerging and few true branches, which were visually distinct). For species with prickles, we removed pickles from both the leaves and stem. For harvesting at both weeks 5 and 15, we divided each harvested individual sapling into leaf, stem, root and spine (for spines, done only for week 15 as spines were not well developed at week 5). Root samples were oven-dried for 48 h at 65°C, whereas leaf, stem and spines were dried for 72 h at 70°C (Perez-Harguindeguy et al. 2013). We combined all samples to obtain the sapling dry mass (g).

We measured RGR ($\text{g g}^{-1} \text{d}^{-1}$) following Tomlinson et al. (2014, 2016) as the difference between the logarithm of final sapling mass ($\ln \text{Mass}_{15}$) and the logarithm of average initial mass at week five ($\ln \text{Mass}_5$) divided by the growth interval (days). We determined RGR for 30 species out of the 42 species as we had no initial mass data for 12 of our species. We estimated specific leaf area (SLA, mm^{-2} leaf area mg^{-1} leaf mass), mass-based assimilation rate (A_m ; $\mu\text{mol CO}_2 \text{g}^{-1} \text{s}^{-1}$) and leaf nitrogen per unit mass (N_m ; mg g^{-1}) following Perez-Harguindeguy et al. (2013). Before removing spines on leaves, we removed up to five healthy, fully expanded leaves from each individual and scanned one-sided surface area, for calculation of leaf area (L_{sa} , cm^2). We computed the mean of these five leaves as the L_{sa} for each individual sapling. Each scanned leaf included midrib, all leaflets (for compound leaves) and petiole. L_{sa} was determined using ImageJ (Abràmoff et al. 2004). SLA was estimated by dividing the total area of scanned leaves by the total dry leaf mass (L_{sm}). Area-based assimilation rate (A_a , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) was measured with an open gas-exchange system at least one week before final harvesting of saplings. Measurements were made on 3–5 individuals for 19 species (due to unavailability of the LICOR during final harvesting of other species). During measurements, PPFD was set at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ with an artificial light source, and vapour pressure deficit in the chamber was $< 0.1 \text{ kPa}$. Measurements were made under ambient CO_2 concentration with flow rates between 400 and $500 \mu\text{mol s}^{-1}$, and readings were taken after photosynthetic rates stabilised. Mass-based assimilation rate (A_m ; $\mu\text{mol CO}_2 \text{g}^{-1} \text{s}^{-1}$) was calculated directly as $A_m = A_a \times \text{SLA}$.

61 Photosynthetic traits were measured in December 2015 and 2016. Leaf nitrogen content (N_m ; mg g^{-1}) was quantified using the Dumas combustion method (Bremner 1965) with an elemental analyser.

65 We determined SPMF (g g^{-1}) as a ratio of total spine dry mass (leaf and stem spine) divided by total sapling dry mass ($n = 26$, as spines were too few to allow accurate estimation of SPMF on four species, Supplementary material Appendix 1 Table A2). For estimation of ADF, ADL and CT (all in %), dried leaves from all individuals of a species were pooled together for subsequent analysis (all spines on leaves were removed, as mentioned above, prior to chemical analysis). This was necessary because several species did not have enough leaf mass to allow for determination of these traits at the individual sapling level. ADF and ADL content were determined using a FOSS TM 2010 semi-automated system. Condensed tannin content was determined following the method described by Mole and Waterman (1987). Specifically, 2 g of dried, powdered leaf material was diluted with 80 ml of distilled water in a 250 ml conical flask. The flask was heated and the mixture allowed to boil for 30 min and subsequently centrifuged (8000 r min^{-1}) for 4 min. The supernatant was collected in a 100 ml volumetric flask and diluted to volume. Five milliliter of Folin–Denis reagent was added followed by 3 ml of sodium carbonate solution and diluted with 100 ml of distilled water. The mixture was thoroughly mixed and allowed to settle, and absorbance was read at 765 nm. All leaf chemical analyses were conducted at the Public Technology Service Centre (Central Laboratory) of Xishuangbanna Tropical Botanical Garden.

95 **Phylogenetic reconstruction**

We constructed a species-level phylogeny for our species to account for the possibility that the examined trait relationships may be driven by shared ancestry (Felsenstein 1985, Garamszegi 2014, Lajeunesse and Fox 2015). Here, phylogenetically-adjusted models were used only to ensure that our conclusions were not driven by relatedness in the sampled taxa and not to infer any evolutionary pattern. This is particularly important for spinescence for which some families, such as Fabaceae, contain a significant proportion of the spiny species and are also known for strategies that enhance their nitrogen acquisition. The current dataset is derived as part of a bigger ongoing project involving comparative trait analyses across sapling of both spiny and non-spiny plants. For this reason, we first constructed the species-level phylogeny for all the ninety two study species. The species-level phylogeny was constructed based on molecular sequence data of chloroplast (*rbcL*, *matK*, *trnH-psbA* and *trnL-trnF*) and combined ribosomal (ITS) gene regions downloaded from www.ncbi.nlm.nih.gov. Sequences for each molecular marker were aligned using MUSCLE (Edgar 2004). Aligned sequences were concatenated for phylogenetic Bayesian inference analysis using BEAST ver. 2.4.7 (Bouckaert et al. 2014). Best substitution models were tested by AIC using jModeltest ver. 2 (Guindon and Gascuel 2003, Darrriba et al. 2015).

0 Four chains were run for 20 000 000 iterations with the first
5 5 000 000 iterations discarded as burn-in and the remain-
ing runs sampled at every 1000th iteration. Priors were set
on some plant lineages to fix the phylogenetic topology
according to the APG IV (APG 2016) after which a consen-
sus tree was constructed. For the current analyses, we
pruned the tree to include the forty two species of interest
(Supplementary material Appendix 1 Table A4). We had no
molecular sequence data for five of the 42 species. We manu-
ally inserted three species as polytomy within the related gen-
era (all are *Berberis* species). We substituted *Gmelina elliptica*
with *G. villosa* and dropped *Cenocentrum tonkinense* (because
it is the only species from its genus; Supplementary material
Appendix 1 Table A4).

15 Statistical analyses

We performed all analyses at the species level (by averaging
traits per species) because individual-level data were unavail-
able for some traits (CT, ADL and ADF, N_m). We first
evaluated how traits were associated in multivariate space
by performing a principal component analysis (PCA) using
the ‘prcomp’ function. We excluded RGR and A_m from the
PCA analysis because of missing values for several species. To
include all species in this analysis, species that did not pro-
duce spines were assigned an SPMF value of ‘0’.

Subsequently, we tested for phylogenetic signals for each
trait using Pagel’s λ (Pagel 1999), which quantifies the degree
to which common descent of species describes the pattern of
trait evolution across species. Pagel’s λ is expected to be ‘0’ if
there is no phylogenetic signal for a given trait but ‘1’ when
there is a strong phylogenetic signal (i.e. trait has evolved
under Brownian motion assumption along the phylogeny)
(Pagel 1999, Kamilar and Cooper 2013). We estimated
Pagel’s λ and assessed if the observed value significantly dif-
fered from ‘0’ and ‘1’ using a maximum likelihood ratio test
implemented in the package ‘carper’ (Orme et al. 2018).
These analyses revealed strong phylogenetic signals in two
traits.

We then examined bivariate relationships among and
between defence (SPMF, ADF, ADL and CT), LES (SLA,
 N_m , A_m), and growth (RGR) traits using both ordinary and
phylogenetic correlation analyses with both approaches pro-
ducing qualitatively similar results (see Results). Ordinary
pairwise trait–trait relationships were examined using the
base R function ‘cor.test’. Phylogenetic correlations were
implemented using the ‘phyl.vcv’ function of the package
‘phytools’ (Revell 2012). To test whether bivariate relation-
ships were contingent on spine type, we implemented phy-
logenetic least square (PGLS) regression models of the form:
 $Y \sim ST \times X$; where Y =response trait (e.g. SPMF), ST =spine
type and X =predictor trait (e.g. RGR) using the ‘pgls’ func-
tion of the ‘carper’ package (Orme et al. 2018). For each
bivariate relationship, we included only species for which
we measured both traits. Three traits (CT, SPMF and RGR)
were significantly skewed whereas other traits showed slight
skewness. We therefore log-transformed all traits to correct

this for all the above analyses. All statistical analyses were con-
ducted in R ver. 3.4.4 (<www.r-project.org>).

65 Results

Emergence and biomass investment in spines

Of the 42 species included in our study 30 produced spines
by the time of harvesting at week 15 whereas eleven thorn-
bearing species (out of 20) and one prickle-bearing species
did not produce spines during our experiment (Fig. 2). Across
species, investment in spines (SPMF) ranged from 0.01% to
9.4% of total sapling mass and was substantially more vari-
able (187-fold) than any other measured traits (varied from
2.8 to 27.6-fold, Supplementary material Appendix 1 Table
A2).

Influence of phylogeny on trait distribution

Two of the LES traits (A_m and SLA) showed strong phyloge-
netic signal (Pagel’s λ for all > 0.9; and likelihood ratio test
indicated that estimated λ was different from 0, $p < 0.001$
but not 1; $p > 0.05$, Fig. 2, Supplementary material Appendix
1 Table A2), suggesting that these traits were not indepen-
dent of phylogeny in our dataset.

Multivariate trait covariations across spiny species

Trait associations in multivariate space suggested that, across
species (using our full dataset, $n = 42$), investment in structural
defence (both leaf structural defence and spines) is negatively
linked to the LES. The first PCA axis (which explained ~46%
of the variation in trait composition; Fig. 3) separated species
with high investment in structural defences (i.e. SPMF, ADF,
ADL) on the right from those with high leaf productivity
traits (i.e. N_m and SLA) on the left. Hence this axis repre-
sents a ‘LES – structural defence’ spectrum with a separation
between species possessing thin leaves with high nitrogen
content (including species such as *Senegalia pennata*, *Vachellia*
farnesiana and *Flueggea virosa*) from species with thick leaves
that had high leaf fibre/lignin content and strongly defended
by spines (e.g. *Berberis* spp, *Flacourtia indica*, *Carissa macro-*
carpa). A second axis (PCA axis 2, explained 22.4% of the
variation in trait composition, Fig. 3) discriminated species
with high investment in condensed tannin content from spe-
cies with both high leaf structural defences (ADF and ADL)
and high leaf productivity (N_m and SLA; Fig. 3).

Bivariate relationships among traits

Pairwise trait–trait analyses showed strong positive covaria-
tions among the LES traits (Table 1). Generally, all LES traits
were significantly (except assimilation rate) and positively
related to sapling growth rate. Investment in spines (SPMF)
was positively (but not significant for phylogenetic correla-
tion analyses) related to all other defence traits whereas fibre

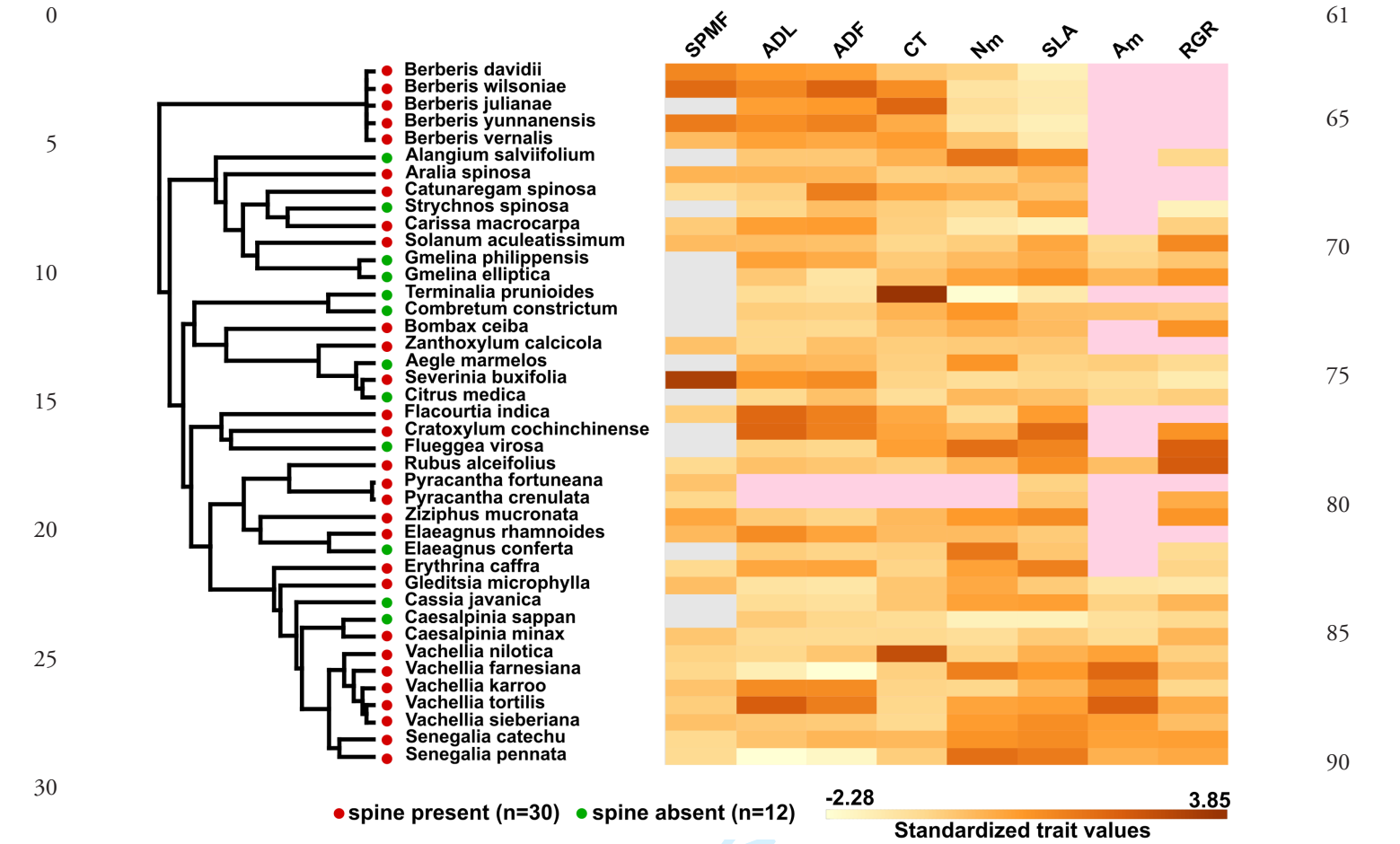


Figure 2. Phylogenetic tree showing trait distribution across the 41 (of 42) species included in this study. Traits values are standardized. Light pink color represent missing data. Light gray color in SPMF represents species that did not produce spines. Traits are: SPMF, spine mass fraction; ADF, acid detergent fibre; ADL, acid detergent lignin; CT, condensed tannins; Nm, nitrogen per unit mass; SLA, specific leaf area; Am, assimilation rate; RGR, relative growth rate. Red and green filled dots on phylogeny represent spiny species that produced or did not produce spines during experimental period.

(ADF) and lignin (ADL) contents were unrelated to condensed tannins (CT) (Table 1). All defence traits were negatively related to the LES traits (except for a non-significant positive correlation between CT and Am), providing further support to the finding that defences tradeoff with leaf productivity (Table 1, Fig. 3). Across species (excluding species with leaf spines which lacked RGR data), growth rate was positively related with condensed tannins content (CT; $r=0.494$; $p<0.005$, for the phylogenetic correlation) but negatively related to all other defence traits (Table 1).

Strategies across spine types

Exploration of the multivariate trait space, using PERMANOVA (Anderson 2017), indicated significant differences in the multidimensional trait space of the different spine types ($F=3.15$, $p=0.002$). Specifically, species possessing leaf spines were separated in ordination space from the other spine types and were more associated with the ‘high structural defence’ end of the spectrum (Fig. 4). We further tested whether bivariate relationships among traits were contingent on spine types (leaf spine, stipular spine, prickles and thorns). While the intercept of few (5 out of 23) trait–trait relationships differed by spine type (i.e. additive effects), none of the slopes of these relationships were significantly different (Table 2).

Discussion

In this study, we examined the links between the leaf economic spectrum (LES), structural defences (spines, leaf fibre and lignin content), quantitative chemical defence (condensed tannins) and sapling growth rate across a diverse set of spiny species (42 species) encompassing a broad taxonomic scope (17 families). Given that spines derived from distinct organs likely have differential effect on leaf morphology and physiology, we also tested whether species with different spine types partitioned out along the LES given. We found two independent trait axes that together explained ~68% of

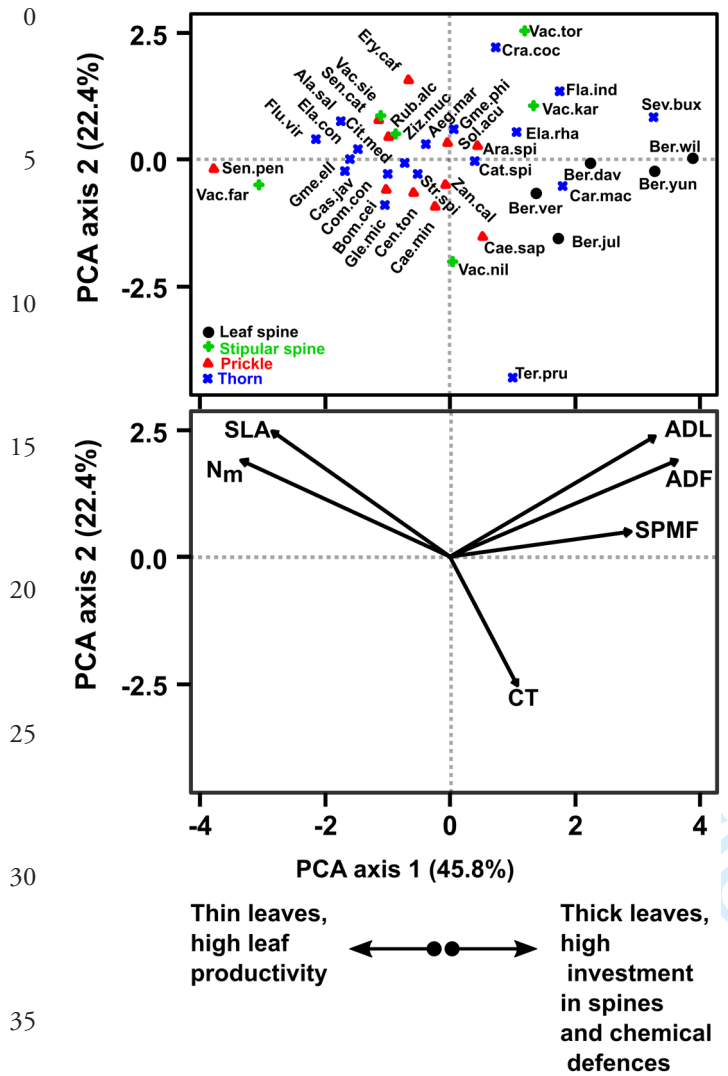


Figure 3. Principal component analysis (PCA) of multivariate trait associations of saplings of 42 spiny species. (a) Biplot of species score on first two PCA axes. Species names are abbreviations of first three letters of ‘Genus.species’ names (e.g. Sen. pen = *Senegalia pennata*). Symbols for different spine types shown in legend. (b) Biplot of factor loadings for traits on the first PCA axes. Leaf productivity traits; specific leaf area (SLA) and Nitrogen content per unit leaf mass (N_m); defence traits; spine mass fraction (SPMF), acid detergent fibre (ADF) and lignin (ADL), condensed tannins (CT).

trait variations across species. The first axis showed that the leaf economic spectrum (LES) and structural defences are linked in spiny plants. The second axis discriminated species with high investment in condensed tannins from species with both high leaf structural defences (lignin and fibre content) and high leaf productivity. Bivariate trait analyses confirmed that LES traits positively covary with sapling growth rate. Importantly, our analyses disclosed positive synergies between spines and leaf structural defence traits (fibre and lignin) whereas leaf structural defence traits were less integrated with condensed tannins (a measure leaf chemical defence). Generally, structural defence traits were negatively

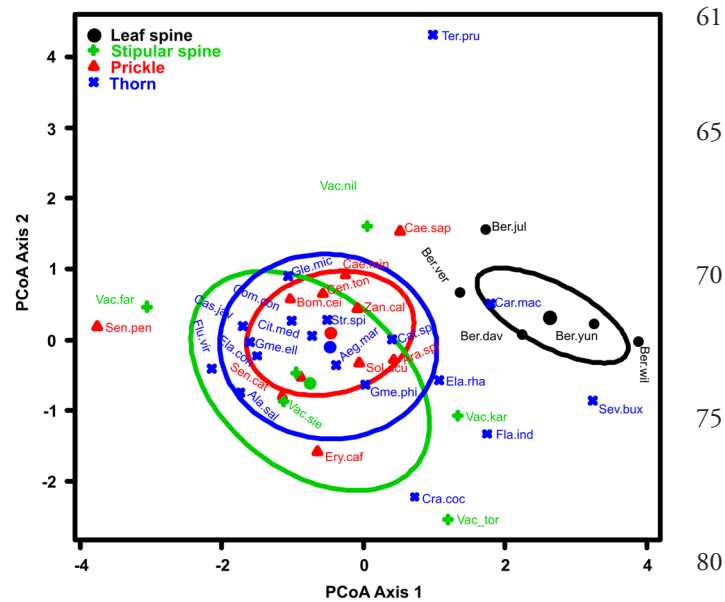


Figure 4. Principal coordinate analysis (PCoA) of multivariate trait associations of saplings of 42 spiny species. Species names are abbreviations of first three letters of ‘Genus.species’ names (e.g. Sen. pen = *Senegalia pennata*). Symbols for different spine types shown in legend. Centroid of each spine type indicated with large filled circles. Broken ellipses enclose 95% confidence intervals of trait space for each spine types. Inserted table is summary results of pairwise comparison of the trait space between spine types. Differences in multivariate trait space are denoted with $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*) and $p > 0.05$ (ns = no significant difference between groups).

related to the LES traits and sapling growth. Thus, the general constitutive growth – LES – structural defence spectrum observed in this study is consistent with predictions from plant defence and the leaf economic spectrum theories that slow-growing species are selected for low leaf productivity and greater investment in constitutive defences (Coley et al. 1985, Herms and Mattson 1992, Stamp 2003, Wright et al. 2004). Across spine types, species with leaf spines were associated with the conservative end of the LES, being characterized by high structural defences and lower leaf productivity relative to other spine types.

General patterns in LES, defence and growth trait covariations across spiny species

Our analyses disclosed that structural defence (spine, leaf fibre and lignin) and LES axis (leaf N, SLA and assimilation rate) were coupled in spiny plants (Fig. 3). This finding is consistent with previous studies reporting that leaf structural traits are physiologically linked to the LES (Hallik et al. 2009, Mason and Donovan 2015, Chauvin et al. 2018). Leaf productivity (e.g. carbon assimilation rate) is determined to a large extent by leaf N and SLA (Wright et al. 2004, Reich et al. 2012, Díaz et al. 2015) and both leaf N and SLA are expected to tradeoff with investment in leaf structural

0 defence (Westoby et al. 2002, Wright et al. 2004, Reich et al.
2012, Onoda et al. 2017). Both lignin and fibre are also
expected to contribute to low SLA (or high leaf mass per
unit area=LMA) (Poorter et al. 2009, Kitajima et al. 2012,
5 Onoda et al. 2017). Consistently, we observed strong positive
correlations between LES traits and negative correlations
between all structural defence traits and LES traits (Table 1).
Condensed tannin (CT) content was less integrated with
the LES-structural defence axis, suggesting that quantitative
10 chemical defences may have evolved independently of
the LES or structural defences in spiny species. Across multivariate
trait space, CT was orthogonal to the LES-structural
defence axis (Fig. 3). Further, bivariate trait analyses (Table
1) showed mostly non-significant correlations between CT
15 and other defence traits. These findings contradict the expectation
that leaf-level allocation to immobile quantitative defences
trades off with leaf productivity (Wright et al. 2004,
Poorter et al. 2009) and should be linked to conservative
strategy (Coley et al. 1985). Similar to our results, a recent
20 study, on 83 moist tropical forest species in Panama, also
observed that tannin content (both condensed and hydrolysable
tannin) was decoupled from the LES (Chauvin et al. 2018).
In contrast, Mason and Donovan (2015) showed that
tannin activity (% tannic acid) was strongly correlated with
25 the LES axis in three *Helianthus* species across ontogeny. It
is possible that within species or clades trade-off between
quantitative defences and leaf productivity may exist, however
across distantly related taxa, fundamental differences in
leaf chemistry may override any general patterns (Moles et al.
30 2013, Agrawal and Weber 2015). Given the limited literature,
further studies across different plant species and from diverse
systems are required to confirm whether chemical defence traits
are coupled or decoupled from the LES.
Investment in spines scaled negatively with leaf productivity
35 (Fig. 3, Table 1) in contrast to previous studies that reported
positive association between spininess and leaf productivity
(Rafferty and Lamont 2007, Wigley et al. 2018). The contrasting
results might stem from differences in the ontogenetic stage
(adult versus saplings) or taxonomic scope (e.g. our study
40 includes only spiny species whereas previous analyses included
both spiny and non-spiny species). For instance, Wigley et al.
(2018) has recently shown that across adult savanna species,
moderate to high investment in spines (measured as average
spine density; spines cm⁻¹) is associated with species with
45 greater available leaf N, suggesting a potential switch in strategy
from saplings to adults. Indeed, Mason and Donovan (2015) have
recently demonstrated that changes in leaf productivity (i.e.
LES strategy) during whole-plant ontogeny, in three sunflower
species, may be driven by changes in leaf physical and chemical
50 defences. Thus, across spiny and non-spiny species greater leaf
N in spiny species may cause positive correlations between
investment in spines and leave productivity. However, within
spiny species, our findings suggest that greater leaf productivity
tradeoff with investment in spines.
60

It is still debated whether alternative defence types (e.g.
structural versus chemical defence) tradeoff or are coordinated
across species (Agrawal and Fishbein 2006, Read et al.
2009, Moles et al. 2013, Barton 2014). Here, our analyses on
a diverse set of spiny plants suggest that different structural
65 defence traits (i.e. spines versus leaf fibre and lignin content)
may be coordinated whereas chemical defence is independently
expressed (Fig. 3). Generally, across spiny species, there is
little evidence of tradeoff between chemical and structural
defences with most studies observing synergies (Twigg and
70 Socha 1996, Hanley and Lamont 2002) or no correlations
(Pisani and Distel 1998, Rafferty and Lamont 2007, Barton
2014). The coordination of spines and leaf lignin and fibre
content in spiny plants may have adaptive value. Firstly,
75 building spines requires fiber and lignin (Hanley et al. 2007,
Kellogg et al. 2011). Thus, species selected for greater investment
in spines may be inherently predisposed to have greater leaf
fiber and lignin content. Secondly, simultaneous expression
of spines and leaf structural defences may be desirable for
80 saplings growing in risky environments (i.e. where cost of
herbivory may be high) (Bryant et al. 1983, Coley et al. 1985,
Read et al. 2009).

Syndromes across spine types

Across multivariate trait space, a spectrum of constitutive
LES – structural defence strategies in spiny saplings emerged
(Fig. 3). At one extreme end of the continuum, the strategy
90 consists of plants with high structural defences and low leaf
productivity, consistent with the ‘low nutrition, high resistance’
syndrome (Agrawal and Fishbein 2006). This strategy was most
characterized by species with leaf spines (Fig. 4). Species on
the opposite end of the spectrum are characterised by a
95 syndrome of low investment in structural defences and high
leaf productivity (consistent with the ‘tolerance and escape’
defence syndrome) (Agrawal and Fishbein 2006). Most species
with prickles, thorns and stipular spines aligned along this
end of the spectrum. These results indicate that spiny species
100 with leaf spines may predominantly adopt the low leaf
productivity and high structural defence strategy (Campbell 1986)
relative to other spine types. However, this result should be
interpreted with caution given that all leaf spiny species in
our dataset are from a single plant Genus (*Berberis*).
105
Generally, the directions (i.e. positive or negative associations)
of the relationship among traits were not contingent on spine
types (Table 2). Even for the trait–trait relationships for which
we observed significant differences (in the intercepts) between
the spine types, the directions of the relationships were always
110 the same. Thus, all spine types (leaf spine, stipular spine,
prickles and thorns) seems to exhibit a common strategy
irrespective of their contrasting anatomical pathways (i.e. modified
leaves, stipules, epidermis or branch). In spite of this, the
intercept of the relationships between ‘growth – leaf N’ and
115 ‘growth – assimilation rate (A_m)’ differed across spine types
(Table 2). Generally, species with prickles had greater growth
rate at a given leaf N or assimilation rate
120

0 relative to species with stipular spines or thorns (data not
shown). This suggests that species with different spine types
may have different growth efficiencies. However, given that
the relationship between growth rate or LES and defence
5 traits did not differ across spine types (Table 2), the differ-
ence in growth efficiencies between the spine types may be
driven by other unmeasured traits (e.g. leaf size or biomass
allocation) (Tomlinson et al. 2014).

10 **Study limitations and recommendations for further
studies**

Our results should be interpreted with caution for two main
reasons. First, species were grown under a single common
15 garden lacking any herbivore. Thus, trait covariations pre-
sented in this study is relevant at the constitutive level. A sub-
stantial body of literature has shown that spines, like most
other defence traits (Cipollini et al. 2003, Heil 2014), are
inducible i.e. greater resources allocated to spines under her-
20 bivory conditions (Milewski et al. 1991, Obeso 1997, Young
and Okello 1998, Gómez and Zamora 2002, Young et al.
2003, Hanley et al. 2007, Barton 2016). Similarly, leaf traits
such as nitrogen content, SLA and assimilation rate can
25 be altered under herbivory conditions (Zhou et al. 2015,
Peschiutta et al. 2018). Second, our analyses focused only
on spiny plants, because one of our aims was to understand
whether and how spines (which function differently from
direct leaf defence traits such as tannins and lignin) are related
30 to the LES. By focusing on spiny plants, our analyses do not
provide information how LES and defence traits covary in
non-spiny plants. However, in nature, spiny and non-spiny
plants co-occur across biomes and experience similar growth
constraints (Grubb 1992, Schmidt et al. 2013, Tindall et al.
35 2016).

Despite the above-mentioned limitations, our results have
elucidated some of the general trends in LES – defence trait
covariations. For instance, although focusing only on spiny
40 plants, our results are consistent with that of Chauvin et al.
(2018), who explicitly studied LES-defence traits in 83 moist
tropical forest species (some species in the study area were
spiny but this study did not consider spininess). While only
few studies have evaluated the links between defence and
45 the LES (Zuest and Agrawal 2017), the convergence of our
results and those of Chauvin et al. (2018) suggests, that across
species, structural defence and the LES are linked whereas
quantitative chemical defence are decoupled from this axis.
50 In spite of this, we recognize that further studies testing the
generality of the trait–trait relationships observed in this
study, under natural conditions, across different biomes and
comparing between plant functional groups will be particu-
larly insightful for developing an integrative framework for
55 understanding LES-defence trait covariations.

Conclusion

60 We conducted a large-scale common garden study to exam-
ine the links between the LES, defence (structural and

quantitative chemical defences) and growth in saplings of 61
spiny species. Generally, our results demonstrate that struc-
tural defence traits (spines, leaf lignin and fibre content) are
coupled with the LES but are decoupled from quantitative 65
chemical defence. Importantly, the most productive spiny
species (high leaf productivity and fast growth rate) were
associated with low constitutive investment in structural
defences. Leaf spiny species in our dataset were more com-
monly associated with the high structural defence and low 70
leaf productivity end of the spectrum relative to other spine
types. Understanding how the observed traits syndromes vary
across biomes, plant functional groups and ontogeny will be
particularly helpful in providing insights for predicting how
75 spinescent species are likely to respond to changes in herbiv-
ory and environmental resource supply.

Data availability statement

Data are available from the Dryad Digital Repository: 80Q
<<http://dx.doi.org/10.5061/dryad.xxxxx>> (Armani et al.
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disclosed by the authors.

Author contributions – KWT and MA conceived the study. MA
and UG collected the data. XY conducted the phylogenetic
reconstruction. MA analysed the data and led the writing of the
manuscript. KWT, UG, TCD, XY and KEB contributed critically
100 to the drafts and gave final approval for publication.

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5	Supplementary material (available online as Appendix oik-	
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- Q3 AQ: Update reference Archibald and Bond 2003
- Q4 Please update the reference 'Armani et al. 2019'.
- Q6 AQ: Publisher for Orme et al. 2018
- Q7 AQ: Update volume for Rooke et al. 2004

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