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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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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

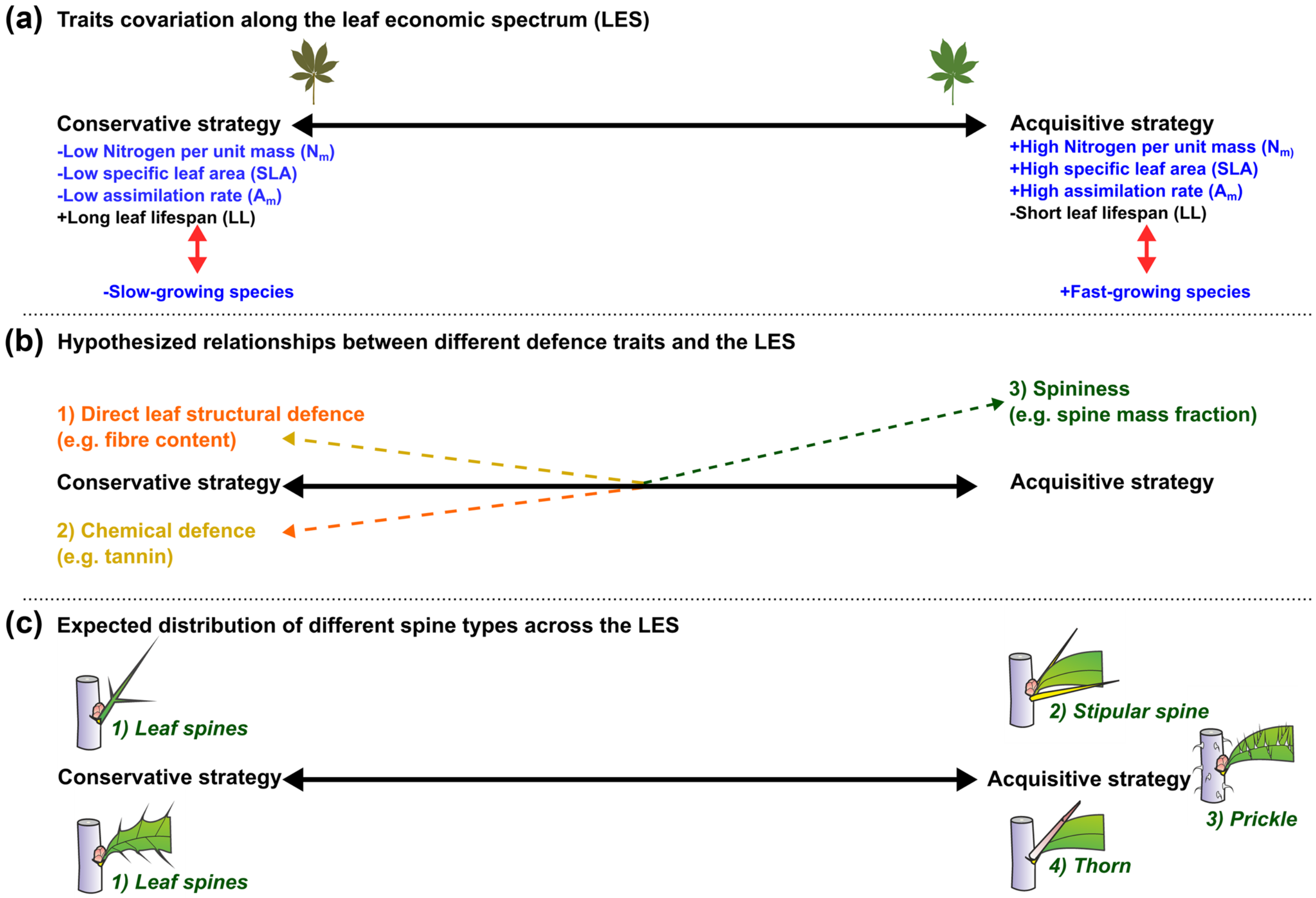

Figure 1. Conceptual framework of the relationships between different defence traits, spine types and the leaf economic spectrum (LES) traits. (a) Acquisitive leaf traits are generally associated with fast-growing species whereas conservative leaf traits are characteristic of slow-growing species. (b) 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.

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 quantitative defences (e.g. tannins), are predicted to be coupled 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 spectrum (Fig. 1b-2). However, evidence for correlation between LES and quantitative chemical defence traits is equivocal. For instance, Mason and Donovan (2015) observed significant correlations between tannin activity and the LES axis in three *Helianthus* species across whole-plant ontogeny. In contrast, Chauvin et al. (2018) observed that tannin content was decoupled from the LES axis in 83 species in a tropical 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 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 function mainly as defences against large mammalian herbivores (Cooper and Owen-smith 1986, Hanley et al. 2007, but see: Kariyat et al. 2017). Spines differ from direct leaf structural 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 (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 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 communities (Grubb 1992) and their evolution in Africa has been shown to be tightly linked to evolution and diversification of mammalian herbivores (Charles-Dominique et al. 2016). If defence is coupled with the LES and constrain leaf productivity in spiny plants, then ongoing changes in large mammalian herbivory pressure (Ripple et al. 2015, Hempson et al. 2017) 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 (Fig. 1b-3; Grubb 1992, Tomlinson et al. 2016, Wigley et al. 2018). Within biomes, spiny species tend to possess nutritious 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 forests 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. rocky slopes or forest edges). Similarly, in African savannas, spiny species tend to possess high-quality fine-leaves often dominating nutrient rich soils (Scholes and Walker 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 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 'spininess', leaf N and specific leaf area (SLA) for saplings 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 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 producing these divergent spine types have differential effects on leaf morphology and physiology and hence their relationship with the LES (Fig. 1c). Spines can be derived from modified 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 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 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 compatible with acquisitive leaf strategies. Similarly, although thorns incur significant biomass allocation cost relative to stipular 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 LES (Fig. 1c) but this notion has never been experimentally tested.

The aim of this study was to test whether different categories of defence traits (leaf structural defence, chemical defence 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 families and contrasted environments; Supplementary material Table A1) and grow them under common garden conditions for trait measurement. We measured eight traits that have been deomonstrated to be important for plant growth and defence (Supplementary material Table A2).

Table 1. Pairwise correlations between defence, leaf productivity and growth traits of spiny saplings grown under common garden conditions for 15 weeks. Pearson correlation coefficients (r) are given for phylogenetically corrected (above diagonal) and non-corrected methods (below diagonal). Significant relationships are (highlighted in bold) are shown as $p < 0.0001 = ***$, $p < 0.001 = **$, $p < 0.05 = *$.

	Spine mass fraction ($g\,g^{-1}$)	Acid detergent fibre (%)	Acid detergent lignin (%)	Condensed tannin (%)	Nitrogen per unit mass ($mg\,g^{-1}$)	Specific leaf area ($mm^2\,mg^{-1}$)	Assimilation rate ($\mu mol\,g^{-1}\,s^{-1}$)	Relative growth rate ($g\,g^{-1}\,d^{-1}$)
Spine mass fraction ($g\,g^{-1}$)		(+) 0.254	+0.269	+0.304	**−0.497***	**−0.400***	**−0.693***	**−0.663****
Acid detergent fibre (%)	+0.374		**+0.908*****	−0.020	**−0.406***	−0.315	**−0.597****	−0.349
Acid detergent lignin (%)	**+0.530****	**+0.875*****		−0.132	−0.213	−0.146	**−0.504***	−0.325
Condensed tannin (%)	+0.016	+0.135	+0.095		**−0.399***	−0.260	+0.251	**+0.494****
Nitrogen per unit mass ($mg\,g^{-1}$)	**−0.627****	**−0.378***	**−0.336***	−0.152		**+0.628*****	**+0.695*****	**+0.678*****
Specific leaf area ($mm^2\,mg^{-1}$)	**−0.579****	−0.279	−0.238	−0.140	**+0.705*****		**+0.546***	**+0.718*****
Assimilation rate ($\mu mol\,g^{-1}\,s^{-1}$)	−0.416	−0.013	+0.058	+0.220	**+0.463***	**+0.621****		+0.400
Relative growth rate ($g\,g^{-1}\,d^{-1}$)	−0.478	−0.157	−0.033	+0.221	+0.331	**+0.449***	+0.374	

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

Relative growth rate (RGR) is widely used as measure of plant performance (Evans 1972, Zuest and Agrawal 2017) and scales positively with acquisitive leaf traits (Lambers and Poorter 1992, Reich 2014). Specific leaf area (SLA), leaf N per unit mass (N_m) and carbon assimilation per unit leaf mass (A_m) are primary traits used in the LES and scale positively with each other (Wright et al. 2004). Spine mass fraction (SPMF) is a measure of biomass investment in spines (Bazely et al. 1991, Pisani and Distel 1998, Gowda and Palo 2003). Acid detergent fibre (ADF) and lignin (ADL) give measures of the least digestible fibre portion of plant material and are commonly used as indices of forage antiquality (Schindler et al. 2003, Rooke et al. 2004, Rafferty and Lamont 2007). Both lignin and fibre also generally contribute to low SLA and high leaf longevity (Poorter et al. 2009, Kitajima et al. 2012). Here, we classified ADF and ADL as structural defence trait. However, ADF and ADL are functionally similar to tannins in that their antiherbivore role involves reduction of digestibility and limiting the digestive energy of forages (van Soest 1982, Moore and Jung 2001). Condensed tannins (CT) are quantitative secondary metabolites (i.e. true chemical defence) that binds to protein making it unavailable to herbivores (Ayres et al. 1997, Wallis et al. 2012). Fibre, lignin and tannins are widespread across plant species (Mole 1993, Moore and Jung 2001), and are suitable common currencies for investigating trait–trait relationship across multiple species.

Specifically, we tested three main hypotheses (Fig. 1a–c). First, we predicted that across the studied taxa, acquisitive leaf traits (such as high leaf N, SLA and assimilation rate) will be associated with fast-growing species and vice versa (Fig. 1a). Secondly, we posited that all defence traits (spines, leaf chemical and structural defences; SPMF, ADF, ADL and CT) are linked to the LES across spiny species (Fig. 1b). However, we expected divergence between leaf structural and chemical defence traits (Fig. 1b1–b2; ADF, ADL and CT) and investment in spines (Fig. 1b3; SPMF) along the LES axis such that greater investment in spines will be associated with acquisitive life-history strategies whereas higher investment in leaf structural and quantitative chemical defences will be associated with the conservative life-history strategies. Finally, we hypothesized that species with different spine types will be associated with different ends of the LES. Specifically, we expected species with stipular spines, prickles and thorns to be more associated with the 'acquisitive end' of the LES whereas species with leaf spines will be characterized by conservative leaf traits.

Material and methods

Seed collection

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

Table 2. Results of phylogenetic least squares (PGLS) models testing for differences in the relationships between defence, leaf productivity and growth rate for different spines types.* Model summary values are F-values derived from phylogenetically – adjusted analysis of variance (ANOVA). Significant relationships are shown in bold as <0.0001 = ***, <0.001 = **, <0.05 = *.

	Model summary					Spine type replicates			
			F-values						
Trait–trait relationship	Y-variable	X × Spine type	X	Spine type	X × Spine type	Leaf	Prickle	Stipular	Thorn
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.

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

field station in Yuanjiang Valley, Yunnan (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 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.

Trait measurements

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 ($g\,g^{-1}\,d^{-1}$) 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: $\mu mol\ CO_2\,g^{-1}\,s^{-1}$) 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 (Lsa, cm^2). We computed the mean of these five leaves as the Lsa for each individual sapling. Each scanned leaf included midrib, all leaflets (for compound leaves) and petiole. Lsa 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 (Lsm). Area-based assimilation rate (A_a, $\mu mol\ CO_2\,m^{-2}\,s^{-1}$) 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 $\mu mol\,m^{-2}\,s^{-1}$ with an artificial light source, and vapour pressure deficit in the chamber was < 0.1 kPa. Measurements were made under ambient CO_2 concentration with flow rates between 400 and 500 $\mu mol\,s^{-1}$, and readings were taken after photosynthetic rates stabilised. Mass-based assimilation rate (A_m: $\mu mol\,CO_2\,g^{-1}\,s^{-1}$) was calculated directly as $A_m = A_a \times SLA$. 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 elementar analyser.

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.

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.nihh.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, Darriba et al. 2015).

Four chains were run for 20 000 000 iterations with the first 5 000 000 iterations discarded as burn-in and the remaining 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 consensus 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 manually inserted three species as polytomy within the related genera (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).

Statistical analyses

We performed all analyses at the species level (by averaging traits per species) because individual-level data were unavailable 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 produce 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 differed 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 producing qualitatively similar 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 relationships were contingent on spine type, we implemented phylogenetic 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' function of the 'caper' 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 conducted in R ver. 3.4.4 (<www.r-project.org>).

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 variable (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 phylogenetic 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 independent 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 represents 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 macrocarpa*). 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 species 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 covariations 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

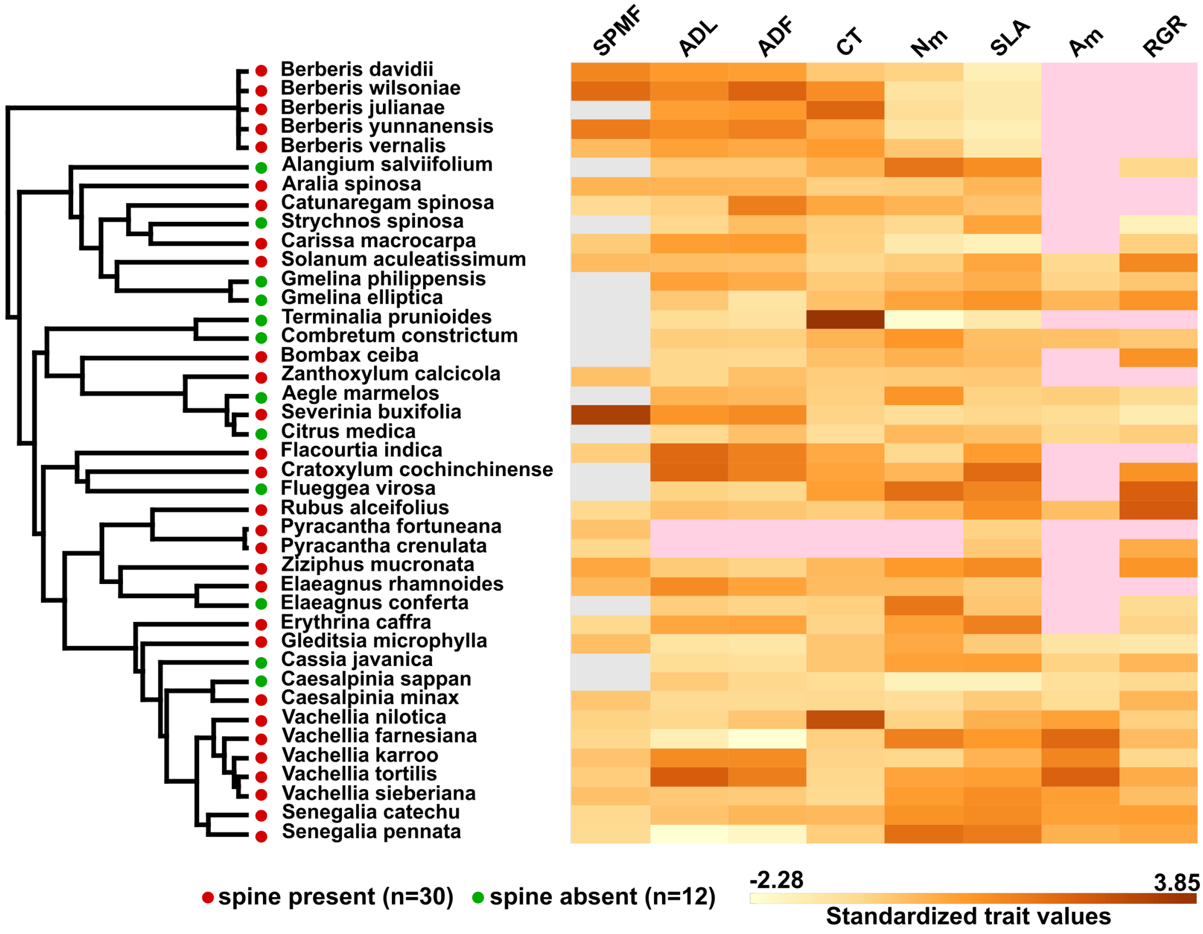

Figure 2. Phylogenetic tree showing trait distribution across the 41 (of 42) species included in this study. Traits values are standardized. Light pink color represent missing data. Light gray color in SPMF represents species that did not produce spines. Traits are: SPMF, spine mass fraction; ADF, acid detergent fibre; ADL, acid detergent lignin; CT, condensed tannins; N_m, nitrogen per unit mass; SLA, specific leaf area; A_m, 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.

correlation analyses) related to all other defence traits whereas fibre (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 A_m), providing further support to the finding that 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

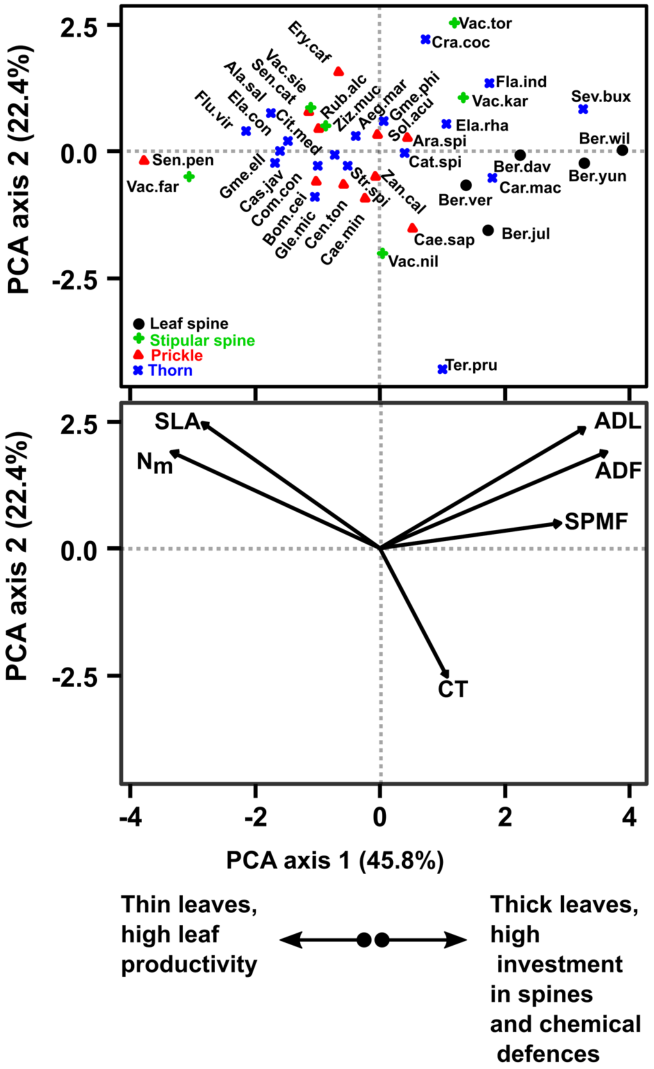

Figure 3. Principal component analysis (PCA) of multivariate trait associations of saplings of 42 spiny species. (a) Biplot of species score on first two PCA axes. Species names are abbreviations of first three letters of 'Genus.species' names (e.g. Sen. pen = *Senegalia pennata*). Symbols for different 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).

two independent trait axes that together explained ~68% of 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 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.

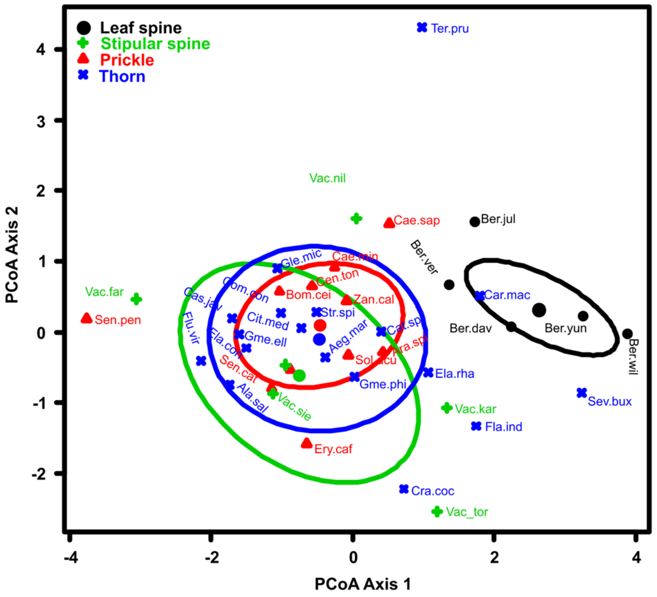

Figure 4. Principal coordinate analysis (PCoA) of multivariate trait associations of saplings of 42 spiny species. Species names are abbreviations of first three letters of 'Genus.species' names (e.g. Sen. pen = *Senegalia pennata*). Symbols for different spine types shown in legend. Centroid of each 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).

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 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, 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 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 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 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 the LES axis in three *Helianthus* species across ontogeny. It is possible that within species or clades tradeoff 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. 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 (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 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 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 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.

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 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 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, 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 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 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 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 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*).

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 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 ‘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 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 traits did not differ across spine types (Table 2), the difference 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).

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 garden lacking any herbivore. Thus, trait covariations presented in this study is relevant at the constitutive level. A substantial 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 herbivory 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 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 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. 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 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 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. 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 particularly insightful for developing an integrative framework for understanding LES-defence trait covariations.

Conclusion

We conducted a large-scale common garden study to examine the links between the LES, defence (structural and quantitative chemical defences) and growth in saplings of spiny species. Generally, our results demonstrate that structural defence traits (spines, leaf lignin and fibre content) are coupled with the LES but are decoupled from quantitative 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 commonly associated with the high structural defence and low 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 spinescent species are likely to respond to changes in herbivory and environmental resource supply.

Data availability statement

Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.wpzgmsbhh> (Armani et al. 2020).

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Conflict of interest – There is no potential conflict of interest to be 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 to the drafts and gave final approval for publication.

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Supplementary material (available online as Appendix oik-06960 at <www.oikosjournal.org/appendix/oik-06960>). Appendix 1.