

Seedling Ecology and Evolution

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Seedling ecophysiology: strategies toward achievement of positive net carbon balance

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8.1 Introduction

Many plant ecophysiological issues, such as trade-offs associated with resource acquisition strategies, are shared between seedlings and larger plants. Yet, seedlings face several unique challenges in their struggle to achieve positive net carbon balance necessary for growth and survival. First, seedlings go through dynamic physiological changes from complete dependency on seed reserves to dependency on light and external nutrients. Second, because seedlings are small, modest changes in carbon allocation patterns will have large consequences on whole plant carbon balance and survival. Third, seedlings experience intense mortality from a wide range of abiotic and biotic factors, including strong asymmetric competition from larger neighbors, herbivory, disease, and disturbance (Moles & Westoby, 2004c; Fenner & Thompson, 2005; Kitajima, 2007; Chapter 10). Finally, phylogeny exerts a particularly strong influence on seed size (Moles *et al.*, 2005b) and morphological traits (Saverimuttu & Westoby, 1996b; Ibarra-Manríquez *et al.*, 2001; Zanne *et al.*, 2005) of young seedlings. These unique aspects undoubtedly influence the evolution of resource allocation strategies in relation to the regeneration niches of species (Grubb, 1977).

In this chapter, we discuss both theoretical and technical issues important in evaluating carbon allocation strategies of seedlings. We place a large emphasis on inter- and intraspecific trade-offs due to constraints in resource allocation. Presumably, such trade-offs reflect specializations in ecological space defined by spatial and temporal heterogeneity, and ultimately restrict the range of environments in which seedlings establish (Chapters 3, 10). We explore key concepts pertaining to carbon balance strategies. Many of our examples are drawn from comparative studies in species-rich tropical forests with high morphological and physiological diversity. Yet, these concepts should be relevant in many plant communities. The topics

roughly follow the ontogeny of a seedling, starting with issues related to seed reserve utilization, followed by sections on interspecific variation in carbon balance strategies and phenotypic plasticity. Our goal is not to provide a comprehensive review of all relevant literature. Rather, we hope to stimulate interest in conceptual issues relevant for carbon balance strategies of seedlings within and across species and communities.

8.2 | Seed reserve utilization

Developing embryos and seedlings depend completely on resources stored in endosperm or reserve-type cotyledons for their energy and nutrient demands until sufficient development of photosynthetic organs and roots. How long seedlings depend on seed reserves is influenced by many factors, including developmental rates, cotyledon functional morphology, and environment (Kitajima, 1996a), as well as the resource in question (e.g. energy vs. nitrogen and other mineral nutrients). Concentrations of various resources stored in seeds are not completely in balance with demands by young seedlings. Fenner (1986) evaluated the relative durations of dependency for mineral elements stored in seeds by comparing the final sizes of seedlings achieved without supply of individual mineral elements. This method, applied to various temperate legume and nonlegume species, revealed that seedlings show a tendency to shift to external dependency on nitrogen before other mineral elements (Fenner, 1986; Fenner & Lee, 1989; Hanley & Fenner, 1997). Although these approaches help us understand the relative order in which seed reserves become exhausted, they cannot be used to quantify the time course of gradual changes from heterotrophic dependency on seed reserves to autotrophic energy gain.

Gradual decreases in seed reserve dependency for energy as well as for mineral elements can be analyzed by comparing growth curves under contrasting resource levels (Fig. 8.1; Kitajima, 2002). While seedlings are completely dependent on seed reserves, seedling growth rates are independent of light and soil nutrients. Once seedlings start acquiring carbon by photosynthesis and mineral nutrients by roots, their growth is influenced by the external availability of these resources. In three tropical woody species in the Bignoniaceae with contrasting cotyledon functional morphologies, dependency on light developed before dependency on soil nitrogen. In addition, the species with photosynthetic cotyledons (Fig. 8.1b) started to utilize light as the energy source earlier than the other two with reserve-type cotyledons (Fig. 8.1d,f). This was expected because thin photosynthetic cotyledons have high net photosynthetic capacity similar to true leaves on a unit-mass basis (Kitajima, 1992b). Active photosynthesis requires high tissue nitrogen concentration for construction of various enzymes and membrane-bound proteins. Yet, duration of dependency on seed nitrogen was not related to cotyledon type

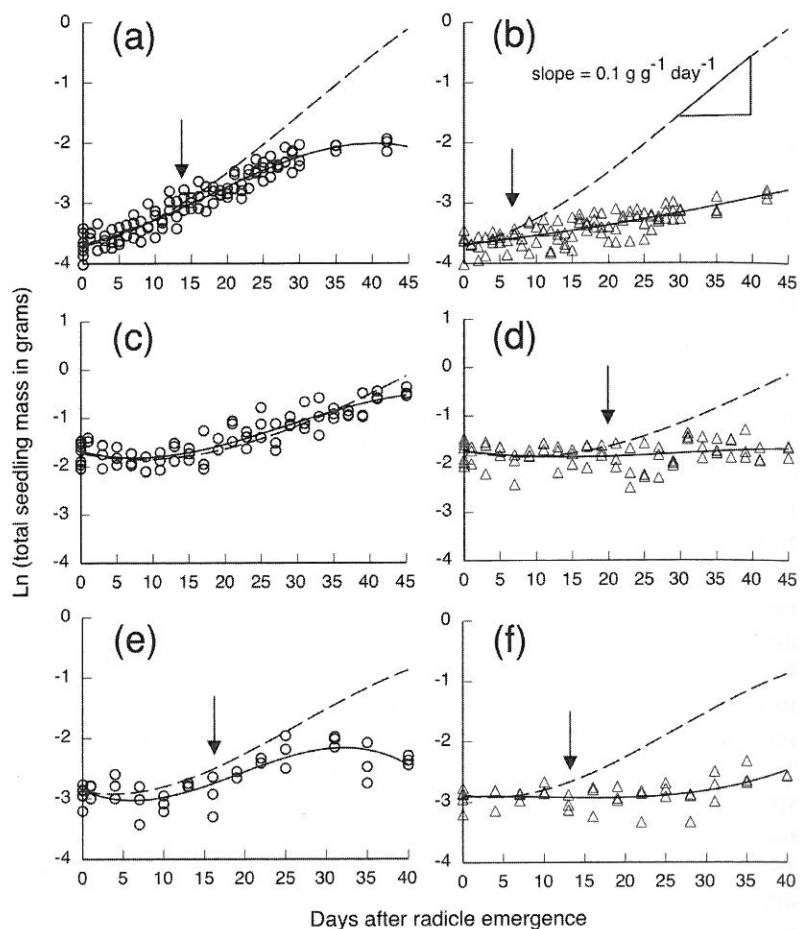


Fig. 8.1 Seedling biomass growth (= natural log of total biomass plotted against time) during the transition from dependency on seed reserves to autotrophy using external resources for three tropical woody species in Bignoniaceae: *Tabebuia rosea* (a, b), *Callichlamys latifolia* (c, d), and *Pithectenium crucigerum* (e, f). In each panel, reference growth of seedlings, individually potted in washed sand under optimal light (27% of total daily photon flux density, PFD) and soil nitrogen supply (complete nutrient solution), is shown by broken line (third-order polynomial fit, individual data points not shown for clarity). The instantaneous slope along the fitted curve is the relative growth rate (RGR); RGR for the linear growth phase under optimal growth supply is indicated in panel (b). The left panels (a, c, e) show the effects of eliminating nitrogen from nutrient solution, whereas the right panels (b, d, f) show the effects of shading (1% PFD). Arrows indicate the end of the complete seed reserve dependency for nitrogen and energy, i.e. the age after which the 95% confidence intervals of the growth curves under single-resource limitation no longer overlap with the reference growth curves. Adapted from Kitajima (1992a; see 2002).

among the three species, but it appears to be influenced by seed nitrogen concentration (Fig. 8.1c for the species with high seed nitrogen concentration, compared to the other two species shown in Fig. 8.1a,e; see Kitajima, 2002). This study shows that a seedling's dependency on seed reserves for a particular resource is influenced not only by

seed size, but also by its concentration in seed, cotyledon functional morphology, and growth constraints by other resources.

Elevated concentrations of mineral elements may have energetic advantages in environments in which the cost of soil nutrient acquisition is high. In infertile soils in Australia, some species in Proteaceae often have very high nitrogen and phosphorus concentrations in seeds (Pate *et al.*, 1985). The prolonged duration of seed reserve dependency for these elements may be advantageous for seedling establishment in infertile sandy soils, especially following fires (Stock *et al.*, 1990; Milberg & Lamont, 1997). In Amazonian caatinga, tall trees have smaller seeds with higher concentrations of phosphorus and magnesium than tall trees on fertile soil in other lowland rain forest sites (Grubb & Coomes, 1997). High seed nitrogen concentration is also found among shade-tolerant tropical woody species with relatively large seeds (Kitajima, 2002), possibly because it may help initial development of deep tap roots or minimize the energy expended on soil nutrient uptake for an extended period. In contrast, a study of 12 congeneric grass species in New Zealand found no differences in mineral nutrient concentrations in seeds of species from fertile and infertile soils (Lee & Fenner, 1989). How elevated mineral nutrient reserves contribute to seedling establishment in contrasting habitats is a research area that deserves further attention.

Seeds of different species exhibit widely different concentrations of starch and lipids, the two forms of energy storage. Higher lipid content results in higher energy content per gram of seed mass, but do elevated lipid concentrations in seeds confer energetic advantages for seedling establishment (Levin, 1974)? Although energy per gram of seed tissue increases with higher lipid concentrations, it probably does not mean prolonged energetic support for seedlings, because energy retrieval from beta-oxidation of lipids in plants is not as efficient as from starch (Chapin, 1989). A 100% increase in energy content per gram of seed tissue by increasing lipid concentrations may result in only a 40% increase in terms of initial seedling mass (Kitajima, 1992a). Hence, storing starch costs less to the parent than storing lipids for a unit amount of energy endowed to the seedling. Furthermore, isotopic analysis suggests that energy transfer from lipids in seeds to developing seedlings may be negligible (Kennedy *et al.*, 2004). Then, why are lipid-rich seeds common among many plant species even though lipids are not used as energy storage in vegetative tissues in general? Perhaps, the dispersal advantage associated with low seed weight outweighs the disadvantage of added energy costs to the parent (Lokesha *et al.*, 1992).

8.3 Ontogenetic trajectories of seedling carbon balance

From an energy balance perspective, early seedling growth is a process of transforming the total energy pool in seeds (embryo plus

endosperm) into seedlings with gradual development of photosynthetic autotrophy. Thus, there is a strong allometric relationship between seed mass and seedling mass within and across species (e.g. Green & Juniper, 2004b; Paz *et al.*, 2005). Departure from a perfect allometry is caused by species difference in net carbon balance and its determinants including cotyledon functional morphology.

Net carbon balance per unit seedling mass can be quantified as relative growth rate (RGR), the instantaneous slope of the natural log of total seedling mass plotted against time (Fig. 8.1; see Poorter & Garnier, 2007, for technical details). RGR continuously changes throughout early seedling development: initially negative or zero, then becoming positive following development of photosynthetic autotrophy. In a constant environment with adequate resource supply, RGR is expected to reach a maximum for some period (log-linear phase in growth curves, Fig. 8.1), after which RGR typically starts to decline. This ontogenetic trajectory of RGR against time after germination can be generalized as a bell-shaped curve (Hanley *et al.*, 2004; Poorter & Garnier, 2007). Unlike RGR, net assimilation rate (NAR), which quantifies net carbon balance per unit leaf area, is ecologically meaningful only after seedlings become fully autotrophic.

Prolonged dependency on seed reserves, especially in species with large storage cotyledons, requires special considerations in the calculation and interpretation of RGR. Regardless of cotyledon types, cotyledon mass must be included as a part of the total seedling mass for the purpose of quantifying net carbon gain. Otherwise, early RGR will be arbitrarily inflated in species with nonphotosynthetic cotyledons. During early development, significant proportions of carbohydrates are transferred from seeds for storage in stems and roots (Kabeya & Sakai, 2003; Myers & Kitajima, 2007). But, large cotyledons that remain attached in some temperate and tropical woody species stop transferring energy reserves to seedlings after the first set of leaves become fully functional (Ichie *et al.*, 2001; Kennedy *et al.*, 2004; Myers & Kitajima, 2007). For purposes other than quantification of net carbon balance, other measures of growth (e.g. RGR of biomass excluding storage carbohydrates, height or total leaf area, and absolute growth rate) may be more ecologically meaningful variables.

Even under constant availability of light and nutrients, an eventual decline of seedling RGR with increases in size is expected for two reasons (Givnish, 1988): (1) a decrease of the ratio of photosynthetic mass relative to nonphotosynthetic mass due to greater allocation to stems for stability of taller stems, and (2) greater degrees of leaf overlap and self-shading in larger plants. For example, Walters *et al.* (1993a) found a decrease in allocation to leaf mass to be the main reason for a decrease in RGR with age and size of temperate tree seedlings. In contrast, Lusk (2004) showed that shade-tolerant evergreen seedlings continue to accumulate leaf area with size and age. For seedlings of an evergreen conifer in Chilean rain forests, Lusk *et al.* (2006) found that increasing leaf area results in greater self-shading and lower light use efficiency, but that light interception efficiency per unit mass remains

similar due to increase in leaf area ratio (LAR, the ratio of leaf area to total plant mass). This result suggests that self-shading increases as new leaves are added, whereas the need to increase stem allocation for greater mechanical stability may not develop until much later in environments in which seedlings grow slowly. In summary, allometric analyses combined with functional growth analyses can help illuminate trade-offs associated with increases in plant size and ontogenetic shifts in seedling carbon balance.

8.4 Species differences in inherent relative growth rate (RGR)

In fully autotrophic seedlings, RGR is a function of species traits (morphology, physiology, and allocation patterns), which may be compromised by suboptimal resource supplies, and biotic and abiotic stresses. Inherent maximum RGR, measured as seedling RGR during the log-linear growth phase under optimal conditions, differs greatly among species, from values less than $0.02 \text{ g g}^{-1} \text{ day}^{-1}$ found in shade-tolerant tree seedlings to values much more than $0.1 \text{ g g}^{-1} \text{ day}^{-1}$ found for herbaceous plants and woody pioneers (Poorter & Remkes, 1990; Kitajima, 1996b; Shipley & Almeida-Cortez, 2003). It is well known that seedling RGR is negatively correlated with seed size across diverse taxa and habitats (see Shipley & Peters, 1990 for a review), for example, pasture grasses and legumes (Fenner & Lee, 1989); species from a variety of climatic conditions in Australia (Jurado & Westoby, 1992; Saverimuttu & Westoby, 1996b; Swanborough & Westoby, 1996); woody plants in temperate (Cornelissen *et al.*, 1996; Walters & Reich, 2000) and tropical climates (Kitajima, 1994; Huante *et al.*, 1995; Valladares *et al.*, 2000; Poorter & Rose, 2005); and Mediterranean annuals (Maranon & Grubb, 1993). In some cases, negative correlations between RGR and seed size is also found within species (e.g. Meyer & Carlson, 2001; Paz *et al.*, 2005).

Although the physiological and genetic bases for the trade-off between RGR and seed size within species are unclear, there are several functional explanations to explain this tradeoff among species.

- (1) Large-seeded species tend to have reserve-type cotyledons (Hladik & Miquel, 1990; Kitajima, 1996a; Wright *et al.*, 2000; Zanne *et al.*, 2005) and delayed development of photosynthetic area relative to biomass.
- (2) Seedlings of large-seeded species tend to develop more slowly, in terms of time between radicle emergence and shoot extension, time until the first leaf expansion, and time between successive leaf development (Kitajima, 1992a).
- (3) Large-seeded species may store greater amounts of carbohydrate in reserve-type cotyledons, stems, and roots (the large-seed-slower-deployment or reserve effect; Saverimuttu & Westoby

1996b; Kidson & Westoby, 2000; Green & Juniper, 2004b; Myers & Kitajima, 2007).

- (4) Large-seeded species tend to allocate more to roots and stems (Kitajima, 1994; Walters & Reich, 2000; Paz, 2003) both in terms of nonstructural carbohydrates and structural mass (Canham *et al.*, 1999).
- (5) Large-seeded species tend to have leaves with low specific leaf area (SLA, the ratio of leaf area to leaf mass) and leaf area ratio (LAR), the two traits that are known to be positively correlated with interspecific differences in RGR under standardized conditions (e.g. Poorter & Remkes, 1990; Kitajima, 1994; Reich *et al.*, 1998; Wright *et al.*, 2000; Shipley & Almeida-Cortez, 2003; Poorter & Garnier, 2007).

Of the explanations above, those due to seed reserve allocation and cotyledon functional morphology are likely to disappear with time, while differences in biomass partitioning (e.g. SLA, LAR, and root allocation), photosynthetic physiology (e.g. NAR), and developmental rates should become increasingly strong predictors of RGR (Poorter & Garnier, 2007). The relative importance of these morphological and physiological traits as determinants of species differences in RGR differ among environments (e.g. Poorter, 1999) and continuously change with size (Walters *et al.*, 1993a; Lusk & Del Pozo, 2002; Delagrangé *et al.*, 2004; Baraloto *et al.*, 2005; Claveau *et al.*, 2005). Yet, life history correlations of seed mass with these functional traits (Reich *et al.*, 2003; Wright *et al.*, 2007) may continue to reinforce the negative correlation between RGR and seed mass, especially in resource-rich environments in which photosynthetic gain per unit leaf area is greater. One interesting functional correlation in seedlings is the positive relationship between cotyledon SLA and true-leaf SLA (Kitajima, 1992a); seedlings with thin photosynthetic cotyledons continue to grow faster even after true leaves become their main source of autotrophic carbon gain. In a meta-analysis of comparative studies of rain forest tree species, Poorter and Rose (2005) found that the negative interspecific correlation between seed mass and RGR is stronger under higher light availability in which growth is faster and seedling size quickly becomes decoupled from seed mass, remaining detectable for 1–2 years. Seed mass is known to be negatively associated even with diameter growth rates of adult trees (in four out of the five neotropical forests, as well as across 219 species in the pooled dataset; Poorter *et al.*, in press).

8.5 Opportunistic versus conservative strategies

Trade-offs between growth rates and survival have been demonstrated for seedlings of various life forms in diverse communities, including, for example, temperate trees (Grime & Jeffrey, 1965; Reich *et al.*, 1998), tropical trees (Kitajima, 1994; Dalling &

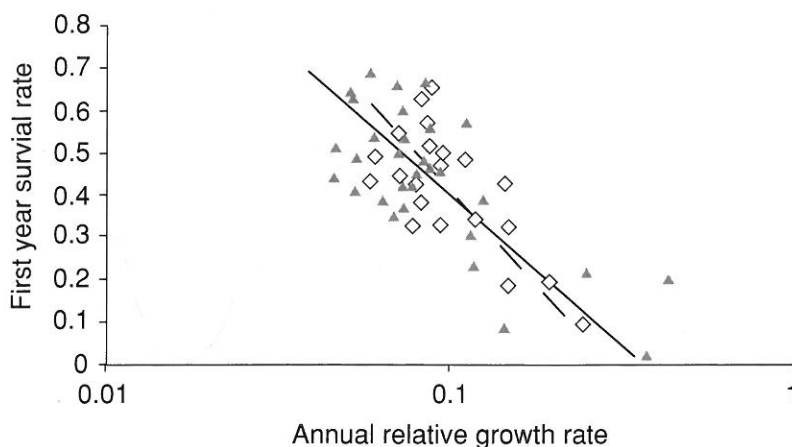


Fig. 8.2 The trade-off between first year survival and relative height growth rates (log-scale) for seedlings of neotropical trees (solid triangle, solid line, $n = 31$ species) and lianas (open diamond, dashed line; $n = 22$ species) on Barro Colorado Island (Panama). From Gilbert *et al.* (2006), with permission from the Ecological Society of America.

Hubbell, 2002), and rosette-forming perennial herbs (Metcalf *et al.*, 2006). Growth-survival trade-offs can vary continuously among coexisting species, as shown for trees and liana species in a neotropical forest (Fig. 8.2) (Gilbert *et al.*, 2006) and for temperate forest trees (Pacala *et al.*, 1996). Such trade-offs are considered to arise from continuous variation in growth, defense, and storage strategies from the very opportunistic (i.e. fast growth, low survival) to the very conservative (i.e. slow growth, high survival) ends of the trade-off continuum. However, the functional traits that underlie growth-survival trade-offs are still debated, mainly because researchers differ in their views about carbon balance strategies that enhance survival (e.g. Sack & Grubb, 2001, 2003; Kitajima & Bolker, 2003; Lusk, 2004).

Maintenance of positive net carbon balance is a prerequisite for survival and growth once seedlings survive beyond the initial period of heavy seed reserve dependency. However, maintenance of positive net carbon balance does not necessarily mean that species are selected to maximize short-term carbon gain rates. Indeed, long-term maintenance of positive net carbon gain may be achieved by either of the two contrasting strategies, opportunistic or conservative, as long as plants are alive with sufficient reserves to recover from traumatic tissue losses, represented by the sharp declines in Fig. 8.3. Opportunistic species emphasize growth over defense in their allocation patterns. As a result, they achieve fast growth rates in the absence of physical and biotic disturbance. However, because of low defense, they suffer severe tissue loss and high mortality when they experience physical damage or attack by natural enemies. In contrast, conservative species that emphasize defense over growth experience less frequent and only moderate tissue loss. One underlying assumption of this scenario is

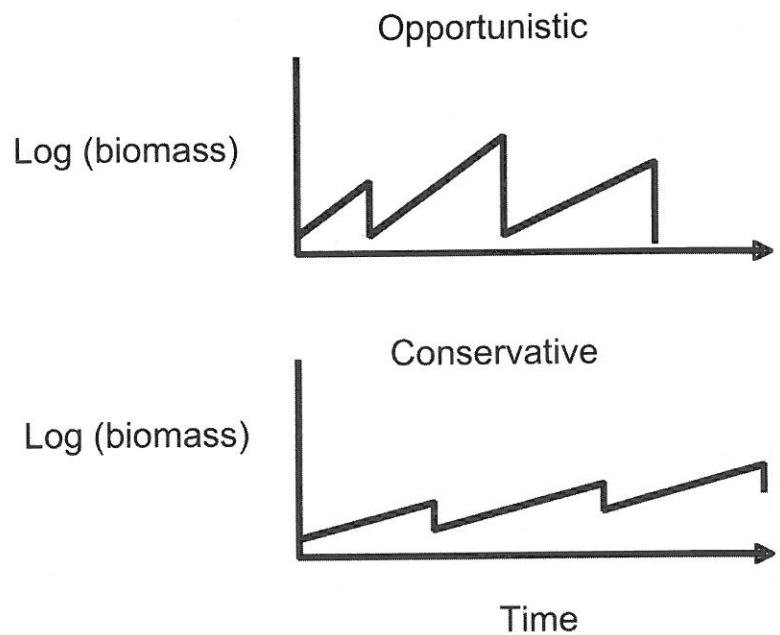


Fig. 8.3 Schematic diagram to contrast opportunistic vs. conservative strategies for maintenance of positive carbon balance, in which loss of tissue to natural enemies or periodic environmental stresses impose negative carbon balance, shown as sudden drops in the trajectories from time to time.

that defense, in particular, physical defense achieved through thick cell walls and tough tissues, is incompatible with fast growth due to allocation constraints.

The opportunistic strategy is represented by a suite of functional traits optimized to achieve fast RGR over the short term (Fig. 8.4). This strategy, analogous to r-strategy in life history theory (Pianka, 1970), is common among many successional species that quickly colonize gaps in vegetation. However, these species may experience large setbacks in biomass from tissue loss to herbivory and disturbance (represented by large declines in Fig. 8.3). As long as they grow in a high resource environment and as long as the tissue loss is not too great, they may recover quickly due to their fast inherent growth rates. However, in low resource environments, such as forest understories and infertile soils, realized carbon income may be too slow for recovery before reserves in remaining tissues are exhausted. This explains the failure of such opportunistic species to persist in low-resource environments, even though their short-term RGR may be greater than conservative species when they are compared under the same low resource environments (Kitajima, 1994; Fine *et al.*, 2004). Indeed, in such low resource environments, species with conservative strategies (K-strategy) dominate despite their slow RGR. As a broad generalization, opportunistic and conservative species exhibit opposite suites of seed and seedling traits (Fig. 8.4). These contrasting trait syndromes

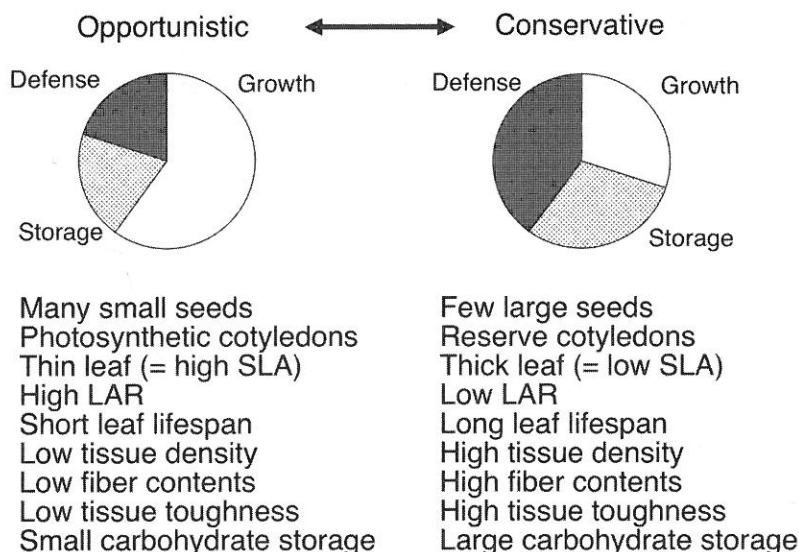


Fig. 8.4 Overall contrasts in allocation patterns associated with opportunistic vs. conservative carbon allocation strategies. Also shown are individual traits of seedlings typically associated with these strategies. (SLA = specific leaf area, LAR = leaf area ratio.)

parallel those between typical early vs. late successional species (Bazzaz, 1982) and ruderal vs. competitive species (*sensu* Grime's life history classification; Grime, 1979; Westoby, 1998).

Which of these two strategies is most likely to achieve long-term maintenance of positive net carbon gain depends on the interplay of resource competition with natural enemies. The role of natural enemies in mediating growth-survival trade-offs among high and low resource specialists was elegantly shown by Fine *et al.* (2004) in the Peruvian Amazon, where fertile alluvial soil and nutrient-poor white sand support contrasting tree communities. In a factorial experiment, they demonstrated that seedlings of alluvial soil specialists grew faster than white sand specialists in both soil types when they were protected from herbivores. However, when they were grown in white sand without protection from herbivory, alluvial soil specialists suffered greater herbivory and achieved less net leaf area growth than white sand specialists. Other studies also found that species that dominate rich soils grow fast in both rich and poor soils compared to those from poor soils when they are protected from herbivores (Huante *et al.*, 1995; Lusk *et al.*, 1997; Schreag *et al.*, 2005). Species from infertile soil tend to have lower SLA and greater leaf life span than species from rich soils (Wright *et al.*, 2002). Low SLA species characteristically have thick leaves with high tissue density and thick cuticles that confer protection against herbivores (Wright & Cannon, 2001). These results suggest that avoidance of tissue loss is selected in resource-limited environments, in which replacement of lost tissue is constrained by limited resources (Coley *et al.*, 1985).

Increased allocation to defense is expected to enhance survival, even though this strategy may result in decreased growth rates. Physical and chemical defenses are important for survival in a wide range of seedlings, from temperate forb seedlings that experience mollusk herbivory (Hanley *et al.*, 1995) to tropical tree seedlings attacked by soil-borne pathogens (Augspurger, 1984a,b). Both physical and chemical defenses may incur carbon costs, and there may be a trade-off between the two (Hanley & Lamont, 2002). Physical defense may be of particular importance for seedlings damaged by fallen litter and disturbance by vertebrates (Clark & Clark, 1989). Yet, comparative studies of seedling physical defense are surprisingly rare. The importance of physical defense for seedling survival was demonstrated for eight neotropical tree species (Alvarez-Clare & Kitajima, 2007); the greater the fracture toughness of seedling stems, the greater the first-year survival in the understory (Fig. 8.5a). This study also found that fracture toughness was highly correlated with tissue density (mass per unit volume) as well as fiber contents (expressed per unit volume or mass). These results, supported by data from an additional 70 species at the same study site (K. Kitajima, unpublished data) and studies in temperate forests (Cornelissen *et al.*, 1996), suggest that seedlings that survive well in the shaded understory are physically well defended with high tissue density in both leaves and stems, even very early in ontogeny. Similar survival advantages associated with high tissue density and stem mechanical strength have also been reported for saplings (Muller-Landau, 2004; Poorter & Bongers, 2006; van Gelder *et al.*, 2006).

Despite patterns of ontogenetic shifts across broad taxa, tissue density of stems and leaves are correlated between young and older seedlings (Alvarez-Clare & Kitajima, 2007), as well as between seedlings and saplings (K. Kitajima & L. Poorter, unpublished data). Mechanical strength obviously prevents stem breakage and herbivory, but it also is important for crown development to achieve efficient light interception, especially in older juveniles to minimize self-shading (Sterck *et al.*, 2006). High tissue density is also important in protecting seedlings against soil-borne pathogens (Augspurger, 1984a). Even well-defended conservative species, however, may experience occasional negative carbon balance due to stress and disturbances (Fig. 8.3).

8.6 Carbohydrate reserves

Carbohydrate reserves not only allow seedlings to survive through periods of negative carbon balance imposed by abiotic and biotic stresses, but also facilitate rapid recovery from tissue losses. In this way, carbohydrate reserves contribute to the maintenance of positive carbon balance in the long term even though allocation to carbohydrate storage may reduce short-term growth rates. For temperate

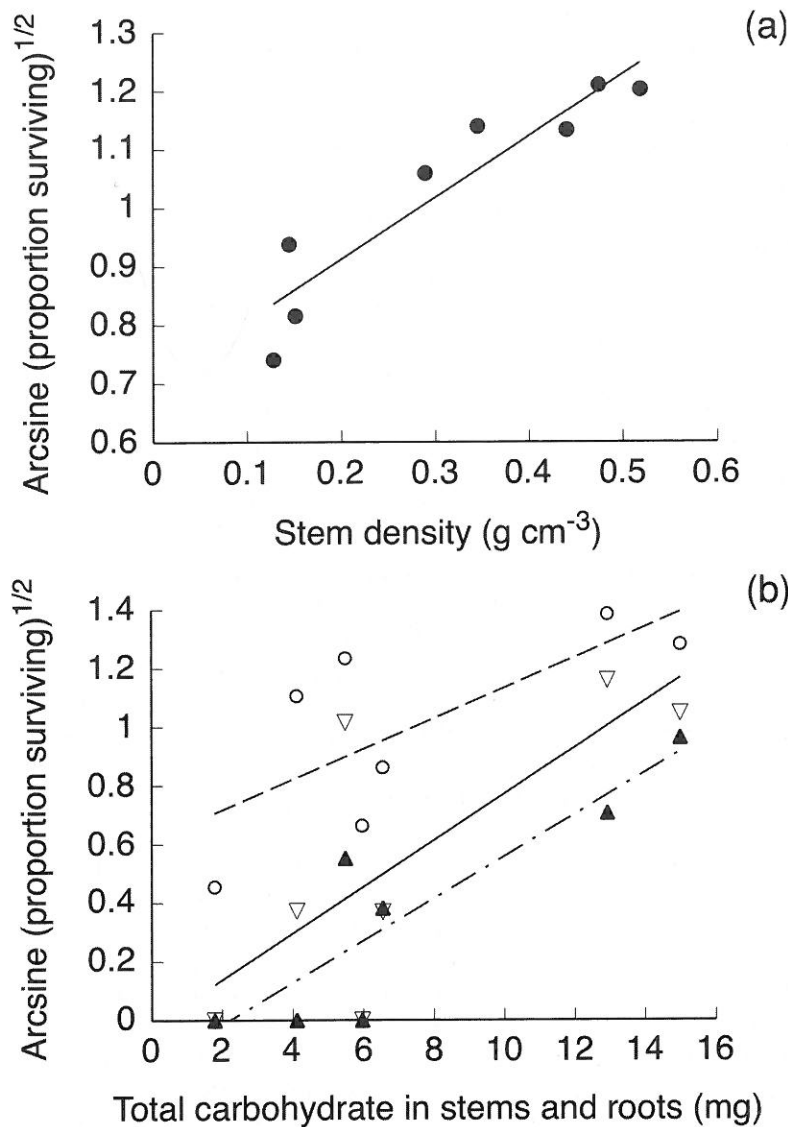


Fig. 8.5 Survival advantage of physical defense and carbohydrate storage in seedling stems and roots of neotropical tree species. (a) Stem density (dry mass per volume) of 6-month-old seedlings is positively correlated with 0–6 month survival in the natural forest understory ($r = 0.93$, $P = 0.008$). Arcsine square root transformation was applied to proportion of seedlings surviving. (b) Seedlings were grown in the forest understory (0.8% PFD) and under three treatments (open circle = control, open triangle = 90% light reduction for 2 months, closed triangle = complete defoliation). The total of nonstructural carbohydrate in the stems and roots (average per plant) at the beginning of treatment was positively correlated with survival ($r = 0.71$, 0.75 , and 0.87 , and $P = 0.07$, 0.04 , and 0.008 , respectively). Adapted (a) from Alvarez-Clare & Kitajima (2007), which also demonstrates high correlation of tissue density with biomechanical strength, and (b) from Myers & Kitajima (2007), reproduced with permission from Blackwell Publishing.

deciduous and evergreen trees, carbohydrate reserves are important for survival of seedlings subjected to stresses of simulated herbivory or winter (McPherson & Williams, 1998; Canham *et al.*, 1999). In tropical savannas, survival and recovery of seedlings subjected to fire depends heavily on carbohydrate reserves (Miyaniishi & Kellman, 1986; Hoffmann *et al.*, 2004). It is less obvious why having large carbohydrate reserves may be advantageous for survival in shade, in which carbohydrate allocation is costly relative to potential photosynthetic income. However, Kobe (1997) argued that opportunity costs (i.e. fitness costs of growth reduction) of carbohydrate allocation is smaller in shade than in treefall gaps. Indeed, saplings of shade-tolerant species have higher stem concentrations of carbohydrate reserves compared to saplings of light-demanding species both in temperate (two deciduous and two evergreen species; Kobe, 1997) and tropical dry forests (85 species, Poorter & Kitajima, 2007).

Seedlings under canopy shade in the humid tropics experience occasional periods of negative carbon balance due to variation in cloudiness, seasonal drought, physical disturbance, and attack by natural enemies. In a comparison of seven neotropical tree species, Myers and Kitajima (2007) found that seedlings of more shade-tolerant species had a greater total amount (but not concentration) of sugar and starch in stems and roots. Furthermore, survival of species that received experimental defoliation and heavy shading was enhanced by large pool sizes of nonstructural carbohydrates (Fig. 8.5b). The majority of the carbohydrate reserves in stems and roots must have been transferred from seed reserves rather than produced by photosynthesis, as the experiment was initiated only 2 weeks after full expansion of the first leaves in deep shade (0.8% of light available above the forest). Interestingly, seedlings apparently did not depend on residual carbohydrates in cotyledons, but only those in stems and roots. This finding contrasts with the results of Kabeya and Sakai (2003), in which clipping of shoots during the initial leaf flush resulted in a substantial draw of carbohydrates from cotyledons. Lusk and Piper (2007) also found a positive effect of carbohydrate concentration in leaves and roots, but not in stems, for survival of seedlings naturally recruited in the shaded understory of a temperate rain forest. Their study also suggested a potential role of carbohydrate reserves in long-lived leaves of shade-tolerant species, which may become more important with increases in biomass allocation to leaves in these species during ontogeny. The shifting relative importance of different storage organs for growth and survival is an understudied aspect of seedling ecophysiology.

Total carbohydrate reserve size is ultimately constrained in young seedlings because of their small size. This may constrain small seedlings from adopting opportunistic allocation strategies in low resource environments. At the sapling stage (e.g. juveniles taller than 1.3 m), however, either opportunistic or conservative strategies can lead to high survival. Some shade-tolerant saplings have leaves with high SLA that are more efficient in photosynthetic light utilization

(King, 2003), whereas high survival of other shade-tolerant saplings can be attributed to well-defended low SLA leaves (Poorter & Bongers, 2005), or carbohydrate reserves (Kobe, 1997; DeLucia *et al.*, 1998). However, for seedlings in both temperate and tropical forests, shade tolerance is associated with low SLA (Kitajima, 1994; Reich *et al.*, 1998; Poorter, 1999). *Alseis blackiana* (Rubiaceae), a tropical tree species with small seeds and high SLA, presents an exceptional case. As seedlings, it is a pioneer species unable to establish and survive in shade, but as saplings, it is a shade-tolerant species that can recover from repeated trauma of shading and stem breakage (Dalling *et al.*, 2001). Carbohydrate storage and its dynamics in changing light environments may offer a better understanding of the functional basis of shade tolerance in this and many other species.

Carbohydrate accumulation is influenced not only by current environmental conditions that affect photosynthetic rates, but also by the history of environments experienced by individual seedlings. Seedlings grown in high light have a higher concentration and total amount of carbohydrate compared to seedlings grown in shade (Johnson *et al.*, 1997; Kitajima *et al.*, 2006; Myers & Kitajima, in prep.). Lusk *et al.* (2007) found that larger seedlings of light-demanding species have higher carbohydrate concentration in roots than shade-tolerant species. This pattern may not only reflect species differences, but also the higher light levels that surviving individuals of light-demanding species must have experienced on average compared to shade-tolerant species. Carbohydrate dynamics of seedlings experiencing changing light environments is an ecologically important topic that has only recently received attention (Veneklaas & den Ouden, 2005; Myers & Kitajima, in prep.).

8.7 | Phenotypic plasticity

Individual seedlings adjust their morphological and physiological traits in response to environmental variation. Because individual seeds and seedlings cannot move to their preferred environments, phenotypic adjustments of morphology and physiology are important in both avoidance and tolerance of undesirable conditions, as well as maximization of carbon gain in preferred environments. Reader *et al.* (1993) quantified increases of maximum rooting depth of unwatered seedlings for 42 temperate herbaceous and woody species and found that species with the highest plasticity in rooting depth sustained shoot growth in unwatered soil. Likewise, seedlings exhibit plasticity in response to soil nutrient regimes within the constraints imposed by ontogeny (e.g. Gedroc *et al.*, 1996; Baraloto *et al.*, 2006). Among environmental factors that are known to cause plastic responses in seedlings, light is perhaps the best studied. Hence, our discussion will focus primarily on plasticity in response to light environments that are extremely heterogeneous at small spatial and temporal scales within vegetation.

It is useful to distinguish qualitative and quantitative responses in phenotypic plasticity. Photoblastic germination and etiolation are examples of all-or-nothing qualitative responses that occur when seedlings sense signals above species- and genotype-specific thresholds. Qualitative responses are often restricted to certain ecological and phylogenetic groups. For example, only seedlings of light-demanding species, but not those of shade-tolerant species, exhibit hypocotyl elongation in response to low red:far-red light ratios (Kitajima, 1994; Khurana & Singh 2006). Such a response makes sense only for opportunistic species and genotypes that specialize in vegetation gaps where shade escape is possible by rapid height growth (Schmitt *et al.*, 2003). Signals other than red:far-red light ratio, such as soil temperature fluctuation, high temperature, flush of nitrate, and smoke, are also used by seeds to detect gaps of different sizes and types (e.g. Thompson *et al.*, 1977; Keeley, 1991; Hillhorst & Karssen, 2000; Pearson *et al.*, 2002; Daws *et al.*, 2006). However, compared to seeds, much less is known about these non-light signals for seedling responses to gap environments.

In seedlings, quantitative responses to light quantity, water availability, and soil nutrient availability are perhaps more ubiquitous than qualitative responses. For example, leaves of both shade-tolerant and shade-intolerant seedlings exhibit acclimation response to light environments in both morphological and photosynthetic traits (e.g. Björkman & Holmgren, 1966; Loach, 1967; Fetcher *et al.*, 1983; Walters *et al.*, 1993a,b; Kitajima, 1994; Lee *et al.*, 1996). From these and many other studies on photosynthetic acclimation, we can generalize that leaves developed under higher light availability tend to have greater thickness, lower SLA, higher nitrogen and carboxylation enzyme concentrations per unit area, higher chlorophyll a/b ratio, and higher rates of respiration and photosynthesis per unit area. These acclimation responses to high light also interact with soil nitrogen availability (Poortsmuth & Niinemets, 2007); under low nitrogen availability, leaves must be protected against potential photoinjury through adjustment of nitrogen allocations to different photosynthetic components (Niinemets *et al.*, 1998; Kitajima & Hogan, 2003).

Levels of phenotypic plasticity are under genetic control, which creates large variations among species, ecotypes, and genotypes (Schlichting, 1986). At the whole seedling level, rates of acclimation should be faster in inherently fast-growing species that produce more leaves per unit time (Newell *et al.*, 1993). Thus, opportunistic species with inherently fast growth rates and shorter leaf life span, including many pioneers and other high resource specialists, should be more plastic (Lortie & Aarssen, 1996). Indeed, many empirical studies demonstrate that early successional species whose seedlings specialize in gaps for regeneration tend to show greater degrees of plasticity than late successional species (e.g. Bazzaz & Carlson, 1982; Straus-Debenedetti & Bazzaz, 1991; Kitajima, 1994; Valladares *et al.*, 2000; Delagrangé *et al.*, 2004; Khurana & Singh, 2006; Poortsmuth &

Niinemets, 2007). The later successional dominants also have reasons to have genetic potential for high phenotypic plasticity because they may experience greater ontogenetic changes of light availability as juveniles (that typically establish in shade) and adults (exposed to full sun). If phenotypic plasticity allows adaptation to a broader range of environments, then species that experience more variable environments in their lifetime, as well as those that have greater geographical distribution, should show higher degrees of phenotypic plasticity. But, the results may be specific to particular traits and environmental factors. For seedlings of two Australian *Acacia* (Leguminosae) species, Pohlman *et al.* (2006) found only leaf traits, but not whole plant allocation and growth, were more plastic for species with wider geographical distributions.

It is also important to consider whether plasticity is always adaptive, because plasticity may incur costs (DeWitt *et al.*, 1998). It is obvious that stem elongation response is maladaptive if it is not sufficient to escape shade. Thus, in shaded forest understory, seedlings minimize the risk of toppling by not responding to red:far-red light ratios. Likewise, regardless of the current moisture availability, initial biomass allocation to roots is greater for shade-tolerant seedlings (Walters *et al.*, 1993b; Kitajima, 1994; Delagrange *et al.*, 2004). This is a strategy with multiple advantages for stress tolerance. Larger root mass allows not only greater carbohydrate storage (Myers & Kitajima, 2007), but it also serves as a preemptive strategy to cope with droughts. Even though seeds in many communities germinate during the rainy season, dry spells during the rainy season as well as seasonal drought kill many seedlings even in humid forests (Pearson *et al.*, 2003a; Engelbrecht *et al.*, 2006). In some cases, apparent phenotypic similarity within a species may be paradoxically a result of phenotypic plasticity. For example, seedlings of two evergreen Mediterranean *Quercus* (Fagaceae) species exhibited low phenotypic variations that result in conservative resource use in fire- and drought-prone environments with high year-to-year variations in light and soil nutrient availabilities (Valladares *et al.*, 2002).

In summary, phenotypic plasticity of seedlings is widely recognized to have adaptive values for specialization to particular environments by facilitating opportunistic use of resource pulses associated with disturbances, or to ensure phenotypic consistency to minimize maladaptive responses when they exploit a wide range of environments. Yet, debates continue about how to quantify the adaptive nature of observed plastic changes (Valladares *et al.*, 2006).

8.8 | Concluding remarks

The diverse seedling carbon balance strategies discussed here are intimately related to issues addressed in other chapters, including morphological diversity (Chapters 2, 4, 6), life history trade-offs

(Chapter 10), habitat specializations, including specialization to stressful environments (Chapter 3), and population and community dynamics (Chapters 11, 12). Symbiotic associations with mycorrhizal fungi and nitrogen-fixing bacteria also have strong implications for carbon balance strategies of seedlings (Chapter 9). For example, mycorrhizae represent a significant carbon sink that influences photosynthetic response of seedlings to elevated CO_2 (Lovelock *et al.*, 1997).

Carbon balance is a common physiological currency for understanding the evolution of diverse seedling morphology and development patterns. However, a few caveats are worth mentioning. Maximization of short-term carbon gain is not necessarily the target of natural selection because it may be in conflict with maintenance of long-term carbon balance and survival. Likewise, greater phenotypic plasticity is not necessarily adaptive. Multiple traits that influence carbon balance may be simultaneously selected in response to a given environmental factor. Conversely, a given trait, such as high tissue density, may confer multiple advantages including high resistance against herbivory, disease, mechanical damage, and drought, even though it restricts rates of growth and development. Some species are selected to be conservative and slow growing, but this strategy is perhaps adaptive only in combination with large seed size and initial seedling size. In contrast, small-seeded species must compensate for their small size through opportunistic carbon balance strategies that combine efficient rapid growth and high phenotypic plasticity, even if this means survival is limited to the most favorable microsites. Finally, given considerably high phylogenetic constraints on seed size and seedling morphologies, recent advances in phylogenetic comparative analyses (Ackerly, 2000) should be useful in untangling the complicated web of seedling trait evolution.

8.9 Acknowledgments

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