

Chapter 3

The Earliest Stages of Tree Growth: Development, Physiology and Impacts of Microclimate

Daniel M. Johnson, Katherine A. McCulloh, and Keith Reinhardt

Abstract Although the germinant and young seedling life stage of trees has the highest mortality rates, our understanding of their fundamental physiology is surprisingly limited in naturally-occurring environments. Field measurements for this life stage are particularly important because of the uniquely harsh environment these small trees experience: their proximity to the soil surface results in an exceptionally dynamic microclimate with large daily shifts in such parameters as temperature, humidity and radiation. The impact of these changing conditions is discussed with respect to seedling survival. Our current knowledge of anatomical and morphological responses of early seedlings to variation in light and water conditions is also reviewed. For example, research has shown that differences in light intensity result in divergence in vessel diameters and number in *Eucalyptus grandis* seedlings. Using these data, we calculated the theoretical hydraulic conductivity for high and low light-grown seedlings. This comparison showed that after 7 weeks the differences in xylem anatomy would result in the high light seedlings having two times the theoretical leaf-specific hydraulic conductivity of the low light seedlings. Future research in this field should include developing new techniques specific to the unique fragility and size of seedlings, determining the adaptive nature of different cotyledon structures, and better quantifying the physiological and developmental characteristics of early germinants.

D.M. Johnson (✉)

Department of Environmental and Plant Biology, Ohio University, Athens, OH 45701, USA
e-mail: johnsod6@ohio.edu

K.A. McCulloh

Department of Wood Science and Engineering, Oregon State University,
Corvallis, OR 97331, USA

K. Reinhardt

Department of Biological Sciences, Idaho State University, Pocatello, ID 83209, USA

Keywords Drought • Facilitation • Mortality • Photosynthesis • Radiation • Seedling • Water relations

1 Introduction

1.1 *Population Dynamics and Seedlings*

The primary determinant of vegetation patterns and species distributions on a global scale is aridity gradients (e.g., Engelbrecht et al. 2007), and moisture regimes (either via precipitation changes or temperature changes and related evaporative demands) have shifted over last 40 years (van Mantgem et al. 2009) and are predicted to continue to change. Species distributions can shift over time through adult mortality and less subsequent regeneration (shrinking) or through seedling establishment beyond current distribution boundaries (expansion). Therefore, a critical component of our ability to predict future species distributions is an understanding of the mechanisms of seedling establishment, which requires a fundamental knowledge of seedling physiology. In fact, the life stage (other than the seed stage) with the greatest mortality, by far, is the germinant seedling stage (Leck and Outred 2008) and young seedling performance may be more important than seed germination for determination of species distributions (e.g. Figueroa and Lusk 2001).

Few studies have addressed the ecophysiology of naturally-occurring current year seedlings (newly emerged) even though this life stage has a high mortality rate (e.g. Alvarez-Buylla and Martinez-Ramos 1992; Whitmore 1996; Masaki and Nakashizuka 2002). Possible reasons for the lack of emphasis on germinant seedling physiology include the difficulty in recognizing and documenting the presence of natural, newly emerged seedlings and the even more difficult task of acquiring physiological measurements (Cui and Smith 1991; Germino and Smith 2001). In general, the coupling of physiological ecology (e.g. carbon gain, water relations) to population biology (e.g. seedling abundance and survival) appears to be an important omission in studies of plant biology and ecology (Ackerly and Monson 2003). Given that the highest mortality of all life stages often occurs during the newly-emerged seedling stage, emphasis on current-year seedling studies may be particularly important as an early and sensitive indicator of community and ecosystem health.

Recent literature suggests that the newly-emerged seedling stage is becoming more in focus as an important life stage for understanding limitations to natural forest regeneration (e.g. Smith et al. 2003; Matthes and Larson 2006; Miina and Saksa 2006). In the southern Appalachian Mountains, there appears to be a consistently high mortality (approximately 95%) of new *Abies fraseri* seedlings on a yearly basis (Johnson and Smith 2005), similar to measurements on *Abies lasiocarpa* in the Rocky Mountains of the western US (Cui and Smith 1991; Germino and Smith 2000, 2001). Studies of first year survival of tropical tree seedlings have also reported high seedling mortality. In 7 out of 9 of the species that were measured at Barro Colorado Island Panama (Augsburger 1984) germinant mortality was between 69% and 100%

and in 50 out of 75 species studies at La Selva, Costa Rica, mortality was greater than 75% (Li et al. 1996). However, studies of the mechanisms of establishment in current-year seedlings are rare in the literature. For example, most studies on *Abies fraseri* seedlings have focused only on the abundance of considerably older, established seedlings, without addressing specific factors influencing the high mortality of newly establishing seedlings (Nicholas et al. 1992; Smith and Nicholas 2000).

The goals of this review are to document the microclimatic conditions of newly emerged seedlings and to discuss young seedling structure and function. This review will focus on woody tree species in particular and will attempt to provide a framework for important omissions and future directions for research on young seedlings. The purpose of this review is not to cover in detail the topics of seedling shade tolerance or seed mass/survival relationships, both of which have been thoroughly covered in the current literature (Moles and Westoby 2004a; Kitajima and Myers 2008).

1.2 Working Definitions of Life Stages

There appears to be a large discrepancy in the heights, diameters or ages of woody plants that are considered seedlings or saplings. For example, trees of up to 2.7 m have been classified seedlings (Whitmore 1996), whereas some researchers reserve the term for plants that still have seed reserves present (Fenner 1987). Throughout this chapter, we will use the term “germinant” or “emergent” for a seedling that is in its first growing season. Our use of the term “seedling” will refer to a young plant in its first few years of growth, which is a more liberal use of the term than many ecologists use (e.g., see Garwood 1996). We also use the phrase “established seedling” to indicate a seedling that has survived beyond its first season of growth.

1.3 Population Range Shifts and Reforestation

Worldwide changes in plant community distributions have been observed over the last decade (Walther et al. 2005) and further shifts are predicted with climate change (e.g. Iversen and Prasad 2001). However, most models predicting future patterns of community distribution rely on correlations between current climate and species current location (a species’ “bioclimate envelope,” Pearson and Dawson 2003). To develop mechanistic models of species and community distribution under climate change scenarios it will be critical to understand the constraints on seedling establishment under natural conditions. For example, recent studies in tropical forests have shown that emergent seedling drought tolerance is correlated strongly with the occurrence of that species along moisture gradients (Engelbrecht et al. 2007; Kursar et al. 2009). These types of studies and data could be used for predictions of future species distributions under changing moisture regimes. Although any complete picture of forest regeneration and migration would include all life stages

(e.g. adult mortality, flower and/or seed production, pollination), forest regeneration and migration into new areas ultimately depends on new seedlings establishing in the understory or beyond the current boundary of the community. Seedling establishment is critical for reforestation after clearcutting, fire or other major disturbance. Successful establishment and subsequent growth beneath canopy gaps is critical for stand regeneration as well, and for preventing encroachment by shrub species that could out-compete understory tree seedlings (White et al. 1985; Pauley and Clebsch 1990).

1.4 Causes of Seedling Mortality

Compared to studies focusing on older age classes, relatively few studies have addressed the causes of mortality during the early establishment phase of new seedlings. In certain communities, herbivory and/or trampling by animals appears to be a significant factor contributing to the mortality of young seedlings (Ehrlen 2003; Moles and Westoby 2004a). In other systems, drought is likely the primary cause of seedling death (e.g. Cui and Smith 1991; Moles and Westoby 2004b; Negussie et al. 2008). In tropical moist forests, pathogens are frequently responsible for a large portion of young seedling mortality (Augspurger 1984), especially in dense shade. Small seedlings are particularly susceptible to uprooting due to frost heaving in areas with frequent frosts (Goulet 1995). Additional causes of mortality can include burial by substrate (e.g. sand, Maun 1998) and litterfall (Clark and Clark 1989; Alvarez-Claire and Kitajima 2009) as well as fire (Plumb 1980; Grace and Platt 1995; Edwards and Krockenberger 2006).

Reductions in carbon gain, which may result in reduced root growth (and possibly desiccation) or even “carbon starvation” (McDowell et al. 2008) can also lead to seedling mortality. For example, current-year seedlings of *A. fraseri* had reduced photosynthetic capacity and sunlight under closed canopies (as compared to open canopies), which also corresponded to increased mortality (Johnson and Smith 2005). Lack of photosynthesis in low light environments (i.e. under closed canopies) appeared to be the primary cause of mortality for *Quercus pyrenaica*, as well (Rodriguez-Calcerrada et al. 2010). In the cases of *A. lasiocarpa* and *Picea engelmannii*, Cui and Smith (1991) found that the high mortality (>90%) in first-year seedlings in the forest understory appeared symptomatically to be due to desiccation, possibly enhanced by a lack of root growth and/or mycorrhizal infection. Additionally, Sacchi and Price (1992) observed high mortality rates in young seedlings of *Salix lasiolepis* in response to limited soil water availability. Carbon gain and survival were also reduced in individual seedlings growing in microsites with either too much or too little sunlight (Germino and Smith 2000; Johnson et al. 2004). This finding of too much or too little sky exposure (degree of canopy openness) was attributed to both positive and negative effects related to sunlight exposure and nighttime sky exposure. Sky exposure was influenced by the overstory tree canopy, plus the vegetation and inanimate structures at ground level. Earlier studies pointed to the possibility that a lack of photosynthetic carbon gain in less favorable microsites (too little or too much sunlight) led to decreased root growth

and desiccation-related mortality (Knapp and Smith 1982; Cui and Smith 1991). Seedling photosynthesis declined in response to low minimum leaf temperatures (predawn) as well as high sunlight the following morning, associated directly with the amount of sky exposure of the seedling microsite (Germino and Smith 2000; Maher et al. 2005). As a result, seedling survival in many species appears strongly associated with microsite facilitation provided by surrounding structures such as overhead canopy, tree islands, and ground vegetation, all of which substantially reduce seedling sky exposure (Hättenschwiler and Smith 1999; Germino and Smith 2001).

The amount of sky exposure strongly influences seedling growth and survival, as well as provides a quantitative measure of microsite and canopy openness. In addition to sunlight exposure and photosynthetic effects, the accompanying exchange of longwave (thermal) radiation emitted from the cold night sky is an important forcing variable for nighttime leaf temperatures, often driving leaf temperature well below air temperature (5–7°C, Jordan and Smith 1995). Other studies have also reported large decreases in downwelling radiation from cold night skies due to vegetative overstories (McLaughlin et al. 1990; Örlander 1993), and cloud patterns such as found at southern Appalachian sites can generate large increases in longwave radiation of up to 100 W m⁻² on nights with fully overcast skies (Feygel'son and Tsvang 1974; Harrison et al. 1993). In addition, cloud immersion in high-altitude southern Appalachian forests has been shown to increase understory sunlight penetration and photosynthesis and decrease transpirational water loss in young seedlings (Johnson and Smith 2008). Substantial decreases in seedling temperatures occur on clear nights and are followed by high sunlight levels in the morning, causing long-term declines (up to several days) in photosynthetic carbon gain (low temperature photoinhibition and/or downregulation of photosynthesis) and, ultimately, reductions in root growth that have been associated with desiccation and mortality in new seedlings (e.g. Knapp and Smith 1982; Ball et al. 1991; Ball 1994; Ball et al. 1997; Cui and Smith 1991; Germino and Smith 2000).

2 Seedling Environments

First-year seedlings experience unique stresses compared to other life stages and are more vulnerable to both biotic and abiotic stresses, compared to older life stages. The unique stresses experienced by seedlings are due to their small size and the fact that they have not escaped the microclimate of the ground surface. The surface environment is dynamic, and can feature large diurnal swings in every environmental parameter (temperature, humidity, radiation, etc.). In contrast to larger, established saplings and adult trees, which have escaped the climate near the ground, emergent seedlings are entirely contained in the near-surface climate at the whole plant level and do not have height or branch complexity to escape the surface environment. Thus, seedling physiological ecology is affected at the whole plant level.

Seedlings emerge from the soil along a continuum of microsites, and it would be difficult to address all situations. Therefore, the approach in this section will be to compare the extremes of possible germinating environments common to seedlings.

The microsites described in the following sections will be a dark, forest understory microsite and an exposed, open canopy microsite, such as might be found for a seedling establishing in an alpine ecotone or during a typical old field succession (this could also be synonymous with forest/shade and pioneer seedling types).

Ultimately, seedling growth and survival depends on adequate carbon capture and processing, avoidance of hydraulic failure (i.e. desiccation), and escaping biota-induced mortality (e.g. trampling, herbivory). Many factors directly influence the carbon and water balance, and thus the survival, of young seedlings.

2.1 *Near-Surface Incident Radiation*

Incident radiation loads on seedlings depend on both radiation intensity and duration. For first year seedlings, there is a delicate balance between too little versus too much sunlight (Germino and Smith 1999; Johnson and Smith 2005). In situations where there is too little sunlight, seedlings may perish because they cannot escape infestation by pathogens on the soil surface and/or seedlings may experience carbon “starvation”, where metabolic needs are not met by adequate carbon assimilation (Givnish 1988). Too much sunlight, especially in combination with low temperatures, can result in decreased carbon gain via photoinhibition (Ball et al. 1991; Germino and Smith 1999; Maher et al. 2005). The optimal incident sunlight regime for a seedling seems to be species-specific, and may co-vary with other factors such as proximity to neighboring plants or rocks, microtopography, and aspect (see further discussion below).

Shaded understory environments can be very dark, with less than 5% of the total radiation at the top of the canopy reaching the forest floor (Baumgartner 1952; Geiger 1965; Lee 1987; Endler 1993; Grant 1997; Johnson and Smith 2005). Net radiation may be less than $21 \text{ W m}^{-2} \text{ day}^{-1}$ ($<1 \text{ MJ m}^{-2} \text{ day}^{-1}$), with the instantaneous quantum flux of photosynthetically active radiation (PAR, $\sim 400\text{--}700 \text{ nm}$) averaging between 10 and $20 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in deep forest shade. Total daily PAR at the ground is often $<20 \text{ mol m}^{-2} \text{ day}^{-1}$, most of which occurs during occasional sunflecks (Young and Smith 1983; Lee and Downum 1991; Combes et al. 2000; Johnson and Smith 2005; Navrátil et al. 2007). Consequently, in the understory near the forest floor, sunlight intensities are rarely above the light compensation threshold for photosynthesis to occur, even for shade-adapted species. This low light availability can strongly impact carbon gain, and thus, allocation to root, stem, and lateral branch development. Seedlings in such light environments may not gain enough carbon for survival before exhausting carbon reserves (Kohyama 1983; Kobe 1997; Givnish 1988; Walters and Reich 1999; Myers and Kitajima 2007). Furthermore, much of the carbon balance of a newly emerged seedling in a forest understory is dictated by ephemeral periods of sunlight. During these periods, seedlings must quickly adjust to a changing light environment to maximize carbon gain. The ability to respond to sunflecks by quickly upregulating photosynthetic mechanisms (i.e. the photosynthetic “induction period”, Chazdon and Pearcy 1986) may be critically important

for seedlings inhabiting dark understories where sunflecks are the primary source of light for carbon gain. Little research has addressed the different responses of photosynthetic tissues (cotyledonous vs. primary leaves) to variable sunlight conditions, though there is some evidence that cotyledons may be more efficient at harvesting sunlight for photosynthesis (Johnson and Smith 2005; Reinhardt et al. 2009). It should also be highlighted that some species are remarkably adapted to low light environments, and seedlings of these species are able to persist for many years in such environments with very little growth, until opportunities for substantial positive carbon balance occur (often referred to as “release” in the literature), via dieback of canopy trees (e.g. Kobe et al. 1995).

On the other extreme, seedlings establishing into open areas of an ecotone (for example, beside a tree island at the alpine-treeline ecotone, into an old-field meadow, or into a large canopy gap) can encounter high radiation loads that affect the whole plant (compared to just a few leaves or branches for plants that are taller and/or with a more complex branch architecture). For many hours of the day in an open-canopy alpine scenario, instantaneous whole-seedling radiation exposure may be $>1060 \text{ W m}^{-2}$, or $>2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (PAR), with daily radiation loads $>30 \text{ MJ m}^{-2} \text{ day}^{-1}$ ($>900 \text{ mol m}^{-2}$ PAR per day; Lee and Downum 1991; Jordan and Smith 1994; Larcher 2003; Reinhardt and Smith 2008). In some situations, such as in snowmelt concavities or in depressions of light-colored ground where there is substantial reflected sunlight, instantaneous irradiance has been reported to be even greater, exceeding $3500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Day et al. 1989; Germino and Smith 2000). Such prolonged exposure to high radiation loads can cause increased leaf temperatures, photorespiration, and high-light photoinhibition. These problems with high radiation loads can be exacerbated if there was frost on the leaves the previous night. Frost occurrence on leaves typically happens via a combination of low air temperatures and radiative cooling (e.g. Ball et al. 1991; Jordan and Smith 1994, 1995; Germino and Smith 1999, 2000; Maher et al. 2005).

2.2 *The Effect of the Boundary Layer on Heat and Mass Fluxes*

Boundary layer effects are paramount to understanding radiation (heat) and mass fluxes, including convection and conduction dynamics, of newly emerged seedlings. In some cases, boundary layers protect small seedlings from harsh environmental conditions and/or large diurnal variations in the environment (e.g. temperature, sunlight intensity, humidity, wind). The boundary layer can be described as the zone of air covering a surface where convection (wind) is both laminar (vs. turbulent) and still (due to viscosity) (Nobel 2005). Conceptually, what is considered the “boundary layer” is a scaling issue and can vary by many orders of magnitude, depending on the system. For example, whereas the boundary layer of a forest is usually considered the area below the canopy (10^1 – 10^2 m), the boundary layer of an individual leaf surface might be on the scale of 10^{-4} – 10^{-3} m . The boundary layer near the ground in open areas is on the order of 10^{-3} – 10^1 m , depending mostly on surface

roughness and wind speed (boundary layer decreases with greater roughness and wind velocity), and is commonly about 5–10 cm over bare ground (Morgan and Rickson 1995). Therefore, small seedlings are often entirely within the near-surface boundary layer.

Within the near-surface boundary layer, heat fluxes of seedlings are greatly reduced relative to taller plants. This difference is because thermal mixing is minimized due to the lack of wind and turbulent eddies, and thermal transfer is dominated by conduction. For example, even with wind velocities at 1 m height in excess of 5 m s^{-1} , wind velocities below 0.5 m height rarely exceed 1 m s^{-1} , with most reported, and modeled, measurements at ground-level less than 0.5 m s^{-1} (Geiger 1965; Elias et al. 1989; Jordan and Smith 1994; Tappeiner and Cernusca 1998). Deacon (1953) and Geiger (1965) showed that the minimum wind velocity for turbulence at 1 m height above the ground is 0.4 m s^{-1} for short grass cover and about 0.8 m s^{-1} for bare ground. As wind velocity decreases logarithmically with decreasing height (Bagnold 1941), the wind velocity in the boundary layer does not induce turbulent mixing the majority of the time. This underscores that newly germinated, and small, seedlings are entirely within the boundary layer of the ground.

The consequences of residing within the boundary layer of still air can be beneficial (protective) or detrimental (exposing). In forest understories, air temperature profiles are more uniform than above the canopy, and have less diurnal variation. The uniformity of these profiles in the understory is because air movement inside the thick forest is low compared to outside the forest, and the understory area is, effectively, one entire boundary layer. Regardless of how large the diurnal range of air temperature is over the course of 24 h, which can be greater than 30°C in temperate forest ecosystems, forest temperatures near the ground in summer typically remain between 15°C and 25°C for the whole day (see Fig. 3.1; similar to the range higher in the canopy), mostly due to trapping and re-radiation of longwave radiation (Geiger 1965; Larcher 2003).

In exposed microsites outside the forest, boundary layer dynamics can be much more variable. In the absence of ground-level convection (as in Fig. 3.1, which was based on a very low wind speed of 0.5 m s^{-1}), leaf temperatures are commonly $\sim 2\text{--}6^\circ\text{C}$ above air temperature (Tranquillini 1979; Hadley and Smith 1987; Germino and Smith 2001; Johnson and Smith 2008), which is well within the limits of tolerance of leaf biochemistry. However, there are reports of leaf temperatures of plants near the ground exceeding air temperature by as much as 23°C inside a krummholz mat in open meadows in the alpine (Hadley and Smith 1987). Midday air temperatures at the ground surface in open-canopy microsites are commonly between 40°C and 50°C , and in some instances can exceed 80°C , especially on dark surfaces (Turner 1958; Tranquillini 1979; Alexander 1987). Prolonged exposure to air temperatures greater than 45°C can cause “heat girdling” of stem cortex tissue in succulent seedlings, resulting in seedling death (see Tranquillini 1979; Alexander 1987 and references therein). Because small seedlings are entirely within the surface boundary layer, with little leaf area to provide mutual shading, there are few structural mechanisms to ameliorate this environment. Moderately high leaf temperatures can lead to heat stress of photosystem II, increased respiration, as well as increased

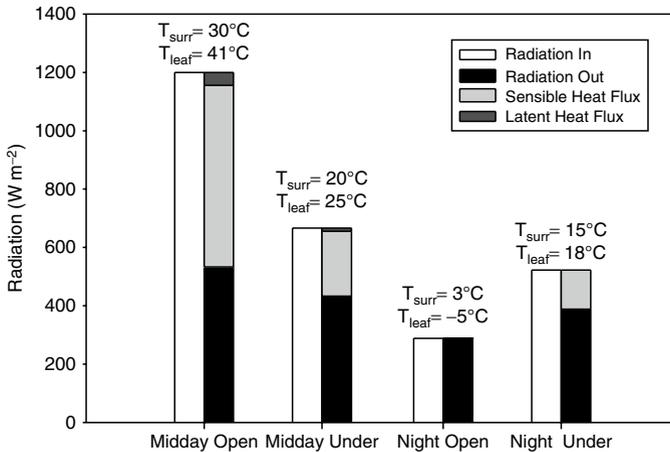


Fig. 3.1 Comparison of seedling energy balance due to microsite. Calculated energy balances of seedlings during midday in open canopy (Midday Open) and understory (Midday Under) microsites, as well as nighttime open canopy (Night Open) and understory (Night Under) microsites. Energy balance calculations are based on the equation: $\text{Radiation}_{\text{in}} = \text{Radiation}_{\text{out}} + G$ (Sensible heat flux) + L (Latent heat flux). Assumptions: leaf absorptance = 0.6, leaf emissivity = 0.97, leaf size = 5 cm, wind velocity = 0.5 m s⁻¹, leaf width = 5 cm, leaf shape = flat, horizontal leaf angle (angle of incidence = 0°). Variables: relative humidity = 30% (Midday Open), 80% (Midday Under), 10% (Nighttime Open), 95% (Nighttime Under); midday stomatal resistance = 2500 s m⁻¹; nighttime stomatal resistance = 10000 s m⁻¹ (no transpiration)

photorespiration, all of which negatively impact seedling carbon balance. Biochemical heat stress of small seedlings may be exacerbated by decreased plant water status and the inability of juvenile root systems to adequately transport water to leaves for transpirational cooling. Root systems of first year seedlings are usually shallow (on the order of centimeters), and not fully developed with few lateral roots for tap-rooted species (Alexander 1987; Greenwood et al. 2008).

At night in open-canopy microsites, evening near-surface temperatures start off relatively warm compared to higher above the ground (due to soil re-radiation of absorbed longwave radiation during the day), but by early morning (pre-dawn) the temperature near the ground is the coldest section in the air profile. This cold temperature is primarily due to the lack of wind in this part of the profile. Such a situation allows for effective radiative cooling, resulting in seedling leaf temperatures that are commonly 2–4°C below air temperature, and occasionally as much as 8°C below air temperature (Fig. 3.1; Hadley and Smith 1987; Jordan and Smith 1994). Leaf temperature depressions can promote frost formation even when air temperatures are above freezing. Thus, frost development on seedlings is likely to occur more frequently than on taller, adult plants and can occur at the whole plant level, not just on individual leaves.

Mass (e.g. CO₂, H₂O) fluxes of seedlings inside the near-surface boundary layer are also different compared to taller plants, though trends may be different between CO₂ and H₂O. Soil respiration, lack of wind, and trapping of CO₂ by overstory

vegetation all act to concentrate CO_2 at the ground relative to upper heights, generating typical CO_2 concentrations of about 450–500 ppm near the ground during evening and morning hours, compared to common ambient values of about 380 ppm (Kimmins 1987; Larcher 2003; Nobel 2005). Greater CO_2 levels at ground level increase the gradient between internal leaf and air CO_2 concentrations, allowing for a greater driving force for CO_2 movement and potentially, photosynthesis. However, the photosynthetic advantage due to increased CO_2 gradients may not be realized in young seedlings due to immature root, leaf, and plant architecture systems. While increased leaf-level photosynthesis under elevated CO_2 concentrations has been observed in seedlings of tropical broadleaved tree species (Holtum and Winter 2003) and temperate conifer trees (Reinhardt and Germino, unpublished data), other researchers have reported no difference, or slight decreases, in photosynthesis in seedlings in both seedbed and greenhouse experiments (Tingey et al. 2007). These decreases in photosynthesis and overall plant carbon balance in seedlings under elevated CO_2 levels may have been due to seedlings altering allocation patterns vs. overall photosynthesis, as well as indirect adverse affects of elevated CO_2 on photosynthesis, caused by drier soils and reduced seed quality. Compared to in the understory, in open-canopy microsites the concentration of ground-level CO_2 is much less and may not differ appreciably from CO_2 concentrations at a height of 1 m (~380–400 ppm), especially in the absence of high soil respiration and/or thick herbaceous mats.

Similar to CO_2 , H_2O vapor concentrations close to the ground also are greater compared to above-ground heights typical of saplings and trees (>1 m), for the same reasons associated with boundary layer as mentioned above. Evaporation of surface soil moisture and transpiration from low growing plants such as seedlings, mosses, and small herbs (“surface evapo-transpiration”) is minimized within the soil boundary layer, and primarily controlled by conduction. In the absence of ground-level (<5 cm) wind and/or radiation, near-surface air humidity is “trapped” at ground level and is much greater compared to higher above the surface for many hours of the day. Sunlight irradiance acts to increase boundary layer conductance and promote turbulence, even at near surface elevations, especially in spatially heterogeneous understory sunlight environments. Nevertheless, surface humidity in forests is greater, and with less diurnal fluctuation, than higher in the canopy for all hours of a typical day. For both deciduous and conifer temperate forests, ground-level relative humidity averages about 80% for a clear summer day, and typically ranges from about 50% to 95% during the day (Geiger 1965; Elias et al. 1989; Tappeiner and Cernusca 1998; Larcher 2003). At heights near and above the forest canopy, daily relative humidity averages about 60%, commonly fluctuating from 30% to 90% during the day. For a conifer plantation in Europe, near surface air humidity was >80% for 14 h of the day, while at 10 m relative humidity was above 80% only 8 h of the day (Geiger 1965).

When air humidity is high, H_2O gradients between inside the leaf (assumed relative humidity near 100%) and the air are smaller (as is the case in still air). Consequently, water fluxes are minimal because the driving force for transpiration is reduced. In mesic forests, near-surface conditions in dark understories can be wet and cool 24 h a day. Such conditions in the southern Appalachian Mountains, USA led to

low seedling water stress (water potentials never less negative than -0.75 MPa) for all times of the day (Johnson and Smith 2005; Reinhardt et al. 2009). However, in the absence of protective snowpack, evergreen seedlings in a mesic forest may be exposed to extended periods of freezing conditions during the winter.

2.3 *Importance of Ecological Facilitation to Seedling Environment*

Although the presence of neighboring vegetation can result in competitive interactions that can adversely affect seedling survival, many species benefit from facilitative interactions. Many of the stressful environmental conditions mentioned above can be ameliorated to some degree by neighboring structures, such as tree canopies, herbaceous species, rocks, or even topographical depressions that provide some degree of protection against exposure (Ball et al. 1991; Berkowitz et al. 1995; Greenlee and Callaway 1996; Callaway and Walker 1997; Hastwell and Facelli 2003; Hughes et al. 2009). Such “facilitation” has been shown to protect young seedlings from high intensity sunlight exposure, radiative cooling at night, low temperature photoinhibition, and damaging winds (e.g. Hadley and Smith 1987; Germino and Smith 1999; Maher et al. 2005; Maher and Germino 2006). In commercial forestry, seedlings are often planted in close association with “nurse trees” which can result in substantially enhanced seedling survival and/or growth (Schlesinger and Williams 1984; Ashton et al. 1997). In natural systems, facilitation plays a large role in primary succession and in ecotone migration (e.g. Connell and Slayter 1977). For example, Germino and Smith (1999) showed that emergent seedling abundance in the alpine ecotone in Wyoming was greatest in microsites located partially under krummholz mats (40–80% sky exposure), and that nighttime warming treatments (simulating protective herbaceous cover) increased *Abies lasiocarpa* seedling photosynthesis by about 400%, and *Picea engelmannii* seedling photosynthesis by about 150%. Furthermore, Maher et al. (2005) demonstrated that *Pinus albicaulis* seedlings had less negative water potentials (less water stress) and greater maximal quantum efficiency (F_v/F_m) in microsites with overstory vegetation cover. Belowground ecological facilitation also has been shown to increase seedling growth and survival. Between 90% and 95% of all plants benefit from associations of mycorrhizal fungi with their root systems. Such associations usually occur within days or weeks of germination (Janos 1980; See and Alexander 1996), and effectively increase the surface area of root systems (100- to 1000-fold) allowing for greater water uptake by plants (Smith and Read 2008). Furthermore, mycorrhizal infection of seedlings results in increased growth rates and greater nutrient uptake in many species (e.g., Janos 1980; Allsop and Stock 1995).

Apparently, proximity to neighboring vegetation can affect the degree of mycorrhizal infection of roots. Hasselquist et al. (2005) showed that colonization of spruce and fir seedling roots by mycorrhizae were fourfold greater in seedlings adjacent to tree islands compared to seedlings ~ 7 m away from tree islands, and that colonization

increased seedling water potentials. Dickie et al. (2005) found that seedlings had greater degrees of mycorrhizal infection near forest edges, but that there was an optimal distance that minimized shading while still providing benefits of a high degree of mycorrhizal colonization. Beyond facilitating macro-nutrient acquisition, recent research suggests that there is also an exchange of carbon, nutrients, and water between facilitating (host) plants and seedlings, occurring on time scales of weeks (He et al. 2006). In stressful environments where carbon gain is limited, this extra source of carbon may be crucial for seedling survival.

3 Early Seedling Development

Seedlings are entirely dependent on seed energy reserves until cotyledon or leaf machinery is developed enough for photosynthetic carbon capture and until radicle development has progressed to be able to supply the photosynthetic tissues with water and nutrients. The function of cotyledons ranges from purely storage of reserves for growth (e.g., *Phaseolus*, *Quercus*; de Vogel 1980; Kitajima 2003) to photosynthetic tissue that is physiologically indistinguishable from that of true leaves. Photosynthetic cotyledons can possess many of the same traits as mature leaves, including stomata and differentiated mesophyll tissue (e.g. Sasaki and Kozłowski 1969). It is noteworthy that the cotyledons of gymnosperms species are always green, even with no exposure to light, whereas eudicot species require light for cotyledon chlorophyll development (Niklas 2008).

Generally, the cotyledons maintain the same function for all seedlings of a species, and all function in the same capacity. However, it has been documented that certain members of the Piperaceae have one cotyledon that functions in storage and transfer of reserves from within the seed coat and one that emerges (Bell 1991) and may be photosynthetic (Leck and Outred 2008). Both types of cotyledons exist in most forest types (Garwood 1996), although boreal and subalpine forests (primarily conifer-dominated), are populated with species with primarily photosynthetic cotyledons. Additionally, small-seeded species tend to have photosynthetic cotyledons (Fenner and Kitajima 1999). Cotyledon size and number can vary dramatically, even within a single family. For example, the number of cotyledons in Pinaceae seedlings ranges from 3 to 18 (Lubbock 1892) and cotyledon size ranges from several mm to over 7 cm (Griffin 1962). Most eudicots simply have two cotyledons (as their name would indicate) and the majority of gymnosperms have between two and six (Leck and Outred 2008). In general however, eudicot cotyledons have a much greater diversity in morphology and function than do those of gymnosperms (Ji and Yi 2003; Niklas 2008).

The structural and functional differences between cotyledon and primary leaves in a tree species have been studied most comprehensively in *Abies fraseri* (see Table 3.1). Johnson et al. (2005) found that in cotyledons and primary needles of *A. fraseri* from the same light environment, there were distinct differences in cotyledon vs. needle structure as well as in light processing properties.

Table 3.1 Anatomical and physiological characteristics of *Abies fraseri* cotyledons and primary needles (Numbers in parentheses are standard errors; data compiled from Johnson et al. 2005 and Reinhardt et al. 2009)

	Leaf thickness	Leaf area palisade (%)	Depth of maximum [Chl]	Stomatal distribution	A _{SAT}	Apparent quantum efficiency	Light saturation point
Cotyledon	355 (11)	14.7 (1.7)	325	Adaxial	2.47 (0.08)	0.031 (0.004)	96.3 (1.1)
Primary needle	331 (24)	24.2 (0.7)	280	Abaxial	2.50 (0.19)	0.014 (0.003)	244.3 (74.2)

Cotyledons of *A. fraseri* had significantly longer palisade cells than those of primary needles (ca. 56 versus 46 μm , respectively), but had less palisade per unit cross sectional area (24% in primary needles, 15% in cotyledons). Additionally, chlorophyll concentrations in cotyledons were shifted toward the abaxial surface as compared to primary needles, as interpreted from chlorophyll fluorescence. This combination of characteristics resulted in deeper propagation of red and blue light into cotyledons of *A. fraseri* compared to primary needles. Reinhardt et al. (2009) found that there were no significant differences in maximum photosynthesis in primary needles versus cotyledons in *A. fraseri* but that the light required to saturate photosynthesis was two to four times greater in primary needles than in cotyledons. In addition, the apparent quantum yield was two times greater in cotyledons than primary needles. Cotyledons of *A. fraseri* appear to be more effective, as compared to primary needles, at utilizing very low levels of sunlight, which would be typical of a closed-canopy forest understory.

The stresses that seedlings are exposed to in the germinating environment are initially experienced entirely by the embryonic tissues and directly affect the tissues that grow from them. In general, many seedlings show a remarkable degree of phenotypic plasticity across environmental gradients (e.g. Greenwood et al. 2008), although certain traits display little variation even when there is substantial environmental variation (see Day and Greenwood 2011). In the rest of this section, we review changes in the structure of organs and allocation of biomass to shifts in the availability of two main resources to seedlings: light and water.

Little work has examined the effect of light regime on the structure of cotyledons, but it has been shown that chlorophyll concentration increases in cotyledons of *Pinus pinaster* germinated and allowed to develop in white light (as compared to dark-grown seedlings, Cánovas et al. 1991) and in cotyledons of *Tabebuia rosea* grown in high light (as compared to low light, Kitajima and Hogan 2003). It is noteworthy that cotyledon photosynthetic potential may be inversely related to cotyledon thickness (Kitajima 1992), which would be the opposite of the trend in true leaves. For true leaves of young seedlings, a meta-analysis of five separate studies with varying light conditions revealed no trends in differences in specific leaf area (SLA; leaf area per mass) or the leaf mass ratio (ratio of leaf to total mass of the plant) for woody species (Wright and Westoby 2001). When different species were compared at a common size, though, all species had greater SLA in low vs. high

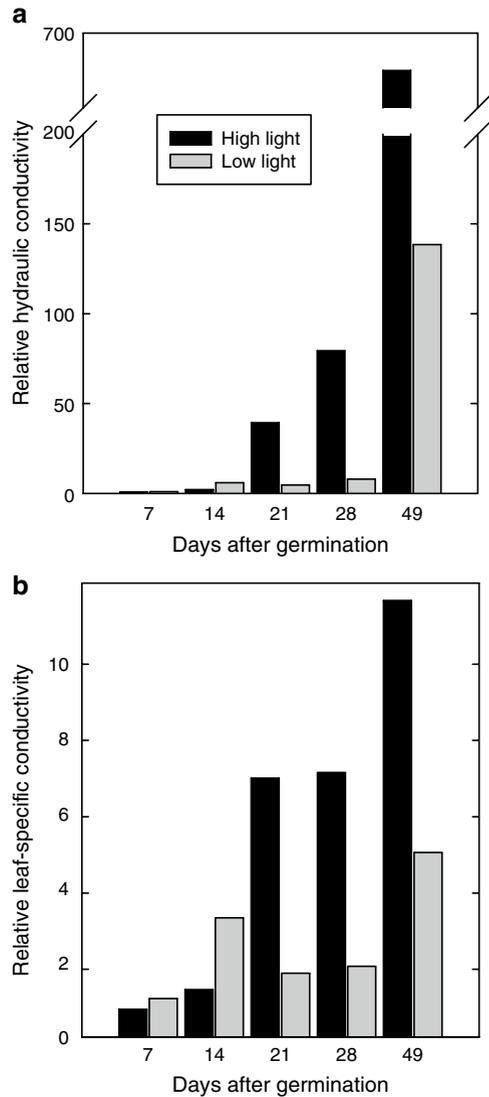
light (Reich et al. 1998), which was also observed across leaf size in three liana and two tree congeners as well as in seedlings of *Tabebuia rosea* (Kitajima and Hogan 2003; Cai et al. 2008).

At the level of the whole seedling, allocation patterns tend to change with differences in light regime. It has been widely observed that species increase shoot mass relative to whole plant or root mass in low vs. high light (Smith 1976; Reich et al. 1998). What has not received as much attention is the anatomical changes in the wood associated with the changes in allocation. In a classic study, Denne (1972) found that under normal growing conditions tracheid diameter and wall thickness increased towards the root tip in *Picea* and *Pinus* seedlings, and that transferring them to shorter day lengths (decreasing integrated daily light) caused the tracheids to narrow throughout the plant due to slower expansion rates. In *Picea* this result was also observed when comparing high and low light intensity treatments rather than day length (Denne 1974). However, Denne (1976) found that after an initial decline in the expansion rate as a response to a decline in light, which lasted approximately 2–3 weeks, the photosynthetic efficiency increased and then tracheid dimensions and number increased again, as well.

Unlike for *Picea*, for *Eucalyptus grandis* seedlings grown at constant low vs. high intensity light regimes, differences in vessel diameter and number continued for weeks (Doley 1979). Using data from Doley (1979) on changes in vessel lumen area, number and total vessel area: leaf area ratios over time, we used the Hagen-Poiseuille equation (Tyree and Zimmermann 2002) to calculate theoretical values of hydraulic conductivity and leaf-specific conductivity (conductivity/leaf area) for seedlings growing in each light regime (Fig. 3.2). Each value of hydraulic function was calculated relative to the maximum day-7 value (7 days after germination, a date at which seedling sizes were not significantly different between treatments, Doley 1978), which for both cases was for the low light intensity seedlings. In fact, the seedlings growing in low light intensity had greater theoretical hydraulic- and leaf-specific conductivity than did those at high light at day 7 and day 14. This trend was reversed by day 21 after which the seedlings growing in high light intensity outpaced the low intensity seedlings in both measures of hydraulic efficiency. By day 49, the high-light seedlings had nearly 600 times the theoretical hydraulic conductivity that the low-light seedlings had had on day 7, and more than four times the value of the low light seedlings of the same age. These increases were caused by the addition of more and wider vessels to the xylem relative to the low light intensity seedlings. Increases in leaf area resulted in smaller differences in hydraulics when compared on a leaf-specific scale (Fig. 3.2b). However, by day 49 the leaf-specific conductivity of the high light grown plants was >11 times the day-7 value of the low light seedlings, and more than double when comparing hydraulic function of the low light plants on day 49.

The functional consequences of these patterns (Fig. 3.2) are apparent when the values from the two panels are compared. For the high light seedlings, the increase in the theoretical hydraulic conductivity between days 21 and 28 is insufficient to compensate for the increase in leaf area, and the leaf-specific value does not increase. The low light grown seedlings exhibit a drop in leaf-specific conductivity because

Fig. 3.2 The change in theoretical values of hydraulic conductivity (a) and leaf-specific hydraulic conductivity (b) over the first 49 days after germination in *Eucalyptus grandis* seedlings grown under high ($12.6 \text{ mol m}^{-2} \text{ day}^{-1}$ photosynthetically active radiation) and low ($2.8 \text{ mol m}^{-2} \text{ day}^{-1}$) light intensity. Hydraulic values are relative to the maximum value for either growth treatment on day 7 (which was the low light value for both panels). Hydraulic values were calculated from anatomical data in Doley (1979)



the hydraulic conductivity did not increase in concert with the leaf expansion. Unfortunately, the data are not available to determine if this disconnect between the hydraulic supply and leaf area resulted in limited carbon assimilation due to stomatal closure.

A number of studies have examined the effect of limited water supply on root and shoot structure. In particular, Padilla et al. (2007) showed that when water is less available, individuals of three Mediterranean shrub species tend to elongate their roots relative to individuals of the same species growing under conditions of

greater soil moisture. This response to grow deeper roots can enhance seedling survival in habitats with prolonged droughts where deeper soil layers may maintain more moisture than the top layers (Padilla and Pugnaire 2007). When congeneric pairs consisting of one species from a high rainfall environment and one species from a low rainfall environment were grown under common and favorable conditions, most low-rainfall species had lower specific root length (length per mass of roots) relative to their high-rainfall congeneric (Wright and Westoby 1999; Nicotra et al. 2002). The root systems of the low-rainfall species also showed greater elongation of the main axis of the root system, but fewer and shorter side branches (Nicotra et al. 2002). Although the theoretical axial hydraulic conductivity of the root systems were calculated and no differences were found between the congenics, it is unclear what impact the thicker roots (i.e., lower specific root length) would have on the radial conductivity and thus the entire hydraulic conductance of the root network. As the plant hydraulic conductance is proportional to the photosynthetic rate (Hubbard et al. 2001; Meinzer and Grantz 1990; Sperry et al. 1993), plants would benefit from maximizing their root conductance, yet the tools and techniques to measure this value in seedlings are not currently developed.

4 Future Research

Due to their small size and fragile nature, it is difficult to measure the physiology of emergent seedlings. For example, it is difficult to clamp young seedlings into traditional gas exchange chambers without destroying the seedling. In addition, it typically requires several individual seedlings inside the same chamber to achieve a signal to noise ratio high enough to make the measurements reliable. Consequently, many seedlings end up being sacrificed for a set of physiological measurements, which is especially problematic when working with limited numbers of naturally-occurring seedlings. It is also difficult to perform water potential measurements, due to the small diameter of the cut end and the difficulty in recognizing the balance pressure. Furthermore, measures of hydraulic conductance on emergent seedlings are nonexistent because our current methods are primarily designed for woody tissues. In addition, naturally-occurring seedlings may only be available only every few years due to natural cycles in reproductive output, so most researchers rely on planted seedlings (germinated in the greenhouse) or greenhouse studies, which may not be reflective of naturally-occurring seedling microclimate and physiology.

However, there are promising strategies and new methods being developed that could be easily adapted to emergent seedling physiology. Techniques that could be adapted to the study of young seedlings include chlorophyll fluorescence for studies of photosynthetic potential (or photoinhibition); using smaller chambers for measuring gas exchange of single cotyledons (e.g. LICOR Arabidopsis chamber); and the rehydration kinetics method (Brodribb and Holbrook 2003), previously used for leaf hydraulic conductance, could be used to measure seedling hydraulic conductance. There are also tools that are independent of size: tissue osmometry for

measuring solute concentrations, use of Lockhart-based analyses of growth, and studies of gene expression and gene product inactivation (e.g. aquaporins) with inhibitory chemicals.

Overarching questions about which little is known and that are likely to be fruitful in the near future include the following. (1) What are the adaptive nature of different cotyledon structures? Much work has been done on classification of seedling types (e.g. Garwood 1996) and on relationships between cotyledon or seed size/mass and survivorship (Kitajima 1996; Bonfil 1998; Moles and Westoby 2004a). However, we know very little about the adaptive significance of cotyledon shape (and why cotyledons are often very different from primary leaves), orientation, internal structure, stomatal distribution, cuticular qualities, etc. An energy balance approach incorporating these cotyledon parameters along with the very low wind speeds found near the ground might be particularly elucidative. (2) What determines the timing of the transition between cotyledonous and the first true leaves, and is this transition a time of greater mortality? Does carbon balance or water status determine whether or not young seedlings flush a new set of primary leaves or is it more closely related to environmental conditions (e.g. temperature degree days, red:far-red ratios, etc.) or determined entirely by genetic cues? (3) What are the hydraulic properties of young seedlings and how are they affected by different types of stress? Measurements of hydraulic conductivity and conductance of various parts of emergent seedlings and their vulnerability to hydraulic dysfunction during drought or freeze-thaw conditions could be particularly informative, especially for predictions of future species or community migration under climate change. A greater understanding of how seedling hydraulics impacts carbon gain and seedling survival will allow more accurate predictions of seedling establishment and population range shifts under different predicted scenarios of future climates.

Acknowledgements The authors would like to thank Kaoru Kitajima for a thoughtful and thorough review that resulted in a much stronger chapter. We are also grateful to Drs. Frederick Meinzer and Barbara Lachenbruch, as well as the entire AvH group for critical discussion of ideas.

References

- Ackerly DD, Monson RK (2003) Waking the sleeping giant: the evolutionary foundations of plant function. *Int J Plant Sci* 164(Suppl):S1–S6
- Alexander RR (1987) Ecology, silviculture, and management of the Engelmann spruce-subalpine fir type in the central and southern Rocky Mountains. Agric. Handb. No. 659. USDA Forest Service, Washington, DC, 144 pp
- Allsop N, Stock WD (1995) Relationship between seed reserves, seedling growth and mycorrhizal responses in 14 related shrubs (Rosidae) from a low-nutrient environment. *Funct Ecol* 9:248–254
- Alvarez-Buylla ER, Martinez-Ramos M (1992) Demography and allometry of *Cecropia obtusifolia*, a neotropical pioneer tree – an evaluation of the climax-pioneer paradigm for tropical rain forests. *J Ecol* 80:275–290
- Alvarez-Claire S, Kitajima K (2009) Susceptibility of tree seedlings to biotic and abiotic hazards in the understory of a moist tropical forest in Panama. *Biotropica* 41:47–56

- Ashton PM, Gamage S, Gunatilleke IAUN, Gunatilleke CVS (1997) Restoration of a Sri Lankan rain forest: using Caribbean pine *Pinus caribaea* as a nurse for establishing late-successional tree species. *J Appl Ecol* 34:915–925
- Augsburger CK (1984) Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps and pathogens. *Ecology* 65:1705–1712
- Bagnold RA (1941) *The physics of blown sand and desert dunes*. Methuen, London, 265 pp
- Ball MC (1994) The role of photoinhibition during tree seedling establishment at low temperatures. In: Baker NR, Bowyer JR (eds.) *Photoinhibition of photosynthesis: from molecular mechanisms to the field*. BIOS Scientific Publishers, Oxford, pp 365–377
- Ball MC, Hodges VS, Laughlin GP (1991) Cold-induced photoinhibition limits regeneration of snow gum at treeline. *Funct Ecol* 5:663–668
- Ball MC, Egerton JGG, Leuning R, Cunningham RB, Dunne P (1997) Microclimate above grass adversely affects spring growth of seedling snow gum. *Plant Cell Environ* 20:155–166
- Baumgartner A (1952) Die Strahlungsbilanz in einer Fichtendickung. *Forstw C* 71:337–349
- Bell AD (1991) *Plant form: an illustrated guide to flowering plant morphology*. Oxford University Press, Oxford
- Berkowitz AR, Canham CD, Kelly VR (1995) Competition vs. facilitation of tree seedling growth and survival in early successional communities. *Ecology* 76:1156–1168
- Bonfil CZ (1998) The effects of seed size, cotyledon reserves and herbivory on seedling survival and growth in *Quercus rugosa* and *Q. laurina* (Fagaceae). *Am J Bot* 85:79–87
- Brodribb TJ, Holbrook NM (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiol* 132:2166–2173
- Cai Z-Q, Poorter L, Han Q, Bongers F (2008) Effects of light and nutrients on seedlings of tropical *Bauhinia* lianas and trees. *Tree Physiol* 28:1277–1285
- Callaway RM, Walker LR (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* 78:958–1965
- Cánovas FM, Canton FR, Gallardo F, Garcia-Gutierrez A, de Vicente A (1991) Accumulation of glutamine synthetase during early development of maritime pine (*Pinus pinaster*) seedlings. *Planta* 185:372–378
- Chazdon RL, Pearcy RW (1986) Photosynthetic response to light variation in rain-forest species. *Oecologia* 69:529–531
- Clark DB, Clark DA (1989) The role of physical damage in the seedling mortality regime of a neotropical rain forest. *Oikos* 55:225–230
- Combes D, Sinoquet H, Varlet-Grancher C (2000) Preliminary measurement and simulation of the spatial distribution of the morphogenetically active radiation (MAR) within an isolated tree canopy. *Ann For Sci* 57:497–511
- Connell JH, Slayter RO (1977) Mechanisms of succession in natural communities and their role in community stability and organisation. *Am Nat* 111:119–144
- Cui M, Smith WK (1991) Photosynthesis, water relations and mortality in *Abies lasiocarpa* seedlings during natural establishment. *Tree Physiol* 8:37–46
- Day ME, Greenwood MS (2011) Regulation of ontogeny in temperate conifers. In: Meinzer FC, Lachenbruch B, Dawson TE (eds.) *Size- and age-related changes in tree structure and function*. Springer, New York
- Day TA, DeLucia EH, Smith WK (1989) Influence of cold soil and snowcover on photosynthesis and leaf conductance in two rocky mountain conifers. *Oecologia* 80:546–552
- de Vogel EF (1980) *Seedlings of dicotyledons: structure development, types, descriptions of 150 woody Malesian taxa*. Center for Agricultural Publishing and Documentation, Wageningen
- Deacon EL (1953) Vertical profiles of mean wind speed in the surface layers of the atmosphere. *Geophys Mem Lond* 11:91
- Denne MP (1972) A comparison of root- and shoot-wood development in conifer seedlings. *Ann Bot* 36:579–587
- Denne MP (1974) Effects of light intensity on tracheid dimensions in *Picea sitchensis*. *Ann Bot* 38:337–345

- Denne MP (1976) Effects of environmental change on wood production and wood structure in *Picea sitchensis* seedlings. *Ann Bot* 40:1017–1028
- Dickie IA, Schnitzer SA, Reich PB, Hobbie SE (2005) Spatially disjunct effects of co-occurring competition and facilitation. *Ecol Lett* 8:1191–1200
- Doley D (1978) Effects of shade on gas exchange and growth in seedlings of *Eucalyptus grandis* Hill Ex. Maiden. *Aust J Plant Physiol* 5:723–738
- Doley D (1979) Effects of shade on xylem development in seedlings of *Eucalyptus grandis* Hill Ex. Maiden. *New Phytol* 82:545–555
- Edwards W, Krockenberger A (2006) Seedling mortality due to drought and fire associated with the 2002 El Niño event in a tropical rain forest in north-east Queensland, Australia. *Biotropica* 38:16–26
- Ehrlen J (2003) Fitness components versus total demographic effects: evaluating herbivore impacts on a perennial herb. *Am Nat* 162:796–810
- Elias P, Kratochvilova I, Janous D, Marek M, Masarovicova E (1989) Stand microclimate and physiological activity of tree leaves in an oak-hornbeam forest. *Trees* 4:227–233
- Endler JA (1993) The color of light in forests and its implications. *Ecol Monogr* 63:1–27
- Engelbrecht BMJ, Comita LS, Condit R, Kursar TA, Tyree MT, Turner BL, Hubbell SP (2007) Drought sensitivity shapes species distribution patterns in tropical forests. *Nature* 447:80–82
- Fenner M (1987) Seedlings. *New Phytol* 106:35–47
- Fenner M, Kitajima K (1999) Seed and seedling ecology. In: Pugnaive F, Valladares F (eds.) *Handbook of functional plant ecology*. Marcel Dekker, New York, pp 589–621
- Feygel'son YM, Tsvang LR (1974) In: Feygel'son Ye M, Tsvang LR, Feygel'son Ye M, Tsvang LR (eds.) *Heat transfer in the atmosphere, translation of teploobmen v atmosfere*. Nauka Press, Moscow
- Figueroa JA, Lusk CH (2001) Germination requirements and seedling shade tolerance are not correlated in a Chilean temperate rain forest. *New Phytol* 152:483–489
- Garwood NC (1996) Functional morphology of tropical tree seedlings. In: Swaine MD (ed.) *The ecology of tropical tree seedlings*. UNESCO and The Parthenon Publishing Group, Paris, pp 59–129
- Geiger R (1965) *The climate near the ground*, 3rd edn. Harvard University Press, Cambridge, MA, 611 pp
- Germino MJ, Smith WK (1999) Sky exposure, crown architecture, and low-temperature photoinhibition in conifer seedlings at alpine treeline. *Plant Cell Environ* 22:407–415
- Germino MJ, Smith WK (2000) Differences in microsite, plant form, and low-temperature photoinhibition in alpine plants. *Arct Antarct Alp Res* 32:388–396
- Germino MJ, Smith WK (2001) Relative importance of microhabitat, plant form and photosynthetic physiology to carbon gain in two alpine herbs. *Funct Ecol* 15:243–251
- Givnish TJ (1988) Adaptation to sun vs. shade: a whole-plant perspective. *Aust J Plant Physiol* 15:63–92
- Goulet F (1995) Frost heaving of forest tree seedlings: a review. *New For* 9:67–94
- Grace SL, Platt WJ (1995) Effects of adult tree density and fire on the demography of pregrass stage juvenile longleaf pine (*Pinus palustris* Mill.). *J Ecol* 83:75–86
- Grant RH (1997) Partitioning of biologically active radiation in plant canopies. *Int J Biometeorol* 40:26–40
- Greenlee JT, Callaway RM (1996) Abiotic stress and the relative importance of interference and facilitation in montane bunchgrass communities in western Montana. *Am Nat* 148:386–396
- Greenwood MS, O'Brien CL, Schatz JD, Diggins CA, Day ME, Jacobson GL, White AS, Wagner RG (2008) Is early life-cycle success a determinant of the abundance of red spruce and balsam fir? *Can J For Res* 38:2295–2305
- Griffin JR (1962) Intraspecific variation in *Pinus sabiniana* Dougl. PhD Thesis, University of California, Berkeley, 274 p
- Hadley JL, Smith WK (1987) Influence of krummholz mat microclimate on needle physiology and survival. *Oecologia* 73:82–90

- Harrison EF, Minnis P, Barkstrom B, Gibson GG (1993) Radiation budget at the top of the atmosphere. In: Gurney RJ, Foster JL, Parkinson CL (eds.) Atlas of satellite observations related to global change. Cambridge University Press, Cambridge, pp 19–38
- Hasselquist NJ, Germino MJ, McGonigle TC, Smith WK (2005) Variability of *Cenococcum* infection and its ecophysiological significance for young conifers at alpine-treeline. *New Phytol* 165:867–873
- Hastwell GT, Facelli JM (2003) Differing effects of shade-induced facilitation on growth and survival during the establishment of a chenopod shrub. *J Ecol* 91:941–950
- Hättenschwiler S, Smith WK (1999) Seedling occurrence in alpine treeline conifers: a case study from the central Rocky Mountains, USA. *Acta Oecol* 20:219–224
- He XH, Bledsoe CS, Zasoski RJ, Southworth D (2006) Rapid nitrogen transfer from ectomycorrhizal pines to adjacent ectomycorrhizal and arbuscular mycorrhizal plants in a California oak woodland. *New Phytol* 170:143–151
- Holtum JAM, Winter K (2003) Photosynthetic CO₂ uptake in seedlings of two tropical tree species exposed to oscillating elevated concentrations of CO₂. *Planta* 218:152–158
- Hubbard RM, Stiller V, Ryan MG, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant Cell Environ* 24:113–121
- Hughes NM, Johnson DM, Akhalkatsi M, Abdaladze O (2009) Characterizing *Betula litwonii* seedling microsites at the alpine-treeline ecotone, central Greater Caucasus Mountains, Georgia. *Arct Antarct Alp Res* 41:112–118
- Iverson LR, Prasad AM (2001) Potential changes in tree species richness and forest community types following climate change. *Ecosystems* 4:186–199
- Janos DP (1980) Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology* 61:151–162
- Ji XB, Yi NG (2003) The seedling types of dicots and their evolutionary relationships. *Acta Phytotaxinomica Sin* 41:447–464
- Johnson DM, Smith WK (2005) Refugial forests of the southern Appalachians: photosynthesis and survival in current-year *Abies fraseri* seedlings. *Tree Physiol* 25:1379–1387
- Johnson DM, Smith WK (2008) Cloud immersion alters microclimate, photosynthesis and water relations in *Rhododendron catawbiense* and *Abies fraseri* seedlings in the southern Appalachian Mountains, USA. *Tree Physiol* 28:385–392
- Johnson DM, Germino MJ, Smith WK (2004) Abiotic factors limiting photosynthesis in seedlings of *Abies lasiocarpa* and *Picea engelmannii* at alpine timberline. *Tree Physiol* 24:377–386
- Johnson DM, Smith WK, Vogelmann TC, Brodersen CR (2005) Leaf architecture, incident light direction, and mesophyll fluorescence profiles inside a broadleaf, conifer needle and cotyledon. *Am J Bot* 92:1425–1431
- Jordan DN, Smith WK (1994) Energy-balance analysis of nighttime leaf temperatures and frost formation in a sub-alpine environment. *Agric For Meteorol* 71:359–372
- Jordan DN, Smith WK (1995) Microclimate factors influencing the frequency and duration of growth season frost for subalpine plants. *Agric For Meteorol* 77:17–30
- Kimmins JP (1987) Forest ecology. Macmillian Publishing Company, New York, 531 pp
- Kitajima K (1992) Relationship between photosynthesis and thickness of cotyledons for tropical tree species. *Funct Ecol* 6:582–589
- Kitajima K (1996) Cotyledon functional morphology, patterns of seed reserve utilization and regeneration niches of tropical tree seedlings. In: Swaine MD (ed.) The ecology of tropical tree seedlings. UNESCO and The Parthenon Publishing Group, Paris, pp 193–210
- Kitajima K (2003) Impact of cotyledon and leaf removal on seedling survival in three tree species with contrasting cotyledon functions. *Biotropica* 35:429–434
- Kitajima K, Hogan KP (2003) Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. *Plant Cell Environ* 26:857–865
- Kitajima K, Myers JA (2008) Seedling ecophysiology: strategies towards achievement of positive carbon balance. In: Leck MA, Parker VT, Simpson RL (eds.) Seedling ecology and evolution. Cambridge University Press, UK, pp 172–188

- Knapp AK, Smith WK (1982) Factors influencing understory seedling establishment of Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) in southeast Wyoming. *Can J Bot* 60:2753–2761
- Kobe RK (1997) Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos* 80:226–233
- Kobe RK, Pacala SW, Silander JA Jr, Canham CD (1995) Juvenile tree survivorship as a component of shade tolerance. *Ecol Appl* 5:517–532
- Kohyama T (1983) Seedling stage of two subalpine *Abies* species in distinction from sapling stage: a matter-economic analysis. *J Plant Res* 96:49–65
- Kursar TA, Engelbrecht BMJ, Burke A, Tyree MT, El Omari B, Giraldo JP (2009) Tolerance to low leaf water status of tropical tree seedlings is related to drought performance and distribution. *Funct Ecol* 23:93–102
- Larcher W (2003) *Physiological plant ecology*, 4th edn. Springer, New York, 513 pp
- Leck MA, Outred HA (2008) Seedling natural history. In: Leck MA, Parker VT, Simpson RL (eds.) *Seedling ecology and evolution*. Cambridge University Press, UK, pp 17–55
- Lee DW (1987) The spectral distribution of radiation in two neotropical rainforests. *Biotropica* 19:161–166
- Lee DW, Downum KR (1991) The spectral distribution of biologically active solar radiation at Miami, Florida, USA. *Int J Biometeorol* 35:48–54
- Li M, Lieberman M, Lieberman D (1996) Seedling demography in undisturbed tropical wet forest in Costa Rica. In: Swaine MD (ed.) *The ecology of tropical tree seedlings*. UNESCO and The Parthenon Publishing Group, Paris, pp 285–304
- Lubbock J (1892) *A contribution to our knowledge of seedlings*. Appleton and Company, New York, 646 pp
- Maher EL, Germino MJ (2006) Microsite differentiation among conifer species during seedling establishment at alpine treeline. *Ecoscience* 13:334–341
- Maher EL, Germino MJ, Hasselquist NJ (2005) Interactive effects of tree and herb cover on survivorship, physiology, and microclimate of conifer seedlings at the alpine tree-line ecotone. *Can J For Res* 35:567–574
- Masaki T, Nakashizuka T (2002) Seedling demography of *Swida controversa*: effect of light and distance to conspecifics. *Ecology* 83:3497–3507
- Matthes U, Larson DW (2006) Microsite and climatic controls of tree population dynamics: an 18 year study on cliffs. *J Ecol* 94:402–414
- Maun MA (1998) Adaptations of plants to burial in coastal sand dunes. *Can J Bot* 76:713–738
- McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yezzer EA (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol* 178:719–739
- McLaughlin SB, Anderson CP, Edwards NT, Roy WK, Layton PA (1990) Seasonal patterns of photosynthesis and respiration of red spruce saplings from two elevations in declining Southern Appalachian stands. *Can J For Res* 20:485–495
- Meinzer FC, Grantz DA (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity. *Plant Cell Environ* 13:383–388
- Miina J, Saksa T (2006) Predicting regeneration establishment in Norway spruce plantations using a multivariate multilevel model. *New For* 32:265–283
- Moles AT, Westoby M (2004a) Seedling survival and seed size: a synthesis of the literature. *J Ecol* 92:372–383
- Moles AT, Westoby M (2004b) What do seedlings die from and what are the implications for evolution of seed size? *Oikos* 106:193–199
- Morgan RPC, Rickson RK (1995) *Slope stabilization and erosion control: a bioengineering approach*. E & F Spon, London, 274 pp
- Myers JA, Kitajima K (2007) Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *J Ecol* 95:383–395

- Navrátil M, Spunda V, Markova I, Janous D (2007) Spectral composition of photosynthetically active radiation penetrating into a Norway spruce canopy: the opposite dynamics of the blue/red spectral ratio during clear and overcast days. *Trees* 21:311–320
- Negussie A, Aerts R, Gebrehiwot K, Muys B (2008) Seedling mortality causes recruitment limitation of *Boswellia papyrifera* in northern Ethiopia. *J Arid Environ* 72:378–383
- Nicholas NS, Zedaker SM, Eagar C (1992) A comparison of overstorey community structure in three southern Appalachian spruce–fir forests. *Bull Torrey Bot Club* 119:316–332
- Nicotra AB, Babicka N, Westoby M (2002) Seedling root anatomy and morphology: an examination of ecological differentiation with rainfall using phylogenetically independent contrasts. *Oecologia* 130:136–145
- Niklas KJ (2008) Embryo morphology and seedling evolution. In: Leck MA, Parker VT, Simpson RL (eds.) *Seedling ecology and evolution*. Cambridge University Press, UK, pp 103–129
- Nobel PS (2005) *Physicochemical and environmental plant physiology*, 3rd edn. Academic Press/Elsevier, Burlington, 567 pp
- Örlander G (1993) Shading reduces both visible and invisible frost damage to Norway spruce seedlings in the field. *Forestry* 66:27–36
- Padilla FM, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Funct Ecol* 21:489–495
- Padilla FM, de Dios MJ, Pugnaire FI (2007) Early root growth plasticity in seedlings of three Mediterranean woody species. *Plant Soil* 296:103–113
- Pauley EF, Clebsch EEC (1990) Patterns of *Abies fraseri* regeneration in a Great Smoky Mountains spruce–fir forest. *Bull Torrey Bot Club* 117:375–381
- Pearson RG, Dawson TP (2003) Predicting the impacts of climate on the distribution of species: are bioclimate envelope models useful? *Glob Ecol Biol* 12:361–371
- Plumb TR (1980) Response of oaks to fire. In: Plumb TR (technical coordinator) *Proceedings of the symposium on the ecology, management, and utilization of California oaks*; 1979 June 26–28; Claremont, CA. Gen. Tech. Rep. PSW-44. Berkeley, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Forest and Range Experiment Station, pp 202–215
- Reich PB, Tjoelker MG, Vanderklein DW, Buschena C (1998) Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. *Funct Ecol* 12:327–338
- Reinhardt K, Smith WK (2008) Impacts of cloud immersion on microclimate, photosynthesis and water relations of *Abies fraseri* (Pursh.) Poiret in a temperate mountain cloud forest. *Oecologia* 158:229–238
- Reinhardt K, Johnson DM, Smith WK (2009) Age-class differences in Fraser fir (*Abies fraseri*) photosynthesis and water relations. *Can J For Res* 39:1–5
- Rodriguez-Calcerrada J, Cano FJ, Valbuena-Carabaña M, Gill L, Aranda I (2010) Functional performance of oak seedlings naturally regenerated across microhabitats of distinct overstorey canopy closure. *New For* 39:245–259
- Sacchi CF, Price PW (1992) The relative roles of abiotic and biotic factors in seedling demography of Arroyo willow (*Salix lasiolepis*: Salicaceae). *Am J Bot* 79:395–405
- Sasaki S, Kozłowski TT (1969) Effects of cotyledon and hypocotyl photosynthesis on growth of young pine seedlings. *New Phytol* 69:493–500
- Schlesinger R, Williams RD (1984) Growth responses of black walnut *Juglans nigra* to interplanted trees. *For Ecol Manage* 9:235–243
- See LS, Alexander IJ (1996) The dynamics of ectomycorrhizal infection of *Shorea leprosula* seedlings in Malaysian rain forests. *New Phytol* 132:297–305
- Smith CJ (1976) Adaptations to environmental change in *Picea sitchensis* seedlings – changes in photosynthesis, growth and substrate availability. *Ann Bot* 40:1003–1015
- Smith GF, Nicholas NS (2000) Size- and age-class distributions of Fraser fir following balsam woolly adelgid infestation. *Can J For Res* 30:948–957
- Smith SE, Read DJ (2008) *Mycorrhizal symbiosis*, 3rd edn. Academic, London, 605 pp
- Smith WK, Germino MJ, Hancock TE, Johnson DM (2003) Another perspective on the altitudinal occurrence of alpine tree lines. *Tree Physiol* 23:1101–1113

- Sperry JS, Alder NN, Eastlack SE (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *J Exp Bot* 44:1075–1082
- Tappeiner U, Cernusca A (1998) Model simulation of spatial distribution of photosynthesis in structurally differing plant communities in the central Caucasus. *Ecol Model* 113:201–233
- Tingey DT, Lee EH, Phillips DL, Rygielwicz PT, Waschmann RS, Johnson MG, Olszyk DM (2007) Elevated CO₂ and temperature alter net ecosystem C exchange in a young Douglas fir mesocosm experiment. *Plant Cell Environ* 30:1400–1410
- Tranquillini W (1979) Physiological ecology of the Alpine timberline: tree existence at high altitudes with special reference to the European Alps. Springer, New York, 137 pp
- Turner H (1958) Maximaltemperaturen oberflächennaher Bodenschichten an der alpinen Waldgrenze. *Wetter Leben* 10:1–12
- Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of sap. Springer Verlag, Berlin
- van Mantgem PJ, Stephenson NL, Byrne JC, Daniels LD, Franklin JF, Fulé PZ, Harmon ME, Larson AJ, Smith JM, Taylor AH, Veblen TT (2009) Widespread increase of tree mortality rates in the western United States. *Science* 323:521–524
- Walters MB, Reich PB (1999) Research review: low-light carbon balance and shade tolerance in the seedlings of woody plants-do winter deciduous and broad-leaved evergreen species differ? *New Phytol* 143:143–154
- Walther G-R, Berger S, Sykes MT (2005) An ecological footprint of climate change. *Proc R Soc B* 272:1427–1432
- White PS, Mackenzie MD, Busing RT (1985) Natural disturbance and gap phase dynamics in southern Appalachian spruce–fir forests. *Can J For Res* 15:233–240
- Whitmore TC (1996) A review of some aspects of tropical rainforest seedling ecology with suggestions for further enquiry. In: Swaine MD (ed.) *The ecology of tropical tree seedlings*. UNESCO and The Parthenon Publishing Group, Paris, pp 3–39
- Wright IJ, Westoby M (1999) Differences in seedlings growth behaviour among species: trait correlations across species and trait shifts along nutrient compared to rainfall gradients. *J Ecol* 87:85–97
- Wright IJ, Westoby M (2001) Understanding seedlings growth relationships through specific leaf area and leaf nitrogen concentration: generalizations across growth forms and growth irradiance. *Oecologia* 127:21–29
- Young DR, Smith WK (1983) Effect of cloudcover on photosynthesis and transpiration in the subalpine understory species *Arnica latifolia*. *Ecology* 64:681–687