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Changes in drought response strategies with ontogeny in *Quercus rubra*: implications for scaling from seedlings to mature trees

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Abstract We investigated scaling of physiological parameters between age classes of *Quercus rubra* by combining in situ field measurements with an experimental approach. In the in situ field study, we investigated changes in drought response with age in seedlings, juveniles, and mature trees of *Q. rubra*. Throughout the particularly dry summer of 1995 and the unusually wet summer of 1996 in New England, we measured water potential of leaves (Ψ_{Leaf}) and gas exchange of plants at three sites at the Harvard Forest in Petersham, Massachusetts. In order to determine what fraction of the measured differences in gas exchange between seedlings and mature trees was due to environment versus ontogeny, an experiment was conducted in which seedlings were grown under light and soil moisture regimes simulating the environment of mature trees. The photosynthetic capacity of mature trees was three-fold greater than that of seedlings during the wet year, and six-fold greater during the drought year. The seedling experiment demonstrated that the difference in photosynthetic capacity between seedlings and mature trees is comprised equally of an environmental component (50%) and an ontogenetic component (50%) in the absence of water limitation. Photosynthesis was depressed more severely in seedlings than in mature trees in the drought year relative to the wet year, while juveniles showed an intermediate response. Throughout the drought, the predawn leaf water potential (Ψ_{PD}) of seedlings became increasingly negative (–0.4 to –1.6 MPa), while that of mature trees became only slightly more negative (–0.2 to –0.5 MPa). Again, juveniles showed an intermediate response (–0.25 to –0.8 MPa). During the wet summer of 1996, however, there was no difference in Ψ_{PD} between seedlings, juveniles and mature trees. During the dry summer of 1995, seedlings were more responsive to a major rain event than mature trees in terms of Ψ_{Leaf}, suggesting that the two age classes depend on different water sources. In all age classes, instantaneous measurements of intrinsic water use efficiency (WUE_i), defined as C assimilation rate divided by stomatal conductance, increased as the drought progressed, and all age classes had higher WUE_i during the drought year than in the wet year. Mature trees, however, showed a greater ability to increase their WUE_i in response to drought. Integrated measurements of WUE from C isotope discrimination (Δ) of leaves indicated higher WUE in mature trees than juveniles and seedlings. Differences between years, however, could not be distinguished, probably due to the strong bias in C isotope fractionation at the time of leaf production, which occurred prior to the onset of drought conditions in 1995. From this study, we arrive at two main conclusions:

1. Different age classes of *Q. rubra* use different strategies for responding to drought. Seedlings resist drought by closing stomata early in the day at the expense of C uptake; mature trees avoid drought conditions by accessing deeper water reserves and adjusting WUE.
2. Only through studies which separate environmental differences from ontogenetic differences can parameters measured on seedlings be scaled to mature trees.

Key words Ontogeny · Drought response · Photosynthesis · Water use efficiency · *Quercus rubra*

Introduction

We investigated ontogenetic changes in C and water relations of red oak (*Quercus rubra*) as a prelude to scaling from seedlings to mature trees. We combined an experimental approach with seasonal measurements in naturally regenerated stands of red oak to understand how physiological and environmental parameters change from the seedling stage to the adult stage. In the first part of the

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study, we quantified differences in photosynthetic capacity between age classes and separated the ontogenetic component of these differences from the environmental component experimentally. In the second part of the study, we took advantage of a naturally occurring drought to investigate how seedlings, juveniles, and mature trees differ in their responses to drought stress in terms of photosynthesis and water relations. Our underlying goal in this study was to work towards developing a method for scaling from seedlings to mature trees and to understand better the difficulties involved.

Red oak was chosen because it is the dominant canopy species in many northern hardwood forests and is, therefore, of particular relevance in questions of C sequestration and regional hydrologic budgets in these forests. Often results of experimental manipulations on tree seedlings are used to predict response patterns of mature individuals, which are more difficult to manipulate experimentally (Woodward et al. 1991; Ziska et al. 1991; Wullschleger et al. 1995). Recent studies have developed a framework for predicting mature tree responses to changes in the environment by using scaling factors that account for differences in responses between seedlings in controlled environments, seedlings grown under mature tree environments in the field, and mature trees in their natural environment (Bassow 1995; Bazzaz et al. 1996). The validity of such approaches is dependent on investigations of how physiological responses to environmental variables change with ontogeny. Studies that help illuminate different behaviors of age classes in response to environmental change will help efforts to scale from seedlings to mature trees.

Differences in plant age and size can reflect different developmental stages and changes in genetic expression (Donovan and Ehleringer 1992). The developmental stage of a plant can also determine its microenvironment. Light and moisture regimes can change significantly through the life of mid-successional trees, such as red oak. Seedlings that emerge in the forest understory must cope with low light levels for long periods of time, but are generally protected from wind desiccation and from photoinhibition due to excessive light (Barber 1994). Canopy leaves of mature trees, on the other hand, experience full sunlight and higher windspeeds and, thus, must cope with greater potentials for desiccation. Seedlings have smaller and shallower root systems than mature trees and occur in soil layers that are most susceptible to soil drying largely from transpiration of neighboring trees. Conversely, mature tree roots can penetrate into deeper soil layers where water may be more abundant (Lyford 1980). Mature trees also have greater biomass allocated to storage tissues which could provide reserve water (Borchert 1994; Holbrook 1995; Holbrook and Putz 1996). Thus, light and moisture regimes influence the ability of individuals at different stages of development to assimilate C and access water. Changes throughout development in the ability to access resources as well as in plant structure and function may cause plants at different stages to rely on different mechanisms for coping with stress.

We were interested in understanding how seedlings and mature trees differ as a result of ontogeny alone, as well as how different age classes, in their natural environments, respond to a common stress. We tested the following hypotheses:

1. Observed differences in photosynthetic capacity between seedlings and adults have both an environmental and an ontogenetic component.
2. Seedlings and juveniles are more affected by drought stress than mature trees in terms of water relations and C gain.
3. Different age classes use different mechanisms to tolerate drought conditions.

Materials and methods

Study site and approach for field measurements

In New England, the summer of 1995 was particularly dry and contrasted sharply with the unusually wet summer of 1996, as can be seen from the differences in soil moisture at 10 cm (Fig. 1). For both summers, we measured water potential of leaves (Ψ_{Leaf}) and gas exchange of seedlings, juveniles and mature trees at three sites along the Prospect Hill tract at the Harvard Forest, a 1200-ha reserve located in the hardwood-white pine-hemlock forest region in central Massachusetts. Red oak is the dominant canopy species providing 60–70% of the canopy cover. At each site, at least five individuals from each of three age classes were measured throughout the season providing measurements for a total of approximately 45 individuals month^{-1}. The age classes were as follows: (1) seedlings, 1–3 years old, typically one flush with four or five leaves; (2) understory juveniles, 1–3 cm basal diameter with canopies in the understory and not yet reproductive; (3) mature trees, crowns fully extended into the canopy and of reproductive age, stems between 20–50 cm diameter at breast height. Although age and size do not necessarily covary, the three stages of development described here were distinct both in terms of age and size.

Individual juveniles and mature trees could be repeatedly measured throughout the season for both summers without compromising the health of the plant. Seedlings, however, were too small to measure repeatedly without causing damage. Therefore, new seedlings were chosen at random within the general site location for each month's measurements.

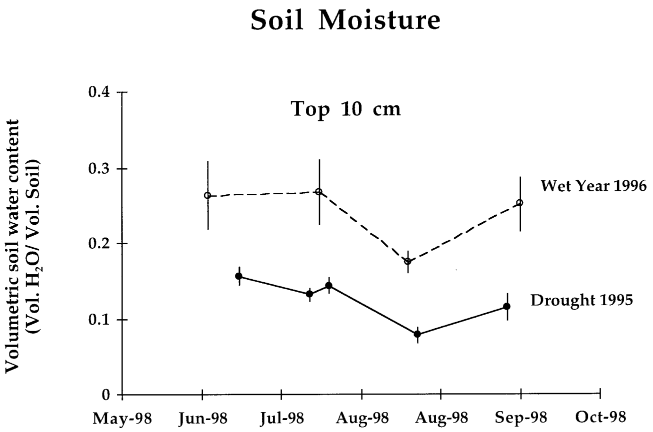

Fig. 1 Volumetric soil water content at the soil surface during the 1995 drought summer and the 1996 wet summer at the Harvard Forest (K. Newkirk, unpublished data). *Jun* June, *Jul* July, *Aug* August, *Sep* September, *Oct* October

Gas exchange

Rates of C assimilation (A), stomatal conductance (g) and ambient CO_2 concentrations were measured with a portable gas exchange system (LI-6200; Li-Cor, Lincoln, Neb.) during the peak summer months (June–September) of 1995 and 1996. Canopies of mature trees were accessed using an aerial lift with a 20-m boom. The aerial lift was not available in June 1996 and these canopy measurements were made on two trees in a nearby location that could be accessed with scaffolding. The seedling and juvenile measurements during June 1996 were made at the same three sites as for all the other months. Measurements of maximum photosynthetic rates (A_{max}), which we refer to as photosynthetic capacity, were taken under saturating light (2000 and 1700 μmol m^{-2} s^{-1} for mature trees and seedlings, respectively) using a detachable LED (Quantum Devices) used with the LI-6200 photosynthesis system or a LI-6400 photosynthesis system with a LED attachment. Light curves were used to verify saturating light levels for different age classes (data not shown).

All gas exchange measurements were conducted in the morning between 0800 hours and 1200 hours on clear days in an effort to measure maximal rates at that point in the season (Bassow and Bazzaz 1997). The light environment in the understory was patchy; in situ gas exchange rates of seedlings and juveniles were, therefore, measured during sunflecks or periods of direct sunlight. Nevertheless, light levels rarely exceeded 700 μmol m^{-2} s^{-1} for juveniles or 500 μmol m^{-2} s^{-1} for seedlings.

Seedling experiment

A seedling experiment was conducted to determine how much of the measured difference in photosynthetic capacity between seedlings and mature trees was due to differences in microenvironment versus ontogeny, following an approach similar to that of Bassow (1995). Red oak seedlings were grown in two light and two watering treatments in a full factorial design to simulate the light and soil moisture regimes experienced by understory seedlings and mature trees. There were 120 seedlings in the experiment, 30 in each of four treatments. Seedlings were grown in a large open clearing (~0.4 ha) at the Harvard Forest that allowed the same radiation regime as at the top of the canopy. Bottomless pots (18×18 cm, 60 cm deep) containing carefully extracted soil divots from the forest floor were placed in holes, dug with a post hole digger, spaced ~1 m apart. Red oak acorns collected from the Harvard Forest were germinated in April in a greenhouse in Cambridge, Massachusetts and transplanted into the pots immediately upon emergence.

Two soil moisture regimes were imposed: in the first, simulating the mature tree environment, plants were individually drip-irrigated in three blocks with automatic watering systems (WTD 1900; Rainbird, San Diego, Calif.). The water source for each of the three blocks was controlled by a separate watering system. Within each block, individual watering tubes were run to each plant. Soil moisture measurements were taken bi-weekly to make sure soil moisture levels compared to those of the deep soil horizons (between 30% and 35% vol. H_2O to vol. soil). Another 60 pots, spatially arranged into three blocks, were not watered, serving as the seedling water regime.

Within each of the watering treatments, there was a canopy and an understory light treatment. Understory light levels in red oak stands were found to be approximately 10% of full sun (data not shown), and these light levels were assumed to correspond to a red/far-red ratio of 0.7 (Lee 1987). Thus, one half of the seedlings were enclosed by wire cages, which attenuated 90% of the ambient light and provided a red/far-red ratio of 0.7 when measured under white light with a spectrophotometer (Beckman DU-20). Cages were covered with shade cloth and strips of plastic theater film (Rosco, Boston), attached vertically to allow air circulation. The other 60 seedlings were exposed to full irradiance (between 1500 and 2000 μmol m^{-2} s^{-1} on clear days) and well-mixed air, simulating the canopy environment. Diurnal light levels and temperatures were measured for 3 days and nights in August, and these compared well with those of the canopy environment. Light-saturated A (i.e., A_{max}) were measured throughout the summer.

ANOVA was carried out to determine the effects of light and soil moisture regimes on A_{max} (μmol CO_2 m^{-2} s^{-1}) of seedlings at the peak of their photosynthetic activity (n=93). Light and water were treated as fixed factors, while block was treated as a random factor. Block was nested within the water treatment. The analysis was done using procedures for general linear models, in SAS, version 6.12 (SAS 1996).

Photosynthetic capacity was then compared for simulated canopy seedlings, simulated understory seedlings, actual understory seedlings, and canopy leaves of mature trees (all measured during the same period) using ANOVA and Scheffe post hoc tests.

Water relations

Water potential

Predawn and midday Ψ_{Leaf} (Ψ_{PD} and Ψ_{MD}, respectively) were measured with a Scholander pressure chamber (PMS Instruments, Corvallis, Ore.) between 0400 and 0500 hours and 1300 and 1400 hours, respectively. These periods corresponded to minimum and maximum Ψ_{Leaf} as determined by diurnal measurements of seedlings and mature trees in July 1995.

Water use efficiency

Two approaches to measuring water use efficiency (WUE) were used. First, an instantaneous measure of intrinsic WUE (WUE_i) was calculated from gas exchange measurements as the ratio of A to g (A/g). By calculating WUE with g rather than transpiration in the denominator, potential differences in vapor pressure deficit between the canopy and the understory are factored out (Ehleringer et al. 1993; Picon et al. 1996).

Second, stable C isotope ratios ($\delta^{13}C$) were measured in leaf tissue. C isotope discrimination ($\Delta\delta^{13}C$ or Δ), which is the difference between foliar $\delta^{13}C$ (δ_l) and $\delta^{13}C$ of atmospheric CO_2 (δ_a), has been empirically correlated with long-term WUE because the discrimination processes involved in A are related to the ratio of C acquisition to water loss (Farquhar et al. 1989). The lighter $^{12}CO_2$ diffuses into the leaf faster than $^{13}CO_2$, causing a 4.4‰ enrichment of $^{12}CO_2$; a further enrichment of $^{12}CO_2$ by circa 27‰ results from the preferential fixation of the lighter isotope by ribulose biphosphate carboxylase. Thus, plant tissues accumulate relatively less ^{13}C than ^{12}C, and the unused ^{13}C in the intercellular spaces of the leaf diffuses out according to the extent of stomatal opening. The inward diffusion and carboxylation of ^{12}C correlates with photosynthesis while the outward diffusion of ^{13}C is correlated with g and transpiration (Kramer and Boyer 1995). Note, however, that other factors which influence the intercellular CO_2 concentration (C_i) including light, vapor pressure deficit, and mesophyll resistance, also influence Δ (Farquhar et al. 1989; O'Leary et al. 1992; Parkhurst 1994; Rao et al. 1995).

δ_l and δ_a analysis

A subsample of leaves harvested for the Ψ_{Leaf} measurements in July and September of 1995 and 1996 were used for isotope analysis. Leaf tissue was oven dried at 65°C and finely ground in liquid N. Samples (200–400 μg) were enclosed in tin capsules for combustion in a Carlo Erba CHN analyzer. C and N were separated via gas chromatography prior to injection in an isotope ratio mass spectrometer (Finnigan, England). ^{13}C content is expressed in delta notation as $\delta^{13}C$ (‰) relative to that of PD belemnite.

To calculate Δ values, we had to estimate δ_a values. Strong correlations between the δ_a and $1/[CO_2]$ of air have been demonstrated in vertical gradients from the forest floor to the forest canopy (e.g., Harwood et al. 1999). This relationship allows the prediction of δ_a from measured CO_2 values. The CO_2

data were converted to δ_a based on the regression equation: δ_a=4320×(1/[CO_2])–20.116 (R^2=0.81), derived from measured CO_2 concentrations and δ_a values at different heights in the canopy at the Harvard Forest in 1991 from Ehleringer and Field (unpublished data). CO_2 concentrations, measured at the same height as the leaves of each age class, did not change significantly throughout the season or between years (data not shown). Therefore, we used the following δ_a values, calculated from average daytime [CO_2] using the regression equation above, for both years and all months: mature trees –7.627, juveniles –8.219, seedlings –8.619. We also calculated different δ_a values from measurements of CO_2 for each month and year, and we tried using the actual δ_a values measured by Ehleringer and Field at different heights in the Harvard Forest (unpublished), but the results did not change significantly.

Soil moisture measurements

Surface soil moisture measurements at the Harvard Forest for 1995 and 1996 (Fig. 1) were supplied by K. Newkirk (unpublished data) and were made using time domain reflectometry (TDR) on probes buried horizontally at 10 cm (in the same plots both years). The soil moisture data at three depths (Fig. 7A) were collected by EJ Humphries (1996) using TDR at 12 sites at the Harvard Forest repeated every 2 weeks during 1995.

Repeated measures analyses

Measurements of gas exchange, Ψ, and Δ were analyzed using SAS version 6.12 in separate ANOVAs with repeated measures using the procedure for mixed models (Littel et al. 1996). Dependent variables were in situ A (μmol CO_2 m^2 s^{-1}), g (mol H_2O m^2 s^{-1}), A_{max} (μmol CO_2 m^2 s^{-1}), intrinsic water use efficiency (WUE_i; A/g), Ψ_{PD} (MPa), Ψ_{MD} (MPa), and Ψ_{PD} before and after a major rain event. Main fixed effects were age (seedlings, juveniles, mature trees) and year, corresponding to the drought summer of 1995 and the wet summer of 1996. Individuals and site were treated as random factors. Month (July, August, September), was nested within year. Tests for fixed effects are reported. Site was not included as a factor in the ANOVA for Ψ_{PD} before and after rain because measurements made after the rain event were conducted at only one site. In the repeated measures ANOVA with Δ as the dependent variable, main fixed effects were age, year, and month nested within year. Site was treated as a random factor.

For all analyses, measurements for juveniles and mature trees were repeated on the same individuals, while measurements on seedlings were always done on different individuals since leaf removal may have damaged the seedlings. An unstructured covariance structure accounted for repeated measures of individuals among months within year. Values for A and g differed dramatically between age classes. Therefore, equivalent absolute changes between drought and non-drought years across age classes represent large relative differences in the responses of those age classes to drought. For this reason, photosynthesis and conductance were log transformed. This allowed the ANOVA to compare differences in the log ratios of means rather than absolute differences in the means for these two parameters.

Results

Photosynthetic capacity of seedlings and mature trees

A_{max} of mature trees were, on average, over 3 times greater than those of seedlings during the wet year and almost 6 times greater during the drought year (Fig. 2). This change between years was due primarily to a decline in seedling A_{max} during the drought to values <4 μmol CO_2 m^{-2} s^{-1}. Repeated measures ANOVA on log-transformed A_{max} showed significant effects of age (P<0.0001) and drought (indicated as “year”, P<0.008; Table 1).

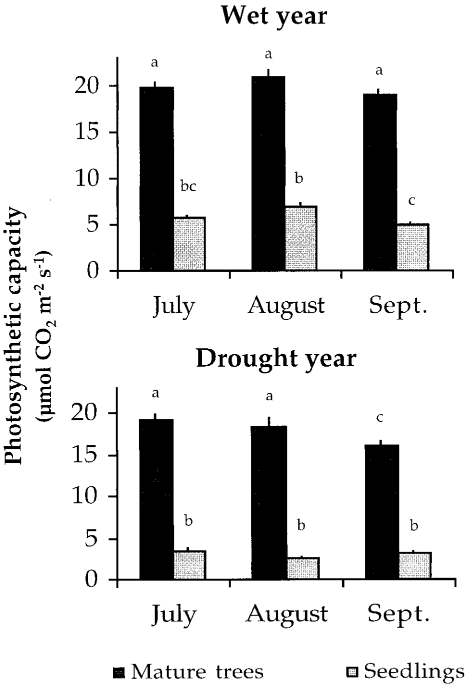

Fig. 2 Photosynthetic capacity of light-saturated leaves for seedlings and mature trees during July, August, and September (*Sept.*) of wet and drought years. *Error bars* are ±1 SE. Within each year, means are shown with different letters if P<0.05

Table 1 ANOVA for maximum photosynthetic capacity (A_{max}; μmol CO_2 m^{-2} s^{-1}) of red oak seedlings grown under a simulated canopy and understory light and water regimes (n=93). *MS* Mean square

Source	*df*	MS	*F*-value	*P*
A_{max} (μmol CO_2 m^{-2} s^{-1})				
Light	1	488.963	69.93	0.0001***
Water	1	84.222	12.04	0.0008***
Block(water)	2	5.92404	0.85	0.4323 ns
Water×light	1	78.301	11.2	0.0012***
Block×light	2	16.689	2.39	0.0982 ns
Error	83	6.992		

***P≤0.001, **P<0.05, *ns* P>0.05

The seedling experiment allowed quantification of this difference between seedlings and mature trees into separate environmental and ontogenetic components. Within the understory light treatment, the photosynthetic capacity of non-irrigated seedlings was not statistically different than that of irrigated seedlings. This lack of difference is due to the fact that the experiment was conducted during a year with high precipitation, and seedlings were not water limited as long as they were in the shade. Within the simulated canopy light treatment, however, non-irrigated seedlings had significantly lower

Table 2 Repeated measures ANOVA for in situ photosynthesis (*A*), in situ stomatal conductance (*g*), intrinsic water use efficiency (WUE_i), photosynthetic capacity (A_{max}), seasonal leaf water potential (Ψ) [predawn (*PD*) and midday (*MD*)], leaf C isotope discrimination (Δ), and Ψ_{PD} during the drought year before and after a major rain event. NDF Numerator *df*, DDF denominator *df*

Dependent				
Source	NDF	DDF	Type III *F*	*P*
In situ *A* (μmol CO_2 m^{-2} s^{-1})				
Age	2	4	78.26	0.0006***
Year	1	2	131.76	0.0075**
Age×year	2	268	41.93	0.0001***
g (mol H_2O m^{-2} s^{-1})				
Age	2	4	93.69	0.0004***
Year	1	2	80.92	0.0121**
Age×year	2	259	15.39	0.0001***
A_{max} (μmol CO_2 m^{-2} s^{-1})				
Age	2	4	610.69	0.0001***
Year	1	2	114.67	0.0086**
Age×year	2	257	26.47	0.0001***
WUEi (A/g)				
Age	2	4	4.18	0.1049 **ns**
Year	1	2	69.16	0.0142**
Age×year	2	258	5.14	0.0065**
Ψ_{PD} (MPa)				
Age	2	4	7.6	0.0434*
Year	1	2	32.13	0.0297*
Age×year	2	254	5.82	0.0034**
Ψ_{MD} (MPa)				
Age	2	4	11.25	0.0228*
Year	1	2	181.27	0.0055**
Age×year	2	241	1.93	0.1478 ns
Leaf Δ				
Age	2	4	8.48	0.0364*
Year	1	2	0.14	0.7470 ns
Month(year)	1	19	0.2	0.6578 ns
Age×year	1	19	0.06	0.8129 ns
Age×year	2	19	0.05	0.9520 ns
Age×year×month	1	19	0.15	0.6991 ns
Ψ_{PD} before and after rain (MPa)				
Age	2	38	52.92	0.0001***
Rain	1	38	33.16	0.0001***
Age×rain	2	38	4.71	0.0149**

***$P<0.001$, **$P<0.01$, *$P<0.05$, *ns* $P>0.05$

mean A_{max} than irrigated seedlings (8.7 versus 12.6 μmol CO_2 m^{-2} s^{-1}; $P<0.001$). Seedlings in the understory light treatment showed a mean photosynthetic capacity of 6.2 μmol CO_2 m^{-2} s^{-1} (SE 0.5) which is not significantly different from that of naturally occurring understory seedlings (6.1 μmol CO_2 m^{-2} s^{-1}, SE 0.3) measured during the same period. Experimental seedlings under all treatments showed increasing *A* throughout the season, reaching a maximum in late August and early September. Therefore, all comparisons were made using data collected during this period. ANOVA (Table 1) showed a highly significant effect of light ($P<0.0001$) and water ($P<0.008$) on A_{max}. A significant interaction of light and water is due to the fact that there was no effect of irrigation within the understory light treatment. Under full sun, however, the non-irrigated seedlings became water limited.

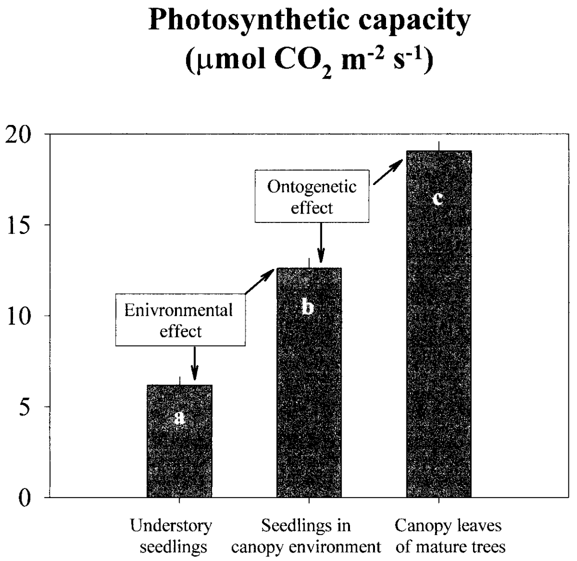

Fig. 3 Differences in photosynthetic capacity between seedlings and mature trees can be broken down into an environmental and an ontogenetic component. Shown here are the photosynthetic capacities of understory seedlings, seedlings grown under simulated canopy conditions, and canopy leaves of mature trees. The difference between the photosynthetic capacity of the canopy seedlings and understory seedlings show the effect of environment, while the difference in photosynthetic capacity between canopy seedlings and mature trees show the effect of ontogeny. *Error bars* are ±1 SE

The effect of environmental differences between mature trees and understory seedlings can be determined by comparing the photosynthetic capacity of the irrigated seedlings in full sun (simulated mature tree environment) with that of actual understory seedlings, measured at the same time in their natural environment. The ontogenetic difference between seedlings and mature trees can then be quantified by comparing the photosynthetic capacity of seedlings in the simulated mature tree environment to that of canopy leaves of mature trees (Fig. 3). Seedlings in the simulated mature tree environment showed significantly higher photosynthetic capacities than understory seedlings ($P<0.0001$), and these values were significantly lower than those of canopy leaves of mature trees measured at the same time ($P<0.0001$, Fig. 3). This shows that, in red oak, the contribution of environment to the difference in photosynthetic capacity between understory seedlings and mature trees is about 50% and the contribution of ontogenetic differences is about 50% under conditions in which water is not limiting. These results are consistent with a those of a previous study in which red oak seedlings were grown on tower platforms at the top of the canopy and their photosynthesis compared with that of canopy leaves of mature trees (Bassow 1995).

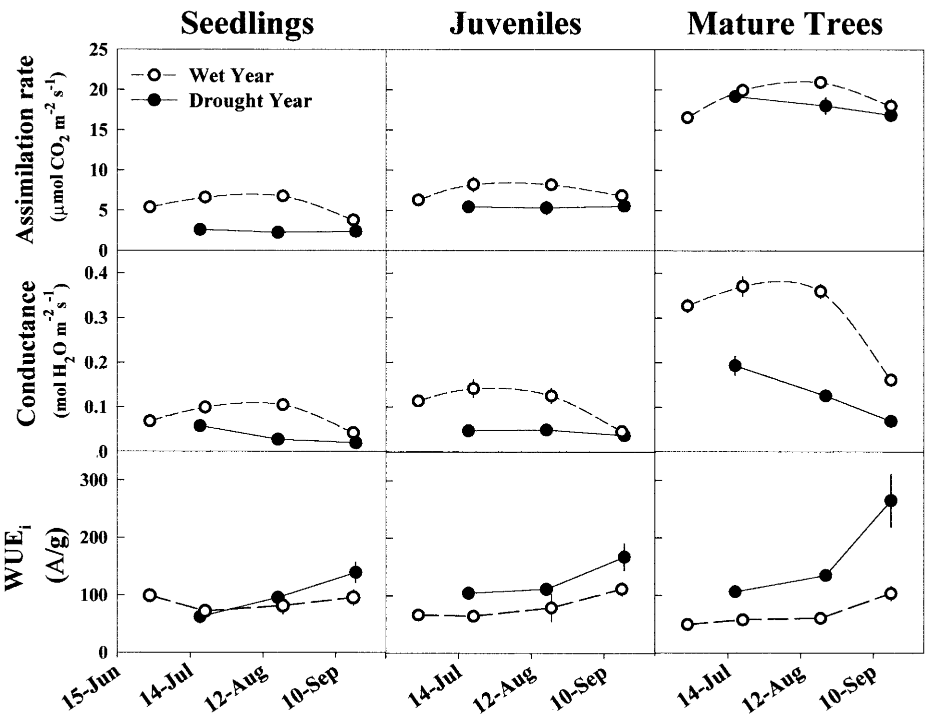

Fig. 4 In situ gas exchange measurements for seedlings, understory juveniles, and mature trees during the drought (*closed circles*) and wet summer (*open circles*). The *upper three panels* show photosynthetic rates (*A*), the *middle three panels* show stomatal conductance rates (*g*), and the *lower three panels* show internal water use efficiency (*WUE_i*, calculated as *A/g*). Measurements were taken on clear days between 0800 hours and 1200 hours in direct sunlight or during sunflecks for understory seedlings and juveniles. Means are shown for 15 individuals of each age class. June measurements for mature trees during the wet year were done on only two individuals (ten leaves each). *Error bars* are ±1 SE. For other abbreviations, see Fig. 1

We attribute these differences in photosynthetic capacity between leaves of seedlings and mature trees growing in similar environments, in part, to differences in leaf anatomy and N content (not measured). Canopy leaves of mature trees had 31% higher leaf mass per area than leaves of seedlings grown in a canopy environment; two palisade layers rather than one; 42% higher relative chlorophyll content; and higher carboxylation efficiency as indicated by steeper initial slopes of the relationship between *A* and C_i (J. Cavender-Bares, unpublished data). These measures, taken together, indicate substantial differences in leaf structure and a higher leaf N content in mature trees relative to seedlings.

Drought responses of different age classes

Seasonal gas exchange rates

Physiological measurements of different age classes of red oak in their natural environment during a wet year and a drought year show that plants at different ontogenetic stages are affected differentially by drought stress. A_{max} (light-saturated) were depressed to a greater extent in seedlings than in mature trees during the drought (Fig. 2), as shown by the significant interaction between age and year (P<0.0001; Table 2). Seedlings showed a 40%, 63%, and 35% decline in July, August, and September of the dry year relative to the wet year. Mature trees showed a decline of only 3%, 13%, and 16% for the same months.

Seasonal in situ *A* and *g*, as well as the WUE_i are shown for the drought and wet years throughout the peak summer months (Fig. 4). During the months of July and August, in situ *A* in seedlings were depressed by 67% and 37%, respectively, in the drought year relative to the wet year, while those of mature trees were depressed by only 4% and 15% for July and August, respectively. During the wet year, in situ gas exchange rates declined in all age classes in August and September relative to July. This trend was less marked during the drought year. Between mid-August and September of 1996 (wet year), temperatures were lower than average and overcast skies and hurricane weather occurred frequently. This was in sharp contrast to 1995 during the same months when the skies were almost always clear and temperatures were warmer. Since photosynthesis in red oak is sensitive to temperature (Bassow and Bazzaz 1998), the observed decline in gas exchange values in September 1996 was probably due, in part, to cooler temperatures, in addition to somewhat lower light levels rather than leaf age alone. For log-transformed in situ *A* and *g*, respectively, ANOVA showed highly significant effects of age class (P<0.0006, P<0.0004) and drought (P<0.0075, P<0.0121) as well as a highly significant drought by age interaction (P<0.0001, P<0.0001; Table 2).

Diurnal gas exchange rates

Diurnal measurements of seedlings and mature trees in early August during both the wet and drought years show a change in diurnal timing of peak photosynthesis for seedlings but not for mature trees (Fig. 5). Seedlings assimilated C at higher rates for a longer period during the day in the wet year relative to the drought year. The difference in diurnal patterns of *g* for seedlings in the drought year relative to the wet year are also striking. In

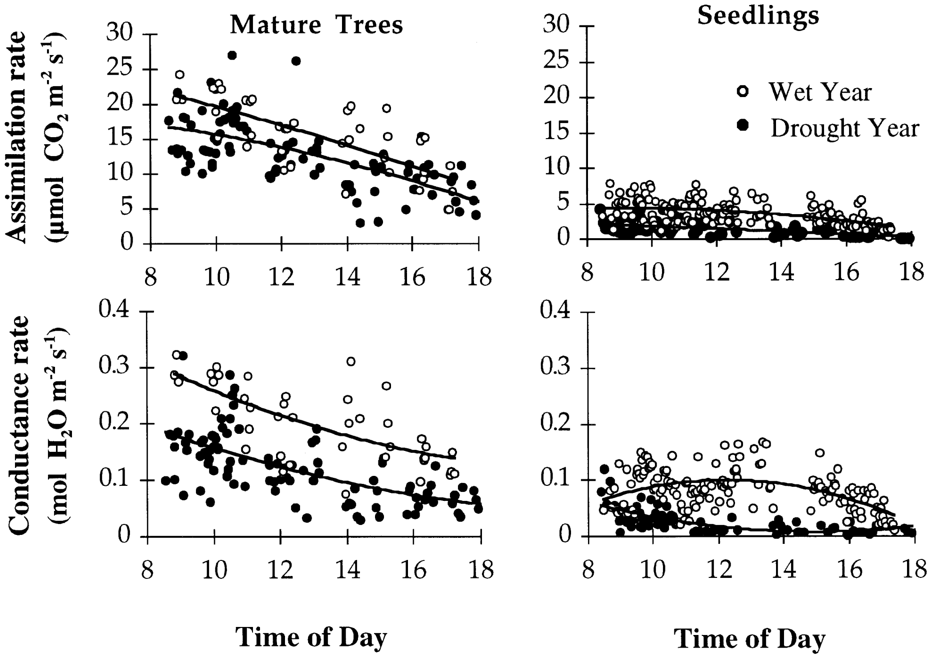

Fig. 5 Diurnal gas exchange measurements during 2 clear days in August for the drought (*closed circles*) and wet summer (*open circles*) for seedlings and mature trees. *Top panels* show in situ photosynthetic rates (*A*) throughout the day, and *bottom panels* show in situ stomatal conductance rates (*g*). *Lines* are second-order polynomials fitted to the data

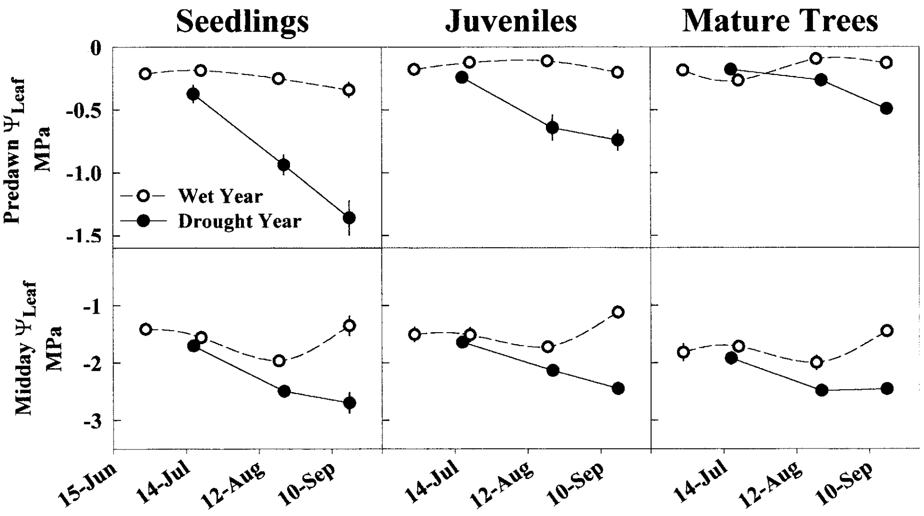

Fig. 6 Predawn (*upper panels*) and midday leaf water potentials (Ψ_{Leaf}) (*lower panels*) for seedlings, understory juveniles, and mature trees during the dry and the wet summer. Measurements were taken at three different sites on Prospect Hill at the Harvard Forest on the same days and on the same individuals as in Fig. 4. Error bars are ±1 SE. For other abbreviations, see Fig. 1

the wet year, seedlings maintained high *g* for most of the day, which decreased as light levels dropped later in the day. During the drought year, however, the stomata closed before noon, reducing C gain in the afternoon after Ψs (see below) reached their minimum. This change in diurnal *A* and *g* is an important difference between seedlings and mature trees that is not reflected in the seasonal measurements since they were conducted only in the morning.

Leaf water potential

Figure 6 shows the change in Ψ_{PD} throughout the summer months for all three age classes during the dry and the wet year. During the dry summer, Ψ_{PD} became increasingly negative as the drought progressed. This trend, however, was more pronounced in seedlings (–0.4 to –1.6 MPa) than in mature trees (–0.2 to –0.5 MPa). Juveniles showed an intermediate response (–0.25 to –0.8 MPa), but still became more stressed by September than mature trees. During the wet year, there was little change in Ψ_{PD} throughout the season and no significant difference between age classes. ANOVA showed significant effects of age ($P<0.04$) and year ($P<0.03$) and a highly significant age by year interaction ($P<0.003$; Table 2).

Ψ_{MD} measurements were similar for all three age classes during the drought. They were also more negative during the drought than during the wet year, with the wet year showing greater within-age class variability. ANOVA showed significant age ($P<0.02$) and year ($P<0.005$) effects, but no significant age by year interaction (Table 2).

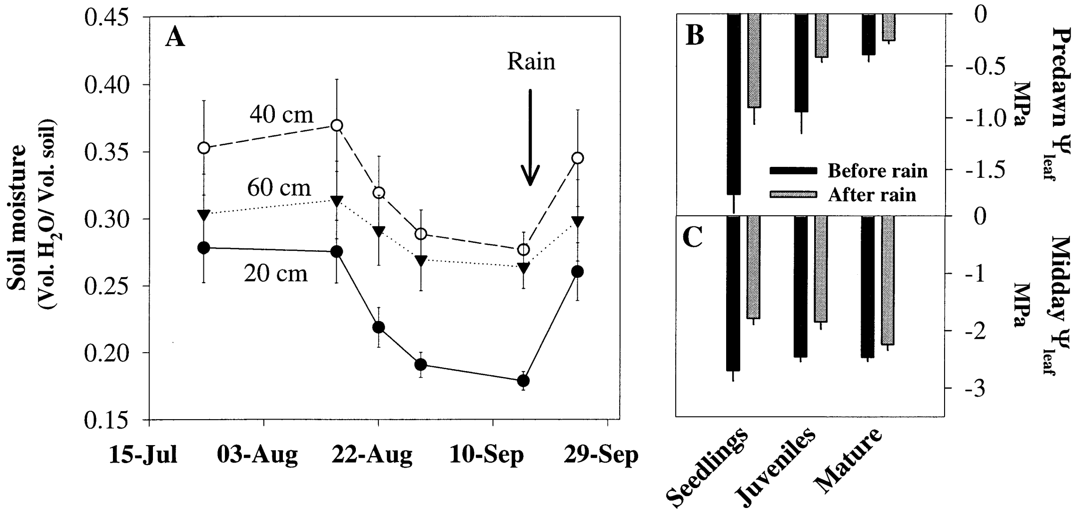

Fig. 7 (**A**) The progression of soil water content at the Harvard Forest, measured at ten sites and at three depths, throughout the course of the 1995 drought and after the drought-breaking rain in late September (EJ Humphries 1996). The *right panels* show predawn (**B**) and midday (**C**) Ψ_{Leaf} for seedlings, juveniles, and mature trees (n=15) at one site the day before and the day after the drought-breaking rain. For other abbreviations, see Fig. 1

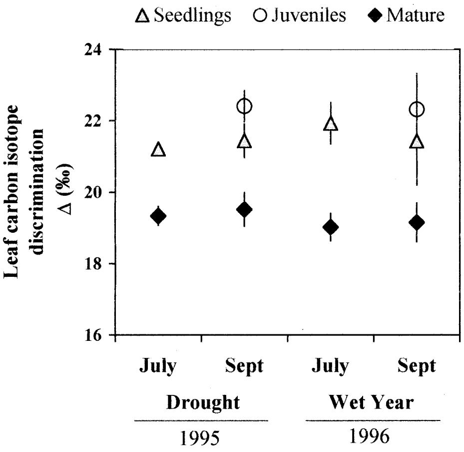

Fig. 8 C isotope discrimination (Δ) in leaves of seedlings and mature trees from July and September, and in juveniles for September only, during the drought year and wet year. Differences among age classes show that seedlings have lower WUE at the time of leaf development than mature trees or juveniles. *Error bars* are±1 SE

During the drought year, Ψs at one of the three sites were measured the day before and the day after the drought-breaking rain in late September (Fig. 7; J. Humphries, J.-F. Yeh, and F. Eltahir, unpublished data). The rain had only a marginally significant effect on the Ψ_{PD} of mature trees, while juveniles, and particularly seedlings, showed large increases in both Ψ_{PD} and Ψ_{MD}, resulting in a significant interaction between the effect of rain and age class for Ψ_{PD} ($P<0.014$, Table 2) and a marginally significant interaction for Ψ_{MD} ($P<0.052$, data not shown).

Instantaneous WUE

Instantaneous measurements of WUE_i indicate that all age classes increased their WUE_i during both the drought and wet years as the season progressed (Fig. 4). In the drought year relative to the wet year, however, mature trees showed a greater than two-fold increase in WUE_i. Seedlings and juveniles showed differences between the 2 years only in the month of September. When seedlings and mature trees were compared, mature trees showed higher WUE_i than seedlings in the drought year, but no difference in the wet year. This resulted in no overall age effect, but a significant effect of year ($P<0.014$) and a year by age interaction ($P<0.0065$).

Stable C isotope analysis

Foliage of mature trees had significantly less negative $\delta^{13}C$ values than juveniles or seedlings (age effect: $P<0.004$). In 1995, mean values were –27.1 (SE 0.5), –30.63 (SE 0.4), and –30.6 (SE 0.6) for mature trees, saplings, and seedlings in September, respectively. In 1996, these values were –26.8 (SE 0.5), –30.5 (SE 0.1), and –30.1 (SE 1.2). There were no differences in leaf $\delta^{13}C$ between July and September in either year and no differences between the drought and wet years Other studies have shown similar patterns of increasing $\delta^{13}C$ with canopy height (Bassow 1995; Gartner 1995; Harwood et al. 1999). Seedlings and juveniles, which are closer to the forest floor, are likely to fix C supplied from soil respiration and decomposition of litter, while mature trees are more likely to fix C in well-mixed air at the top of the canopy. This is supported by our estimates of δ_a, which are more negative for seedlings and juveniles than mature trees (see Materials and methods). Using these values to calculate leaf Δ removes the effect of changing δ_a values at different heights.

Figure 8 shows foliar Δ values for seedlings and mature trees in July and September of 1995 and 1996, as well as juveniles in September of the 2 years. Δ values are shown rather than δ^{13}C values, as the former values reflect only fractionation processes in the leaf without the influence of source air. Mature tree foliage showed lower Δ than juveniles or seedlings, as shown by a significant age effect ($P<0.036$, $n=27$; Table 2). However, there was no difference between July and September in either year and no difference between the drought and wet years. While Δ values can largely be interpreted as a measure of WUE, several microenvironmental factors may contribute to the higher Δ in seedlings and juveniles relative to mature trees. Lower light levels and lower evaporative demand in the understory may increase Δ in seedlings and juveniles by increasing C_i (Jackson et al. 1993). Also, increased thickness of leaves of mature trees could increase mesophyll resistance, thereby decreasing C_i and Δ in mature trees (Parkhurst 1994).

Discussion

We found significant changes in C and water relations of red oak as seedlings develop into mature trees. Previous studies on other species have also shown that physiological parameters related to C and water relations may change with plant age and developmental stage (Donovan and Ehleringer 1991, 1992; Epron and Dreyer 1993; Putz et al. 1995; Farnsworth and Ellison 1996).

In the present study, red oak seedlings had lower photosynthetic capacities than mature trees. This difference is due, in part, to the fact that seedlings and mature trees occupy different microhabitats with contrasting light and soil moisture regimes. Independent of the developmental stage of the plant, acclimation to sun versus shade would be expected to give rise to significant differences in photosynthetic capacity (Boardman 1977; Björkman 1981; Murchie and Horton 1997). When seedlings were grown under a simulated mature tree environment, thereby factoring out major environmental differences, the seedlings still had 30% lower $A_{\max}$ than canopy leaves of mature trees. The high photosynthetic capacities of mature individuals could not be achieved by seedlings, regardless of environmental conditions. This indicates that a scaling factor, reflecting the ontogenetic effect, should be incorporated into models attempting to scale from seedlings to mature trees.

In addition to the clear ontogenetic differences in photosynthetic capacity, we also found that the degree to which different age classes were affected by drought, as well as the mechanisms they used for responding to it, were distinct. Under natural conditions, mature trees, in general, were less affected by the drought in terms of C gain and Ψ_{PD} than juveniles and seedlings. The small decrease in A during the drought by mature red oaks (Fig. 4), which are the dominant canopy species at the Harvard Forest, helps to explain the relatively small decrease in overall forest C uptake during the drought as determined from eddy correlation measurements at the Harvard Forest (Goulden et al. 1996). A were reduced disproportionately in seedlings relative to mature trees under drought conditions. Diurnal photosynthetic measurements suggest, moreover, that the number of hours during the day in which seedling leaves were assimilating C was greatly reduced during the drought (Fig. 5). In mature trees, there was no change in the period of C gain between the 2 years. This difference in diurnal patterns of gas exchange between age classes has implications for scaling from seedlings to mature trees, but has been left relatively unexplored in previous work.

Gas exchange rates were clearly coupled to the water status of the plants. During prolonged drought, mature trees were able to restore a high Ψ_{PD} overnight, while seedlings and juveniles became increasingly stressed. Seedlings, whose roots extend only about 20 cm deep (Lyford 1980), had their roots in the driest soil horizons (Fig. 7A; EJ Humphries 1996). Soil moisture close to the surface is evaporated first and depleted quickly by competing roots since this is the region of greatest root biomass (Lyford 1980). Due to their shallow rooting, seedlings would be expected to rely solely on precipitation as their water source. This is supported by the responsiveness of their Ψ_{PD} and Ψ_{MD} to a major rain event (Fig. 7). Mature trees were less responsive to precipitation during drought than seedlings, as shown by smaller proportional changes in Ψ_{PD} after rain. This indicates that mature trees do not rely solely on precipitation at the soil surface to restore their high Ψ_{PD}. A significant hard pan starting at about 2 m below the surface exists in most regions of the Harvard Forest (Lyford 1980). Mature trees with deep and penetrating roots, such as red oak, may have access to soil moisture, possibly groundwater, beneath this hard pan.

Finally, mature trees were able to increase their WUE to a greater extent during drought than were seedlings. Seedlings and saplings showed a smaller range in the ratio of A/g than mature trees. The flexibility to adjust WUE increased from seedlings to mature trees. Higher WUE in mature individuals relative to juveniles has been found by other researchers (Donovan and Ehleringer 1992). Donovan and Ehleringer (1991) suggested that, in seedlings, a lower WUE may be favored during establishment if it is accompanied by increased growth and proportional allocation to root biomass. Increased allocation to roots would increase the probability of reaching deeper water stores sooner, thereby increasing the chances of survival during drought. In mature trees, however, a high WUE helps maintain near maximal C uptake rates while preventing excessive water loss, and possibly limits xylem embolism.

Lower Δ in mature trees relative to juveniles and seedlings is consistent with the higher WUE in mature trees determined from gas exchange measurements. The lack of change in the δ^{13}C and Δ between the wet year and drought year, however, indicates a strong bias towards C assimilation at the time of leaf production and expansion, which occurred in May and June before the onset of the drought. This bias may make determination of seasonal WUE problematic in species, such as red oak, which typically flush once during the growing season. Damesin et al. (1997) tracked $\delta^{13}C$ of leaves throughout the growing season for *Quercus pubescens* and *Quercus ilex*, and found that once leaves mature there is no longer a change in the $\delta^{13}C$ of the leaves. Thus, the Δ values shown (Fig. 8) should give an indication of WUE during the early part of the growing season, but not the latter part.

A framework for scaling seedling responses to mature trees was developed by Bazzaz et al. (1996). This approach incorporates an environmental scaling factor, which takes into account environmental differences between the growth environment of seedlings and of mature trees, as well as an ontogenetic scaling factor, which takes into account ontogenetic differences between seedlings and adults. The response of seedlings to some imposed change in the environment can then be scaled to mature trees. From this study, it is apparent that some environmental changes, such as drought, have differential effects across age classes: seedlings of red oak are more affected by drought than mature trees, both in terms of *A* and the number of hours per day that assimilation is possible. This demonstrates that the ontogenetic scaling factor may be different under different environmental conditions. Environmental changes that affect all age classes equally are more likely to have a constant ontogenetic factor, making scaling more tractable.

Ontogenetic scaling factors are also likely to differ across species. In species where ontogenetic effects are not significant, scaling should be possible as long as the mature tree environment can be appropriately simulated. In species where ontogenetic effects are significant, such as red oak, the feasibility of scaling will depend on a thorough evaluation of these effects and how they vary under different environmental conditions. Species that experience similar environments as seedlings and adults are likely to show relatively small ontogenetic effects. Evolution of similarity in traits across developmental stages would be expected if selection factors tended to be relatively constant throughout ontogeny. For example, early successional tree species, which have limited shade tolerance as seedlings and are shallow-rooted as adults, may show a smaller ontogenetic effect than red oaks and other later successional species, which have fairly high shade tolerance as seedlings and are deep-rooted as adults. This prediction could be tested by comparing ontogenetic effects across species (e.g., early versus late-successional species) under different environmental conditions.

We conclude that the difference between seedling and adult photosynthetic capacities for red oak under field conditions comprises both an environmental and an ontogenetic component. About 50% of this difference is ontogenetic and 50% environmental under conditions which are not water limiting. Both of these components need to be addressed when scaling from seedlings to mature trees. Smaller age classes are more affected by drought than mature trees, due both to their shallower rooting as well as their inability to fix C at low Ψ. Seedlings resist drought by closing stomata early in the day at the expense of C uptake. Mature trees avoid drought conditions by accessing deeper water reserves and adjusting WUE, sacrificing C gain only marginally. Ontogenetic differences in the way that conspecifics respond to environmental stresses present a challenge when scaling from seedling experiments to mature trees. However, understanding the nature and degree of ontogenetic effects may allow the introduction of scaling factors into models which take these effects into account. Studies such as these will help efforts to scale experimental manipulations of seedlings to predict responses of mature trees under changing environments.

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