# HISTORICAL CO$_2$ GROWTH ENHANCEMENT DECLINES WITH AGE IN *QUERCUS* AND *PINUS*

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*Abstract.* Despite experimental evidence showing that elevated CO$_2$ levels increase growth in most plants, the isolation of a signal consistent with anthropogenically caused increases in atmospheric CO$_2$ from the dendrochronological record has shown mixed results. Our extensive sets of tree ring data from the Ozark Mountains in Missouri showed that since 1850, *Quercus velutina* Lam., *Quercus coccinea* Muench., and *Pinus echinata* Mill. trees increased in stem growth coincidently with increases in atmospheric CO$_2$. Those long-term increases in radial growth appear unrelated to historical disturbance levels for the region, to long-term changes in relevant climatic variables, or to productivity of sites sampled for the purpose of creating a time sequence of tree ring growth. It is still unclear what the potential role of nitrogen deposition might have been for tree growth. We cross-dated a large number of increment cores and aligned the ring width data by pith date for accurate age constant assessments of growth over the past 150 years. Thus, we circumvented changes in growth trend associated with differences in physiological functioning during development, as well as the need for statistical detrending that removes an unknown degree of long-term environmental signal, the so called segment length curse that applies to standard dendrochronological investigations. When the positive relationship between CO$_2$ and ring width was examined at different ages, an ontogenetic decline in the rate of growth stimulation was found. Specifically, both the pooled *Quercus* spp. and *P. echinata* were characterized by a negative exponential pattern of response over a developmental sequence through age 50. Further knowledge of an intrinsic decline in CO$_2$ sensitivity with tree age or size such as this may be important for increased accuracy in estimating terrestrial carbon stocks across successional landscapes.

*Key words: carbon dioxide; climate change; CO$_2$; dendrochronology;* Pinus echinata; Quercus coccinea; Quercus velutina; *red oak; segment length curse; shortleaf pine.*

## INTRODUCTION

Tree rings have recorded numerous environmental signals that would be too costly or impossible to recreate through experimentation. For example, tree rings have been used to track leaf-internal CO$_2$ concentrations under experimental conditions enriched in CO$_2$ (Leavitt et al. 2003) and in natural populations of trees responding to rises in atmospheric CO$_2$ levels (Feng 1998, 1999). It has been documented by observation and experimentation that photosynthetic capacity, as well as leaf and stomatal development, have been regulated by atmospheric CO$_2$ for C3 plants, as CO$_2$ has ranged from <200 ppm during past glacial maxima through highly enriched concentrations nearing 1000 ppm, which may not have occurred for millions of years (Idso and Kimball 1993, Polley et al. 1993, Ehleringer and Cerling 1995, Beerling 2005). Given the demonstrable linear increase in photosynthesis, in addition to the indirect

effect of increased water use efficiency (WUE) under elevated CO$_2$ (Wullschleger et al. 2002), we should logically expect to be able to detect and thus predict increases in growth of natural populations of trees. However, the now common measures of instantaneous leaf level gas exchange and photosynthetic rates cannot be simply related to long-term growth of trees. That is made clear by age-related changes in hydraulic architecture and photosynthesis in individual trees as well as net primary production at the stand level (Bond 2000, Ryan et al. 2004). Problems such as those would add error to already tenuous estimates of long-term terrestrial carbon fluxes due to the complex feedbacks in hydrologic budgets and nutrient cycling expected as CO$_2$ rises (Amthor 1999, Aber et al. 2001, Schäfer et al. 2002, Medlyn and McMurtie 2005).

Dendroecological methods can indeed provide a context of interannual variability to periodic forest growth inventories (Biondi 1999). In addition, long-term growth patterns from tree ring data may yet prove a useful intermediary to help link top-down and bottom-up scaling methods for the prediction of large-scale terrestrial carbon fluxes across topographically complex or successional landscapes (sensu Canadell et al. 2000). A major obstacle to the use of tree rings to isolate such a low-frequency response related to increasing atmospher-

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ic $CO_2$ has been the segment length curse (Cook et al. 1995). Standard techniques have been developed to isolate particular environmental signals such as temperature or precipitation from tree rings. Most often, each tree ring series is statistically detrended with one or two "curves" to remove age-related decline in ring widths and other unwanted signals. This standard process does not, however, differentiate between low-frequency climatic changes or atmospheric-$CO_2$ concentrations, but reduces by an unknown degree those important environmental influences from each individual tree ring series (i.e., the segment-length curse). If extremely old trees with a stable age-response were to occur regularly across many ecosystems, the interpretation of dendrochronological data would certainly be much simpler. Since this is not the case, and experimental data on mature forests is limited, other approaches might help fill gaps in how we understand the consequences of rising $CO_2$ for forest productivity.

Tree stem growth would seem a simple measurement, but the attribution of a stem growth response to anthropogenic increases in atmospheric $CO_2$ has yet to be established since being reviewed by Jacoby and D'Arrigo (1997) and more recently by Norby et al. (2005). Long-term studies of $CO_2$ enrichment on trees are limited, but a few studies have reported a decreasing enhancement of relative growth rate from elevated $CO_2$ during development or during the time after initial exposure (Hättenschwiler et al. 1997, Idso 1999, Wang et al. 2006). This decline in response during ontogeny may differ with species' relative growth rates and longevity. For instance, exposure of fast-growing *Populus* spp. to $CO_2$ resulted in progressively smaller increases of gross primary productivity as the canopy reached three years of age (Wittig et al. 2005). In contrast to the fast-growing *Populus*, mature and slower-growing hardwood species showed no consistent stem growth response to enriched $CO_2$ conditions (Körner et al. 2005). No doubt, most gains in plant biomass documented from smaller and younger trees were due largely to increased rates of ontogenetic development during $CO_2$ enrichment (Norby et al. 1999). Great variability exists in reports of $CO_2$ enhancement of above-ground carbon fixation of woody species, from 4% to 242% (Medlyn et al. 2001). Free-air carbon exchange experiments (FACE) have provided much-needed perspective on this variability, and the overall pattern is toward a substantial increase in growth of most plants (Ainsworth and Long 2005). However, at this relatively early stage, FACE experiments cannot yet fully address longer-term biotic feedbacks (Luo and Reynolds 1999) nor the effects of stand species compositions, ages, and structures across complex landscapes (Norby et al. 1999, Körner et al. 2005).

Thus far, dendroecological studies have shown mixed results in ascertaining a common growth response related to elevated $CO_2$ (Kienast and Luxmoore 1988, Nicolussi et al. 1995, Hättenschwiler et al. 1996). One unique set of examples comes from investigations of radial growth near geothermal vents that have provided long-term elevated $CO_2$ levels. Hättenschwiler et al. (1997) found that total stem growth was increased near vents by 12% over the first 25–30 years but that this increase was largely due to gains during early development. There seem to be a number of questions about using those single sites without knowledge of past $CO_2$ emissions from the spring (Saurer et al. 2003) or about whether those $CO_2$-enriched trees around the vents have grown faster at all (Tognetti et al. 2000).

The use of tree rings for studying $CO_2$ fertilization was first proposed by LaMarche et al. (1984). A later study by Graumlich (1991) found no consistent $CO_2$ signal in three conifer species of northern California, USA. In an expansive study of subalpine conifers, low-frequency patterns from tree ring chronologies suggested a widespread increase in ring widths across the western United States that was consistent with $CO_2$ fertilization (Graybill and Idso 1993). Consistent with increasing atmospheric $CO_2$ and WUE, recent applications of the Graumlich (1991) method, which used a range of tree ages, showed that trees growing on drier sites displayed positive trends in residual tree ring growth not explained by temperature and precipitation (Knapp et al. 2001, Wang et al. 2006). Wang et al. (2006) emphasized that younger stages of development included greater growth increases than older developmental stages. To date only three studies provide precedents for increased growth attributed to atmospheric-$CO_2$ fertilization in trees not located at high elevations or in semiarid regions, and those studies are not directly comparable (West et al. 1993, Bascietto et al. 2004, Wang et al. 2006). Taken together, tree ring studies have demonstrated the possibility of a widespread increase in the growth of *some* tree species under *some* site conditions. After considering their mixed results, the question remains: do the studies simply provide a qualitative reassurance that some of the $CO_2$ response demonstrated from earlier experimentation on small trees can exist in natural populations? Since tree stems are (1) the most readily available part of trees to study; (2) often proportional to many other pools of plant tissue; and (3) responsible for a great amount of global carbon storage, an understanding of the range of possible responses in stem growth to increasing $CO_2$ is critical. We used extensive sets of tree ring data from the Missouri Ozarks to avoid the segment length curse and infer how a long-term signal attributed to $CO_2$ might change with tree age and stand development.

## METHODS

### Site selection and study area

For most of the data presented, two methods of site selection were used. First, we used geographic information system software (Arcview version 3.2, ESRI Incorporated, Redlands, California, USA) to randomly generate an initial set of 300 coordinates centered on the upper Current River watershed in southeast Missouri
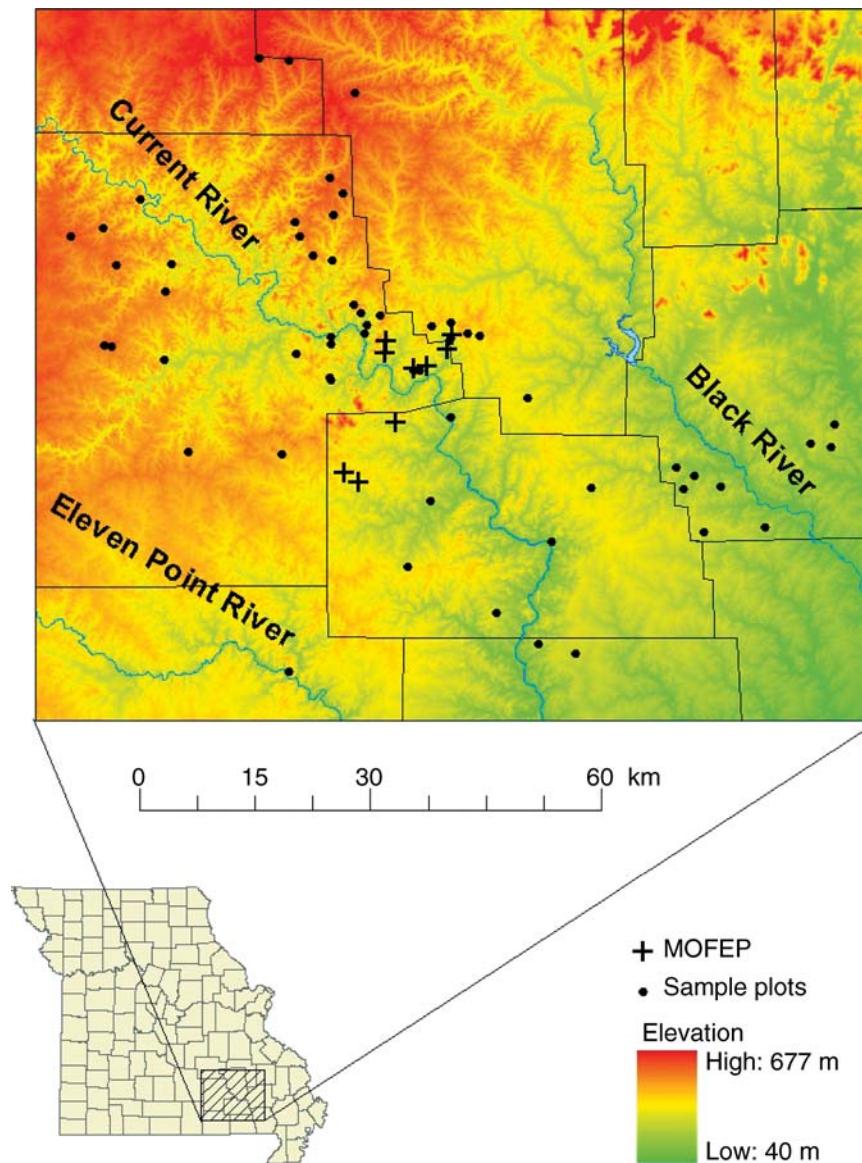
FIG. 1. Distribution of sample plot locations across a region of the Ozarks in southeast Missouri. A total of 576 trees were used from 59 outlying plots, and 876 trees were used from 88 plots located near the nine MOFEP (Missouri Ozark Forest Ecosystem Project) site locations shown here.

(37° N, 91° W) (see Fig. 1). We generated a larger set of coordinates than could be sampled because we knew our specifications for sampling a stand would preclude many randomly located points. After the exclusion of points mapped in nonforested areas, travel and time constraints limited the final sample size to 59, 0.083 ha plots (Fig. 1). The second selection of plots used an existing forest mensurational data set from the Missouri Ozark Forest Ecosystem Project (MOFEP) to randomly select 88, 0.2 ha plots across the nine MOFEP sites (Fig. 1). This procedure allowed us to characterize a centrally located area that was representative of the parent material and range of soil conditions of the broader region. Within the MOFEP sites, the original spatial

arrangement of plots also was random. This paired extensive and intensive sampling was intended to minimize site level artifacts in our age sequence data that could influence classic chronosequence studies from a single transect or from a few sites differing in age (sensu Lorimer and Frelich 1998). As criteria for site selection, we systematically avoided stands that had evident disturbance to overstory trees, stands with <33% basal area in *Q. velutina* and *Q. coccinea*, and stands <50 years old. Also, we limited our sample to closed-canopy forest stands.

*Q. velutina* and *Q. coccinea* dominate the overstory in this region, and the two species account for >50% of stem basal area at MOFEP control sites. Other common

canopy dominants include, in descending rank according to overstory basal area, *Quercus alba* L., *P. echinata*, *Quercus stellata* Wangenh, *Carya ovata* K. Koch., *Carya texana* Buckley*, and Carya glabra* Sweet. Upland soils of this region are Ultisols or Alfisols with variable but often high-gravel content (Kabrick et al. 2000). Patchy fragic layers are common and the upper soil horizon pH normally ranges from ~4 to 6. Mean annual precipitation for the area is 116 cm, though summer droughts occur frequently (S. Voelker, *unpublished data*). Mean daily minimum temperature in January is −6°C and mean high temperature in July is 32°C.

Prior to Euro-American settlement, vegetation in the Missouri Ozarks was greatly influenced by frequent, intentional burning by Native Americans (Batek et al. 1999, Guyette et al. 2002). Conditions following settlement were characterized by increased logging, disturbance, and frequent fires through ca. 1940. The sequence and synergy of disturbance resulted in the loss of much of the historically dominant *P. echinata* and replacement by even-aged stands dominated by *Q. velutina* and *Q. coccinea* (Beilmann and Brenner 1951, Cunningham and Hauser 1989, Voelker 2004).

### Data collection and analysis

A single increment core extracted at breast height (1.3 m) perpendicular to the aspect of the slope was kept for each tree sampled in this study. An increment core, diameter at breast height (dbh), and height measurements were obtained for each overstory *Q. velutina, Q. coccinea,* and *P. echinata* tree on the smaller 58 outlying plots. A maximum of 20 overstory trees of the same three species were randomly selected to be sampled from the larger MOFEP plots that included an average of ~38 overstory trees per plot. Tree height was estimated to the nearest 0.5 m using a clinometer and nylon tape stretched perpendicular to the slope. If a tree was judged to be sound but the initial core taken was too far from the pith, up to two more cores were extracted and the one closest to the pith kept for analysis. Cores from dead or partially rotten trees were excluded. Of the original 614 live trees cored from the 59 outlying plots and 984 trees from the remaining 88 MOFEP plots, we used 1443 tree ring series for this investigation. Each core was stored, labeled, and later mounted on a wooden stave. Once mounted, each core was sanded with progressively finer sandpaper (150, 250, 400, and occasionally 600 grit). Tree rings were measured to the nearest 0.01 mm using an electronic transducer and binocular microscope fixed over a moving stage (John Roberts Incorporated, Model 1, Columbia, Missouri, USA). Before measurement, ages were determined for all cores and visually cross-dated using two or more distinctive growth patterns from tree rings formed during alternating wet and dry growing periods. Those signature periods were known from the instrumental data record over the past 100+ years and numerous regional dendrochronological studies previously undertaken by the authors. Visual cross-dating was checked on each tree ring series with the program COFECHA in the tree ring data program library (Holmes et al. 1986). Ring width data used to relate interannual stem growth patterns were detrended and standardized following established procedures (Cook et al. 1990). Detrending used the standard settings of the CRONOL program from the dendrochronology program library (*available online* from the Laboratory of Tree-Ring Research, University of Arizona, Tuscon, Arizona, USA),[2] which accommodates highly replicated time series data. Other ring width or radial growth data were not detrended or standardized. Conversion of ring widths to basal-area increment was not used because doing so would have unnecessarily added error to age-constant data through the cumulative calculation of this variable from an often uncertain pith location and innermost ring width values. Where ring width data are shown by cambial age, age 1 refers to the first tree ring produced outside the pith at the height the sample was taken.

In order to assess the influence of climatic patterns and whether some long-term trends in important climatic variables might exist, we obtained monthly data of precipitation, temperature, and Palmer drought severity index values (PDSI), and for the nearest climatic division, reconstructed annual values of PDSI (Cook et al. 1999) using data from the National Oceanic and Atmospheric Administration, or NOAA (*available online*).[3] PDSI is a commonly applied regional index of relative drought (negative values) or wetness (positive values), which is calculated from a number of physiologically relevant atmospheric variables as monthly means (Palmer 1965). We used the program DENDROCLIM (Biondi and Waikul 2004) to determine if the current growing season mean PDSI (May–August in the year of tree ring formation) was a temporally consistent predictor of ring widths for *Q. velutina, Q. coccinea, Q. alba,* and *Q. stellata* (M. Stambaugh and S. Voelker, *unpublished data*). Climate response in terms of tree ring growth for *P. echinata*, the only conifer species investigated here, can be considered more complex than for the *Quercus* spp. investigated, although current growing season PDSI is still an important predictor of growth (Stambaugh and Guyette 2004).

Those tree ring series in our database that were visually judged (Applequist 1958) to be >5 years from the pith were excluded from the analysis. To estimate ring widths for increment cores with ≤5 years missing, a least-squares linear regression model was developed for both the pooled *Quercus* spp. and for *P. echinata*. The mean ring width of the first 10 cambial ages from increment cores with the pith intact were used to establish a linear model describing change in ring width for that short sequence of growth. Modeled ring widths at each cambial age from 1 to 5 years for both groups

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[2] ⟨http://www.ltrr.arizona.edu/software.html⟩
[3] ⟨http://www.ncdc.noaa.gov/paleo/pdsiyear.html⟩

were subsequently compared to the samples for which the pith was intact. No significant differences were found for any of the five cambial ages compared in the *Quercus* spp. or *P. echinata*, ($t$ tests, $P$ values ranged from 0.14 to 0.99 for the 10 comparisons).

Despite our efforts to sample a representative selection of plots on the landscape, it could be argued that older trees are more likely to occur on low-productivity sites where logging has been less frequent. However, this potential source of error was inherently minimized by our sampling procedures, which would rarely have sampled the ends of the site productivity continuum for this region. To determine whether or not there was a trend associated with site productivity, we plotted tree heights by pith date for the same trees investigated for growth rates. If there was a distinct bias to our sampling, mean height should have been significantly greater in younger stands, at least in portions of the sample that were old enough to have neared their asymptotic tree height.

Linear regression was used to assess possible trends over time in means of ring width, tree heights, monthly divisional precipitation, monthly divisional temperature, and PDSI. Linear regression was also used to investigate the relationship of mean ring widths by cambial age to an estimate of historical trends in $CO_2$ reported by McCarroll and Loader (2004). The original $CO_2$ data contributing to the trends reported by McCarroll and Loader (2004) since 1850 were from Antarctic ice cores (Etheridge et al. 1996) as reported by Robertson et al. (2001). As indicated in Robertson et al. (2001), the Keeling instrumental record at Mauna Loa, Hawaii, USA, was used for the period after 1958. Both monthly and yearly average $CO_2$ concentration data from Mauna Loa are available online from the U.S. Department of Energy.[4]

We added a final comparison of growth rates to help independently verify that our samples from older trees were not from inherently slow-growing individuals. No representative data of the shorter-lived and relatively fast-decaying wood of *Quercus* spp. were available. However, preservation of the resinous heartwood of *P. echinata* has made stumps and snags from earlier growth periods and $CO_2$ conditions relatively common in many parts of southeastern Missouri. Those remnants from past logging and natural tree deaths have provided a wealth of information regarding disturbance history and climatic patterns for the region (Guyette et al. 2002, Stambaugh and Guyette 2004, Voelker 2004). For our comparison we used three data sets from Missouri (*available online* from the international tree ring databank, ITRDB).[5] The data sets included collections from Shannon County and the Missouri Ozark Forest Ecosystem Project, contributed by R. Guyette and M. Stambaugh, as well as the Lower Rock Creek collection,

contributed by D. Duvick. Because pith dates are uncertain for data derived from the preserved *P. echinata* remnants, only tree ring series displaying a negative exponential age-related pattern common to open-grown trees were selected. The resulting data set would be unlikely to include tree ring series missing much of the early growth period. Two characteristics of the data make this comparison quite conservative for our purposes: (1) the older *P. echinata* series were often taken from stumps (e.g., 0.2–0.8 m height) with taper or butt-swell, which would tend to increase mean ring widths in comparison to our increment cores of recently living trees taken at 1.3 m height; (2) only negative exponential patterns of ring widths that usually characterize open-grown trees were selected, putatively biasing this older set of trees toward greater overall growth. As the final criterion for inclusion in the historical *P. echinata* data set, the tree ring series needed to start before 1850. Of the 178 total tree ring series from the ITRDB, only 59 fit this criterion. Since response to $CO_2$ has been reported to decline with tree age (Hättenschwiler et al. 1997, Idso 1999), the ring widths from older trees should exhibit a much smaller response to the modest range in $CO_2$ levels investigated here. To more accurately align the pre-1850 *P. echinata* chronology along the cambial-age axis, we compared the last 10 cambial-age ring widths to similar cambial ages of the post-1850 chronology. Even when the pre-1850 cambial-age chronology was aligned directly with the current cambial-age chronology, the ring widths for cambial ages 41–50 were visually and statistically indistinguishable. This implied that usually there were few tree rings missing at the beginning of the series owing to increment cores inaccurately taken or to heartrot at the center of stem cross-sections. As a conservative measure we shifted the pre-1850 series to start at age 5, which would assume an average of four missing rings.

## RESULTS

Individual *P. echinata* trees established over the most recent 100 years have more frequently attained greater diameters for the same cambial age as those established in previous centuries (Fig. 2A). When compared to our randomly collected increment core data, the ring width responses are similar, showing an overall trend in ring width from ~1.25 mm prior to 1850 to nearly two times that in more recent years (Fig. 2B). The developmental pattern of ring widths is similar between *Q. velutina* and *Q. coccinea* (Fig. 3A), justifying our pooling of growth data from the *Quercus* spp. over at least the initial 50 years of development. The developmental curves of ring widths for both groups of *P. echinata* trees, established before or after 1850, exhibit much greater growth during the early, juvenile period than that of the *Quercus* spp. (Fig. 3B). The same data indicate that *P. echinata* trees established prior to 1850 grew less while younger than did their more recently established counterparts (Fig. 3B).
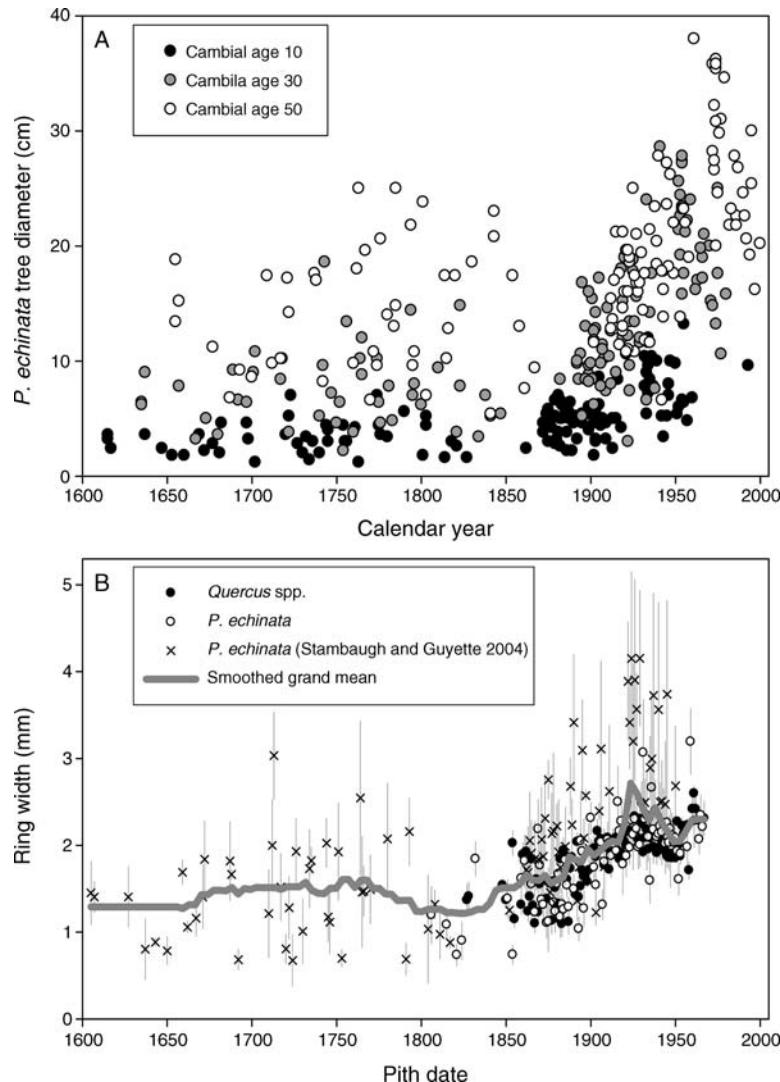
FIG. 2. (A) Diameters of individual *Pinus echinata* calculated from ring widths starting at the pith for three cambial ages (after Stambaugh and Guyette [2004]). (B) Mean ring width responses by pith date. The means (±SE) were calculated across 11 equally spaced cambial ages from 1 to 50, for randomly sampled *Quercus* spp., and *P. echinata* (see *Methods: Data collection and analysis*). The means for *P. echinata* (Stambaugh and Guyette 2004) were calculated from the three cambial ages shown in the top panel but were shifted to the pith date for accurate intercomparisons among data sets. The smoothed grand mean was calculated across each of the closest 14 ring widths.

Growth rates have increased over the past 150 years for *P. echinata* and the *Quercus* spp. (Figs. 2, 3B, 4). We sought to quantify how their growth rates changed with tree age. All slope coefficients from linear regressions for the calculated diameters from the *Quercus* spp. were significant, $P < 0.0001$ (Fig. 4), and varied directly with cambial age. When the slopes were divided by their respective ages the responses were similar (age 1 = 0.0016, age 5 = 0.0015, age 10 = 0.0016, and age 20 = 0.0016). However, this apparently common signal among cambial ages appears to be fortuitous (to be discussed later in this section). Fig. 3 shows that age-constant ring width responses are not similar across tree

ages when adjusted for tree form, growth $CO_2$ levels, and developmental stage.

For each of cambial ages 1–20, a major growth pulse occurs between about 1910 and 1930 (Fig. 4). The continued dominance of these pioneer trees after this period of disturbance and resource release is still evident at cambial age 20. On the graph for cambial age 20, we plotted the frequency of establishment (pith dates) for all *Quercus* spp. and *P. echinata* from the random sample of trees across the region. The steady increase in establishment frequency just preceded the large increase in growth over the common period of reliable establishment data for the tree ages we sampled (ca. 1860–1945). The timing of this growth pulse is consistent with the
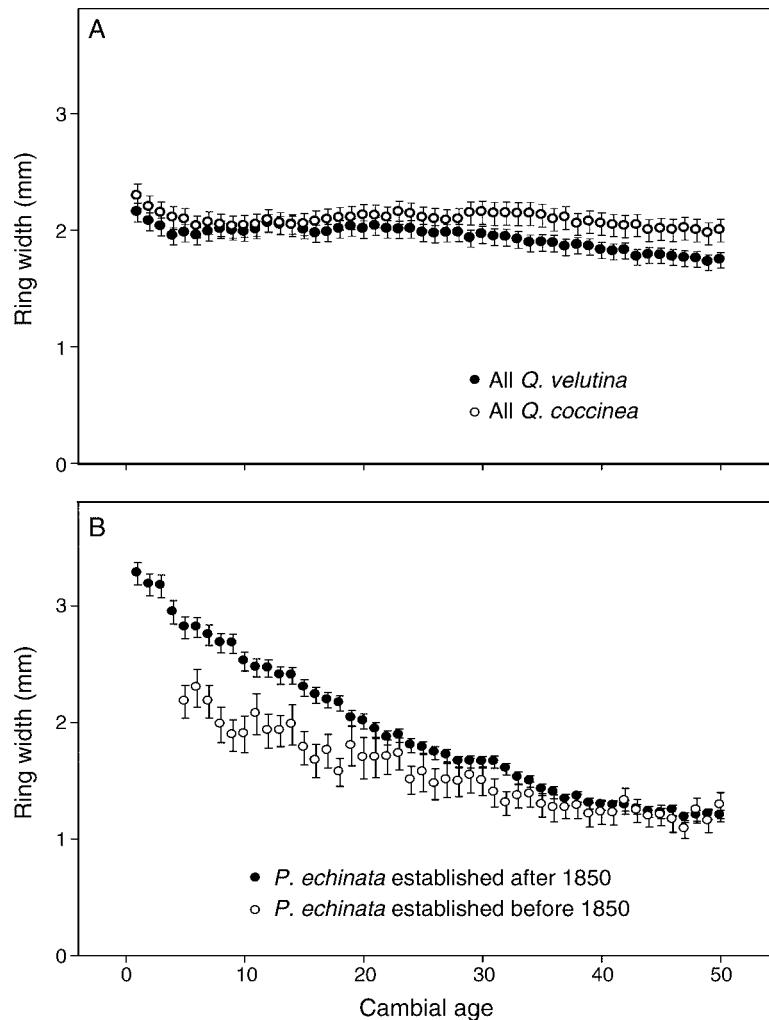
FIG. 3. (A) Ontogenetic trends in ring width (mean ± SE) of *Q. velutina* and *Q. coccinea* and (B) two groups of *P. echinata* that differ by period of establishment and growth. Open symbols in panel B were calculated from previous dendrochronological studies of *P. echinata* in the Missouri Ozarks (*available online* from the ITRDB; see footnote 5).

period of intense logging and fire disturbances in the region. Similarly smoothed *P. echinata* data match the low-frequency "disturbance response" from the *Quercus* spp. from Fig. 4 as indicated by a linear regression model relating these two variables ($R^2 = 0.78$, $P < 0.0001$).

Trends in site productivity were evaluated by plotting heights of mature *Quercus* spp. and *P. echinata* by their pith dates. This method evaluates potential bias because for most species, the maximum or age-specific tree height can be used as a metric of site productivity. Mean tree height showed no significant trend when plotted by pith date, indicating no bias due to site productivities sampled (Fig. 5). Though the age at which trees reach a nearly asymptotic height may be less when higher growth rates associated with increased CO₂ levels are exhibited, we know of no direct evidence to suggest that potential maximum height increases appreciably. There-

fore we expect no trend in asymptotic tree height to influence this assessment of site productivities.

The long-term increases in atmospheric CO₂ have a significant relationship with growth at each cambial age for *Quercus* and *P. echinata*, though the strength of that relationship lessens with age (Table 1). When the slope coefficients from each cambial age in Table 1 were plotted as a percentage increase from the current ontogenetic ring width patterns in Fig. 3, a developmental relationship for each group was found (Fig. 6).

In order to readily compare the relative magnitudes of possible sampling bias, we plotted the predicted *P. echinata* ring width for a given value of atmospheric CO₂ during the years 1850 and 1970 from the data in Table 1 and the mean of ring widths for *P. echinata* established prior to 1850 (Fig. 7). Those calendar years and developmental periods were chosen because they were common to both data sets for cambial ages 5–50. Altogether the data reinforced the conclusion that *P.*
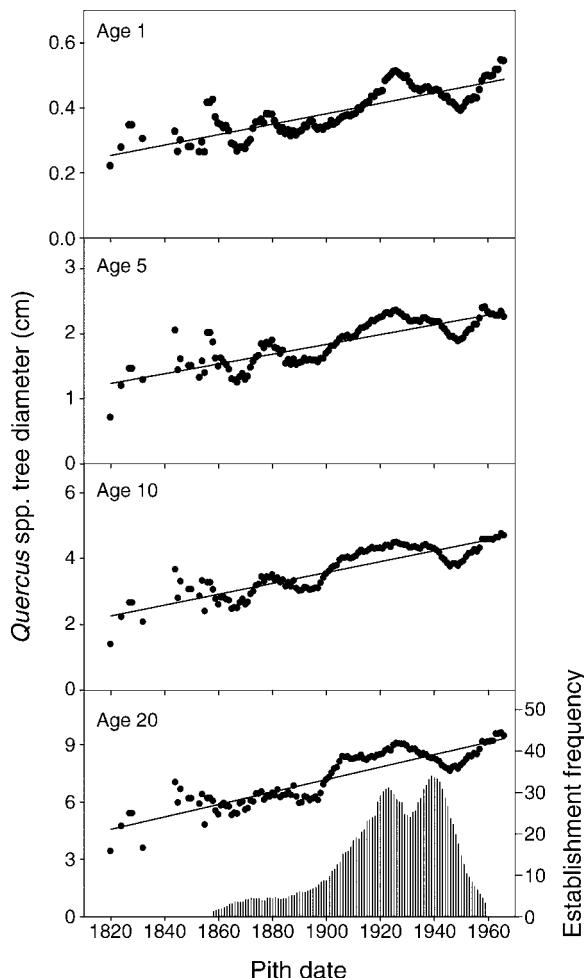
FIG. 4. Calculated diameters from ring widths and "establishment frequency," plotted by pith date for the pooled *Quercus* spp. at four cambial ages. To more clearly show lower-frequency variation in growth, we applied a seven-year moving window that averaged the ring widths on three years on each side of any calendar year. Linear regressions of tree diameter vs. the year of pith date were all significant ($P <$ 0.0001), with or without smoothing applied to the data. The bottom panel includes a seven-year moving average of establishment frequency, or the frequency of pith dates, at dbh from all *Quercus* spp. and *P. echinata* trees from randomly located plots. The establishment frequency falls sharply after 1940 because our methods only sampled stands older than ~50 years. Estimates of variation in diameter for a calendar year are not shown due to the smoothing technique we used.

*echinata* had increased in growth since 1850, a result close to our predictions from the relationship of ring width by cambial age and atmospheric-$CO_2$ levels. There is however some level of directional bias, as can be seen in Fig. 7 (see *Discussion*).

To present a more complete picture of the possible influence of longer-term changes in regionwide environmental signals, we plotted annual values of relevant climatic data as well as the atmospheric $CO_2$ levels used and some tree ring growth responses for the region (Fig.

8A–E). Interannual variability in ring widths of the *Quercus* spp. respond most strongly to growing-season wetness as indicated by PDSI, and no long-term trends in precipitation, temperature, or PDSI values were found to explain long-term growth increases.

## DISCUSSION

Our results clearly show that stem growth of *Quercus* spp. and *P. echinata* of the Missouri Ozarks region has greatly increased over the past 150 years. This is the first evidence we are aware of that shows increases in growth largely consistent with rises in historical $CO_2$ levels (1) for conifers and hardwoods across a productive region and (2) across a developmental sequence through canopy closure and maturity. Given the demonstrated interspecific variation in $CO_2$ effects (Körner et al. 2005, Norby et al. 2005), the common rates of growth enhancement among the two different growth forms investigated may be fortuitous. Also, the analysis and data provide a starting point for comparing age-constant growth rates in other systems.
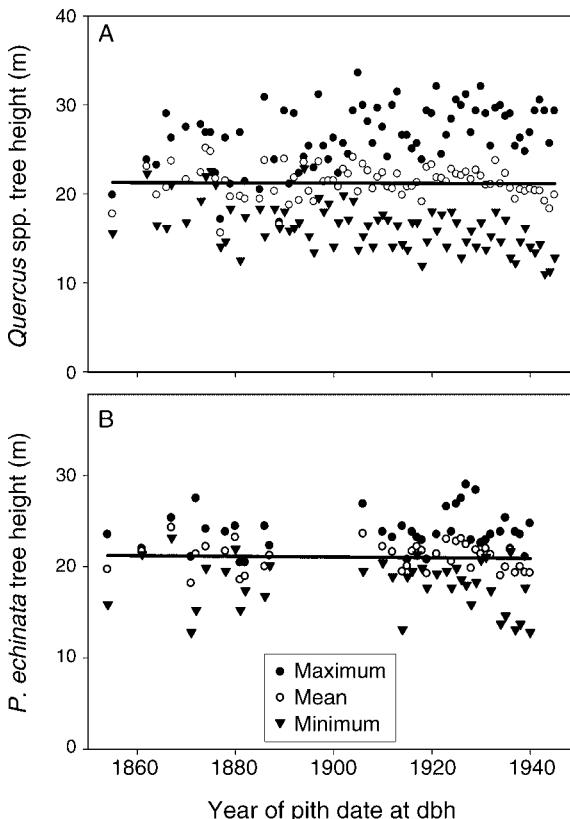


FIG. 5. Mean height of (A) the two *Quercus* spp. and (B) *P. echinata* for the period investigated for years in which there were at least two observations. The linear regression model fit to mean height was not significant ($P > 0.7$) for either plot. *Quercus* spp. established since 1945, and *P. echinata* since 1940, were excluded, as these trees had not yet approached their height potential when measured in 2002 and 2003.

TABLE 1. Characteristics for age-related changes in linear regression models that relate ring width of two *Quercus* spp. and *P. echinata* to mean annual atmospheric CO$_2$ levels since 1850.

| Species | Age in years | df | Tree ring series | Range in years | Range in CO$_2$ (ppm) | Slope coefficient | F | Adjusted $R^2$ | P |
|---|---|---|---|---|---|---|---|---|---|
| *Q. velutina* and *Q. coccinea* | 1 | 1, 103 | 1200 | 1850–1967 | 285.2–322.0 | 0.0269 | 42.9 | 0.29 | <0.0001 |
| | 5 | 1, 104 | 1200 | 1853–1972 | 285.5–327.3 | 0.0226 | 33.5 | 0.24 | <0.0001 |
| | 10 | 1, 104 | 1200 | 1858–1977 | 286–333.7 | 0.0212 | 40.3 | 0.27 | <0.0001 |
| | 20 | 1, 104 | 1200 | 1858–1987 | 286–348.8 | 0.0158 | 27.7 | 0.20 | <0.0001 |
| | 30 | 1, 104 | 1200 | 1858–1997 | 286–363.8 | 0.01 | 18.0 | 0.14 | <0.0001 |
| | 50 | 1, 98 | 1123 | 1867–2002 | 287.6–371.5 | 0.002 | 14.6 | 0.12 | 0.0002 |
| *P. echinata* | 1 | 1, 80 | 243 | 1851–1966 | 285.3–321.2 | 0.049 | 25.3 | 0.23 | <0.0001 |
| | 5 | 1, 80 | 243 | 1856–1971 | 285.8–326.2 | 0.038 | 16.9 | 0.16 | <0.0001 |
| | 10 | 1, 80 | 243 | 1861–1976 | 286.5–332.0 | 0.025 | 9.1 | 0.09 | 0.0035 |
| | 20 | 1, 80 | 243 | 1871–1986 | 288.4–347.0 | 0.018 | 11.1 | 0.11 | 0.0013 |
| | 30 | 1, 80 | 243 | 1881–1996 | 291.1–362.7 | 0.012 | 13.5 | 0.13 | 0.0004 |
| | 50 | 1, 79 | 227 | 1891–2002 | 294–371.5 | 0.005 | 3.9 | 0.03 | 0.0531 |

We attribute the pulse of growth and establishment (Fig. 4) to concurrent peaks in anthropogenic fires and disturbance levels. Besides increased irradiance available to sprouts and newly established trees under such conditions, warmer temperatures and less soil water transpired after completion of harvest or major disturbance would have increased mineralization rates and short-term releases of nutrients bound in the litter layer. Though fire has long played a role in the Ozarks, levels of logging activity, land-clearing, burning, and later land abandonment were greatest between 1880 and 1940 (Guyette et al. 2002). The overall peak in establishment from ~1920 to 1940 is best explained by those disturbances. We can only speculate that the intervening trough between the maxima for establishment frequency may be due to frequent droughts during the "dust bowl" period that coincided with lower levels of logging after

the stock market crash of 1930 and a depressed U.S. economy prior to World War II.

There are a number of potential influences for the long-term growth rates, and their relative importance will be assessed. First, tree ring studies of long-term growth rates may have inherent biases due to the selection of only larger, dominant trees (Cherubini et al. 1998, 1999). These biases are most likely to occur in longer-lived species that are characterized by a greater shade tolerance, where some individuals may differ widely in terms of canopy position during stand development. Such nonrepresentative sampling is not likely to have occurred with the species we investigated, which are intolerant of understory conditions and rarely showed release events related to gap dynamics.

The data presented in Fig. 6 is near the higher end of previously reported experimental studies for rates of growth stimulation per unit of CO$_2$ increase (from Medlyn et al. 2001, calculated as percentage change in
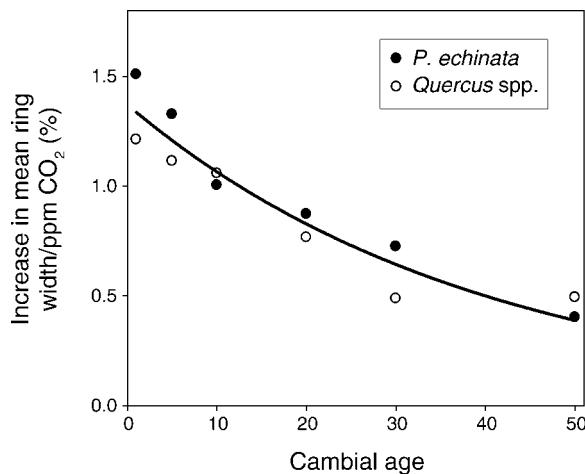


FIG. 6. Percentage increase in ring width with ambient CO$_2$ levels since 1850, plotted by dividing the slope coefficients from the linear regression models (as in Table 1) by the mean ring width for a specified cambial age for *P. echinata* and the *Quercus* spp. (×100; for each group shown in Fig. 3). The nonlinear regression, % increase $= 1.37 \times e^{(-0.0252 \times \text{age})}$, yields a declining relationship during ontogeny across both groups of species since 1850 (adjusted $R^2 = 0.90$, $F = 104.0$, $P < 0.0001$).
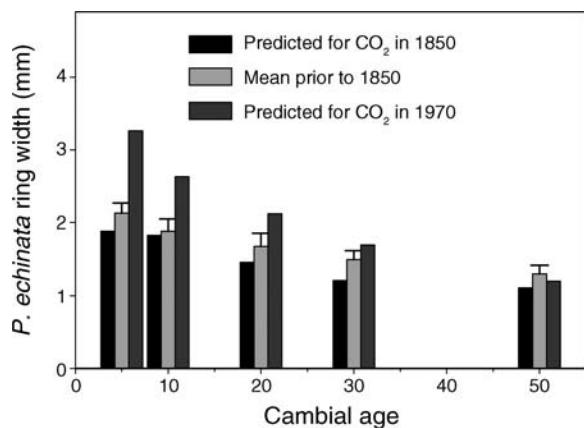


FIG. 7. Predicted and observed ring width data of *P. echinata* for three different periods. Predicted ring widths were from linear regression models presented in Table 1. Mean ring widths ($\pm$SE; $n = 59$) prior to 1850 were from the ITRDB with criteria that required that each tree ring series display an "open-grown" negative exponential trajectory in ring width (see *Methods: Data collection and analysis*).
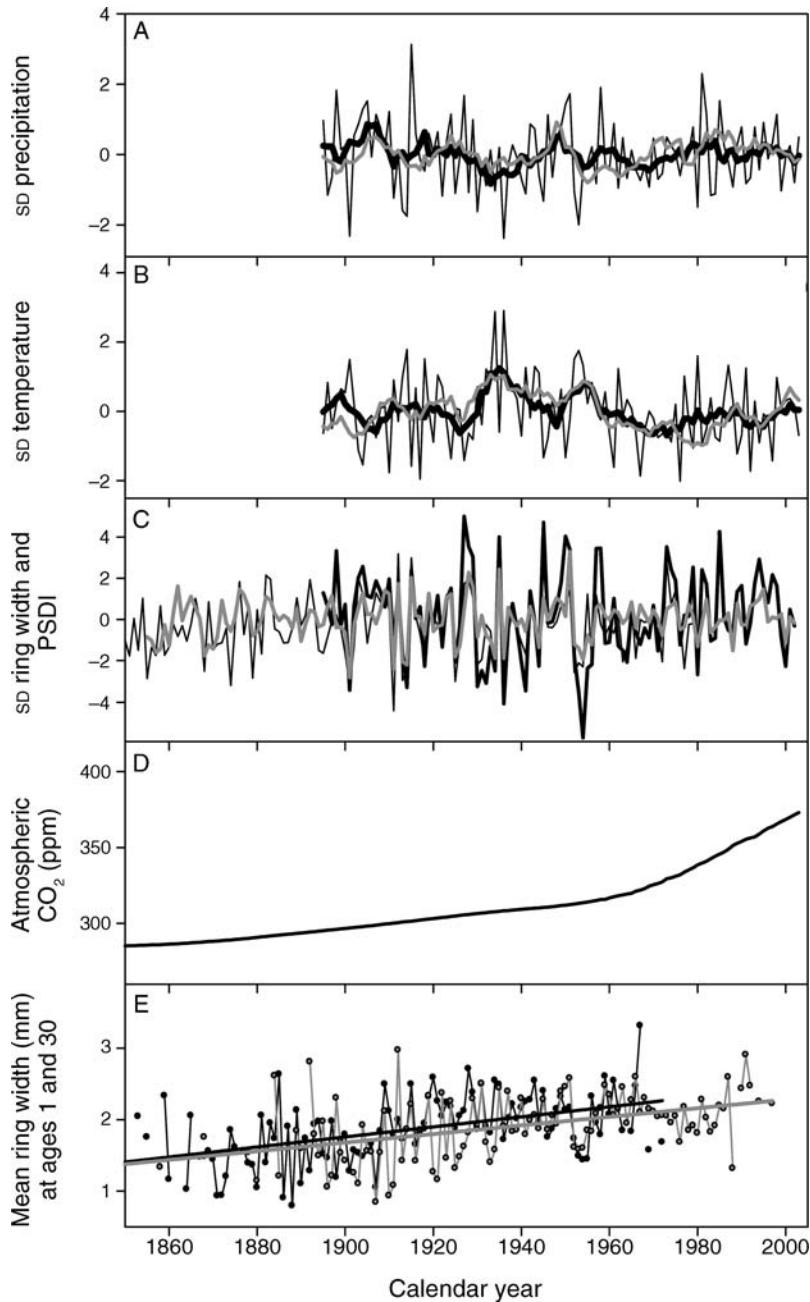
Fig. 8. (A) Standard deviations (sᴅ) from mean precipitation and (B) standard deviations from the mean temperature, where the bold black line is a seven-year moving average for the summer growing season (May–August), the gray line is a seven-year moving average for the yearly total, and the fine black line shows annually resolved summer precipitation. (C) Standard deviations from the mean annual summer Palmer drought severity index (PDSI; bold black line), the detrended stationary mean annual ring width index of all *Quercus* spp. sampled (gray line), and an independent reconstruction of PDSI from this region (fine black line; after Cook et al. [1999]; data from the NOAA web site ⟨http://www.ncdc.noaa.gov/paleo/pdsi.html⟩). (D) The trend in atmospheric $CO_2$ levels used for this study (after McCarroll and Loader [2004]). (E) Mean ring widths for cambial ages 1 (black circles and regression line) and 30 (gray circles and regression line); lines connecting data points are from adjacent years.

$CO_2$ enrichment). The data from our observational study, however, should be expected to differ from past experimental studies in more than a few respects. Recent dominance by the intolerant overstory trees we sampled has resulted from past selection against their

slower-growing competitors. Presumably, such trees were generally the fastest growing and received less light, water, nutrients, or rooting space early in development. Therefore, we would have selected the individuals most capable of capturing the greatest

direct $CO_2$ effects and increases in ontogenetic development. Such selection contrasts with $CO_2$ effects as determined from a population mean of all individual seedlings, fast or slow growing, in experimental studies. In addition, past $CO_2$ chamber studies used much greater absolute treatment $CO_2$ levels, which could exaggerate rooting-space constraints or N dilution from a constant amount of space or mineral nutrition supplied to both control and $CO_2$-treated plants that gain biomass faster. Those conditions could limit the upper range of $CO_2$ effects observed (Farage et al. 1998, Idso 1999). An interesting question about scaling and tree species effects arises. Would it be less biased to scale the average short-term experimental $CO_2$ effects across competitive abilities to natural populations of shade-intolerant tree species that quickly "self-thin" and are increasingly composed of the least limited individuals of a population, or to scale the effects from a retrospective study, after past competition has eliminated the slow growers? To answer this question would require using both approaches to quantify those biases. Our study attempted the latter, retrospective approach. It is interesting to note that in terms of experimental $CO_2$ responses of species closely related to those we investigated, Q. alba tended to have the greatest stimulation of biomass increment to $CO_2$ enrichment, whereas growth stimulation of Pinus spp. was nearer the middle of the responses for the tree species reviewed by Norby et al. (2005). When adjusted for leaf area, which is not possible with our data, the same review (Norby et al. 2005) indicated species-specific responses were more consistent, and growth stimulation was considerably less.

### Drought-mediated selection as an alternative hypothesis for growth stimulation

Owing to the observational nature of our data, it is important that we address in detail the possible sources of error that could have influenced our results and interpretation. During another investigation of growth rates, we found that early patterns of basal-area increment from declining and dead Quercus spp. regularly exceeded that of recently healthy concomitants in all age groups investigated (Voelker 2004). Therefore one competing hypothesis that could explain the long-term increases in growth is that fast-growing Q. velutina and Q. coccinea were more susceptible to drought-induced embolism, branch dieback, subsequent decline, and premature death. As a result, one could argue that older trees might exhibit a conservative pattern of growth by way of a progressive selection against fast growers. This alternative hypothesis may still merit consideration of other processes; however, it can be rejected here as an important influence because the drought-tolerant P. echinata increased in stem-radial growth over the same period and by nearly the same magnitude as the Quercus spp.

### Possibility for sampling bias as an alternative hypothesis for growth stimulation

The great effort we devoted to sampling a large number of plots randomly located over the region would tend to minimize any systematic bias in our assessment of growth trends over the past 150 years. Our evidence showed that maximum heights plotted by pith date did not decrease in progressively younger stands (Fig. 5), which could be interpreted initially as an indication of some directional sampling bias. However, the fact that maximum heights did not decrease in younger stands is a result of fewer old trees being sampled (see establishment frequency from Fig. 4) and less chance of sampling extremes in the distribution occurring for those years. That age-related trend in sampling intensity and resulting height patterns are evident for the Quercus spp. in Fig. 5 where, with the exception of the oldest year investigated, the maximum and minimum heights both trended away from the mean in progressively younger and more intensively sampled tree ages.

The data from Fig. 7 indicate that some consistent but small error was present for two reasons, neither of which is exclusive: (1) predicted ring widths for 1850 were underestimated, (2) ring widths from the preserved P. echinata remnants (stumps likely to have larger rings near the base of the tree) exaggerated growth during this period. The data in Fig. 7 suggests a slight overestimate of predicted growth in 1970 for ages 5 and 10 when compared to ring widths shown in Fig. 3B. However, the difference was not surprising, since most of the trees used for Fig. 3B established long before 1970 under much lower $CO_2$ levels. An error in growth estimates could only have changed the direction of the relationships for P. echinata at cambial age 50, where the variance in the interannual values was the greatest, and the purported $CO_2$-related growth enhancement was weakest. Altogether the evidence for a substantial increase in stem growth of P. echinata is strong (Fig. 2). Notwithstanding our assessment of bias (Fig. 7), which shows some degree of error for the relationship in Fig. 6, any adjustments would likely be minor in comparison to the growth stimulations we report.

### Long-term climatic changes as an alternative hypothesis for growth stimulation

Annually resolved or other lower-frequency patterns in the climatic data for the whole year or for the summer growing season included no significant long-term trend and did not appear to have included a signal with the ability to influence the patterns of increasing growth apparent in both Quercus and Pinus. Pearson's product moment correlation ($r$) between Palmer drought severity index, an independent PDSI proxy, and the Quercus spp. chronology were all significant ($P > 0.0001$), ranging from $r = 0.73$ to 0.69. The high correlations among these variables are indicative of the consistent coupling of radial growth of Quercus spp. in this region to current-year wetness during the summer growing season. The

ring width data that made up the *Quercus* spp. chronology and the PDSI proxy could not indicate long-term trends because of the statistical detrending used and segment length curse. Summer PDSI, a measure of the growing-season wetness that determines much growth for *Quercus* spp. in this region, shows a slightly increasing but nonsignificant trend ($P = 0.14$). In contrast, atmospheric $CO_2$ and growth over the same period show long-term trends (Figs. 2, 8D, E). Linear regression models of ring width by calendar year were included in Fig. 8E for visual comparison, but were of limited value because of the nonlinear nature of atmospheric $CO_2$ levels over the more recent period.

Evidence from other studies suggests that warmer temperatures may have affected tree growth as well as other patterns of vegetation in higher latitudes or elevations (Briffa et al. 2001, IPCC 2001: Chapter 2, Esper et al. 2002, Esper et al. 2003, Naurzbaev et al. 2004, Büntgen et al. 2005, Root et al. 2005). In those cooler areas, growing season temperatures are generally most limiting to tree growth. The temperature regime for the Missouri Ozarks region is certainly not a constant. Oscillations in surface temperatures of the United States as a whole and of our region are recorded by the instrumental record, but the record shows no steady warming trend, as has been seen globally (Fig. 8B, Hansen et al. 2001). Warming would affect a number of biological or ecological processes. For example, increases in nighttime minimum temperatures would increase tissue respiration rates and might also increase mineralization rates through most of the year. Increases in daytime temperatures during dormant periods could stimulate earlier budbreak and a longer growing season, but the same magnitude of increase during the growing season would increase vapor pressure deficit and more quickly limit transpiration and photosynthesis. Given those offsetting costs and benefits of warming at the low elevations and mid-latitudes of the region, we consider past oscillations in temperature (Fig. 8B, Hansen et al. 2001) to have affected tree growth as a second-order variation, with greater frequency than has the long-term influence of consistently rising $CO_2$ over the past 150 years.

### Changes in nitrogen availability as an alternative hypothesis for growth stimulation

Three extensive studies of conifer tree rings in Europe all showed increasing age-constant stem growth patterns over the past 100–200 years (Nicolussi et al. 1995, Bert et al. 1997, Rolland et al. 1998). Indirect evidence from Rolland et al. (1998) suggests a decreased rate of age-constant growth stimulation with increasing tree age. Though increasing $CO_2$ may have been important for this increased growth, soil conditions and N deposition have been shown to have great influence on trees of the region (Hättenschwiler et al. 1996), and warmer temperatures or a longer growing season at the higher elevations of the region could have added to the growth stimulation.

Indirect evidence suggests that soil N, inferred from organic matter at sites across central Missouri, has undergone little observable change from 1930 to 1980 (Walters 1981). Also, a dendrochemical study showed no apparent trend in N concentrations of heartwood samples from long-lived *Juniperus virginiana* L. trees that grew on rocky glades in the region over the past 300+ years (R. Guyette, *unpublished data*; $n = 75$, 20-year tree ring groups with Kjeldahl analysis of N concentrations from 6 trees). The abrupt heartwood–sapwood transition and low moisture contents ($<25\%$) in the heartwood of *J. virginiana* decreased the radial mobility of nutrients (sensu Elhani et al. 2005) and made this species a good choice for dendrochemical reconstructions of past tree nutrient uptake (Cutter and Guyette 1993). However, because N was likely limiting on these sites and highly conserved within trees, it would be difficult to establish whether small atmospheric N additions existed even though nonlimiting sulfur and calcium concentrations from the same tree rings included trend and variability, which were clearly due to anthropogenic influences over the same period (Guyette et al. 1989, 2002).

Mean fire return intervals from 27 sites in the study region ranged from 2.3 to 45 years prior to 1850, from 1.5 to 6.8 years between 1851 and 1890, from 1.7 to 19 years between 1891 and 1940, and from 6.8 to 50 years between 1941 and 1990 (Guyette et al. 2002). Though we attribute a pulse of growth in newly established trees to disturbance-released resources from 1880 to 1940 (Fig. 4), the fires are likely to have led to N losses from erosion, leaching, and volatilization (Wan et al. 2001). Given those repeated fires and disturbances, it seems unlikely that N deposition could have increased enough, cumulatively, to satisfy microbial uptake and storage in the soil and to have progressed enough to significantly decrease the degree of N limitation of tree ring growth consistently throughout the past 150 years. However, without knowledge of past and present pool sizes, or of gross and net N cycling rates, there is no way to quantify potential changes in N availability.

The mode of reproduction and tree form are other important considerations for possible limitations in the low N concentrations needed to add wood increment to stems. The relatively large root systems of sprout origin trees can store considerable photosynthate and N during early development, which permits greater photosynthesis and growth (sensu Kruger and Reich 1993a, b, Lockhart et al. 2003, Peña-Rojas et al. 2004). Hence, those stores along with access to deeper soil water help explain why the *Quercus* spp., often originating from sprouts, did not decrease in ring width as steadily as was seen in *P. echinata* (Fig. 3). Despite the differences in reproduction and available resources between the *Quercus* spp. sprouts and young *P. echinata,* the age-constant growth stimulation is consistent between these disparate growth forms and strategies. The lack of differentiation of growth stimulation suggests either a highly dominant or an insignificant role for N as a co-

limiting factor in the youngest growth stages, with the latter seeming more likely. Altogether, though N deposition has likely increased over the past 50–100 years, its availability to the trees is surely of less magnitude and does not coincide with the inception of the stem growth increases ~150 years ago.

### Patterns of ontogenetic response

Due to the existence of multiple influences on age-constant growth over the past 150 years, it is nearly certain that our data do not represent an entirely "clean" CO$_2$ effect. Moreover, a small exaggeration of the purported CO$_2$ effect may have resulted from the cumulative effect of unquantifiable factors that alone would not have been easily observed as a significant influence on the growth stimulation noted. Nevertheless, a decreasing rate of CO$_2$-induced growth stimulation with age was not unexpected, being consistent with other studies (Hättenschwiler et al. 1997, Idso 1999, Wang et al. 2006). The interpretation of the average response to CO$_2$ we report during stand development is not entirely straightforward because the older developmental ages occured over a more recent period with greater absolute atmospheric CO$_2$ concentrations and greater rates of CO$_2$ increase. However, photosynthetic response to such a range of CO$_2$ concentration is linear, so it should not have undue influence over our results. Given the inevitably rising CO$_2$ concentrations and concomitant ontogenetic advances in growth, the relationship shown in Fig. 6 may become steeper yet as N and other nutrients limit productivity after being fixed in stems and other recalcitrant tissues. In other words, as CO$_2$ increases and trees grow faster, the maximum height potential will be reached earlier in stand development, and the developmental decrease in relative growth rate will be greater despite greater absolute growth.

Also, it may be instructive to consider the purported CO$_2$ effect relative to age by species. That can be done by extrapolating from the relationship shown in Fig. 6, as applied to rough estimates of maximum ages attained by the species investigated (~130 years for *Q. coccinea*, ~200 years for *Q. velutina,* and ~250 years for *P. echinata*). This relationship, even under substantially increased CO$_2$ conditions, would constitute a diminishingly small increase in biomass increment as these species aged past half of their maximum ages. If this range of response were similar among species, then it should not be surprising if past dendrochronological investigations of old trees had established no consistent response (Jacoby and D'Arrigo 1997) and that experimental CO$_2$ enrichment of mature trees did not find any significant increases in growth, although more total carbon was fixed and more quickly transferred from the canopy to the soil (Körner et al. 2005). One major implication of these findings is that the complex biological controls regulating tree growth later in development are far from being understood, if carbon becomes less important as a co-limiting factor.

A number of influences may combine to account for the age-related decline in sensitivity to increasing CO$_2$ levels. As indicated above, ontogenetic advances in tree size require a steeper decline in relative growth rate if maximum potential height does not increase to an equal degree. Second, the lack of soil N limitation after disturbance and possible N storage for sprouts that dominate early development of forests in the region would tend to maximize CO$_2$-induced tree biomass gains. In contrast, the concomitant N losses commonly documented after disturbances, in combination with increased bound N in woody tissue, could have caused a partial down-regulation of photosynthesis (Stitt and Krapp 1999). Saurer et al. (2003) thought the same phenomenon occurred in the *Quercus* stands they investigated around a natural CO$_2$ spring. Third, leaf area index declines with tree age for the *Quercus* spp. (S. Voelker, *unpublished data*). Combined with a probable decrease in N availability during stand development, the decrease in leaf area per unit ground area would limit the profitability of increased CO$_2$ without proportional increases in light-use efficiency. Finally, CO$_2$ concentrations have generally been found to be negatively correlated with stomatal densities (Beerling 2005). That morphological adjustment can be signaled quickly through whole-plant water use and the transport of key hormones that regulate stomatal development (Miyazuma et al. 2005).

### Implications for future tree ring studies

Due to the potential for confounding the effect of rising CO$_2$ with increased temperatures, caution is urged in the interpretation of recent increases in tree ring growth attributed to warming alone. Some studies have used a curve fit to a regional growth chronology (as shown in Fig. 4) to detrend tree ring series and isolate a temperature signal (sensu Briffa et al. 1996, Esper et al. 2002, Esper et al. 2003, Naurzbaev et al. 2004, Büntgen et al. 2005). A marked change, however, can be found in a climate reconstruction by using detrending curves from different tree age groupings (Büntgen et al. 2005). For most sites with long-lived trees, the creation of a regional growth chronology that could entirely separate low-frequency trends in temperature from CO$_2$ seems nearly impossible. To avoid this bias, we suggest that regional growth chronologies and sample tree ring series selected for estimating temperature trends over the most recent century exclusively use older individuals. The use of tree rings formed after more than one-third the maximum age for a species and site, should preclude the greatest potential for CO$_2$ effects on stem growth. Furthermore, unprecedented climate changes may increasingly challenge the principle of uniform tree climate responses, as CO$_2$ concentrations continue to unprecedented levels, and other effects of global warming qualitatively change growth-limiting factors and climatic response thresholds (Barber et al. 2000, Wilmking et al. 2004).

Future attempts to isolate a $CO_2$ signal from age-constant growth patterns should focus on mid- to lower-altitude and latitudinal regions where past N levels have been consistently low. Ideally, past changes in temperature and precipitation should be known to have shown no low-frequency signal that could obfuscate potential $CO_2$ effects. In order to capture the range of tree ring response during ontogeny over a recent sequence of $CO_2$ concentrations, data should be collected from a species across its entire age range including data from young seedlings and saplings that result from a disturbance and establishment regime consistent with those historically known for a region.

## CONCLUSIONS

The data presented here are unique because they are the first evidence to show regional long-term increases in growth of hardwood and conifer species consistent with fertilization by atmospheric $CO_2$, as well as a similar ontogenetic decline in an apparent $CO_2$ growth response for both tree growth forms investigated. It is unlikely that early growth of historically established stands was greatly influenced by N limitation, though the ontogenetic decline in growth stimulation likely was due partly to progressive N limitation during stand development. The sum of other influences, in addition to a potentially decreased N limitation in more recent years, cannot be ruled out as contributing to the absolute levels of growth stimulation observed. Nonetheless, extrapolation of even some of this growth stimulation attributed to $CO_2$ fertilization across multi-aged forest landscapes could help account for spatial and temporal variation in the global "missing" carbon sink and help mitigate some future atmospheric carbon increases, at least to the extent that nutrient availability would permit (Hungate et al. 2003).

In the relatively drought-prone western edge of the deciduous forest biome of the eastern United States, increases in $CO_2$, WUE, and continued N deposition will likely stimulate further increases in the rates of stand development and carbon storage. Those increases could lead to a greater rate of carbon cycling (sensu Körner et al. 2005) but not necessarily an increase in total carbon stocks. If growth rates continue to rise at the rates documented for the past 150 years, then the net ecosystem carbon balance for the region might be most influenced by timber rotation periods and ensuing lateral transfers of carbon out of the region as products. Secondarily, if Ozark forests are to have fewer fires and are less limited by $CO_2$, N, and soil water deficits with increased WUE, it is possible that some tree species historically selected against in the uplands of this system (*Acer rubrum* L. or *Acer saccharum* Marsh.) could compete more effectively. Over the long term, those species' effects could influence total stocks of carbon through increased light-use efficiency, leaf area index, and a change in cycling and storage rates of carbon and nutrients from the canopy to the soil.

Further knowledge of regional, age-constant growth rates are needed to put the interpretation of our data into perspective. In addition to the free-air carbon exchange (FACE) experiments that might experimentally test more combinations of tree age and species combinations, the inclusion of well-planned tree ring studies across adequately replicated tree age sequences could help clear up uncertainties in species-specific and age-related responses to increasing atmospheric $CO_2$. Despite the limitations of nonexperimental studies, extensive data sets like those should better inform predictions of productivity and rates of carbon storage for a number of forest systems where long-term and large multisite experiments have not been feasible.

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