

physiology

Growth, Photosynthesis, and Cold Tolerance of *Eucalyptus benthamii* Planted in the Piedmont of North Carolina

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Inadequate cold tolerance of *Eucalyptus* spp. has limited a broader deployment beyond subtropical regions of the United States. We examined growth, photosynthetic rate, and cold tolerance of a cold-hardy Eucalypt (*Eucalyptus benthamii*) planted in North Carolina and compared the results with native *Pinus taeda*. After two growing seasons, *E. benthamii* surpassed *P. taeda* in both height (5.06 m, 1.34 m) and diameter (5.0 cm, 0.7 cm) growth, respectively. From April through November, *E. benthamii*'s mean photosynthetic rate (Ps) (13.82 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) was more than twice that of *P. taeda* (6.21 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$). Similarly, mean Ps during winter months was 6.09 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ for *E. benthamii* compared to 2.73 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ for *P. taeda*. Laboratory assessments of cold tolerance demonstrated that, unlike *P. taeda*, *E. benthamii* had a limited ability to acclimate to seasonal temperature changes. The coldest temperature (T_m) where >50% of the tissue was damaged and recovery unlikely for *E. benthamii* was -13.4°C for leaves and -14.3°C for stems, whereas for *P. taeda* it was -29.9°C for leaves and -36.5°C for stems. The exceptional productivity of *E. benthamii* was not without trade-offs as all trees died after experiencing air temperatures of -12.8°C on January 7, 2014.

Keywords: freezing injury, relative electrolyte leakage, plantation, exotic

Intensive silviculture in the southern United States has historically been focused on southern pines (*Pinus palustris*, *P. echinata*, *P. elliotii*, *P. taeda*, *P. virginiana*) with considerable focus on *P. taeda*. With genetic improvement and intensive culture (e.g., site preparation, weed control, irrigation, and fertilization), southern pine productivity has improved markedly since the 1950s (Stanturf et al. 2003). Recent interest and projections of the future role of bioenergy in the United States indicate that other feedstocks may have advantages over pine and that the diversification of species utilized will be necessary to realize the potential of bioenergy. *Eucalyptus* spp. have the advantages of rapid growth, short rotations, multiple harvests from coppice silviculture, high density, desirable energy conversion ratios, as well as a

biochemical composition favorable to ethanol production (Gonzalez et al. 2011). Gonzalez et al. (2011) and Dougherty and Wright (2012) reported positive economic forecasts for the development of *Eucalyptus* plantations, specifically for bioenergy in the southern United States. *Eucalyptus* spp. may also serve to augment native hardwood pulp log inventories needed for kraft paper production. In the southern United States, mixed hardwood logs have become supply-limited regionally and seasonally, as they are sourced from native stands and not usually plantations (Hart and Nutter 2012). Laboratory pulping and bleaching converted the wood of several cold-hardy *Eucalyptus* spp. grown in the southern United States (*E. macarthurii*, *E. benthamii*, *E. urograndis*, *E. camaldulensis*, *E. dunnii*, *E. viminalis*, and *E. dorrigoensis*) into an

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adequate replacement for native mixed hardwood pulp at blends of up to 50% *Eucalyptus* (Hart and Nutter 2012). Due to the potential for much higher production than realized with southern pines, both research and commercial *Eucalyptus* plantations were established in the southern United States. Planting *Eucalyptus* beyond its natural range is not without controversies, including concerns about invasiveness (Gordon et al. 2011, Stanturf et al. 2013) and high water use compared with native pines in the southern United States (Maier et al. 2017).

The genus *Eucalyptus* is native to Australia, New Zealand, Papua New Guinea, the Philippines, and Indonesia, and it is found in a diversity of climates and elevations across its native range (Sellers 1910, Kellison et al. 2013, Stanturf et al. 2013). There are more than 500 *Eucalyptus* species adapted to many ecological niches and environmental conditions, from tropical lowlands to high elevations in Pacific islands near the snow line (Kellison et al. 2013). The species' ability to naturalize in marginal soil conditions and persist in drought while also exhibiting rapid growth and producing desirable wood properties for pulp and structural timber make it seem to be an attractive option for planted forests (Stanturf et al. 2013). Since their "discovery" by Europeans in the late 1700s, *Eucalyptus* spp. have been introduced to tropical and subtropical regions around the world for wood, fiber, and fuel production. Attempts to introduce and improve various *Eucalyptus* spp. and varieties are not new to the southeastern United States. Improvement efforts in Florida have been ongoing for more than 50 years (Rockwood 2012), and from 1971 to 1985 the North Carolina State University Hardwood Research Cooperative evaluated the viability of more than 100 species across locations in Louisiana, Mississippi, Alabama, Georgia, Florida, and South Carolina (Kellison et al. 2013). Much was learned about seed sources, species differences, propagation, and silvics, though a noted limitation was the species' marginal freeze tolerance; after successive freezes in 1983, 1984, and 1985, the Hardwood Cooperative's program was ended. It is widely recognized that *Eucalyptus* spp. are susceptible to injury from cold temperatures, and a good deal of variation in cold hardiness within and among species exists (Kellison et al. 2013). Sensitivity to cold and rapid swings in temperature remain the largest obstacle to the introduction of *Eucalyptus* spp. in warm/temperate climates, and this has been the focus of research for decades (Sellers 1910, Paton 1981). Suitability for specific climates and the prediction of lethal temperatures for different species have been assessed through provenance testing, the use of cold rooms with seedlings, and relative electrolyte leakage (REL) assessments of excised tissue.

Eucalyptus benthamii has been identified as a relatively cold-hardy species with excellent growth and survivorship (Arnold et al. 2004, Arnold et al. 2015) that has adequate wood properties and is conducive to plantation production in the American South (Hart and Nutter 2012). Since the hard freezes of the 1980s, mean and minimum annual temperatures have trended upwards, and the planting of cold-tolerant *Eucalyptus* in the interior South away from the coasts has drawn renewed interest. Deployment of freeze-tolerant *Eucalyptus* spp. was found to be successful in the southern Gulf States and the southern portions of South Carolina during the past decade (Gonzalez et al. 2011). The purpose of this study was to assess growth, photosynthetic rate, and cold tolerance of *E. benthamii* planted in Raleigh, NC, north of the range of prior *Eucalyptus* spp. trials. A key component of this analysis was the quantification of seasonal cold hardiness using REL assessments of excised tissue to better identify temperature risk thresholds. This assessment provided a more thorough assessment of the risks and rewards of moving a fast-growing species further north.

Methods

Site and Plant Material

A screening trial was established on the Schenck Memorial Forest (35.8213 N, -78.7393 W) of North Carolina State University in Raleigh, North Carolina to evaluate the adaptation and growth of *Eucalyptus* spp. (*E. benthamii* among others) for the southeastern United States. The USDA Plant Hardiness Zone Map identifies this site as a 7b with minimum annual temperatures (based on a 30-year average) ranging from -12 to -15°C. The site is a typical upland piedmont site with a Cecil soil series (fine, kaolinitic, thermic Typic Kanhapludults). Soil preparation was completed using a skid-steer-mounted soil auger to reduce soil density. Complete weed control was implemented from establishment through the first year of growth. Fertilization with 70 g/tree (175 kg/ha) of triple superphosphate (0-46-0; 46% P₂O₅) was applied at establishment. The native range of *E. benthamii* is very limited, existing in four populations along the Nepean River southwest of Sydney Australia, where fewer than 7,000 individuals exist in four populations (Butcher et al. 2005). Seedlings were produced from an open-pollinated seed orchard mix from the Paraná state in Brazil. Containerized seedlings were planted April 19, 2012 at a spacing of 2 × 2 m resulting in 2,500 trees/ha. To give context to the *E. benthamii* results, loblolly pine (*Pinus taeda*), a locally adapted species widely employed in intensive silviculture, was also analyzed. For the present study, a subset of 12 *E. benthamii* trees (4 trees in 3 plots) and 4 *P. taeda* (one adjacent plot) were selected to examine the cold tolerance and physiology of these species during the winters of 2012–2013 and 2013–2014.

Growth

Beginning November 17, 2012, the 16 trees were measured for total height (ht) and diameter at breast height (dbh: 1.37 m above ground level) every 2 weeks until March 21, 2014. Five of the 12 *E. benthamii* trees were tall enough to begin dbh measurements during the first measurement on November 17, 2012, and all *E. benthamii* trees had reached ≥1.37 m by June 10, 2013. The first *P. taeda* tree could be measured for dbh only when it reached

Management and Policy Implications

The rapid growth of *Eucalyptus benthamii* makes it a tempting alternative to *Pinus taeda* for intensive silviculture in the southern United States. However, it is important for practicing foresters to know whether *Eucalyptus* spp. plantations in the Piedmont region are at high risk of failure from freezing injury. The exceptional productivity of *E. benthamii*, which was considered freeze tolerant, was not without trade-offs, as all trees died after experiencing the coldest air temperatures in 18 years (-12.8°C) in January 2014. Despite having a relatively warm climate, the southeastern United States is subject to rapid temperature swings that may be detrimental to *Eucalyptus* spp. Even though winters are generally becoming milder (IPCC 2014), freezing events and sudden temperature swings still occur that can damage exotic trees whether planted for fiber or fuel (e.g., *Eucalyptus*) or as a food crop (e.g., citrus). Considering this, it seems prudent to screen exotic species and varieties using relative electrolyte leakage methodologies and other risk assessment tools to evaluate winter hardiness relative to long-term climate histories and projections of future temperature regimes.

the appropriate size on September 27, 2013, and three of the four *P. taeda* trees had reached ≥ 1.37 m by November 1, 2013. One *P. taeda* tree never reached 1.37 m in height.

Photosynthesis

Photosynthesis (P_s , $\mu\text{mol m}^{-2} \text{s}^{-1}$) of *E. benthamii* and *P. taeda* foliage were measured 11 times from December 2012 to November 2013, capturing a range of ambient air temperatures from -1 to 28°C . Fully expanded, unshaded leaves were analyzed with a portable photosynthesis system (LI-6400, LI-COR, Inc., Lincoln, NE, USA). Photosynthesis measurements were made between the hours of 10:00 am and noon, reference CO_2 concentration was maintained at 400 ppm, while light intensity (photon flux density) within the chamber was held constant at $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$. Temperature was controlled to match ambient environmental conditions and humidity was maintained at $>50\%$. Data were logged when chamber CO_2 and H_2O concentrations reached equilibrium,

and P_s measurements were recalculated based on either the projected (*Eucalyptus*) or total (pine, Maier et al. 2008) leaf area within the cuvette. *Eucalyptus* leaves that did not entirely fill the cuvette were excised, and the leaf area within the cuvette was measured using a scanner with WinRHIZO Basic 2013 software (Regent Instruments Inc. Quebec City, Canada).

Cold Tolerance

Cold tolerance of both leaves and shoots of *E. benthamii* and *P. taeda* were assessed in the laboratory by exposing plant tissue to stepwise decreases in temperature and quantifying REL from cells damaged by freezing. The approach has been widely applied to predict cold tolerance of plant tissue and has been successfully used on both angiosperms (Gurney et al. 2011, Saielli et al. 2014) and conifers (Strimbeck et al. 1995, Schaberg et al. 1999, Schaberg et al. 2005, Schaberg et al. 2008). Current year foliage and shoots were collected in the field (January, February, March, April, October,

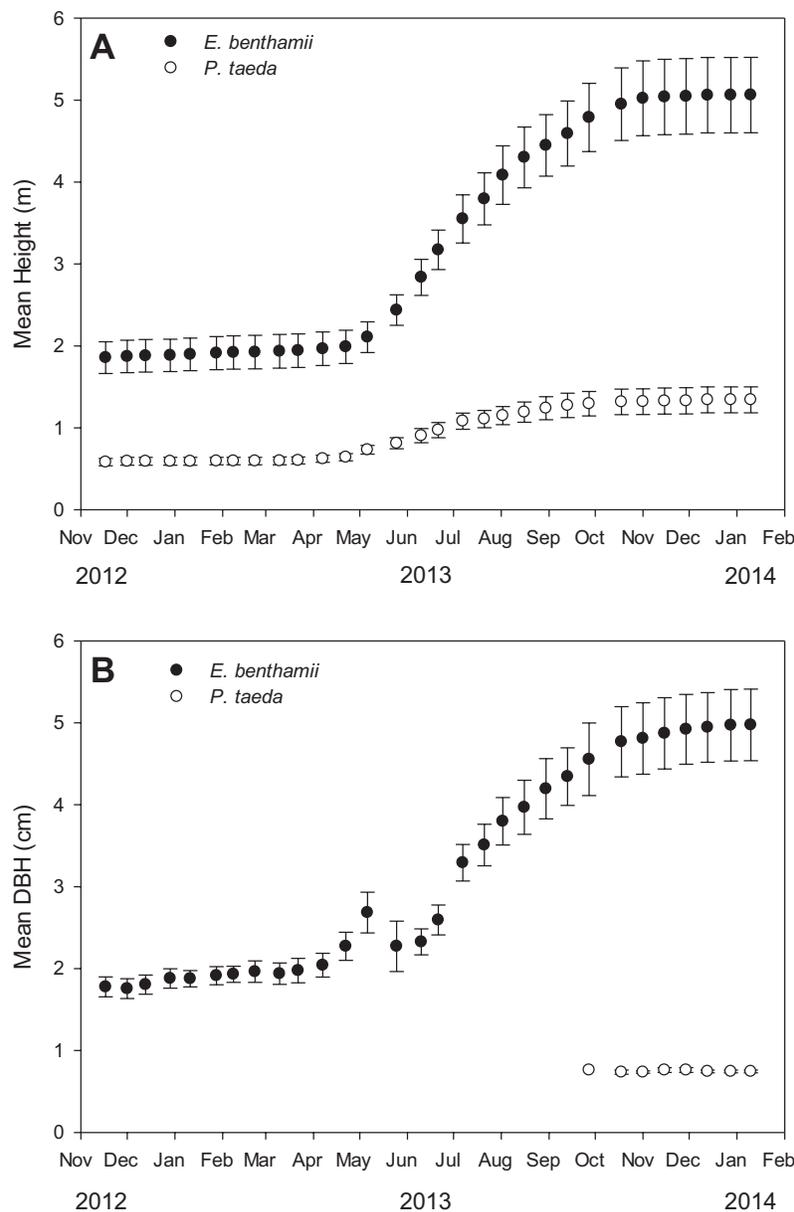


Figure 1. Mean height (A) and dbh \pm SE (B) of *E. benthamii* and *P. taeda* measured biweekly from 200 days after planting to 689 days after planting at the Schenck Memorial Forest, near Raleigh, North Carolina.

2013; January, 2014) and shipped overnight on ice for processing at the University of Vermont on the day after collection. Tissue was rinsed in distilled water and dried with paper towels. Stems of both species and *P. taeda* needles were cut into 5-mm-long sections, while *E. benthamii* leaves were cut into 5-mm circles with a hole punch. The details of stepwise freezing of tissue and assessment of REL were previously described by Gurney et al. (2011) and were conducted in the same laboratory with the same equipment. In brief, tissues received 30-minute exposure to 17 test temperatures ranging from +5° to -90°C, with steps between test temperatures ranging from 2°C (around temperatures projected to induce increased freezing injury) to 12°C (near the asymptote of maximum low temperature injury). After low temperature exposures, a mild detergent was added to samples, and the electrical conductivity of sample-solution mixtures was measured. The more damage to cells from freezing, the higher the resulting conductivity from the release of electrolytes. REL is calculated by comparing the electrical conductivity after each test temperature with maximum conductivity after drying tissues at 40°C to kill the tissue and release the remaining electrolytes (Strimbeck et al. 1995, Schaberg et al. 1999, Schaberg et al. 2005). The relationship between REL and temperature is characterized by a sigmoidal curve with a midpoint (T_m), which represents the temperature where 50% of tissue has been damaged and the potential for repair is limited. T_m values were calculated using the equation and approach described by Schaberg et al. (2008) based on the prior work of Anderson et al. (1988) with JMP Pro 12 software (SAS Institute, Cary, NC, USA).

Experimental Design and Statistical Analysis

The *E. benthamii* seedlings were planted in three blocks of four trees, nested within a larger planting trial, while the *P. taeda* seedlings were planted in one block of four trees. The *P. taeda* seedlings served as a locally adapted point of reference for production and physiology but cannot be jointly analyzed due to lack of replication in the same experiment. Sample collection for REL analysis would have been detrimental to the continued growth of the

comparatively small *P. taeda* seedlings, so four adjacent volunteer *P. taeda* seedlings were destructively sampled to assess cold tolerance. Growth and Ps were measured on the four *P. taeda* within the plot. The effect of sampling date on Ps and T_m was analyzed using the Proc MIXED repeated option in SAS/STAT 9.4 software (SAS Institute, Cary, NC, USA.). Nonlinear regression (Proc NLIN) was used to analyze the parabolic relationship between Ps and air temperature, while stepwise linear regression (Proc REG) was used to analyze air temperature variables related to T_m .

Results

Growth

Throughout the biweekly measurement period from 200 to 689 days after planting, *E. benthamii* outperformed *P. taeda* in both mean height and diameter growth (Figure 1). In early January 2013, *E. benthamii* mean height (1.93 ± 0.03 m) was 3.2 times greater than *P. taeda* (0.61 ± 0.04 m) and increased to 3.8 times taller by January 2014 (5.06 ± 0.46 m versus 1.34 ± 0.16 m). In January 2014, *E. benthamii* dbh (5.0 ± 0.4 cm) was 7 times greater than *P. taeda* (0.7 ± 0.02 cm). The apparent decline in *E. benthamii* dbh from May to July 2013 (Figure 1) represents the addition of smaller trees that did not previously have a recorded dbh, temporarily reducing the overall mean.

Photosynthesis

Photosynthetic rate (Ps) of *E. benthamii* and *P. taeda* varied seasonally, with *E. benthamii* exhibiting higher rates on most days (Figure 2). Using repeated-measures analysis, Ps of both *E. benthamii* ($F = 31.27$, $P < .0001$) and *P. taeda* ($F = 5.93$, $P = .0003$) were affected by measurement date, and both species were photosynthetically active throughout the winter when temperatures were above freezing. During the warmer months of April through November, *E. benthamii*'s mean Ps ($13.82 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was more than twice that of *P. taeda* ($6.21 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Similarly, mean Ps during the winter months was $6.09 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for *E. benthamii* and $2.73 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for *P. taeda*.

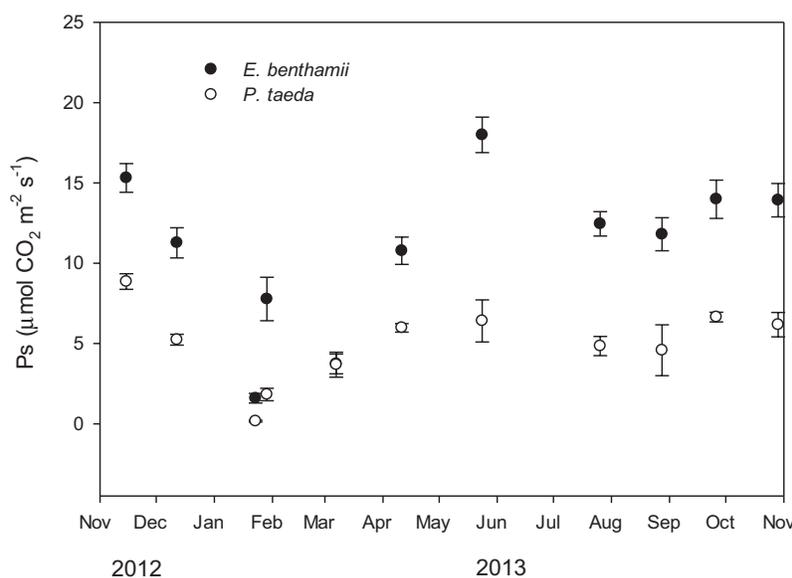


Figure 2. Mean photosynthetic rate (\pm SE) of *E. benthamii* and *P. taeda* measured between November 2012 and November 2013 at the Schenck Memorial Forest, near Raleigh, North Carolina.

(Figure 2). For both species, P_s increased with air temperature at the time of measurement and then declined as temperatures approached 30°C (Figure 3A). Comparing the coefficients of determination and the slopes of the Gaussian regression lines, *E. benthamii* exhibited greater sensitivity to air temperature than *P. taeda*. Recent temperature history quantified as the minimum air temperature in the past 24 hours explained more variation in P_s than did temperature at the time of measurement (Figure 3B). Using the slopes of regressions as proxies for temperature sensitivity, P_s in both species were more sensitive to the minimum air temperature in the past 24 hours than at the time of measurement (Figures 3A and B).

Cold Tolerance

Daily minimum and maximum air temperatures for the duration of the experiment are presented in Figure 4A. Arrows indicate the six periods when REL was assessed from 2013 to 2014. Leaf

and stem T_m of both species varied significantly over the course of year ($P < .001$), with higher values in the warm months and lower values in the winter. In the absence of below-freezing temperatures in May and November 2013, foliar T_m values for *E. benthamii* and *P. taeda* overlapped, ranging from -8.8 to -10.4°C (Figure 4B). After acclimation to colder air temperatures, *E. benthamii* foliar T_m decreased by a few degrees to a minimum mean temperature of -13.4°C , while *P. taeda* foliar T_m dropped to a minimum value of -29.9°C (Figure 4B). Woody stem tissue REL was assessed three times during the experiment, and there were no consistent patterns in T_m between stem and foliar tissues in either species (Figure 4B). After a high temperature of 16.3°C on January 6, 2014, the temperature fell to -12.8°C the next day, REL was assessed on January 14, 2014, and all of the *E. benthamii* in the study were found to have unreparable injury that resulted in tissue mortality. Additional cold periods occurred later in the month, culminating in minimum air temperatures of -11.5°C on January 24 and -13.3°C on January

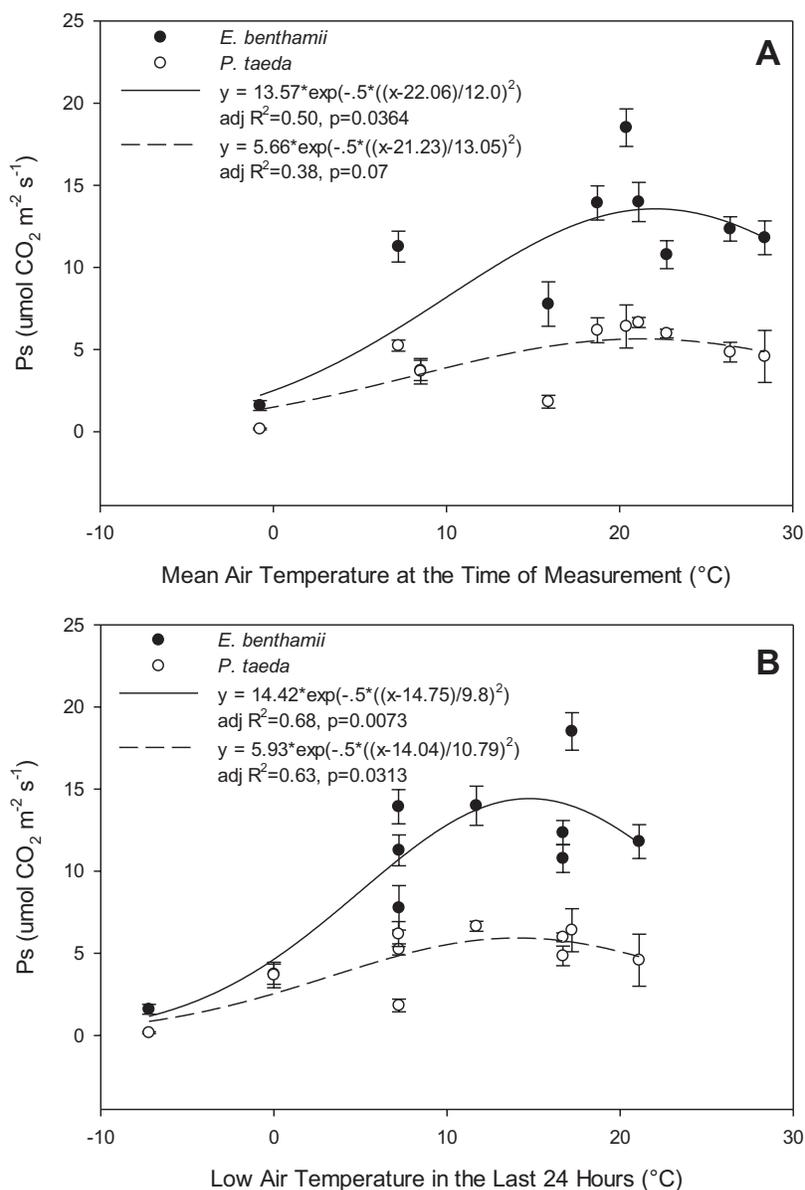


Figure 3. Nonlinear Gaussian function regression analyses of mean photosynthesis (\pm SE) for *E. benthamii* and *P. taeda* measured between November 2012 and November 2013 at the Schenck Memorial Forest, near Raleigh, North Carolina with mean air temperature at time of measurement (A) and low air temperature during the previous 24 hours (B).

30. In the spring of 2014, it was confirmed that all 12 trees had died and no resprouting was observed.

To gain insight into the role of environmental conditions leading up to temperature acclimation, step-wise linear regression was used to identify the period best correlated to T_m . Dependent variables of *E. benthamii* and *P. taeda* T_m were regressed with independent variables of minimum air temperature and mean air temperature in the preceding 3, 7, and 14 days. Variation in *E. benthamii* leaf T_m was best explained by minimum air temperature in the last 7 days, while *E. benthamii* stem, *P. taeda* leaf, and *P. taeda* stem T_m variation was best modeled by mean air temperature in the 3 days preceding REL analysis (Figure 5).

Laboratory-based estimates of cold tolerance are good for comparative analyses, but historically overestimate the true cold tolerance of tissues because they take place in the absence of wind or other adverse conditions (e.g., rapid freezing and freeze-thaw cycles) found in the field that enhance damage. That stated, our mean T_m values ranged from -9.3 to -13.4°C during the measurement period, followed by complete destruction of *E. benthamii* in the field when air temperatures rapidly dropped from 16.4°C on January 6, 2014 to -12.8°C the following day (Figure 4). It was the lowest minimum

temperature recorded in the past 18 years, though as recently as 1985, a minimum of -21.7°C was recorded nearby (Figure 6).

Discussion

Height growth and P_s of *E. benthamii* far exceeded those of locally adapted *P. taeda* planted in the North Carolina Piedmont. While greater productivity of *E. benthamii* is in part due to higher P_s , the species also differ in seasonal growth patterns. Maier et al. (2017) found that *E. benthamii* trees maintained diameter growth into the late fall and winter, whereas *P. taeda* ceased annual growth in early fall. *E. benthamii*'s P_s rates were similar to those reported in the literature for other *Eucalyptus* spp. grown commercially in Tasmania: the highest mean P_s rate observed in the field was $18.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 20.4°C compared to measurements of *E. globulus* $-15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 20°C and *E. nitens* $-20 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 20°C (Battaglia et al. 1996). Optimum temperatures for P_s were reported to shift seasonally, being higher in the warm summer months and lower in the winter (Battaglia et al. 1996). Temperature response curve data for both *E. globulus* and *E. nitens* show that air temperatures of 5°C still yield P_s rates $>10 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ but are sharply reduced at 35°C (Battaglia et al. 1996). Our analysis lacked higher temperatures,

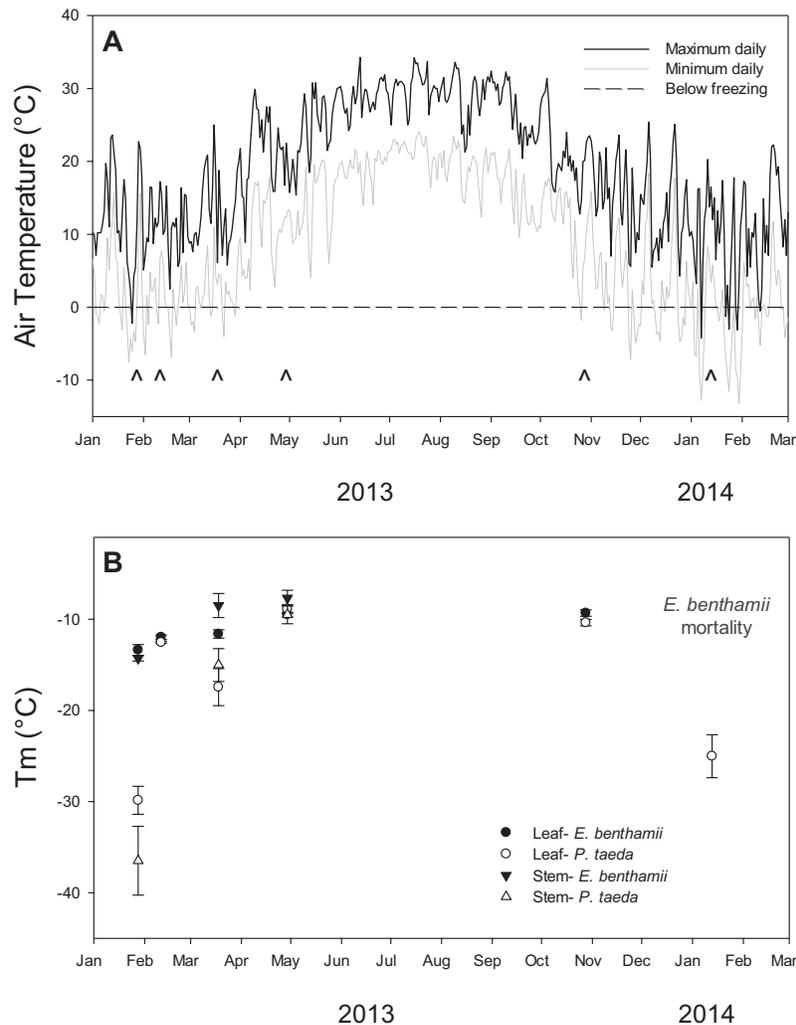


Figure 4. Minimum and maximum daily air temperature recorded at the nearby Reedy Creek Field Laboratory, west of Raleigh, North Carolina (A). Arrows indicate days when REL was assessed. Mean T_m values (\pm SE) represent the temperatures where 50% of the tissue has been damaged and potential for repair is limited (B). Temperature data provided by the State Climate Office of North Carolina, Raleigh, North Carolina.

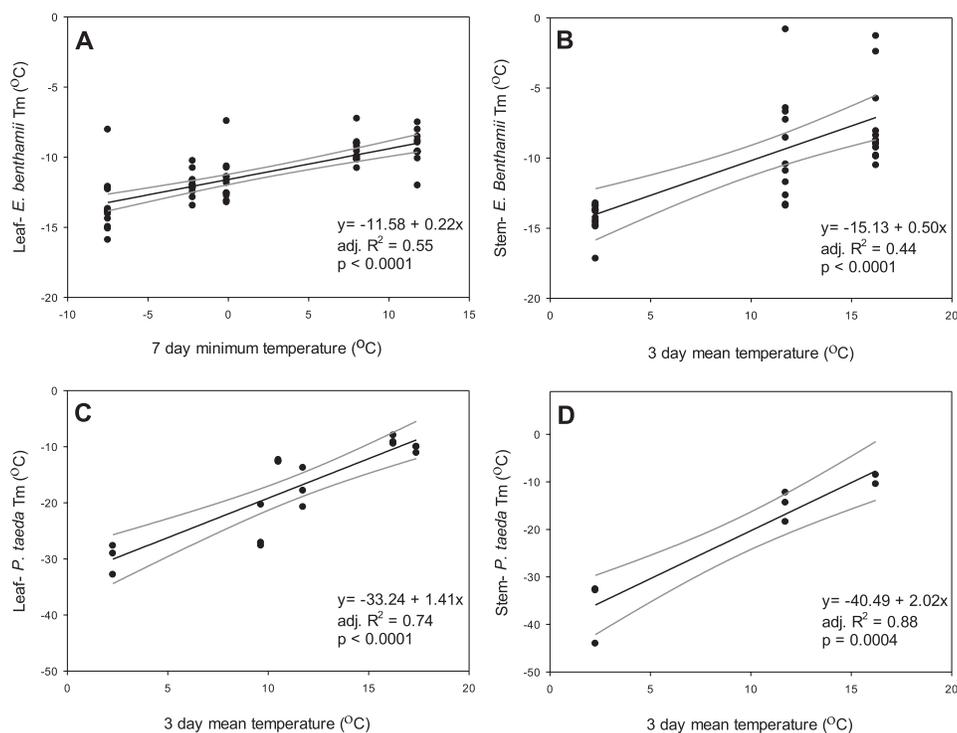


Figure 5. Results of stepwise linear regression of leaf and stem T_m of both species with independent environmental variables of minimum air temperature and mean air temperature in the preceding 3, 7, and 14 days. For each species/tissue type, only one temperature variable remained in the model after the stepwise selection process. Variation in leaf T_m of *E. benthamii* was best described by minimum air temperature in the past 7 days (A), while variation in stem T_m for *E. benthamii* and both tissue types for *P. taeda* were best described by the mean temperature of the last 3 days (B–D).

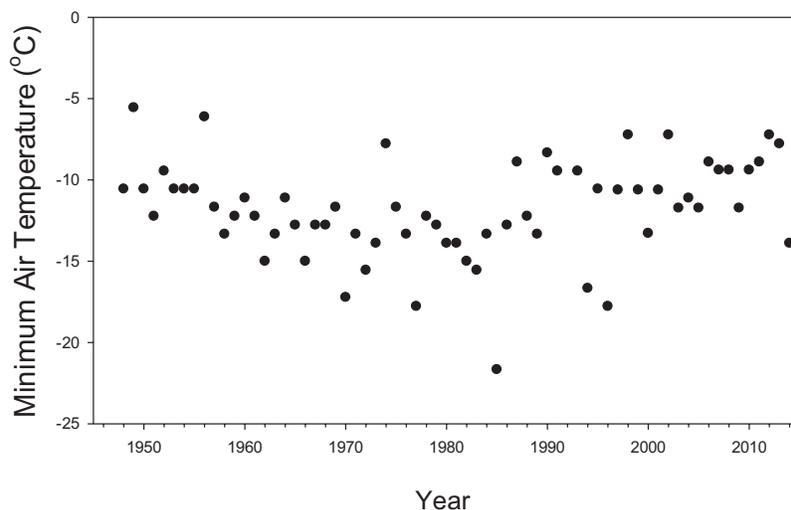


Figure 6. Minimum annual air temperature recorded at the Raleigh-Durham International Airport from 1948 to 2014. The last year of the study (2014), experienced the lowest minimum air temperatures in the past 18 years. Data provided by the State Climate Office of North Carolina, Raleigh, North Carolina.

which may have led to pronounced Ps inhibition, though by fitting a Gaussian function that models a symmetrical rise and fall after a peak it becomes evident that Ps declines are likely as temperatures reach the upper 20s°C (Figure 3). For both *E. benthamii* and *P. taeda*, the minimum air temperatures in the previous 24 hours explained more variation in Ps rates than air temperatures at the time of measurement (Figure 3). Overnight frost has been shown to depress Ps and require several days for Ps to recover to pre-frost levels in both *E. globulus* and *E. nitens* (Davidson et al. 2004) and in pines (Teskey et al. 1994).

Our results imply that not only frost conditions, but cooler nighttime temperatures may limit Ps the following day. Lower Ps following cold nighttime temperatures are likely due to stomatal limitations. Day et al. (1991) demonstrated that moderate chilling of loblolly pine seedling roots (7–15°C) reduced leaf water potential and stomatal conductance causing a 20% to 50% reduction in Ps.

Seasonal acclimation of leaf and stem tissues to low temperatures are the culmination of complex interactions of genetics, physiology, phenology, and developmental processes for many long-lived plants,

especially for those in temperate and boreal environments (Howe et al. 2003). Hardening and de-hardening can reduce productivity if there is a mismatch between hardiness and environmental conditions (Loehle 1998). Even within a species, differences among provenances from different climate zones may exhibit tradeoffs between cold tolerance and productivity. For example, in a study of American chestnut (*Castanea dentata*) out planted at the limit of its range in Vermont, sources from warm and moderate climates exhibited greater height and diameter growth but suffered more winter injury than cold climate sources (Saielli et al. 2014). *E. benthamii* has a very limited range in New South Wales, Australia, where it is classified as a species that is vulnerable to extinction (Butcher et al. 2005). Species with limited geographical ranges generally have limited gene flow, resulting in low genetic diversity (Hamrick et al. 1992). For these reasons, distinct climate-related provenances are unlikely to be associated with natural populations of *E. benthamii*.

Over the course of REL measurements in the present study, mean *P. taeda* *T_m* values varied 21°C for leaf tissue and 27°C for stems seasonally, while *E. benthamii* varied only 4°C for leaves and 7°C for stems. The relative lack of seasonal hardening supports high growth rates when conditions are favorable but entails an elevated risk of freezing injury over time as hard frosts eventually occur. In well-adapted species, the highest risk of cold injury is in the spring during bud break and shoot elongation (Howe et al. 2003), as most temperate and boreal species have mid-winter hardiness levels that far exceed environmental conditions (Sakai 1960, Howe et al. 2003, Strimbeck et al. 2007) in the absence of thaws or de-hardening events (e.g., Strimbeck et al. 1995). Clearly the relatively high mid-winter *T_m* values of *E. benthamii* (minimum value -13.4°C, Jan. 28, 2013) coupled with the minimal capacity to acclimate seasonally to USDA plant hardiness zone 7b (-15 to -12.2°C; the 30-year range of mean low temperatures) indicate that the species is maladapted relative to current conditions in central North Carolina. While all the *E. benthamii* trees in this study died, some coppicing was observed on other *Eucalyptus* at the Schenk Forest. The severe frost in January 2014 affected much of the United States and took a heavy toll on other *Eucalyptus* spp. plantings in the southeast. In southeastern Texas, temperatures of -8°C were observed and trees were covered with ice, but despite some broken branches, a 5-year-old *E. benthamii* planting suffered no permanent damage (Hart et al. 2016). Another 5-year-old plantation of *E. benthamii* located at the Solon Dixon Forestry Center near Andalusia, Alabama, survived temperatures of -7.4°C in 2011 but succumbed to two consecutive nights of lows to -11.3°C during the January 2014 event (Yu and Gallagher 2015). These results show excellent correspondence between REL predictions and observations in the field, where winter cold tolerance in the field may be closer to -9°C to -10°C. Despite being ill-suited for planting in North Carolina, *E. benthamii* has been identified as a high performer with excellent survival, growth rate, and good form in warmer regions of the world (e.g., Yongzhou, Hunan Province with a minimum temperature of -5.2°C; Arnold et al. 2015), southern Brazil with minimum temperatures ranging from -3°C to -6°C (Higa and Carvalho 1990), and higher elevation sites (1,100–1,600 m) prone to frost in South Africa (Swain and Gardner 2002).

Conclusions

E. benthamii had superior growth and Ps in central North Carolina, surpassing those of locally adapted *P. taeda*. However, this

exceptional productivity was not without a significant trade-off, as all the subject trees died after experiencing the coldest air temperatures in 18 years in January 2014. Despite having a relatively warm climate, the southeastern United States is subject to rapid temperature swings that may be detrimental to *Eucalyptus* spp. Even though winters are generally becoming milder (IPCC 2014), freezing events and sudden temperature swings still occur that can damage exotic trees whether planted for fiber or fuel (e.g., *Eucalyptus*) or as a food crop (e.g., citrus). Considering this, it seems prudent to screen exotic species and varieties using REL methodologies and other risk assessment tools to evaluate winter hardiness relative to long-term climate histories and projections of future temperature regimes. While being relatively hardy with good physical properties, *E. benthamii* was pushed beyond its cold tolerance threshold in central North Carolina.

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