INCREASED VPD DUE TO HIGHER TEMPERATURE LEADS TO FASTER MORTALITY OF TREE SEEDLINGS COMMON TO THE FOREST-GRASSLAND ECOTONE DURING DROUGHT

By

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Abstract: My objective was to assess the effects of an increase in VPD on plant water use strategy, seedling establishment, and persistence of multiple species common in Oklahoma along the forest-grassland ecotone under predicted climate change conditions. We tested seedlings of ten species, post oak (*Quercus stellata*), Shummard Oak (*Quercus shumardii*), bur oak (*Quercus macrocarpa*), redbud (*Cercis canadensis*), American plum (*Prunus americana*), persimmon (*Diospyros virginiana*), eastern redcedar (*Juniperus virginiana*), hackberry (*Celtis occidentalis*), mulberry (*Morus rubra*), and sycamore (*Platanus occidentalis*), to see if a 3°C increase in temperature and a 3°C decrease in dewpoint during a time of drought would increase water use and hasten mortality. To test the effects of temperature, we exposed seedlings to day/night temperature regimes of 30/25°C and 33/28°C while maintaining a dew point of 21°C at all times by controlling relative humidity. To test the effect of dewpoint, we held a constant temperature of 30/25°C (day/night) and varied the relative humidity to create a day-time dewpoint of 21°C (59% RH) and 24°C (70% RH). These temperature/humidity combinations resulted in a constant day-time dewpoint of 21°C with a VPD of 1.74 kPa, for the temperature study and day-time dewpoints of 24°C (VPD of 1.26 kPa) and 21°C (VPD of 1.74 kPa) for the humidity study. These treatments resulted in a 45% higher day-time VPD in the 33/28°C chambers in the temperature study and a 27% higher day-time VPD in the humidity study. The higher VPD in each study lead to a significantly greater transpiration rates in all trees in this study. Water potential was also significantly more negative in the non-watered trees grown at a higher temperature but not at the lower dewpoint. Mortality was significantly hastened by the increase in VPD with increased temperature but not with decreased dewpoint. These results indicate that additional water stress along with increased VPD during prolonged drought periods and increased temperatures consistent with climate change scenarios may lead to large shifts in tree species distribution along the forest-grassland ecotone of the central United States.

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CHAPTER I

INTRODUCTION

By the end of the 21st century, the mean annual temperature in the central United States is predicted to increase 2.5 to 4.0 °C (IPCC 2007). Over that time, little change in total precipitation is predicted for the same region, but there may be longer periods of drought and an increase in intensity of rainfall events. If absolute humidity of the air does not change, the increased temperatures will lead to increased saturation vapor pressure and hasten transpiration and evaporation from plants and soil, respectively. Dry soils tend to be hydrophobic. The tendency for hydrophobicity combined with increased rainfall intensity could cause greater runoff and less infiltration of water into the soil when precipitation events occur. These combined effects of increased drought and higher temperatures in the central United States will likely cause widespread vegetation change to many ecosystems (Peters and Lovejoy 1992).

Increased temperatures alone are assumed to provoke a northward migration of plant species. The relaxation of low temperature restrictions would allow southern species to expand northward and the increased heat stress would push the southern extent of less heat tolerant species north (Davis 1989, Peters and Lovejoy 1992). This north/south movement in response to changing climate is often identified as the route of large-scale change in the composition of North American ecosystems. However, the precipitation gradient that stretches from the wetter east coast of the United States to the drier Front Range of the Rocky Mountains could also be a front for vegetation change. In the middle of this precipitation gradient is an ecotone (transition zone) where there is a large shift in moisture over a relatively short distance. This ecotone runs from Minnesota to Texas, spanning the entirety of the United States from north to south. This rapid decrease in precipitation mirrors a shift in vegetative composition, going from tree dominated landscapes of the east to grassland dominated landscapes in the west (Sims and Risser 2000). The narrow width of this forest-grassland ecotone could make it highly susceptible to global climate change, leading to vegetation shifts between tree and grass dominated ecosystems. The forest-grassland ecotone occurs throughout the southern Great Plains. For instance, in the state of Oklahoma over 90 species of trees reach the edge of their range as the average total annual precipitation decreases from approximately 145 cm to 38 cm across a span of 250 km (Little 2002).

The direct effects of water availability and temperature are major drivers of plant species distribution along the ecotone (Sims and Risser 2000). However, temperature also has an indirect effect on plant water use through its effects on vapor pressure deficit (VPD). Vapor pressure deficit is the difference between the vapor pressure at ambient conditions and the vapor pressure at saturation point for the same temperature. Because saturation vapor pressure increases exponentially with temperature, VPD increases with temperature exponentially if absolute humidity is held constant. This deficit is the driving force for transpiration in plants, i.e., transpiration ~ VPD/stomatal resistance. The net effect is that transpiration increases exponentially with temperature in the absence of a change in stomatal resistance. Depending on the starting temperature, a 3°C increase in temperature can result in nearly a 50% increase in VPD (Figure 1), and potentially increase of transpiration of similar magnitude. This response of tree survival in response to the indirect effect of temperature on VPD during drought is not known. Increased temperature combined with a drought could create a condition where a tree becomes water stressed and potentially dies. Prolonged drought under these conditions could lead to large scale die off of trees and could lead to a major change within the current ecotone and/or a shift in ecotone location.

Plants are able to respond to water stress by regulating water loss through opening or closing their stomata. The guard cells in the plant's stomata shrink the stomatal opening which reduces the amount of water that can escape (Kozlowsk and Pallardy 1997). The degree to which species actively regulate water loss and their stomatal sensitivity to water stress varies. Isohydric plants have greater stomatal control, limiting transpiration to maintain relatively constant day-time leaf water potential and thus limiting cavitation in the xylem, which is a break in the water column that prevents movement of water and nutrients (Kozlowski and Pallardy 1997). Anisohydric species have less stomatal control causing large variation in day-time leaf water potential (Tardieu and Simonneau 1998).

There is a trade-off between water conservation and carbon uptake because stomatal closure restricts carbon uptake for photosynthesis. If the stomata close tightly enough to effectively limit or stop water loss and carbon uptake for a long enough period of time, a plant can die from carbon starvation. Recent studies of tree species in the intermountain region of the United States attributed mortality from prolonged drought partially to carbon starvation, a process accelerated by increased temperatures due to the drastic decrease in stomatal conductance resulting in minimal carbon uptake and eventual depletion of non-structural carbon (Breshears et al. 2005, Breshears et al. 2008, Adams et al. 2009). Adams et al. (2009) found that respiration increased with temperature, which increased carbon usage and hastened mortality of trees grown under elevated temperatures compared to those grown under ambient temperature. Alternatively, plants can die during drought due to cavitation (Anderegg et al. 2012).

Given the various strategies plants may employ in response to water stress, different species react differently to drought in the same environment. For example, when exposed to the same drought conditions, the isohydric piñon pine (*Pinus edulis*) eventually succumbed to carbon starvation while one-seed juniper (*Juniperus monosperma*) persisted through extended drought (Breshears et al. 2009). Compared to piñon pine, juniper exhibited more anisohydric behavior demonstrating less ability to regulate water loss, leading to more negative water potentials and thus increased cavitation. Utah Juniper (*Juniperus osteosperma*) when compared to piñon pine also exhibited anisohydric traits (West 2007). Though the anisohydric tendencies of the juniper species increased the potential for mortality through hydraulic failure, the juniper species were, however, able to survive longer than their counterparts. This could be due to a series of physiological and morphological advantages that these juniper species have compared to piñon pine, including deeper root depth (West et al. 2007), ability to limit cavitation causing 100% stem cavitation to occur at a higher water potential (Linton et al. 1998, West et al. 2007), and greater foliar water uptake from rainfall (Breshears et al. 2008).

The ability to react to what types of rainfall may occur can also be important. Each species' reaction to a short rainfall event highlights this difference. Piñon pine can take up water as it becomes available due to the limited cavitation in the stem allowing embolized roots to refill quickly. This is in contrast to the Utah juniper, which needs more time with moist soil conditions to repair the water column in the stem as well as the roots and cannot take advantage of the sporadic summer rains that occur in arid climates (West et al. 2007). These different physiological adaptations of some western species could be similar to tree species found in parts of the forest-grassland ecotone. As temperature and time between rainfall events increase, a species' physiological adaptation to water stress and response to water events may dictate its location and abundance on the landscape.

Under predicted climate change conditions, with increased periods of drought, many physiological adaptations can influence a species placement on the landscape. Root to shoot ratio, leaf area, leaf-level transpiration rates, and hydraulic conductance at the leaf and stem levels, as well as many other traits have the potential to influence the survival of an individual plant and a species. All of these have an impact on to what degree an individual plant can withstand water stress conditions. Seedlings are of particular concern due to a lack of extensive root systems or carbohydrate reserves (Hanson and Weltzin 2000). Seedling establishment and eventual recruitment of seedlings into the overstory is necessary to maintain a species on the landscape. Additionally, seedling establishment is the causal mechanism for migration and expansion of species at the leading edges of its range.

The objective of this study was to assess the ability for seedlings of ten native Oklahoma tree species that occur along the forest-grassland ecotone to survive increased temperatures and VPD during drought conditions. I hypothesized that a 3°C increase in temperature and subsequent increase in VPD will cause increased mortality rate during drought by increasing transpiration and water use. Furthermore, I hypothesized that a change in VPD caused by a 3°C increase in dew point will have the same effect on mortality as the change in temperature.

CHAPTER II

METHODOLOGY

Commercially available one-year-old, bare-root seedlings of ten tree species were selected that are native to Oklahoma, United States. The ten species selected were post oak (*Quercus stellata* Wangenh.), bur oak (*Quercus macrocarpa* Michx), Shumard oak (*Quercus shumardii* Buckley), sycamore (*Platanus occidentalis* L.), red mulberry (*Morus rubra* L.), American plum (*Prunus americana* Marsh.), hackberry (*Celtis occidentalis* L.), common persimmon (*Diospyros virginiana* L.), eastern redbud (*Cercis canadensis* L.), and eastern redcedar (*Juniperus virginiana* L.). All species except post oak came from the Oklahoma Department of Agriculture, Food, and Forestry Division nursery in Goldsby, OK. The post oak seedlings were purchased from the Senter's Nursery in Whitehouse, TX. In late March 2009, before bud break, the seedlings were planted in 7.6 liter pots with an outside diameter at the top of 21.6 cm and a depth of 21.6 cm. Five 1.25 cm diameter holes were drilled in the bottom for water drainage. The pots were filled with a mix of half local soil and half medium sand to promote slow and steady drainage over time. The local soil was a loam (52% sand, 38% silt, 10% clay) and had a pH of 7.2 before mixing. The final, mixed soil was a loamy sand (76% sand, 19% silt, 5% clay). Once planted, the seedlings were placed under a rainout shelter, watered at least every. other day and fertilized with a liquid complete fertilizer (12-4-8) (Miracle-Gro® LiquaFeed®,The Scotts Miracle-Gro Company, Marysville, OH) three times a week with approximately 0.08 g N, 0.06 g P, 0.04 g K, 0.0004 g Mn, and 0.0004 g Zn applied per pot at each application.

For the experiments, the seedlings were moved into four growth chambers. The growth chambers were 2.49 wide × 1.37 deep × 2.35 m tall (interior dimensions). Chambers had 3.3 m^2 growth area with 2.05 m potential growth height (Conviron, Winnipeg, Canada model PGW 36). The light sources within the chambers were a combination of florescent and incandescent light bulbs that were capable of producing up to 960 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR). The bulbs were located in a light bank, the height of which was adjustable to control intensity. For this experiment, the light bank was adjusted to reach a PAR near 500 μmol m^{-2} s^{-1} 0.67 m above the chamber floor. When first moved to the chambers, baseline conditions were temperature 30°C and relative humidity 59% for the period when the lights were on (day-time, 14 hours per day) and temperature 25°C and relative humidity 79% when the lights were off (night-time, 10 hours per day). These settings gave a constant day-time and night-time dewpoint of 21°C with a day-time VPD of 1.74 kPa and a night-time VPD of 0.66 kPa. Temperature and relative humidity were recorded by a WatchDog data logger (Spectrum Technologies, Inc, Plainfield, IL, Model A150) every 15 minutes throughout each experimental period. The WatchDog data loggers were placed at the center of each chamber.

Experiment 1.Temperature to control VPD

Experiment 1 tested the combined effects of a rise in VPD caused by an increase in air temperature and terminal drought on the ten species.

For each of two experimental periods, two trees from each species were moved into each of four growth chambers (eight total of each species per experimental period). Experimental period 1 was conducted from July 26, 2009 to August 13, 2009 and period 2 was conducted from August 17, 2009 to September 4, 2009. The two trees that were placed in a given chamber were paired based on size. Prior to the start of each experimental period, the trees used in that period were placed in the chambers under baseline conditions (described above) for at least seven days and watered as needed to acclimate to the chamber environment. One tree of each species in each chamber was assigned to the well-watered treatment, while the other of that species was assigned to the terminal drought treatment. All trees were watered two to four hours before the chamber lights turned on. The preliminary measurement of leaf gas exchange (net photosynthetic rate, leaf conductance, and leaf transpiration) was conducted on fully expanded leaves using a LI-6400 portable photosynthesis system with the 6400-02B blue-red LED Light Source (Li-Cor Inc, Lincoln, NE) two to four hours after the chamber lights turned on. The conditions in the leaf chamber were set to keep a constant airflow of 500 µmol s^{-1}, reference CO_2 was maintained at 400 µmol mol^{-1}, and PAR set to 1600 µmol m^{-2} s^{-1}. Temperature and relative humidity were controlled to exactly match the set points of the chamber (temperature ± 0.2 °C, relative humidity ± 0.2%). The same measurement timing relative to lights on and leaf chamber settings were used for all subsequent measurements.

Following the preliminary gas exchange measurements, two of the four chambers were set to a day-time temperature of 33°C with a relative humidity of 49% (VPD of 2.56 kPa) and night-time temperature of 28°C with a relative humidity of 66% (VPD of 1.28 kPa). These conditions maintained a constant day-time dewpoint of 21°C for all chambers, but a different VPD due to the different temperatures, i.e., 1.74 vs. 2.56 kPa day-time. Once the temperature was changed in the two chambers, water was withheld from the trees assigned to the terminal drought treatment until they died. Death was determined by leaf color, leaf retention, and leaf and branch desiccation, depending on the species. Once the experimental period was over, non-watered trees were placed back under the rainout shelter and watered to check for resprouting. Initial gas exchange measurements were taken on July 26, 2009 (Period 1) and August 17, 2009 (Period 2). In addition to the initial gas exchange measurements, measurements were taken approximately every other day after treatment imposition. In most cases, measurements were conducted on the same leaf for a given individual. Conditions inside the leaf chamber were maintained at the same temperature and humidity as the chamber set points. For the non-watered trees, gas exchange measurements continued until intercellular CO_2 concentration was greater than 400 μmol CO_2 mol^{-1} which indicated respiration. Gas exchange on the watered trees was measured one interval longer than the non-watered tree in the same chamber.

Four days (Period 1) or three days (Period 2) after applying treatments, xylem water potential was measured for each living seedling using a pressure chamber (Scholander et al. 1965). On the same day water potential readings were taken; leaf gas exchange was measured on all seedlings at both the ambient condition within their assigned chambers and also the environmental conditions of the reciprocal treatment. After measurement in ambient conditions, the environmental conditions were changed in the chamber. The chamber conditions were then allowed to stabilize. Ten to twenty minutes after conditions in the chamber were stable; the conditions in the leaf chamber were adjusted to match the chamber and another set of gas exchange measurements were recorded.

For a given chamber, the reciprocal temperature treatment was employed during the second experimental period and chamber served as the blocking variable. This procedure allowed for the statistical removal of chamber effects and possible chamber bias. Given the two experimental periods, sample size was four for temperature treatment and eight within species. Since watering treatments were randomly assigned to trees, the sample size for testing water stress effects and the interaction between water stress and temperature was eighty.

Experiment 2. Relative humidity to control VPD

Experiment 2 tested the combined effects of a rise in VPD caused by a decrease in humidity and of terminal drought on eight species

The same species were also used as in the previous experiment except for bur oak as there were no suitable individuals available at that time. Data for red mulberry could not be collected as non-watered seedlings died within 48 hours due to excessive leaf area and water use. Data from only three chambers were analyzed for this experiment because one chamber lost the ability to sufficiently control humidity during the experiment.

Again, there were two experimental periods. Experimental Period 3 was conducted from September 8, 2009 to September 28, 2009 and Period 4 was conducted from October 12, 2009 to October 23, 2009. Again, each experimental period included six seedlings from each species, paired based on similar physical characteristics with one pair of each species placed in the same chamber. Three chambers were used, and were again set to baseline conditions with temperature of 30/25°C (day/night) and a relative humidity of 59/79% (day/night), which resulted in a constant day-time dewpoint of 21°C and a VPD of 1.74 kPa. Two of each species were placed in each chamber for at least seven days during an acclimation period and watered regularly. Initial gas exchange measurements were recorded with temperature for all the chambers remaining at 30°C. After these initial readings, the relative humidity in two of the chambers was set to 70% when the lights were on and 84% while the lights were off, while the other two chambers remained at 59% with the lights on and 70% while the lights were off. These environmental regimes resulted in day-time dewpoints of 24°C (VPD of 1.26 kPa) and 21°C (VPD of 1.74 kPa) respectively. Initial gas exchange measurements were taken on September 8, 2009 (Period 3) and October 12, 2009 (Period 4). Gas exchange measurements were then taken every other day following the same method outlined in the temperature study. Reciprocal gas exchange measurements and water potentials were taken as described above two days after treatments were applied. Recordings ceased and death was assessed using the criteria used in Experiment 1.

Statistics

The experiment was a split-plot design that was blocked by growth chamber to partition variability due to chamber. In other words, consecutive experimental runs in the same chamber, but at different treatment levels served as a block. Temperature (or relative humidity) treatments served as the whole-plot factor (n = 4 or 3). The factorial combination of species (n = 16 or 12) and watering treatments (n = 80 or 64) served as the split-plot factors. Seedling mortality data were log transformed to normalize and then analyzed using the Proc Mixed (SAS 9.2) with a model testing for differences in the fixed effects of temperature, species, water stress as well as their interactions. Water potential data were tested using the Proc Mixed using a similar method. The ratio of reciprocal gas exchange measurements was generated by dividing the higher VPD growth condition by the lower VPD growth condition (33°C/30°C or 59% RH/70% RH) for each tree. For each gas exchange measurements this ratio was analyzed using Proc Mixed to test for differences between growth conditions, watering treatment, species, and any interactions. After this analysis, a t-test was used to test whether the ratio was different from one, i.e., a ratio of one indicates measurements were similar among reciprocal environments. Different t-tests were conducted for each factor that contained significant differences based on the Proc Mixed.

The repeated gas exchange measures were analyzed using Proc Mixed. To account for inherent variation between trees in gas exchange rates, the data were relativized using pretreatment data, i.e., for each individual tree, each measurement was divided by the initial measurement for that tree. The ratio of the water stressed individual and the well watered individual of the same species in each chamber for each measurement date was calculated to serve as the experimental unit. The variance of the dry/wet ratios was homogenized using logarithmic transformation. Each of the gas exchange variables were then independently analyzed and tested for differences among temperature treatment, species, and water stress as well as interactions with measurement date. The variance for each time series was managed using a heterogeneous variance model.

CHAPTER III

RESULTS: TEMPERATURE STUDY

During the study, the chambers successfully maintained the desired treatment separation. During the first experimental period, Chamber 1 kept an average day-time temperature of 30.1°C (standard deviation of 0.5), Chamber 2 kept an average day-time temperature of 33.8°C (standard deviation of 1.1), Chamber 3 kept an average day-time temperature of 30.4 °C (standard deviation of 0.5), and Chamber 4 kept and average day-time temperature of 33.4°C (standard deviation of 1.1) (Table 1). During the second experimental period, Chamber 1 kept an average day-time temperature of 32.8°C (standard deviation of 1.1), Chamber 2 kept an average day-time temperature of 30.4°C (standard deviation of 0.9), Chamber 3 kept an average day-time temperature of 33.1 °C (standard deviation of 1.1), and Chamber 4 kept and average day-time temperature of 31.2°C (standard deviation of 2.1) (Table 1).

Reciprocal gas exchange measurements

All seedlings were measured at each growth temperature on the fourth day (Period 1) or the second day (Period 2) after the treatments was applied. The ratio of net photosynthesis measured at 33°C divided by that measured at 30°C from the same seedling was calculated to determine the short-term effects of temperature and VPD. A 45% increase in VPD associated with the increase in measurement temperature decreased the ratio of net photosynthesis by 12% indicating a reduction in net photosynthesis with increased temperature. The ratio was significantly less than a value of 1 ($p<0.0001$). While the decrease in the ratio was greater for seedlings grown at 33°C than for those grown at 30°C, the effects of growth temperature were not significant ($p = 0.20$) (Figure 2). Likewise short-term effects of temperature on net photosynthesis were consistent among the water stress treatments, species, and all interactions ($p > 0.10$).

In contrast, the ratio of transpiration measured at 33°C divided by that measured at 30°C on the same seedlings on the same day was greater than one, indicating greater transpiration at the higher temperature and VPD. However the effects of measurement temperature were larger for seedlings grown at 30°C than those grown at 33°C (growth temperature; $p = 0.04$). The ratio was 1.4 for seedlings grown at 30°C ($p < 0.0001$) and 1.2 for seedlings grown at 33°C ($p < 0.0001$) (Figure 3). Short-term effects of temperature on transpiration were consistent among species, water stress treatments, and for their interactions ($p > 0.12$). These increases in transpiration were less than predicted from a 45 % increase in VPD in part because stomatal conductance declined on average by 14% for seedlings grown at 33°C ($p < 0.0001$) (Figure 4) which experienced smaller increases in transpiration. In contrast, stomatal conductance was not significantly affected by measurement temperature for seedlings grown at 30°C (3 % change) ($p = 0.49$) (Growth temperature effect; $p = 0.05$). No other effects (temperature effect, species effect and all other interactions) were significant ($p > 0.1$).

Xylem water potential

Water potential measurements were made on the fourth day (Period 1) or the second day (Period 2). The range for well-watered trees in the 30°C treatment was -0.46 MPa for mulberry to -0.86 MPa for American plum and -0.43 MPa for mulberry to -1.0 MPa for bur oak in the 33°C treatment. The range for the non-watered trees was -0.6 MPa post oak to -1.25 MPa American plum for the 30°C treatment and -0.78 MPa post oak to -1.65 American Plum for the 33°C treatment (species effects; $p < 0.0001$). Across species, water potentials were more negative in the non-watered compared to the watered trees ($p<0.001$), and this effect was consistent for the various species (species × growth temperature; $p = 0.53$) (Figure 5). The non-watered trees in the 33°C treatment had the most negative water potentials, however growth temperature did not have a significant effect ($p=0.15$). There was a significant interaction between water regime and growth temperature ($p=0.004$) which resulted from the larger difference between the temperature regimes for the well watered trees (growth temperature 30°C mean of -0.66 MPa, growth temperature 33°C mean of -0.74 MPa for all species combined) and the smaller difference between the growth temperatures from the non-watered trees (growth temperature 30°C mean of -0.97 MPa, growth temperature 33°C mean of -1.29 MPa for all species combined).

Change in transpiration over time

When the change in transpiration was relativized based on the pretreatment measurements, the rate of decline in transpiration did not differ between the trees grown at 30°C and 33°C ($p = 0.02$). However, the majority of the species have relative declines in transpiration rate for seedlings grown at 33°C than those grown at 30°C seedlings (Figure 6). There was a significant difference between species ($p < 0.0001$) with no species by growth temperature interaction ($p = 0.21$). There was a significant growth temperature ×day interaction ($p = 0.023$) with no growth temperature×species×day interaction ($p = 0.342$). When species were analyzed separately, redbud was the only species that exhibited a significantly faster relative decline in transpiration for seedlings grown at 33°C than those grown at 30°C ($p = 0.05$).

Mortality

The average days until death of the non-watered seedlings varied greatly between species, with a range of 5.9 (mulberry) to 24 days for (eastern redcedar) (species effect; $p < 0.0001$). The mean days until death for the 33°C seedling was almost 2 days sooner than the 30°C seedlings (30° mean of 14.7 days, 33°C mean of 12.8 days) (growth temperature effect; $p = 0.005$) (Figure 7). Overall, the effects of growth temperature were consistent across species (species × growth temperature; $p =0.54$). Only one well-watered seedling (redbud) died during this study.

CHAPTER IV

RESULTS: HUMIDITY STUDY

During the study, all but one chamber successfully maintained the desired treatment separation. During the third experimental period, Chamber 1 kept an average day-time relative humidity of 63.0% (standard deviation of 3.4), Chamber 2 kept an average day-time relative humidity of 61.8% (standard deviation of 5.4), Chamber 3 kept an average day-time relative humidity of 60.2% (standard deviation of 3.6), and Chamber 4 kept and average day-time relative humidity of 52.1% (standard deviation of 5.4) (Table 2). During the second experimental period, Chamber 1 kept an average day-time temperature of 69.5% (standard deviation of 3.0), Chamber 2 kept an average day-time temperature of 55.4% (standard deviation of 7.4), Chamber 3 kept an average day-time temperature of 68.0% (standard deviation of 3.6), and Chamber 4 kept and average day-time temperature of 51.5% (standard deviation of 7.8) (Table 2). The gas exchange, water potential, and mortality data from the trees in Chamber 4 were removed from analysis due to the chamber conditions being nearly identical. While, conditions in Chamber 3 did not meet the set conditions, there was still a difference in dewpoint of almost 3°C (21.9°C for the third experimental period and 24.1°C for the fourth experimental period). The target was to have a 3°C difference in dewpoint between experimental period, and data was blocked by chamber to counteract just such differences between chambers.

Reciprocal gas exchange measurements

The ratio of net photosynthesis, for each seedling, measured at a dewpoint of 21°C (59% relative humidity) divided by that measured at a dewpoint of 24°C (70% relative humidity) was calculated to determine the short-term effects of a change in dewpoint. The 3°C increase in dewpoint decreased VPD by 27%, and had no statistically significant effect on the ratio of net photosynthesis of the seedlings ($p = 0.35$). The effects of growth dewpoint conditions, species, and watering treatment as well as interactions were not significant ($p > 0.30$) (Figure 8). In contrast, the ratio of transpiration calculated by dividing the measurements at 21°C dewpoint by the measurements at 24°C dewpoint taken on each seedling on the same day, was significantly different than 1 ($p< .0001$). However, there was a significant interaction between species and growth dewpoint ($p = 0.01$). When each species× growth dewpoint combination was analyzed separately, the ratio for eastern redcedar, hackberry, post oak, Shumard oak, and sycamore at 24°C dewpoint and American plum and Shumard oak at 21°C dewpoint were significantly greater than 1 ($p < 0.10$ (Figure 9). Stomatal conductance also exhibited a growth dewpoint × species interaction. The only significant species × growth humidity combinations that had ratios significantly different than 1 were hackberry at dewpoint of 24°C (ratio greater than 1; $p = 0.03$) and redbud at 24°C (ratio less than 1; $p = 0.03$) (Figure 10).

Xylem water potential

Water potential measurements were made two days after imposing treatments. The range for well-watered trees in the 21°C dewpoint treatment was -0.46 MPa for sycamore to -1.29 MPa for hackberry and -0.47 MPa for syamore to -0.90 MPa for redbud and Shumard oak in the 24°C dewpoint treatment. The range for the non-watered trees was -0.88 MPa post oak to -1.75 MPa persimmon and redbud for the 21°C treatment and -0.77 MPa post oak to -1.82 redbud for the

24°C dewpoint treatment (species effects; p = 0.0009). Across species, water potentials were more negative in the non-watered versus watered trees (p<0.001), and this effect was consistent for the various species and growth humidities (species ×growth dewpoint; p = 0.50, watering treatment × growth humidity; p = 0.75). Growth humidity was not significant (p = 0.12) (Figure 11).

Mortality

The average number of days until death of the non-watered seedlings varied greatly between species with a range of 6.7(redbud) to 29 days (Shumard oak) (species effect; p <0.0001)(Figure 12). No watered seedling died during this study. There was no significant difference in days to mortality for non-watered seedlings grown at the different dewpoints (p = 0.22) or species × growth dewpoint interaction (p = 0.20).

Table 1. Day-time set points and actual environmental measurements from the chambers during the two temperature experimental periods. Standard deviations were calculated based on data recorded once every 5 minutes.

	Set T (^{o}C)	Set RH (%)	Act. T (^{o}C)	S.D. T (^{o}C)	Act RH (%)	S.D. RH (%)
Ch 1, P 1	30.0	59.0	30.1	0.5	62.0	2.8
Ch 1, P 2	33.0	49.0	32.8	1.1	53.8	3.6
Ch 2, P 1	33.0	49.0	33.8	0.7	51.4	6.7
Ch2, P 2	30.0	59.0	30.4	0.9	58.1	5.1
Ch 3, P 1	30.0	59.0	30.4	0.5	65.3	5.3
Ch 3, P 2	33.0	49.0	33.1	1.1	55.0	7.3
Ch 4, P 1	33.0	49.0	33.4	1.1	54.5	7.6
Ch 4, P 2	30.0	59.0	31.2	2.1	60.3	5.2

Table 2. Day-time set points and actual environmental measurements from the chambers during the two humidity experimental periods. Standard deviations were calculated based on data recorded once every 5 minutes. Chamber 4 was removed from study

	Set T (°C)	Set RH (%)	Act. T (°C)	S.D. T (°C)	Act RH (%)	S.D. RH (%)
Ch 1, P 3	30.0	59.0	30.1	0.3	63.0	3.4
Ch 1, P 4	30.0	70.0	30.9	0.3	69.5	3.0
Ch 2, P 3	30.0	70.0	30.1	0.3	61.8	5.4
Ch2, P 4	30.0	59.0	28.8	1.5	55.4	7.4
Ch 3, P 3	30.0	59.0	30.4	0.9	60.2	3.6
Ch 3, P 4	30.0	70.0	30.6	0.5	68.0	3.6
Ch 4, P 3	30.0	70.0	30.8	0.9	52.1	5.4
Ch 4, P 4	30.0	59.0	30.8	1.0	51.5	7.8

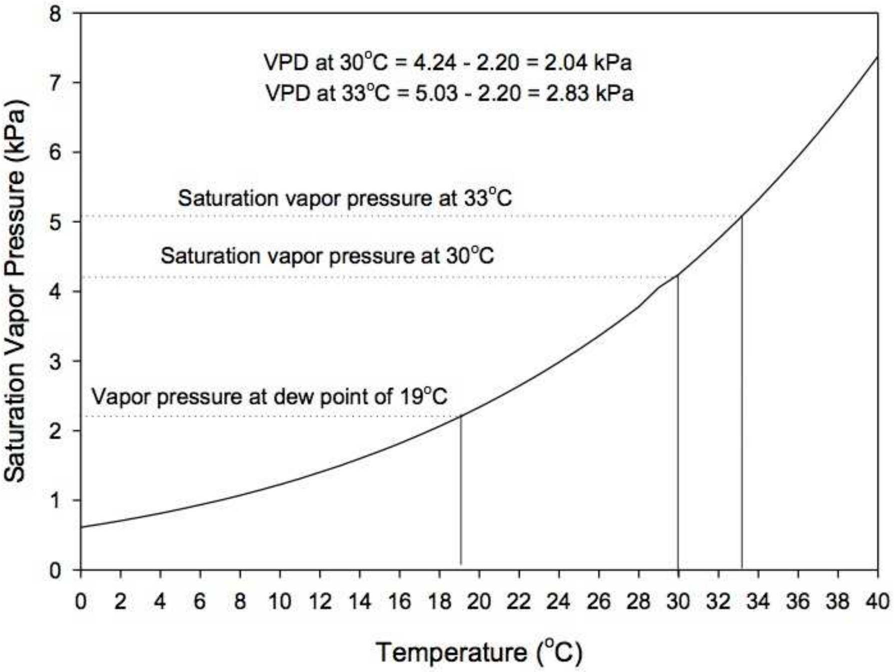

Figure 1. Vapor pressure deficit change with increase in temperature from 30°C to 33°C when vapor pressure of the surrounding air is held constant (e.g. dewpoint 19°C). VPD increases by 0.79 kPa or by 39% with a 3°C increase in temperature during typical summertime temperatures.

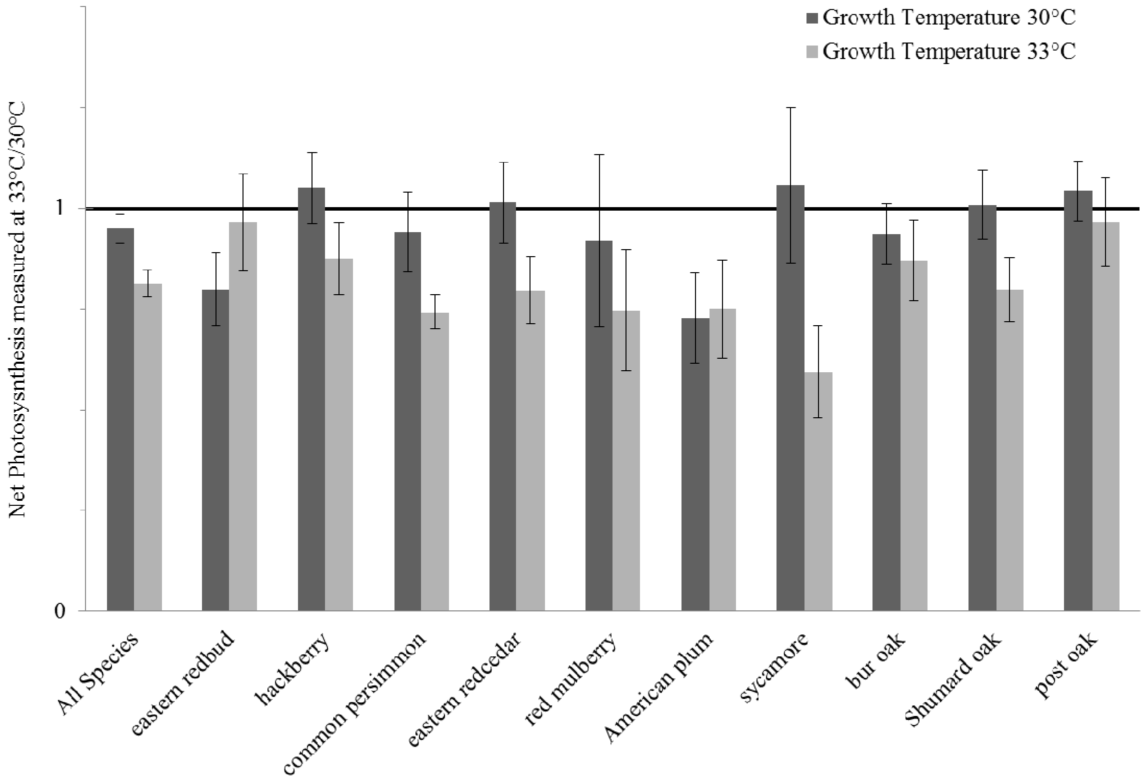

Figure 2. The ratio of net photosynthesis measured at 33°C divided by that measured at 30°C for seedlings of ten tree species. Seedlings were grown at either 33°C or 30°C. Vertical bars represent standard errors (n = 8).

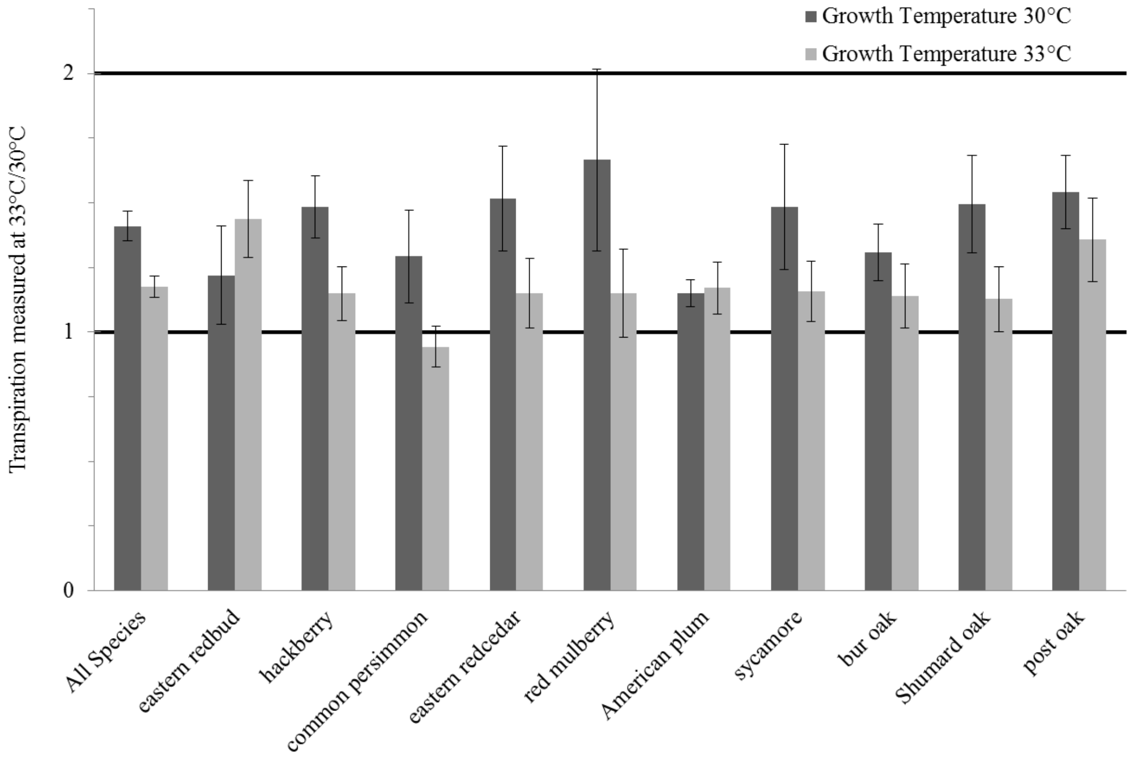

Figure 3. The ratio of transpiration measured at 33°C divided by that measured at 30oC for seedlings of ten tree species. Seedlings were grown at either 33°C or 30°C. Vertical bars represent standard errors (n = 8).

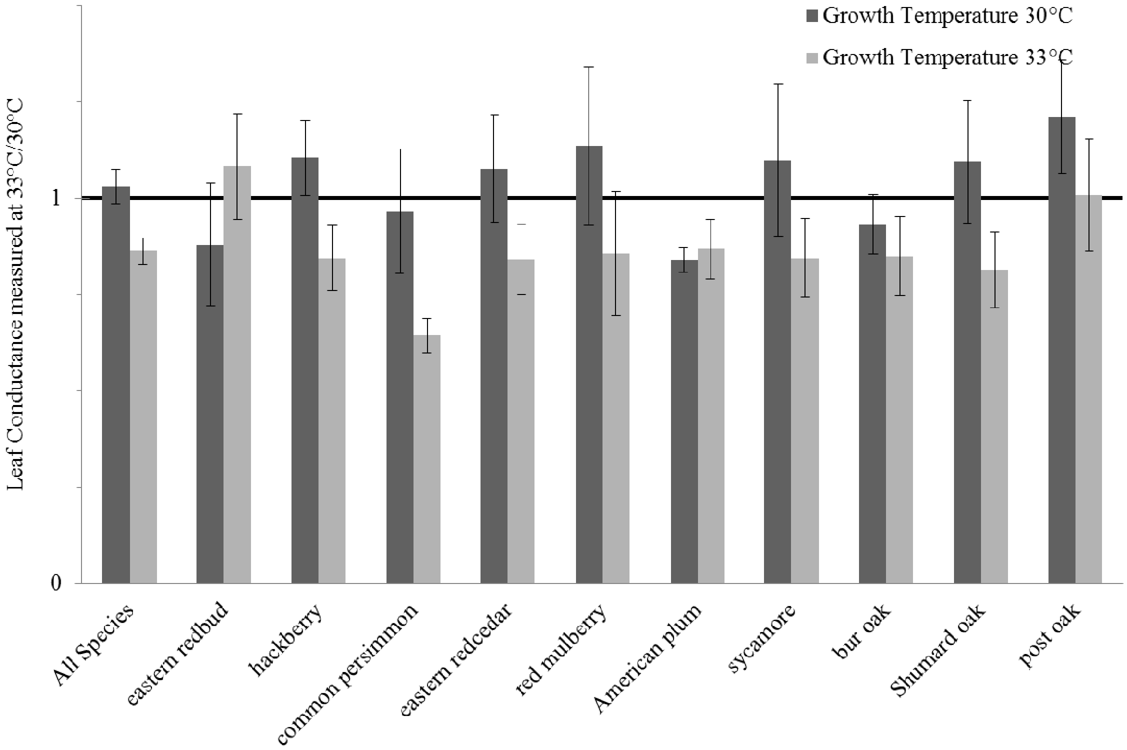

Figure 4. The ratio of leaf conductance measured at 33^{o}C divided by that measured at 30^{o}C for seedlings of ten tree species. Seedlings were grown at either 33^{o}C or 30^{o}C. Vertical bars represent standard errors (n =4).

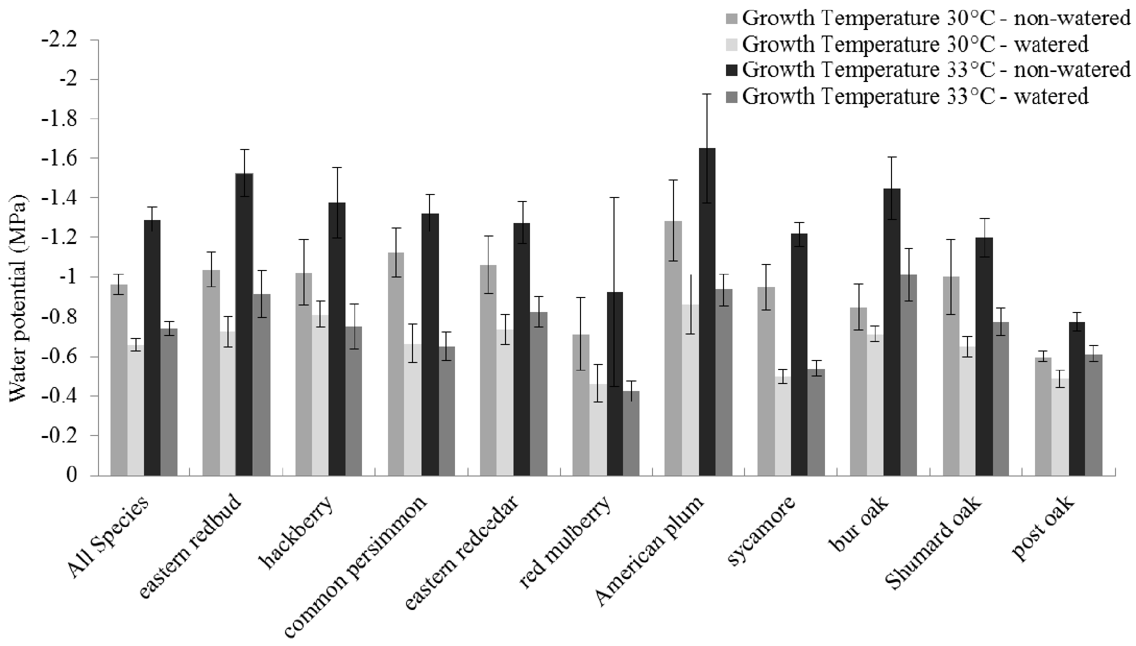

Figure 5. The average water potential (MPa) for seedlings of ten tree species. Seedlings were grown at either 33^{o}C or 30^{o}C. Vertical bars represent standard errors (n = 4).

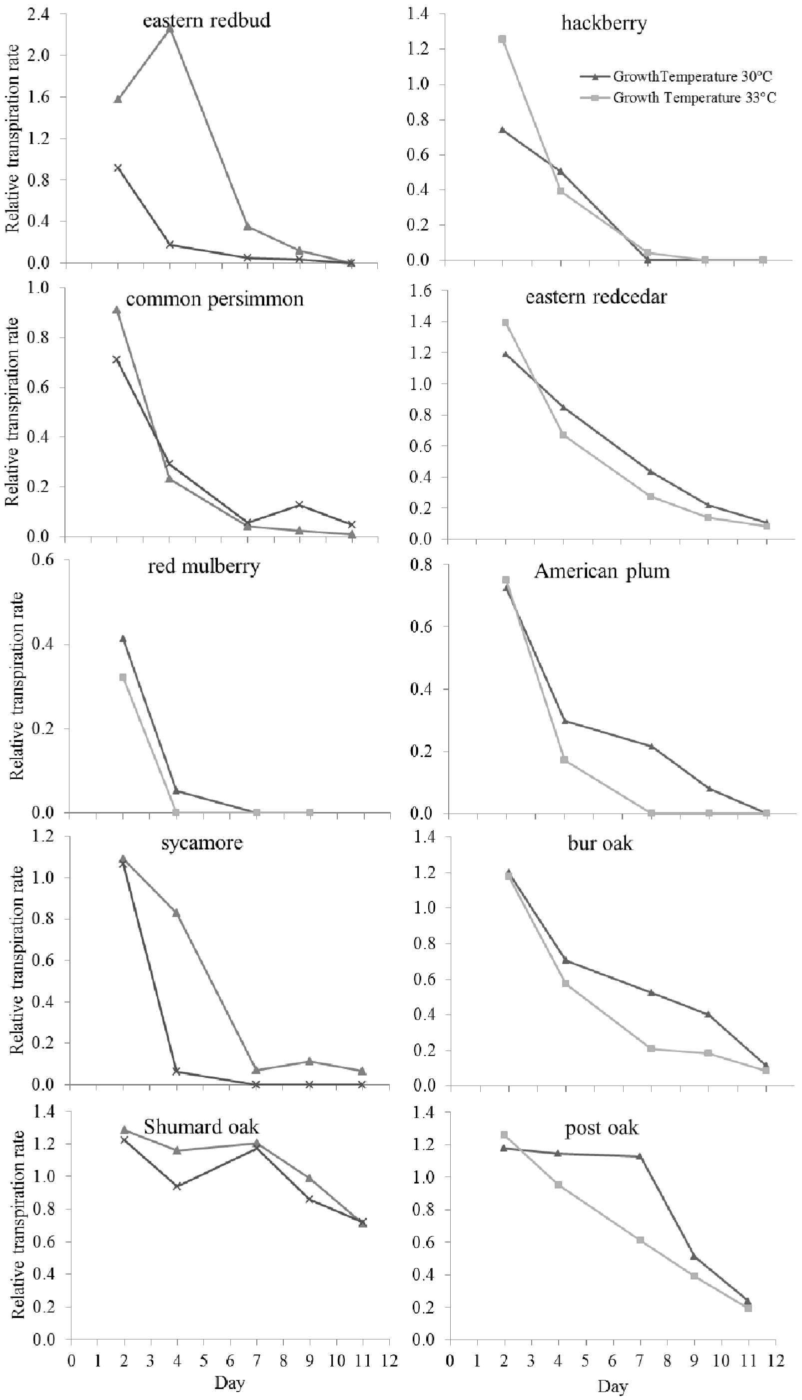

Figure 6. Change in transpiration over time for ten species of tree seedlings. Measurements were relativized based on measurements before imposition of growth temperature treatments. After the pre-measurement for all seedlings at 30°C, seedlings were measured at their growth temperature of 30 or 33°C. Only seedlings from which water was withheld are included in this analysis (n=4).

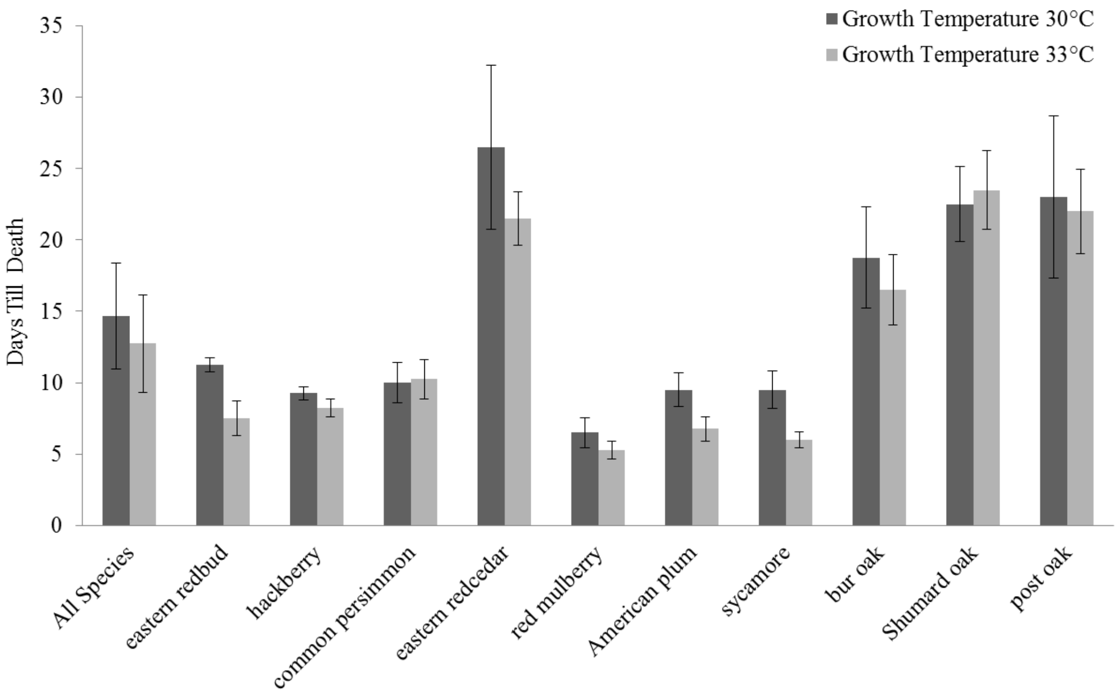

Figure 7. Average days till death for seedlings of ten tree species that were not watered. Seedlings were grown at either 33°C or 30°C. Vertical bars represent standard errors (n = 4).

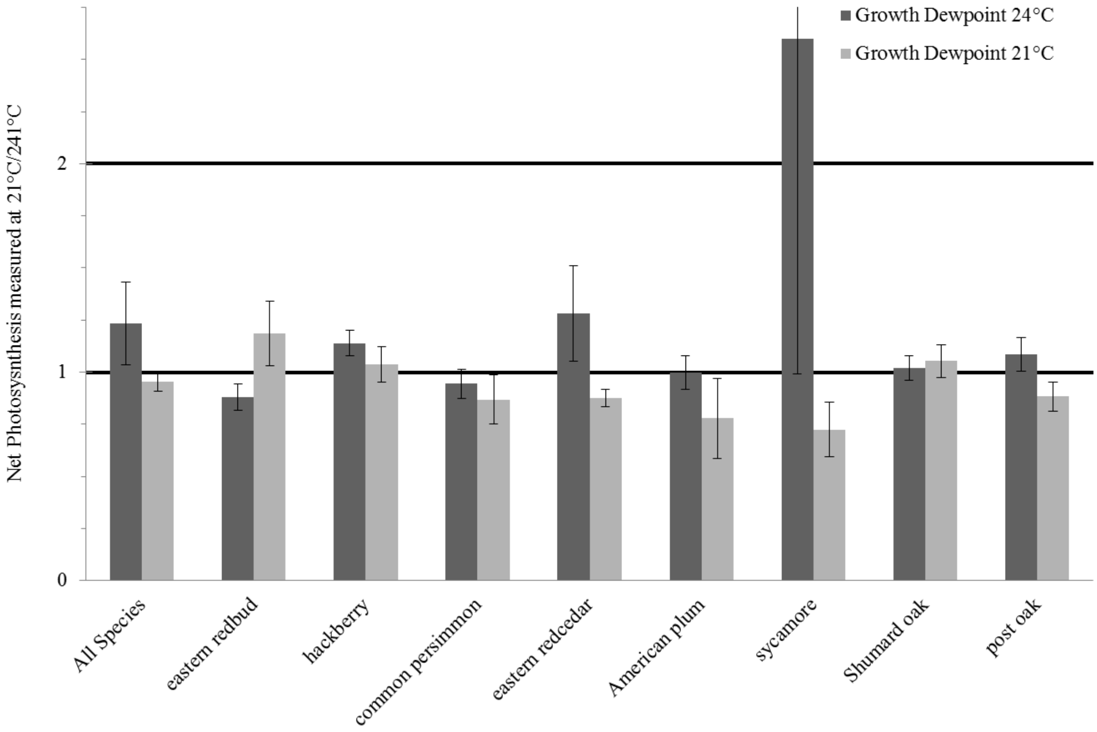

Figure 8. The ratio of net photosynthetic rate measured at dewpoint 21°C divided by that measured at dewpoint 24°C for seedlings of eight tree species. Seedlings were grown at dewpoints of either 21°C or 24°C. Vertical bars represent standard errors (n = 6).

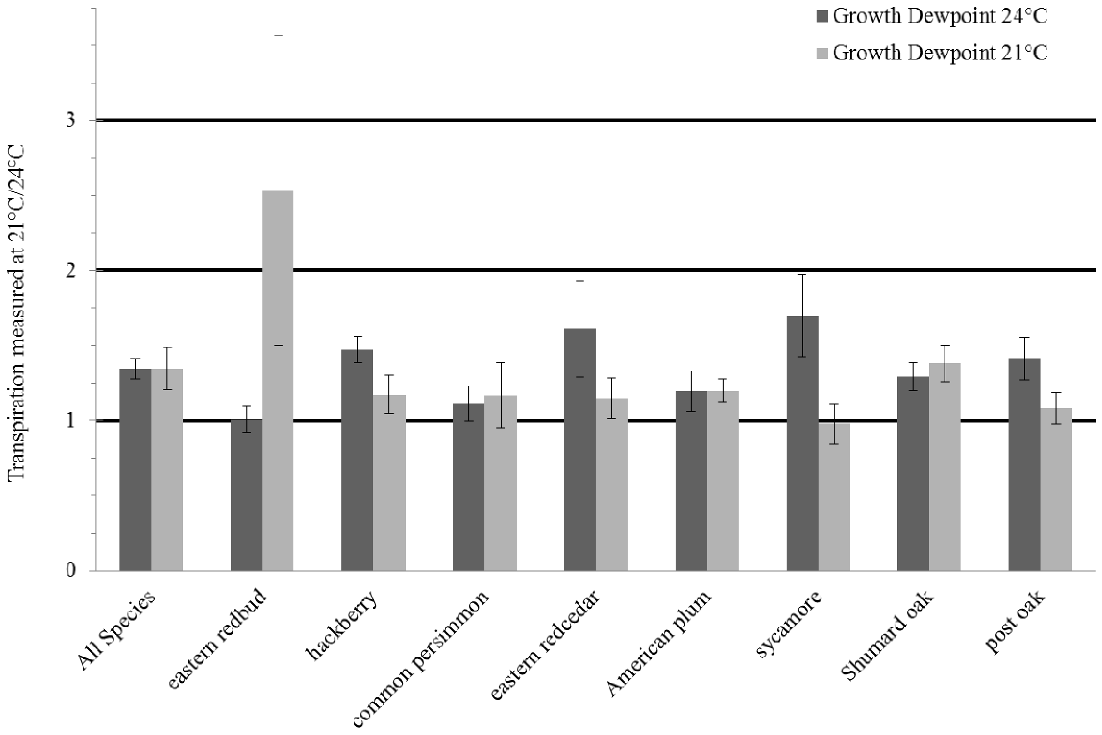

Figure 9. The ratio of transpiration rate measured at dewpoint 21°C divided by that measured at dewpoint 24°C for seedlings of eight tree species. Seedlings were grown at dewpoints of either 21°C or 24°C . Vertical bars represent standard errors (n = 6).

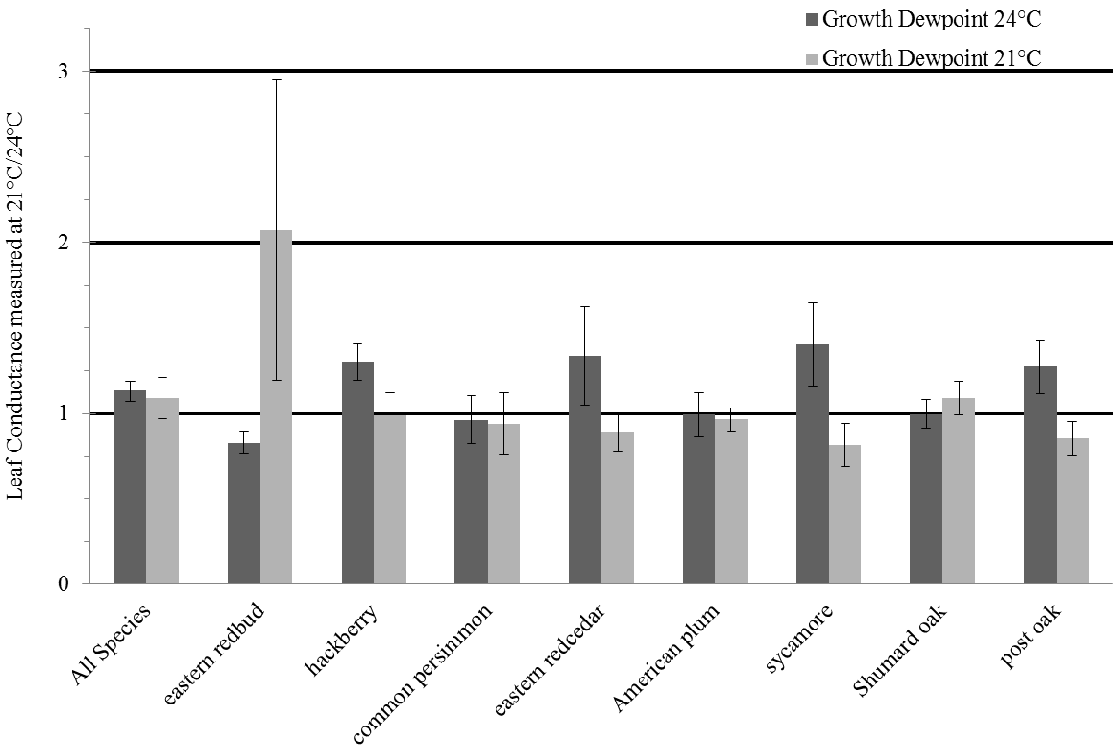

Figure 10. The ratio of leaf conductance rate measured at dewpoint 21°C divided by that measured at dewpoint 24°C for seedlings of eight tree species. Seedlings were grown at dewpoints of either 21°C or 24°C. Vertical bars represent standard errors (n = 6).

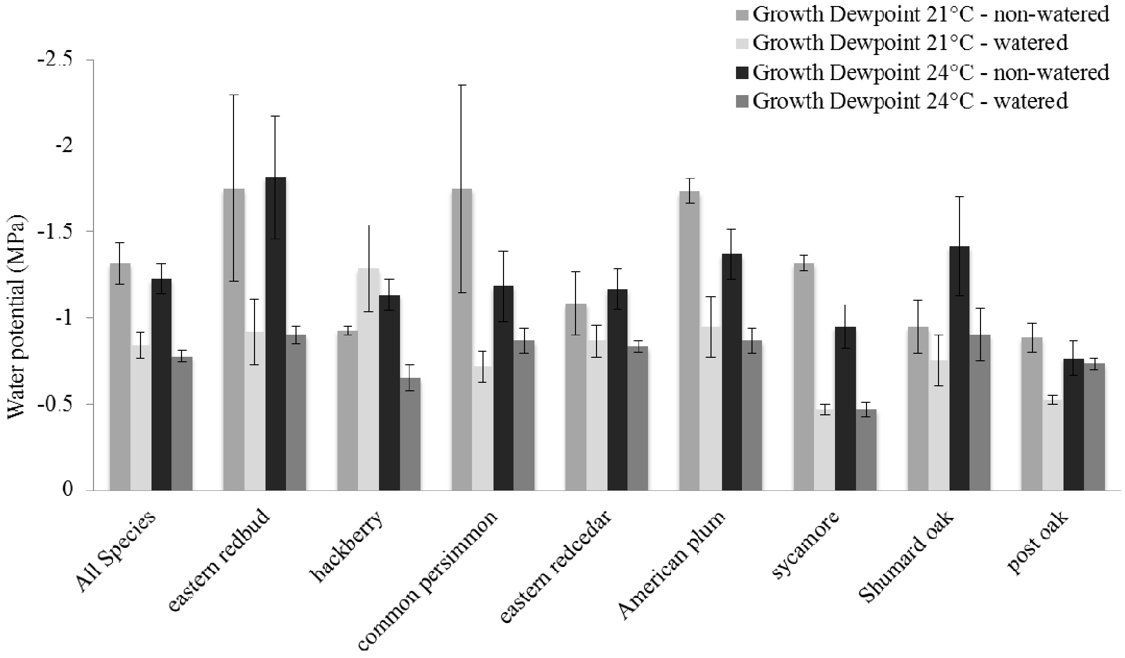

Figure 11. Average water potential (MPa) for seedlings of eight tree species. Seedlings were grown at dewpoints of either 24^{o}C or 21^{o}C. Vertical bars represent standard errors (n = 3).

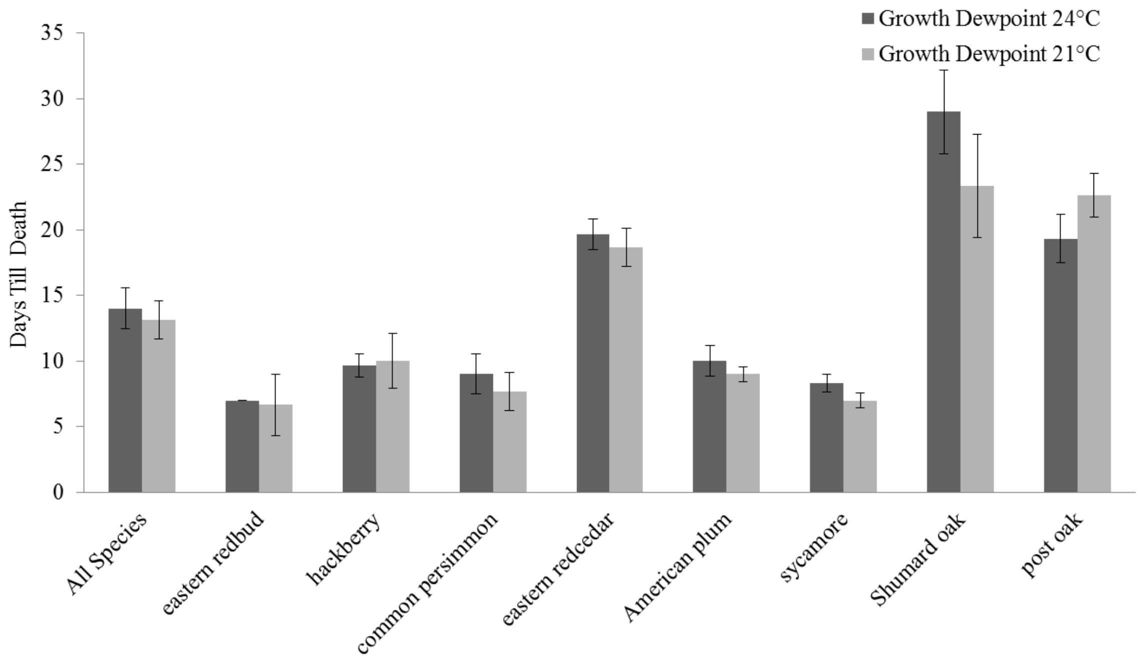

Figure 12. Average days till death for seedlings that were not watered of eight tree species. Seedlings were grown at dewpoints of either 24°C or 21°C. Vertical bars represent standard errors (n = 3).

CHAPTER V

DISCUSSION

Temperature study

The results of these experiments support the hypothesis that an increase in temperature will increase transpiration and water use of seedlings due to increased VPD. The reciprocal portion of the study showed that there was a rapid, short-term increase in transpiration. Decreased conductance moderated the effects of VPD to an extent. For instance, VPD increased by 45%, but transpiration only increased by 29%. The effect of VPD was stronger in the seedlings grown at 33°C than those grown at 30°C, perhaps indicating greater stomatal sensitivity associated with plants growing in higher temperature. The increase of stomatal sensitivity due to higher temperature with drought conditions has also been reported with longleaf pine (*Pinus palustris* Mill.) in a forest setting (Addington et al. 2004). These results and trends show that along the Oklahoma portion of the forest-grassland ecotone, increased VPD caused by increased temperatures combined with prolonged drought may hasten mortality of native seedlings and the shift in the ranges of tree species in Oklahoma eastward, as has been demonstrated in the western United States (Breshears et al. 2005, van Mantgem et al. 2009).

The ability for stomatal closure to moderate water loss is an important adaptation, especially for isohydric species, as it is the mechanism by which water potential is maintained. In this study, some hints are provided as to the water management strategy (isohydric/anisohydric) of the species. Transpiration for most species decreased sharply by the fourth day without water. This trend was generally intensified by increased temperature. However, post oak, bur oak, Shumard oak, and eastern redcedar maintained roughly the same transpiration rate until day seven, when they began a gradual decline. This suggests that post oak, bur oak, Shumard oak, and eastern redcedar are anisohydric based on their increased, but steady transpiration rates, while the other species in the study are more isohydric, as they close their stomates rapidly reducing transpiration and conductance.

For most species in the study, the transpiration rate of the seedlings exposed to the greater temperature decreased at a faster rate than those at the lower temperature. An increase in VPD increases short-term transpiration; the long-term effects of higher VPD is to use soil water faster and hasten water stress and stomatal closure. These long-term effects were seen as a significant increase in the relative transpiration rates for the seedlings exposed to a higher VPD as the transpiration rate for seedlings under a higher VPD decreased more rapidly over time. Other studies conducted on trees and agricultural crops have shown VPD affecting transpiration and conductance (Teskey et al. 1986, Bunce 1996).

However, the species' ability to regulate water loss would influence how rapidly this decline takes place. Generally, more isohydric species would decrease transpiration initially, and then maintain a low level transpiration until they run out of water or carbon, while the anisohydric species would remain steady initially, followed by a sharp decrease and death as the cavitation occurs due to lack of available water and the seedling dies of hydraulic failure (West et al. 2007). The label of isohydric and anisohydric is relative (Maseda and Fernández 2006). In general, all seedlings from this study exhibit isohydric properties to some extent, but some show more anisohydric properties relative to others.

Most of the species included in this study tended to be more isohydric. The relative transpiration rate tended to decline in the first few days without water. Post oak, Shumard oak, and to an extent eastern redcedar did not exhibit these tendencies. Over time the transpiration rate of Shumard oak stayed relatively steady, until it began a gradual decrease, as did post oak. Eastern redcedar was a little different in that it did not stay steady or drop sharply; instead it gradually declined over time. The tracheids of Juniper species, along with other conifer species, are less prone to cavitation because they are narrower and have thicker walls that take greater force to collapse (Bowyer et al 1996; Pitterman et al. 2006).

The oaks and eastern redcedar lived longer under terminal drought conditions than the other species in the study. While it was not measured, it appeared that the species with the greatest leaf area died the fastest. As other elements of this study have demonstrated, the oaks and juniper seem to be more anisohydric, able to withstand greater water stress better than the isohydric species in the study, especially mulberry and sycamore. In this experiment, the anisohydric approach to drought conditions may have delayed mortality. This assessment is consistent with one-seed juniper (*Juniperus monosperma*) and Utah juniper (*Juniperus osteosperma*), which is the same genus as eastern redcedar, was shown to be anisohydric and lived longer than *Pinus edulis* (West 2007; Breshears et al. 2009).

Humidity Study

The humidity study was designed to increase VPD using different methods. However, limitations in seedling availability of some species reduced the number of species used in the humidity study. Variable conditions of one chamber caused the elimination of one of the four blocks, resulting in a smaller sample size. In addition, the increase in VPD for the humidity study was 27% due to limitations in chamber set points. This is less than the comparable 45% difference in VPD as created in the temperature study and makes it more difficult to determine effects of VPD.

Subjecting seedlings to lower humidity increases transpiration and can cause a reduction in stomatal conductance (Kozlowsk and Pallardy 1997). This study demonstrated a short-term effect of a change in humidity on the transpiration rate as well as a difference in water potential, as in the temperature study. However, longer-term trends could not be established for gas exchange in the humidity as the non-watered plants had a negative net photosynthetic rate when not watered after only a few sampling dates.

The difference in number of days until death was not significant in the humidity study. However there was a trend of the seedlings grown under less humid conditions did die before those grown at normal higher humidity. The days until death of individual seedlings in the humidity varied more in the humidity study than the temperature study. In both studies the species differed. While it was not measured, it was observed that the species with the greatest leaf area died the fastest. For mulberry in the humidity study, 3 of the 4 seedlings were dead before the first day of gas exchange measurements (2 days without water) and had to be dropped from that portion of the study.

While the seedlings in this portion of the study did not die at a significantly faster rate, there was increased transpiration and water potential consistent with the temperature study. This reinforces that the increased VPD stressed these seedling as it did in the temperature study and showed trends that VPD was the cause of the increased mortality in the temperature study, not the increase in temperature alone.

General

Species differences

While species differences were not a significant factor in many parts of these studies, the species did react differently to the changes in VPD with varied levels of increased transpiration and stomatal closure. Breshears et al. (2009), Adams et al. (2009), McDowell et al. (2008) and others have pointed out the differences in isohydric and anisohydric species, as well as the potential for carbon starvation caused by these strategies. The general thought is that the more isohydric a species is, the more likely that drought induced mortality is due to lack of available carbon to offset that used by respiration, barring biotic influences. The study presented here used more mesic species than the ones in the intermountain region of the US. McDowell et al. (2008) and McDowell (2011) argue that anisohydric species can be just as drought tolerant as isohydric species because they evolved to recover from hydraulic failure quickly and more efficiently as they stay so close to the edge of hydraulic failure as part of their drought tolerance strategy. Many species native to eastern Oklahoma do not experience frequent or intense droughts compared to those in the intermountain region. This puts many of the more isohydric trees in this study at a disadvantage, as they are not necessarily equipped to recover from hydraulic failure. The mesic species include almost every species in the study except for the oaks and eastern redcedar. The oaks and redcedar are more xeric species and represent the western front of trees in Oklahoma with both ranges spanning nearly the entire state. The more mesic/isohydric species tended to die faster while the xeric/anisohydric species lived longer, in this study. Of course there are many other adaptions and environmental elements that influence the long term survival of these trees, such as rooting distribution/depth and competition for water. This study was conducted in pots where rooting was ignored and competition was purposely avoided.

Ecological ramifications

While it is hard to discern the true cause of mortality in this study, as hydraulic failure and carbon starvation tend to reinforce each other (McDowell 2011), it is obvious that increased temperatures will increase VPD, leading to increased transpiration of tree seedlings along the forest-grassland ecotone. Tree species have different ways of mitigating this demand and survive when adequate water is available. However with prolonged drought, the seedlings cannot survive and those exposed to higher temperature will likely die sooner. In Oklahoma, most tree species are already at the margin of their range due to moisture gradient across the state. If climate change type temperature and droughts increase in the future, this could create an eastward range shift as the mature members of the species die and are not replaced. Allen and Breshears (1998) modeled similar results for a forest-woodland ecotone in New Mexico. The results of this study indicates that there is a potentially huge shift in tree species distribution and the loss of current ecosystems across the ecotone of the central United States under predicted global climate change scenarios.

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