THE EFFECT OF ELEVATED [CO_2] AND ELEVATED TEMPERATURE ON TREE GROWTH AND PHYSIOLOGY

by

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(Under the Direction of ROBERT O. TESKEY)

ABSTRACT

Increasing atmospheric CO_2 concentration ([CO_2]) is predicted to increase mean air temperature, which in turn is anticipated to affect precipitation. Expected changes in [CO_2], temperature, and soil moisture availability may have a substantial effect on tree physiology and species distribution. Changing environmental conditions, especially temperature, may have a variable effect on physiological processes depending on where an individual is located within the species distribution. The hypothesis that an increase in temperature will boost growth in sub-optimal temperature environments, while an increase in temperature in supra-optimal temperature environments will reduce growth was tested. To test this hypothesis two studies were conducted. The first study investigated the effect of elevated temperature, elevated [CO_2] and decreased soil moisture availability on loblolly pine physiology and growth at sites located near the northern and southern edge of the species range. Based on the findings from the loblolly pine study, a second study was conducted to investigate the combined effect of elevated temperature and [CO_2] on northern red oak seedlings grown near the southern edge of its distribution.

Net photosynthesis and biomass accumulation of one-year-old loblolly pine seedlings increased with exposure to elevated [CO_2] and temperature at both the northern and southern sites within the species distribution. A decrease in soil moisture availability significantly reduced accumulation, regardless of growing location or growing conditions. These findings are in conflict with assumptions of models that utilize environmental conditions to predict species distribution. These models predict that an increase in temperature will result in a northward shift in species distributions. Our study suggests that changes in climatic conditions may increase growth across the loblolly pine distribution and may result in an expansion of the species distribution. Northern red oak, unlike loblolly pine, was negatively affected by elevated temperature. The response of oak to elevated temperature suggests that an increase in growing temperature has the potential to negate the boost that elevated [CO_2] may have on net photosynthesis and growth at ambient temperatures. The different responses of these two species to predicted future climatic conditions suggest that models utilized to predict future distributions require species specific verification.

INDEX WORDS: Elevated [CO_2], elevated temperature, water stress, climate change, species distribution, net photosynthesis, respiration, biomass accumulation, *Quercus rubra*, *Pinus taeda*

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Foliar Gas Exchange

Due to anthropogenic sources, atmospheric CO_2 concentration ([CO_2]) is expected to increase to between 590 to 970 μmol $mo1^{-1}$ by the year 2100 (IPCC 2001: Model A1B). Rising [CO_2], along with other greenhouse gases, is expected to cause an increase in mean global temperature of between 1.7 and 4.4°C by 2100 (IPCC 2007a: Global Climate Projections). Annual temperature in eastern North America is expected to increase between 3 and 6°C by 2100 (IPCC 2007b: Regional Climate Projections). While an increase in air temperature is expected to alter precipitation, predictions in shifts of precipitation differ. An increase in air temperature, to the scale predicted by the A1B model, is predicted to result in a slight increase, approximately 7%, in mean precipitation in eastern North America (IPCC 2007b: Regional Climate Projections). Sun *et al*. (2007) also predicts a shift in precipitation for the Southeastern USA, with an increase in total rainfall along with a change in storm frequency. They predict that most rainfall is expected to occur in a few large events, and a substantial amount may be lost as runoff and unavailable for plant use. Precipitation models by Trenberth (1998) predict that future changes in the climate may lead to a slight decrease in the amount of precipitation in the Southeastern USA.

The effect of elevated [CO_2] on net photosynthesis in trees has been well documented (i.e.: Saxe *et al.* 1998; Ainsworth & Long 2005; Korner 2006; Huang *et al.* 2007). On average, net photosynthesis in trees exposed to elevated [CO_2] (approximately doubled over ambient) for an extended period was 61% higher in deciduous broadleaf trees and 41% higher in conifer evergreen trees, compared to trees exposed to ambient [CO_2] (Ceulemans & Mousseau 1994). Net photosynthesis in loblolly pine (*Pinus taeda* L.) and northern red oak (*Quercus rubra* L.) follows a similar trend. Net photosynthesis in loblolly pine trees and seedlings has been demonstrated to be boosted by both short and long term exposure to elevated [CO_2]. In a branch chamber experiment elevated [CO_2] was demonstrated to increase net photosynthesis in mature loblolly pine trees over one growing season (Teskey 1995). Tissue *et al.* (1997) observed, in 4 year old loblolly pine saplings grown in open top chambers, a significant increase in net photosynthesis in the elevated [CO_2] treatment compared to the ambient [CO_2] treatment. Net photosynthesis was increased in the elevated [CO_2] treatment by 60-130% in the summer and 14-44% in the winter. Additionally, a long term boost (occurring over four years) in net photosynthesis with elevated [CO_2] has been demonstrated in loblolly pine trees (Crous & Ellsworth 2004). An increase in net photosynthesis of northern red oak seedlings with exposure to elevated [CO_2] has also been reported (Anderson & Tomlinson 1998). An increase in net photosynthesis with exposure to elevated [CO_2] has also been reported in *Quercus mongolica* (Wang *et al.* 2008), *Quercus ilex* (Arena *et al.* 2005) and *Quercus pubescens* (Rapparini *et al.* 2004). While acclimation of net photosynthesis to elevated [CO_2] has been reported (Gunderson & Wullschleger 1994; Lewis *et al.* 1996; Griffin *et al.* 2000), it is typically associated with nutrient limitation (Conroy *et al.* 1990; Tissue *et al.* 1993; Thomas *et al.* 1994; Stitt & Krapp 1999; Iversen & Norby 2008) and sometimes water stress (Sholtis *et al.* 2004). There is also evidence of enzymatic down regulation with long term exposure to elevated [CO_2] (Ainsworth & Rogers 2007), which may be induced by increases in cellular carbohydrate concentrations (Long *et al.* 2004).

The short term effect of changes in air temperature on net photosynthesis has been well documented. As air temperature increases from sub-optimal to optimal, net photosynthesis increases regardless of light intensity or [CO_2] (Berry & Bjorkman 1980). The optimal temperature range of net photosynthesis can be broad or narrow and depends upon both species and growing location (Cunningham & Read 2002). As air temperature increases above an optimum, net photosynthesis decreases due to decreased CO_2 solubility and a concurrent increase in enzyme kinetics (Farquhar *et al.* 1980; Jordan & Ogren 1984). Increased air temperature beyond the optimum range further reduces net photosynthesis due to increasing rates of mitochondrial respiration. While a short-term exposure to elevated temperature may lead to a suppression of net photosynthesis, at least some tree species may able to partially acclimate to elevated temperatures (Sage & Kubien 2007). A few studies have demonstrated acclimation of net photosynthesis to the local growing environment: e.g. *Pinus radiata* D. Don, (Rook 1969) and *Eucalyptus pauciflora* (Slatyer 1977), while others have demonstrated acclimation of net photosynthesis to elevated temperature: eg. *Pseudotsuga menziesii* (Lewis *et al.* 2001), *Picea sitchensis* (Neilson et al. 1972) and *Plantago* (Atkin *et al.* 2006). However, in studies of other tree species, including loblolly pine (Teskey & Will 1999), net photosynthesis did not acclimate to different temperature regimes (Wayne *et al.* 1998; Nagy *et al.* 2000; Ro *et al.* 2001; Ow *et al.* 2008a; Ow *et al.* 2008b). The lack of consistency in the ability of species to acclimate to growing temperature is likely due to the temperature range a species can grow over, how broad a temperature range net photosynthesis is optimal for a species and the degree to which temperature increases. Even with acclimation of net photosynthesis, the response of a species to elevated temperature may differ across the species range. Net photosynthesis in trees growing in sub-optimal temperatures may increase with elevated air temperatures, while net photosynthesis in trees growing in supra-optimal temperatures may decrease with further increases in air temperature.

Regardless of the potential of net photosynthesis to acclimate, an increase in air temperature has had conflicting effects on net photosynthesis. Net photosynthetic rates of *Pinus ponderosa* (Callaway *et al.* 1994), *Pinus sylvestris* (Wang *et al.* 1995), *Betula alleghaniensis* (Wayne *et al.* 1998) and *Malus domestica* (Ro *et al.* 2001) all declined with an increase in growing temperature. However, an increase in net photosynthesis with an increase in growing temperature has been reported in *Pinus sylvestris* (Beerling 1997) and *Pseudotsuga menziesii* (Lewis *et al.* 2001). In addition, some studies have reported a lack of effect of an increase in air temperature on net photosynthesis (Teskey *et al.* 1987). The variable responses to elevated temperature reported in the literature can be due to various factors including the growing site location (sub or supra-optimal growing temperature), the amount temperature increases, the sensitivity of a species to an increase in temperature, and the ability of a species to acclimate net photosynthesis to a change in temperature.

Exposure to elevated [CO_2] and temperature may have an interactive effect on net photosynthesis. Long (1991) proposed that a concurrent increase in air temperature and [CO_2] would increase the optimal temperature of net photosynthesis by increasing enzyme kinetics and CO_2 availability, thereby reducing photorespiration. While fewer studies have investigated the effect of both elevated [CO_2] and temperature, there are reports of both positive and negative responses in different studies. In a branch bag experiment on mature *Picea abies*, the optimal temperature of net photosynthesis increased in the elevated [CO_2] treatment (Roberntz 2001). In loblolly pine seedlings, the optimal temperature for net photosynthesis in trees grown under elevated [CO_2] was greater compared to trees grown in ambient [CO_2] (Lewis *et al.* 1996). However, studies by Wang *et al*. (1995), on mature *Pinus sylvestris*, and Tjoelker *et al*. (1998), on *Populus tremuloides*, *Betula papyrifera* and *Picea mariana,* report a decrease in net photosynthesis with an increase in growing season temperature under both ambient and elevated [CO_2] suggesting that there was no temperature by [CO_2] treatment interaction.

Plants grown in elevated [CO_2] often have lower stomatal conductance, and therefore lower transpiration rate (Pearson *et al.* 1995; Bettarini *et al.* 1998; Medlyn *et al.* 2001), thus suggesting that long-term exposure to elevated [CO_2] may make a plant less susceptible to drought stress. Johnson *et al*. (2002) observed in *Salix sagitta* and *Populus trichocarpa* × *deltoides* saplings exposed to a short-term drought that net photosynthesis and stomatal conductance were maintained nearer to unstressed rates in the elevated [CO_2] treatment compared with the ambient [CO_2] treatment. However, Ellsworth (1999) did not observe a significant difference in stomatal conductance in loblolly pine trees exposed to elevated [CO_2] compared with trees exposed to ambient [CO_2] when a summer drought occurred, though rates of net photosynthesis were generally higher in trees exposed to elevated [CO_2], suggesting a higher water use efficiency (WUE) in those trees. While elevated [CO_2] may decrease stomatal conductance, elevated temperature has been demonstrated to increase stomatal conductance (Wang & Kellomaki 1997; Maherali & DeLucia 2000; Lewis *et al.* 2002), due in part to an increase in vapor pressure deficit. An increase in transpiration with an increase in temperature may also be a mechanism trees use to dissipate heat and relieve thermal stress. This suggests that if a tree is exposed to both elevated [CO_2], which generally decreases stomatal conductance, and elevated temperature, which may induce thermal stress, the trees may be damaged to a greater extent by thermal stress.

Biomass accumulation

The effect of elevated [CO_2] on tree biomass production has been well characterized. In general, under optimum growing conditions (i.e. adequate nutrients, water and light), long term exposure to elevated [CO_2] (approximately double ambient [CO_2] for one season or more) can increase biomass by up to 130% in conifer and 49% in deciduous trees (Saxe *et al.* 1998). Curtis & Wang (1998) reported that, on average, biomass production was stimulated by approximately 31% in loblolly pine grown in elevated [CO_2]. Long term exposure to elevated [CO_2] at the Duke FACE site has been reported to increase biomass in loblolly pine trees by an average of 28% over 9 years (McCarthy *et al.* 2010). A similar result has been observed for northern red oak (Anderson & Tomlinson 1998). However, the effect of [CO_2] on biomass production is influenced by growing temperature. When temperature is sub-optimal the effect of increased [CO_2] availability on biomass production is often dampened (Poorter & Perez-Soba 2001; Zhang & Dang 2007; Cao *et al.* 2008). At low temperatures growth rate, not carbon fixation, is more often limited in tree species (Rawson 1992; Korner 1998). Conversely, at supra-optimal temperatures elevated [CO_2] not only increases net photosynthesis and reduces photorespiration (Farquhar *et al.* 1980), it also increases the optimum temperature for net photosynthesis (Long 1991).

Previous studies investigating the effect of an increase in air temperature on biomass production have yielded conflicting results. Some studies have reported a decrease in biomass production with an increase in growing temperature. Biomass of *Ecalyptus grandis*, grown in environmentally controlled growth chambers maintained at temperatures from 10 to 35°C, was reduced when grown in temperatures above 25°C (Thomas *et al.* 2007). In another growth chamber experiment, *Picea mariana* biomass production was reduced with an increase in air temperature of 8°C (Way & Sage 2008). In open top chamber experiments, biomass production was less in *Acer rubrum* and *Acer saccharum* grown in elevated temperature chambers (+4°C) compared to trees grown in ambient temperature chambers (Norby *et al.* 2000). In a study looking at growth of *Pinus taeda* throughout its native range, Neldo *et al.* (2009) observed a decrease in biomass with increasing growth temperature. However, other studies have reported an increase in biomass production with an increase in temperature. In growth chamber experiments, both Xiao *et al*. (2003) with *Caragana indermedia*, *Hedysarum mongolicum*, and *Artemisia ordosica* and Hoch and Korner (2009) with *Larix decidua* and *Pinus mugo* reported greater biomass production in seedlings grown in the elevated temperature treatment (+3°C and +6°C, respectively). In a series of close topped chamber experiments, where air temperature was increased by 3 to 5°C, an increase in biomass was observed in *Pinus sylvestris* (Peltola *et al.* 2002), *Betula pendula* (Kuokkanen *et al.* 2004), *Pseudotsuga menziesii* (Olszyk *et al.* 2005) and *Picea mariana* (Bronson *et al.* 2009). A similar response was observed in open top chamber experiments with *Picea glauca* (Danby & Hik 2007), *Picea asperata* and *Abies faxonian*a (Yin *et al.* 2008). It should be noted that in some studies elevated temperature had no effect on biomass production. In a growth chamber experiment, elevated temperature (+5°C) had no effect on *Pinus ponderosa* biomass production (Maherali & DeLucia 2000); additionally, in a close top chamber experiment, elevated temperature had no effect on biomass production in *Pinus sylvestris* or *Betula pubescens* (Rasmussen *et al.* 2002).

While water stress generally leads to significant reductions in biomass production, in some studies exposure to elevated [CO_2] has been shown to partially relieve water stress, mainly by reducing stomatal conductance (Morison & Gifford 1983; Aranda *et al.* 2006; Li *et al.* 2007). However, in a loblolly pine stand subjected to a naturally occurring drought, leaf level stomatal conductance was not lower in trees exposed to elevated [CO_2] compared to those exposed to ambient [CO_2] (Ellsworth 1999). Elevated temperature is predicted to exacerbate water stress by increasing vapor pressure deficit; plants may compensate for water stress by decreasing transpiration, thus limiting photosynthetic capacity and reducing biomass production.

Species Distribution

Increasing air temperature, caused by rising [CO_2], is predicted to significantly affect tree net photosynthesis and biomass production, with the largest impact expected to occur at the coldest and the warmest portion of a species range (He *et al.* 2005; Thuiller *et al.* 2008), where sub- and supra-optimal growing temperatures are believed to limit carbon fixation and subsequently biomass production (Xu *et al.* 2007). Even a small increase in air temperature may positively affect growth in sub-optimal temperature environments and negatively affect growth in supra-optimal temperature environments, and if the effect is large enough species distribution may be altered. In current sub-optimal temperature regions it is commonly assumed that an increase in temperature will have a positive impact on growth by raising air temperature closer to the optimum for net photosynthesis (Kirschbaum 2000) and increasing the length of the growing season (Koca *et al.* 2006; Bronson *et al.* 2009). In current supra-optimal temperature environments an increase in growing season air temperature is expected to apply thermal stress that may reduce net photosynthesis, increase respiration and damage foliage resulting in a reduction in biomass production.

An increase in temperature near the cold limited region of a species generally leads to an increase in biomass (Penuelas *et al.* 2007; Reich & Oleksyn 2008; Yin *et al.* 2008). This increase is attributed to both the longer growing season (Bronson *et al.* 2009) and/or the increase in seasonal mean air temperature closer to the optimum temperature for net photosynthesis (Xu *et al.* 2007). Previous studies have demonstrated that an increase in air temperature at northern latitudes results in an increase in growth, both by lengthening the growing season and by raising the air temperature closer to the optimum for net photosynthesis (Peltola *et al.* 2002; Wilmking *et al.* 2004; Wieser & Stohr 2005; Danby & Hik 2007; Penuelas *et al.* 2007; Thomas *et al.* 2007; Yin *et al.* 2008; Hoch & Korner 2009; Wieser *et al.* 2009).

A negative effect of increased temperature on biomass production near the edge of a species range has been demonstrated in a few studies. Elevated temperature (+4°C) reduced biomass production of *Acer saccharum* seedlings, planted near the southern border of the species distribution (in North America), grown in both ambient and elevated [CO_2], though the reduction was greatest in ambient [CO_2] (Norby *et al.* 2000). Reich and Oleksyn (2008) reported that an increase in air temperature (+1 to +4°C) caused a significant decrease in height growth and an increase in mortality of *Pinus sylvestris* at the southern limit of its European distribution. However, several studies have suggested that warm temperature *per se* does not define a species border (Woodward 1987; Bonan & Sirois 1992). An increase in temperature, even in supra-optimal environments, may not necessarily have a negative effect on net photosynthesis or biomass production. Some tree species demonstrate at least partial acclimation of net photosynthesis to increases in growing temperature (Sage & Kubien 2007). Additionally, elevated temperature may result in a longer growing season and additional time in the growing season to accumulate carbon. Increases in air temperature will be driven by an increase in [CO_2] availability. A doubling of [CO_2] is predicted to shift the optimal temperature for net photosynthesis by up to +5°C (Long 1991), suggesting that a concurrent increase in [CO_2] and temperature may actually boost net photosynthesis by both reducing photorespiration and subjecting foliage to temperatures which may be more optimum for net photosynthesis (Xu *et al.* 2007).

Modeling efforts to predict the effect of elevated temperature and [CO_2] on species distribution have yielded conflicting reports. Most models, including envelope models, process-based models and dynamic models, have suggested that the majority of tree species in North America will see an expansion of the northern distribution and a contraction of the southern distribution (Iverson & Prasad 2001, 2002; McKenney *et al.* 2007; Morin *et al.* 2008; Tang & Beckage 2010). Estimations in the shift of optimal latitude of growth with an increase in temperature range from 100 km (Prasad *et al.* 2006) to 1000 km (Hamann & Wang 2006). The large variation demonstrate both the uncertainty in predicting the response of species to changing climates and the potential variability in species response to changes in climate.

Two studies were conducted to test the hypothesis that tree growth and physiology will respond differently to an increase in temperature and [CO_2], when grown in different temperature conditions. These studies will provide information to improve the accuracy of models that predict the effect of changing climate conditions on species range, as well as help interpret the results in the literature regarding the effect of an increase in temperature on net photosynthesis and biomass accumulation. The first experiment investigated the effect of elevated temperature, elevated [CO_2] and water stress (applied singly and in combination) on loblolly pine growth at three sites in the species distribution. The sites were located at Blairsville, GA (near the northern edge of the species range), Athens, GA (the growing season temperature was similar to the middle of the species range) and Tifton, GA (the growing season temperature was similar to sites located near the southern edge of the species range). *In situ* net photosynthesis and xylem pressure potential were measured at each site throughout the growing season (Chapter 2). In addition, temperature response curves of net photosynthesis and foliar dark respiration as well as sequential harvests were conducted throughout the growing season to determine the effect of elevated [CO_2] and temperature at both the Northern and Southern region of the species range (Chapter 3). At the end of the growing season, the effect of predicted climate conditions (elevated temperature, elevated [CO_2] and low soil moisture availability) on total biomass accumulation and relative growth rate were compared to seedlings grown in current climate conditions (ambient temperature, ambient [CO_2] and high soil moisture availability) at sites with different climatic conditions within the species range (Chapter 4). Based on the findings of the loblolly pine study, a second study was run to determine the effect of elevated temperature on northern red oak net photosynthesis and biomass accumulation at a site near the southern edge of the range (Chapter 5).

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CHAPTER 2

THE INFLUENCE OF ELEVATED TEMPERATURE, ELEVATED ATMOSPHERIC CO_2 CONCENTRATION AND WATER STRESS ON NET PHOTOSYNTHESIS OF LOBLOLLY PINE (*PINUS TAEDA* L.) AT NORTHERN, CENTRAL AND SOUTHERN SITES IN ITS NATIVE RANGE[1]

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Abstract

We investigated the effect of elevated [CO_2] (700 µmol mol^{-1}), elevated temperature (+2 °C above ambient) and decreased soil water availability on net photosynthesis (A_{net}) and water relations of one-year old potted loblolly pine (*Pinus taeda* L.) seedlings grown in treatment chambers with high fertility at three sites along a north-south transect covering a large portion of the species native range. At each location (Blairsville, Athens and Tifton, GA) we constructed four treatment chambers and randomly assigned each chamber one of four treatments: ambient [CO_2] and ambient temperature, elevated [CO_2] and ambient temperature, ambient [CO_2] and elevated temperature, or elevated [CO_2] and elevated temperature. Within each chamber half of the seedlings were well watered and half received much less water (1/4 that of the well watered).

Measurements of net photosynthesis (A_{net}), stomatal conductance (g_s), leaf water potential and leaf fluorescence were made in June and September, 2008. We observed a significant increase in A_{net} in response to elevated [CO_2] regardless of site or temperature treatment in June and September. An increase in air temperature of over 2 °C had no significant effect on A_{net} at any of the sites in June or September despite over a 6 °C difference in mean annual temperature between the sites. Decreased water availability significantly reduced A_{net} in all treatments at each site in June. The effects of elevated [CO_2] and temperature on g_s followed a similar trend. The temperature, [CO_2] and water treatments did not significantly affect leaf water potential or chlorophyll fluorescence. Our findings suggest that predicted increases in [CO_2] will significantly increase A_{net}, while predicted increases in air temperature will have little effect on A_{net} across the native range of loblolly pine. Potential decreases in precipitation will likely cause a significant reduction in A_{net}, though this may be mitigated by increased [CO_2].

Introduction

Reports by the International Panel on Climate Change (IPCC) suggest that atmospheric CO_2 concentrations ([CO_2]) may increase to between 590 and 970 μmol mo1^{-1} by the year 2100 (IPCC, 2001: Model A1B). The rise in [CO_2] and other greenhouse gases is expected to cause an increase in mean global temperature of 2.9 °C and cause an increase in the annual temperature in eastern North America of 3.6 °C (IPCC, 2007: Regional Climate Projections). An increase in air temperature is predicted by the A1B model to also result in a slight increase, approximately 7%, in mean precipitation in eastern North America. However, modeling work by Trenberth (1998) predicts that future changes in the climate may lead to a slight decrease in the amount of precipitation in the southeastern USA.

The effect of elevated [CO_2] on net photosynthesis (A_{net}) has been studied in many tree species, and on average A_{net} in trees exposed to elevated [CO_2] (approximately doubled over ambient) for an extended period of time is 61% higher in deciduous trees and 41% higher in conifer trees than in ambient [CO_2] (Ceulemans & Mousseau, 1994; Ainsworth & Long, 2005). A similar response of A_{net} to elevated [CO_2] has been reported for loblolly pine. In loblolly pine saplings grown for 4 years in open top chambers, A_{net} was 60–130% higher in the summer and 14–44% higher in the winter in the elevated [CO_2] treatment compared with the ambient [CO_2] treatment (Tissue *et al.*, 1997). In a free air CO_2enrichment study, loblolly pine trees exposed to ambient +200 μmol CO_2 mol^{-1} sustained a significant increase (60±11%) in light saturated A_{net} over a four year observation period (Crous & Ellsworth, 2004).

The short-term effect of an increase in air temperature on A_{net} in plants has been well described. As air temperature increases from sub-optimum to optimum, A_{net} increases due to an increase in enzyme kinetics (Berry & Björkman, 1980). As air temperature increases above optimum, A_{net} decreases due to decreased CO_2 solubility and related changes in enzyme kinetics, leading to higher rates of photorespiration (Jordan & Ogren, 1984). Increased air temperature further reduces A_{net} due to increasing rates of mitochondrial respiration. While a short-term exposure to elevated temperature may lead to a suppression of A_{net}, it appears that some tree species may able to partially acclimate to elevated temperatures, e.g. *Pinus radiata* D. Don, (Rook, 1969) and *Eucalyptus pauciflora* Sieb. ex Spreng (Slatyer, 1977). However, in studies of other tree species, including loblolly pine (Teskey & Will, 1999), A_{net} did not acclimate to different temperature regimes (Wayne *et al.*, 1998; Nagy *et al.*, 2000; Ro *et al.*, 2001; Ow *et al.*, 2008a, b). Without acclimation of A_{net} the response of a species to elevated temperature may differ across the species range. Photosynthesis in trees growing in suboptimum temperatures may increase with elevated air temperatures, while photosynthesis in trees growing in supraoptimum temperatures may decrease with further increases in air temperature.

The combined effect of elevated [CO_2] and elevated temperature may have an interactive effect on A_{net}. While fewer studies have investigated the effect of both elevated [CO_2] and temperature, there are reports of both positive and negative responses in different studies. In mature *Picea abies* ((L.) Karst.) (Roberntz, 2001) and in loblolly pine seedlings (Lewis *et al.*, 1996) there was an increase in the optimum temperature for A_{net} in trees grown under elevated [CO_2]. However, studies by Wang *et al.* (1995) on mature *Pinus sylvestris* (L.) and Tjoelker *et al.* (1998) on *Populus tremuloides* (Michx), *Betula papyrifera* (Marsh.) and *Picea mariana* (Mill.) observed a decrease in A_{net} with an increase in growing season temperature under both ambient and elevated [CO_2] suggesting that there was no temperature by [CO_2] treatment interaction.

Plants grown in elevated [CO_2] often have lower transpiration because of decreased stomatal conductance (g_s) (Pearson *et al.*, 1995; Bettarini *et al.*, 1998), suggesting that long-term exposure to elevated [CO_2] may make a plant less susceptible to drought stress. Johnson *et al.* (2002) observed in *Salix sagitta* and *Populus trichocarpa*×*deltoides* saplings exposed to a short-term drought that A_{net} and g_s were maintained nearer to unstressed rates in the elevated [CO_2] treatment compared with the ambient [CO_2] treatment. However, Ellsworth (1999) did not observe a significant difference in g_s in loblolly pine trees exposed to elevated [CO_2] compared with trees exposed to ambient [CO_2] when a summer drought occurred, though rates of net photosynthesis were generally higher in trees exposed to elevated [CO_2], suggesting a higher water use efficiency (WUE) in those trees.

While the effects of elevated [CO_2], elevated temperature and water stress on A_{net} of tree species have been investigated separately, or in two factor combinations, to our knowledge, the combined effects of all three factors (elevated [CO_2], elevated temperature and water stress) on A_{net}has not been investigated in a tree species. Nor has there been an investigation of the influence of these environmental factors on A_{net} across a large portion of a species native range. The objective of this study was to examine the influence of elevated [CO_2], elevated temperature and decreased water availability (separately and in combination) on A_{net} of loblolly pine seedlings at three sites in Georgia, USA. The sites spanned a large portion of the North-South distribution of the species. We hypothesized that elevated [CO_2] would significantly increase A_{net} across the species range, while elevated temperature would increase net photosynthesis at the coolest site near the northern limit of the species range and suppress net photosynthesis at the warmest site in the southern portion of the species range. Regardless of the treatment, we hypothesized that water stress would significantly reduce A_{net} across the species range, though water stress would be mitigated by exposure to elevated [CO_2].

Materials and Methods

Study sites

Three study sites were selected in Georgia along a North–South transect from the upper piedmont to the lower coastal plain. The three sites span a significant portion of the temperature variation across the native range of loblolly pine. The sites were located at University of Georgia Agricultural and Forestry Experiment Stations and were named for the nearest community. From North to South the sites were: Blairsville (34°87'N, 83°95'W, elevation 582 m), Athens (33°57'N, 83°19'W, elevation 230 m) and Tifton (31°29'N, 83°32'W, elevation 108 m) (Fig. 2.1). The mean annual temperature at the three sites over the previous 30 years was 12.6 °C, 16.5 °C, and 18.7 °C, respectively [National Climatic Data Center, 2009, http://www.ncdc.noaa.gov (accessed 1 January 2009)].

Four treatment chambers, half-cylinder in shape and measuring 3.65 m length by 3.62 m width by 2.31 m height, were constructed at each site. Chambers were built with lumber bases and PVC pipe frames supporting 6 mil clear polyethylene film (GT Performance Film, Green-Tek Inc., Edgerton, WI, USA) (Boyette & Bilderback, 1996). The treatment chambers were placed in open areas, spaced 3.7 m apart to prevent shading and oriented facing south to maximize daily sun exposure. Each of the twelve chambers had the same dimensions, the chambers were oriented in the same direction at each site and all equipment within the chambers was located at the same spot to minimize any potential chamber effect.

Plant material

One-year-old nursery-grown bare root seedlings of loblolly pine (*Pinus taeda* L.) were planted in February 2008 in 8 L pots in potting medium (Fafard Nursery Mix, Conrad Fafard Inc., Agawam, MA, USA). Seedlings were grown from a mixed seed source of open pollinated families from the Georgia Piedmont region (Georgia Forestry Commission, Atlanta, GA, USA). This provenance represents genotypes found in the middle of the study region, similar in climate to the Athens study site. Average stem height and diameter of the seedlings at planting were 0.27m and 0.39 cm, respectively. High soil moisture was maintained in the pots from February to May by hand watering. Each pot was fertilized with 30 g of 15-9-12 extended release fertilizer (Osmocote Plus #903286, Scotts-Sierra Horticultural Products, Marysville, OH, USA) in March and August and 0.2 g of chelated iron (Sprint 138, Becker Underwood, Ames, IA, USA) in May and August. In May approximately 0.04 mL Imidacloprid was applied topically to the soil in each pot to control pests (Bayer Advanced 12 months tree and shrub insect control; Bayer; Monheim am Rhein, Germany).

Experimental design and treatments

At each site the chambers were randomly assigned one of four treatment combinations: ambient [CO_2] and ambient temperature ($C_A T_A$), elevated [CO_2] and ambient temperature ($C_E T_A$), ambient [CO_2] and elevated temperature ($C_A T_E$) or elevated [CO_2] and elevated temperature ($C_E T_E$). Ambient and elevated atmospheric [CO_2] treatments were 380 and 700 μmol CO_2 mol^{-1}, respectively. To maintain [CO_2] at the desired levels, a nondispersive infrared CO_2 sensor (Model GMT222, Vaisala Inc., Woburn, MA, USA) in each chamber continuously measured [CO_2] and directly controlled a solenoid valve which released CO_2 into the chambers as necessary from a cylinder of industrial grade compressed 100% CO_2 (Airgas National Welders, Toccoa, GA, USA). An oscillating fan was placed within each chamber to disperse the CO_2 throughout the chamber. The CO_2 treatments were initiated immediately after bud burst.

The target air temperature in the elevated temperature chambers was 2 °C above ambient (day and night). Chamber temperature was controlled using a differential thermostat (Model DSD-2, Kera Technologies Inc., Mississauga, Ont, Canada) which continuously compared the air temperature inside each chamber with the outside temperature measured with matched thermistors. Each thermistor was housed in a ventilated radiation shield (Model SRS100, Ambient Weather, Chandler, AZ, USA) mounted on a pole 1 m above the ground. The differential thermostat controlled an air conditioner (Model FAM186R2A, Frigidaire, Augusta, GA, USA) to maintain the treatment air temperatures. Additionally, in the elevated temperature chambers the thermostats controlled heaters (Model 3VU33A, Dayton Electric, Niles, IL, USA) to increase air temperature at night and on cloudy days. Air temperature was also measured with thermocouples within the radiation shields inside each chamber and outdoors 1.45 m south of the chambers every 3 min and averaged and recorded every 15 min using a datalogger (Campbell 23X, Logan UT, USA). The temperature treatments were initiated as soon as the seedlings were placed within the growth chambers. Vapor pressure deficit was not measured or controlled.

Photosynthetic active radiation (PAR) was measured at each site outdoors and inside one chamber at each site with quantum radiation sensors (Model LI-190SZ, LiCor Biosciences, Lincoln, NE, USA). PAR sensors were mounted on top of the leveled radiation shields. All PAR sensors were connected to the datalogger and recorded at the same frequency as temperature.

Eighty seedlings in each chamber were randomly assigned to one of 10 blocks; five blocks were assigned to a high water treatment and five were assigned to a low water treatment. Pots were evenly spaced within the chamber. In July blocks, and pots within blocks, were randomly rotated within the houses to minimize any potential chamber effect. Owing to freezing concerns the water treatment was not initiated until May. Beginning in May, the water treatments were applied using automated drip irrigation systems. A 2.2 L h^{-1} drip emitter (Model Supertif, Plastro Irrigation Systems, Kibbutz Gvat D. N. Ha'Amakim, Israel) was placed in each pot half way between the stem and the edge of the pot. Watering amounts were customized for each site and continually adjusted through the summer because of the differences in the size of the seedlings at the three sites. Seedlings in the high water treatment were irrigated as follows: May–June: three times a day, 4 days per week, for 6 min at Blairsville, 7 min at Athens and 8 min at Tifton; June–July: four times every day for 6 min at Blairsville, 7 min at Athens and 8 min at Tifton; August–October: four times every day for 10 min at Blairsville, 11 min at Athens and 12 min at Tifton. Seedlings in the low water treatment were given 25% of the water that those in the high water treatment received. Well-watered seedlings were watered to saturation with each watering.

Measurements

Measurements were conducted twice during the growing season (June 26–July 9 and September 3–10) on four seedlings from each of the eight treatment combinations (temperature, [CO_2], and water) at each site. Seedlings were kept in the treatment chambers while measurements were conducted. The measurements were conducted on two consecutive days at each site, with two seedlings from each treatment combination measured each day. Different seedlings were used on the second day of measurements. Additionally, different seedlings were chosen for measurements in September. Needles from the first growth flush were used in June and needles from the second flush were used in September. All measured needles were fully expanded and appeared visually healthy. Measurements were made on sunny or mostly sunny days.

Before sunrise, leaf xylem pressure potential (Ψ_{PD}) was measured on a single detached needle from each plant using a pressure chamber (Model 600, PMS Instrument, Corvallis, OR, USA). Dark-acclimated leaf fluorescence (F_v/F_m) was concurrently measured on a single detached fascicle with a portable photosynthesis system fitted with a fluorescence chamber (Model LI-6400, LiCor Biosciences, Lincoln, NE, USA). Net photosynthesis (A_{net}), stomatal conductance (g_s) and light-acclimated leaf fluorescence (F_v'/F_m') were subsequently measured on the same plants with the portable photosynthesis system on a single attached fascicle three times throughout the day (09:00, 12:00 and 15:00 hours). Leaf chamber conditions (block temperature, relative humidity, [CO_2], and light intensity) were controlled to mimic current conditions in each treatment chamber. Each needle was allowed to stabilize in the chamber for 5 min before A_{net} and g_s values were recorded. F_v'/F_m' was then measured on the same fascicle. Immediately following each gas exchange and fluorescence measurement, the entire fascicle was harvested, and leaf xylem pressure potential was measured on a single needle from the fascicle. Measured gas exchange values were adjusted to actual leaf area enclosed in the cuvette, which was calculated using the method described by Fites & Teskey (1988).

Data analysis

We averaged the measurements of A_{net} and g_s made at 09:00, 12:00, and 15:00 hours on each seedling to obtain a mean daily value of A_{net} and g_s for each seedling. Instantaneous water use efficiency (WUE_I) was calculated for each seedling at each measurement time by dividing the rate of A_{net} by the rate of transpiration and averaging to obtain a daily value for WUE_I.

Measurements of predawn leaf xylem pressure potential (Ψ_{PD}) and leaf xylem pressure potential at 15:00 hours (Ψ_{MIN}), as well as F_v/F_m and F_v'/F_m' at 15:00 hours were analyzed separately.

There were a total of 24 treatment combinations in this study (3 sites × 2 [CO_2] treatments × 2 temperature treatments × 2 water treatments). Physiological parameters were analyzed using a four-way ANOVA treating temperature, [CO_2], water and site as fixed factors on a physiological parameter (i.e. A_{net}, g_s, WUE_I, Ψ_{PD}, Ψ_{MIN}, F_v/F_m and F_v'/F_m'), three-way ANOVA to compare the effects of temperature, [CO_2] and site within a water treatment, two-way ANOVA to compare the effects of temperature and [CO_2] within site and within a water treatment, and one-way ANOVA to compare the effects of a single fixed factor on a physiological parameter. All statistical tests were performed using proc mixed in SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Environmental conditions

Mean air temperature from May 1, when the treatments were fully installed, to September 10, the last day of measurements for this experiment, was 21.2 °C at Blairsville (the northern site), 24.5 °C at Athens (the central site) and 25.8 °C at Tifton (the southern site) (Table 2.1). The elevated temperature treatments averaged 2.1, 2.3 and 2.3 °C above ambient at the Blairsville, Athens and Tifton sites, respectively. Mean daily atmospheric [CO_2] was maintained near 380 μmol mol^{-1} in the ambient [CO_2] chambers and near 700 μmol mol in the elevated [CO_2] chambers, with an exception at the southern site, where both values were slightly higher. The difference between ambient and elevated CO_2treatments was very similar at all three sites, averaging 316, 307 and 299 μmol mol^{-1} at the northern, central and southern site, respectively.

Mean ambient air temperature for the 2-day measurement periods in June–July at the northern, central and southern site was 22.6, 23.3 and 25.6 °C, respectively (Fig. 2.2). Incoming PAR at the northern, central and southern site was 49.2, 53.1 and 43.5 mol m^{-2} day^{-1}, respectively. For the September measurement period, mean air temperature was 21.1 °C at the northern site, 24.7 °C at the central site and 27.2 °C at the southern site. Incoming PAR at the northern, central and southern site was 48.0, 44.1 and 33.7 mol m^{-2} day^{-1}, respectively.

Net photosynthesis

Averaged across all sites and temperature treatments, net photosynthesis (A_{net}) was significantly higher in the elevated [CO_2] treatment compared with the ambient [CO_2] treatment in both water treatments in June and September (Fig. 2.3a and c). In the high water treatment A_{net} was 43% higher in June and 59% higher in September in elevated [CO_2], compared with the ambient [CO_2] treatment ($P<0.001$) (Fig. 2.3a). In the low water treatment A_{net} was 79% higher in June and 28% higher in September in elevated [CO_2] ($P<0.001$) (Fig. 2.3c). This pattern was consistent at each site. In June and September A_{net} was higher in elevated [CO_2] in both high and low water treatments (Fig. 2.3b and d), and the difference was statistically significant in all but one instance.

Our measurements indicated that the elevated temperature treatment did not have a significant effect on A_{net} compared with the ambient temperature treatment in either high or low soil water conditions in June or September (Fig. 2.4a and c). The lack of response of A_{net} to an increase in air temperature of over 2 °C was consistent across all sites and water treatments in both June and September (Fig. 2.4b and d), with one exception: at the central site in June, the elevated temperature treatment caused a significant reduction in A_{net} in the high water treatment (site × temp × water interaction, $P = 0.032$). However, this response was not evident in September.

There was no significant [CO_2] by temperature interaction on A_{net} at any site in either water treatment in June or September ($P = 0.239$ and 0.714). This indicates that A_{net} responded to elevated [CO_2] in a similar manner in both temperature treatments. There was a significant site by temperature by water interaction in June ($P = 0.0327$), but not in September ($P = 0.875$).

In June mean A_{net} was 23% lower in the low water treatment compared with the high water treatment ($P < 0.001$). The significant reduction in A_{net} in the low water treatment was observed for every [CO_2] and temperature treatment combination (Table 2.2). In September A_{net} was 16% lower in the low water treatment compared with the high water treatment ($P = 0.004$), but the difference was statistically significant only in the elevated [CO_2] treatments. The elevated [CO_2] treatment compensated for a depression of A_{net} in the low water treatment. In June, A_{net} in the elevated [CO_2]-low water treatment was significantly higher than in the ambient [CO_2]-well water treatment at the central (8.50 vs. 10.87 μmol m^{-2} s^{-1}; $P = 0.02$) and southern sites (9.00 vs. 11.40 μmol m^{-2} s^{-1}; $P < 0.001$), and not significantly different at the northern site (11.75 vs. 12.62 μmol m^{-2} s^{-1}; $P = 0.274$). A similar result was observed in September, where A_{net} in the elevated [CO_2]-low water treatment was significantly higher than A_{net} in the ambient [CO_2]-high water treatment at the southern (5.97 vs. 7.56 μmol m^{-2} s^{-1}; $P = 0.013$) and northern sites (6.17 vs. 8.45 μmol m^{-2} s^{-1}; $P = 0.045$) and not significantly different at the central site (5.70 vs. 6.38 μmol m^{-2} s^{-1}; $P= 0.122$).

Stomatal conductance

The effect of elevated [CO_2] on stomatal conductance (g_s) was smaller and less consistent than its effect on A_{net}. In June g_s was significantly lower in the elevated [CO_2] treatment compared with the ambient [CO_2] treatment in the high water treatment ($P < 0.001$), but not in the low water treatment ($P = 0.15$) (Fig. 2.5a and c). In September, the opposite pattern was seen; the reduction in g_s was significant in the low water treatment ($P < 0.001$) but not in the high water treatment ($P = 0.43$). Among the sites, the effect of elevated [CO_2] on g_s was not consistent in June. By September g_s was typically lower in the elevated [CO_2] treatment, though the effect was not always significant (Fig. 2.5b and d).

The elevated temperature treatment did not have a significant effect on g_s in the high water treatment in June or in the low water treatment in June and September. In September g_s was significantly higher in the elevated temperature treatment ($P < 0.001$) (Fig. 2.6a and c). However at the individual sites, temperature had no significant effect on g_s in either the high or low water treatments (Fig. 2.6b and d), with one exception. At Athens, in June, in the high water treatment g_s was significantly reduced in the elevated temperature treatment ($P < 0.001$).

Averaged across all sites, g_s in June was significantly lower in the low water treatment compared with the high water treatment for all treatments (Table 2.2). In September, g_s was lower in the low water treatment in the elevated [CO_2] treatment, but not the ambient [CO_2] treatment. There was no significant [CO_2] by temperature interaction on g_s at any site, in either water treatment in both June and September, with one exception. In the high water treatment in June at the northern site there was a significant interaction ($P = 0.0301$).

Instantaneous water use efficiency

WUE_I was significantly higher in the elevated [CO_2] treatment compared with the ambient [CO_2] treatment in both high and low water treatments ($P < 0.001$) (Fig. 2.7a and c). WUE_I significantly increased in the elevated temperature treatment at all sites in June and

September (Fig. 2.7b and d), with two exceptions: the increase was not significant at the central site in June in the low water treatment and in September in high water treatment.

Averaged across all sites and [CO_2] treatments, WUE_I was not significantly different between temperature treatments in June in either water treatment (Fig. 2.8a and c). However, in September WUE_I in the elevated [CO_2] treatment was 21% lower in the high water treatment ($P = 0.062$) and 26% lower in the low water treatment ($P = 0.016$) compared the ambient temperature treatment. The effect of temperature on WUE_I was not consistent across the three sites in June or September (Fig. 2.8b and d). Averaged across sites, WUE_I was higher in the low water compared with the high water treatment for all [CO_2] and temperature treatment combinations in June, but the difference was not significant in C_AT_A (Table 2.2). In September, WUE_I was higher in the low water treatment, but the difference was significant only in C_ET_A. No significant temperature by [CO_2] interaction on WUE_I was observed (high water treatment: $P = 0.064$; low water treatment: $P = 0.88$).

Leaf water potential and fluorescence

Predawn leaf xylem pressure potential (Ψ_{PD}) was not significantly affected by [CO_2] or temperature treatments in June or September, however the high water treatment significantly increased Ψ_{PD} (June: $P < 0.001$; September: $P < 0.001$). This was observed at all sites in June and September, with one exception: the northern site in September (Table 2.3). Averaged across all sites, Ψ_{PD} decreased from June to September in both the high and low water treatments (−0.55 to −0.70 MPa; $P < 0.001$).

There was no significant effect of [CO_2] or temperature treatments on leaf xylem pressure potential measured at 15:00 hours (Ψ_{MIN}) in June or September, with one exception. In September Ψ_{MIN} in the high water treatment was significantly lower in the elevated temperature treatment compared with the ambient temperature treatment (*P* = 0.012). In June, there was no significant effect of water treatment on Ψ_{MIN} at any site. However, in September there was a significant decrease in Ψ_{MIN} in the low water treatment at Athens ($P < 0.001$) and Tifton ($P < 0.001$), but not at Blairsville ($P = 0.49$).

Dark-acclimated leaf fluorescence (F_v/F_m) in June was not significantly affected by the [CO_2], temperature, or water treatments. In September F_v/F_m was significantly reduced by the elevated temperature treatment (P=0.017). There was no significant effect of water treatment on F_v/F_m in June or September at any site (Table 2.3). In June, light-acclimated leaf fluorescence at 3 p.m. (F_v'/F_m') was not significantly affected by the [CO_2], temperature or water treatments. However, in September F_v'/F_m' was significantly reduced by the low water treatment (P=0.005), but not by the [CO_2] or temperature treatment. Averaged across all sites, there was a significant increase in F_v'/F_m' from June to September (0.36–0.43; $P < 0.001$).

Discussion

In this study net photosynthesis (A_{net}), measured on 1-year-old loblolly pine seedlings grown in treatment chambers, responded in a similar manner to elevated [CO_2] (approximately 700 μmol mol^{-1}) and elevated temperature (approximately+2.3°C above ambient) at all three sites along a North to South transect in Georgia in June and September, 2008. The response of A_{net} to elevated [CO_2] and temperature was consistent at two soil moisture contents. We observed significantly higher A_{net} in the elevated [CO_2] treatment compared with the ambient [CO_2] treatment across all sites and both water treatments. This indicates that net photosynthesis in loblolly pine responds strongly to elevated [CO_2] over a large range of temperatures, in this case a 3 °C range in June in mean daily temperature from the northern site (Blairsville) to the southern site (Tifton) and a 6.1 °C range in September. There was also no significant effect of a 2.3 °C increase in air temperature on A_{net} at any site in either June or September, further indicating that A_{net} in loblolly pines was largely insensitive to a wide range of growing season temperatures. While only a single seed source was utilized within this study, Hamrick (2004) suggests that most of the genetic diversity of tree species is found within populations, rather than among populations.

The positive response of A_{net} to elevated CO_2 was consistent with previous studies in loblolly pine. In a free air CO_2 enrichment study, loblolly pine trees exposed to ambient +200 µmol CO_2 mol^{-1} sustained a significant increase (60 ± 11%) in light saturated A_{net} over a 4-year observation period (Crous & Ellsworth, 2004). Loblolly pine seedlings in open top chambers also exhibited enhancement of A_{net} over multiple years of measurement (Tissue *et al.*, 1997). Our observations indicate that photosynthesis in loblolly pine will respond strongly to long-term exposure to elevated [CO_2] across a variety of growing conditions.

In some studies, it has been reported that an increase in temperature (3 – 5°C) resulted in an increase in A_{net} in Douglas fir (Lewis *et al.*, 2001). Other studies have reported a decrease in A_{net} with an increase in air temperature in Ponderosa pine (Callaway *et al.*, 1994), Scotts pine (Wang*et al.*, 1995), yellow birch (Wayne *et al.*, 1998) and dwarf apple (Ro *et al.*, 2001). In this study, the lack of response to a 2.3 °C increase in ambient temperature may be attributable to the broad temperature optimum of A_{net} in loblolly pine. In an early study, light saturated A_{net} in loblolly pine seedlings, grown at a single location, was reported to vary little between 20 °C and 30 °C and only declined gradually at higher temperatures, with A_{net} at 40 °C 45% that at 30 °C (Decker, 1944). Similarly, in a more recent study it was reported that A_{net} of loblolly pine seedlings did not significantly differ across a 15 °C range from 20 °C to 35 °C (Teskey *et al.*, 1987). In a study investigating eight tree species from varying climates in Australia, the temperature optimum of A_{net} was also broad, with the span of temperature in which A_{net} was at least 80% of A_{net} at optimum ranging from 8.6 °C to 15.7 °C (Cunningham & Read, 2002). In *E. pauciflora*, Slatyer (1977) observed that, regardless of growing temperature, the optimum temperature of A_{net} spanned a 10 °C range. The broad temperature optimum of loblolly pine and other species indicates that a 2 °C increase in air temperature will have little effect on A_{net}. Perhaps more surprising was that A_{net} was similar at the three sites in almost all instances. This finding may mean that, during the growing season, air temperature does not significantly limit A_{net} across a large portion of the loblolly pine range. Temperatures during the measurement periods in this study had little influence over instantaneous A_{net} and there was no evidence of damage to photosynthetic capacity resulting from long-term exposure to the different temperature regimes of the three sites.

In some species temperature acclimation can contribute to an insensitivity of A_{net} to different temperature regimes. In three *E. pauciflora* populations growing across a 730 m elevation gradient, the optimum temperature for A_{net} shifted with elevation (Slatyer, 1977). In *P. radiate* grown at 33°C/28°C and 15°C/10°C an 8 °C shift in the optimum temperature for photosynthesis was observed (Rook, 1969). However, in a number of other tree species A_{net} does not acclimate to different temperature regimes (Wayne *et al.*, 1998; Nagy *et al.*, 2000; Ro *et al.*, 2001; Ow *et al.*, 2008a, b). Although acclimation of A_{net} was not specifically tested in this study, it was not observed in a previous study in loblolly pine (Teskey & Will, 1999), suggesting it was not a contributor to the insensitivity of A_{net} to temperature.

Long (1991) proposed that a concurrent increase in air temperature and [CO_2] would increase the optimum temperature of A_{net} by increasing enzyme kinetics and CO_2 availability, thereby reducing photorespiration. Of the studies investigating the combined effect of elevated [CO_2] and temperature on A_{net} in trees, some have shown a positive relationship, while others have shown a negative relationship. In a branch bag experiment on mature *Picea abies*, the optimum temperature of A_{net} increased in the elevated [CO_2] treatment (Roberntz, 2001). However, Tjoelker *et al.* (1998) failed to observe an enhancement in A_{net} in seedlings grown in elevated [CO_2] with increasing growth temperatures (18/12, 21/15, 24/18, 27/21, and 30/24°C day/night temperature) in *P. temuloides, B. papyrifera, Larix laricina* (Du Roi) and *Pinus banksiana* (Lamb.). Conversely, light saturated A_{net} in an elevated [CO_2]-elevated temperature treatment was lower than A_{net} in an elevated CO_2-ambient temperature treatment in mature *P. sylvestris* (Wang *et al.*, 1995).

We observed a significant reduction in A_{net} in the low water treatment compared with the high water treatment in all temperature and [CO_2] treatment combinations in June. By September, the reduction in A_{net} in the low water treatment was only observed in the elevated [CO_2] treatments, though we had expected to see a significant reduction in A_{net} in the low-watered, compared with the well-watered, plants for all treatments. Our results were similar to findings in other studies, which reported an increase in photosynthesis in elevated [CO_2] compared with ambient [CO_2] for both well watered and water stressed loblolly pine saplings, however photosynthesis was substantially lower in water stressed seedlings compared with well watered seedlings (Groninger *et al.*, 1996; Will & Teskey, 1997). During a naturally occurring drought at the Basel, Switzerland FACE site, Leuzinger *et al.* (2005) observed sustained higher photosynthesis in elevated [CO_2] rings compared with surrounding plots in sessile oak, European beach, European hornbeam, hedge maple and big leaf linden.

Despite the reduction in A_{net} in the low water treatment, A_{net} in the elevated [CO_2]-low water treatment was not significantly lower than in the ambient [CO_2]-high water treatment at Blairsville in June and Athens in September, and was significantly higher at Athens and Tifton in June and Blairsville in September. A similar trend was reported by Groninger *et al.* (1996) in loblolly pine and red maple. Seedlings grown in the elevated [CO_2] and low water treatment, which was one-half the amount of water of the high water treatment, had higher photosynthesis than seedlings grown in the ambient [CO_2] and high water treatment. These findings suggest that predicted increases in atmospheric [CO_2] may mitigate declines in net photosynthesis during periods of drought, or if precipitation patterns change and cause a decrease in soil water availability.

Field *et al.* (1995) suggested that, across a wide range of deciduous and conifer tree species, g_s is reduced on average by 23% in elevated [CO_2]. In our study, across all sites, temperature treatments and measurement periods, g_s was reduced by 9% in the well watered treatment but by 21% in the low water treatment. Previous studies in loblolly pine (Teskey, 1995; Murthy *et al.*, 1997; Pataki *et al.*, 1998; Ellsworth, 1999) and *Pinus pinaster* (Ait) (Schwanz *et al.*, 1996), either grown in elevated [CO_2], or exposed to elevated [CO_2] for an entire growing season, reported no difference in g_s in elevated [CO_2] compared with ambient [CO_2]. We did not observe a sizeable effect of elevated temperature on g_s in June or September, which is similar to observations in European beech, where g_s in seedlings grown in growth chambers in ambient, +2.5°C or +5°C air temperatures was not significantly different (Leverenz *et al.*, 1999). As expected, g_s was significantly lower in the low water treatment compared with the high water treatment in June for every [CO_2] and temperature treatment. Our findings are similar to findings by Kolb & Robberecht (1996) who observed a correlation between soil water availability and g_s, but no correlation between air temperature and g_s in Ponderosa pine.

Across the entire study we observed a significant increase in WUE_I in the elevated [CO_2] treatment and no significant impact of elevated temperature treatment on WUE_I. Previous studies have reported an increased WUE_I under elevated [CO_2] due to either an increase in A_{net} or a decrease in g_s or a combination of both (Eamus, 1991; Drake *et al.*, 1997; Saxe *et al.*, 1998). In this study, we observed a consistent increase inA_{net} and a slight decrease in g_s in the elevated [CO_2] treatment that we did not observe with elevated temperature.

Conclusion

Results of this study suggest that predicted increases in [CO_2] will significantly increase A_{net}, while an expected 2–3 °C increase in air temperature will have little or no effect on A_{net} throughout the native range of loblolly pine. This suggests that an increase in air temperature of 2 °C will not mitigate the [CO_2]-induced increase in A_{net} in the warmer portions of the loblolly pine range, nor will A_{net} benefit from a combination of elevated temperature and elevated [CO_2] at the cooler portions of the native range. It should be noted that loblolly pine has a broad temperature optimum and other species with a narrower optimum temperature range for A_{net} may respond differently to increases in temperature. Decreased precipitation, predicted to occur in concert with increased temperature, will likely cause a significant reduction in A_{net} under current [CO_2]. However, our findings suggest that increases in [CO_2] may at least partly mitigate reductions in A_{net} if precipitation decreases in the future.

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Table 2.1. Mean (SE) air temperature (°C) and atmospheric [CO_2] (μmol mol^{-1}) in the four treatment chambers and ambient air temperature (Ambient T) at Blairsville, Athens and Tifton Georgia, USA during the experimental period (May 1 through September 10). The treatments are: ambient [CO_2] and ambient temperature (C_A T_A), elevated [CO_2] and ambient temperature (C_E T_A), ambient [CO_2] and elevated temperature (C_A T_E) and elevated [CO_2] and elevated temperature (C_E T_E).

	Blairsville		Athens		Tifton	
Treatment	T_{Season}	[CO_2]	T_{season}	[CO_2]	T_{Season}	[CO_2]
C_A T_A	21.5 (0.2)	385.2 (3.3)	24.7 (0.2)	396.7 (2.4)	25.9 (0.2)	429.9 (3.7)
C_E T_A	21.6 (0.2)	685.5 (9.5)	24.5 (0.2)	705.2 (6.0)	26.2 (0.2)	731.5 (6.5)
C_A T_E	23.4 (0.2)	400.7 (3.0)	26.6 (0.2)	392.2 (2.5)	28.4 (0.2)	427.3 (3.9)
C_E T_E	23.9 (0.2)	699.4 (5.6)	27.2 (0.2)	698.4 (7.1)	28.3 (0.2)	757.9 (7.9)
Ambient T	21.2 (0.3)	-	24.5 (0.3)	-	25.8 (0.2)	-

Table 2.2 Mean (SE) rates of A_{net} (µmol m^{-2} s^{-1}), g_s (mol m^{-2} s^{-1}) and WUE_I (µmol m^{-2} s^{-1} / mol m^{-2} s^{-1}) in loblolly pine seedlings for the high water (W_H) and low water (W_L) treatments averaged across all sites in June and September, 2008. The treatments are: ambient [CO_2] and ambient temperature (C_A T_A), elevated [CO_2] and ambient temperature (C_E T_A), ambient [CO_2] and elevated temperature (C_A T_E) and elevated [CO_2] and elevated temperature (C_E T_E). Significant difference ($p < 0.05$) between W_H and W_L treatments in either June or September depicted by asterisk.

		June		September	
Parameter	Treatment	W_H	W_L	W_H	W_L
A_{net}	C_A T_A	10.17 (0.38)	6.34 * (0.52)	6.12 (0.25)	5.53 (0.52)
	C_E T_A	14.72 (0.67)	12.71 * (0.58)	9.25 (0.37)	7.63 * (0.56)
	C_A T_E	9.33 (0.39)	6.70 * (0.48)	5.78 (0.39)	5.19 (0.40)
	C_E T_E	13.24 (0.64)	10.58 * (0.71)	9.67 (0.43)	7.36 * (0.49)
g_S	C_A T_A	0.25 (0.011)	0.13 * (0.015)	0.16 (0.013)	0.15 (0.021)
	C_E T_A	0.23 (0.013)	0.13 * (0.013)	0.16 (0.13)	0.092 * (0.012)
	C_A T_E	0.24 (0.013)	0.13 * (0.014)	0.19 (0.018)	0.16 (0.019)
	C_E T_E	0.20 (0.014)	0.10 * (0.011)	0.17 (0.014)	0.096 * (0.014)
WUE_I	C_A T_A	1.90 (0.12)	2.26 (0.28)	2.57 (0.36)	2.76 (0.27)
	C_E T_A	2.89 (0.14)	4.54 * (0.28)	3.39 (0.33)	5.50 * (0.65)
	C_A T_E	1.63 (0.07)	2.20 * (0.15)	1.47 (0.11)	1.72 (0.081)
	C_E T_E	3.04 (0.23)	4.53 * (0.32)	3.32 (0.39)	4.69 (0.38)

Table 2.3. Mean (SE) predawn leaf xylem pressure potential (Ψ_{PD}, MPa), leaf xylem pressure potential at 3pm (Ψ_{MIN}, MPa), dark acclimated leaf fluorescence (F_v/F_m) and light-acclimated leaf fluorescence at 3 pm (F_v'/F_m') in the high water treatment (W_H) and low water treatment (W_L) in loblolly pine seedlings grown at Blairsville, Athens and Tifton, Georgia, USA in June and September, 2008. Significant difference ($p < 0.05$) between water treatments depicted by asterisk; ns indicates no significant difference.

		June			September		
Parameter	Water treatment	Blairsville	Athens	Tifton	Blairsville	Athens	Tifton
Ψ_{PD}	W_H	-0.55 ^{ns} (0.05)	-0.45 * (0.05)	-0.45 * (0.05)	-0.65 ^{ns} (0.10)	-0.55 * (0.05)	-0.65 * (0.05)
	W_L	-0.65 (0.04)	-0.80 (0.10)	-0.55 (0.05)	-0.75 (0.10)	-1.00 (0.05)	-0.85 (0.05)
Ψ_{MIN}	W_H	-1.66 ^{ns} (0.10)	-1.65 ^{ns} (0.10)	-1.55 ^{ns} (0.05)	-1.55 ^{ns} (0.05)	-1.35 * (0.05)	-1.50 * (0.05)
	W_L	-1.55 (0.10)	-1.90 (0.15)	1.70 (0.10)	-1.65 (0.10)	-1.50 (0.1)	-1.95 (0.10)
F_v/F_m	W_H	0.83 ^{ns} (0.01)	0.8 ^{ns} (0.01)	0.83 ^{ns} (0.01)	0.83 ^{ns} (0.002)	0.82 ^{ns} (0.003)	0.85 ^{ns} (0.003)
	W_L	0.83 (0.01)	0.82 (0.02)	0.83 (0.01)	0.83 (0.002)	0.83 (0.003)	0.85 (0.003)
F_v'/F_m'	W_H	0.42 * (0.04)	0.34 ^{ns} (0.03)	0.36 ^{ns} (0.05)	0.53 ^{ns} (0.01)	0.51 ^{ns} (0.01)	0.58 ^{ns} (0.01)
	W_L	0.36 (0.04)	0.34 (0.06)	0.35 (0.06)	0.50 (0.00)	0.46 (0.01)	0.55 (0.01)

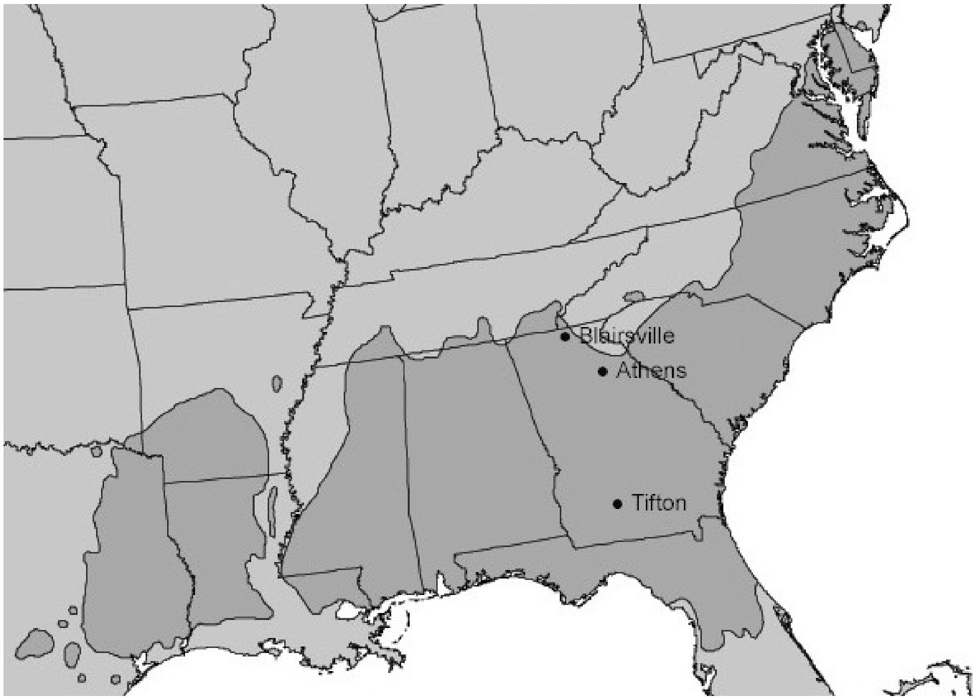

Fig. 2.1. The location of the three study sites: Blairsville, Athens and Tifton, GA USA. The native range of loblolly pine is shaded dark grey. Distribution map taken from: http://esp.cr.usgs.gov/data/atlas/little/pinutaed.pdf.

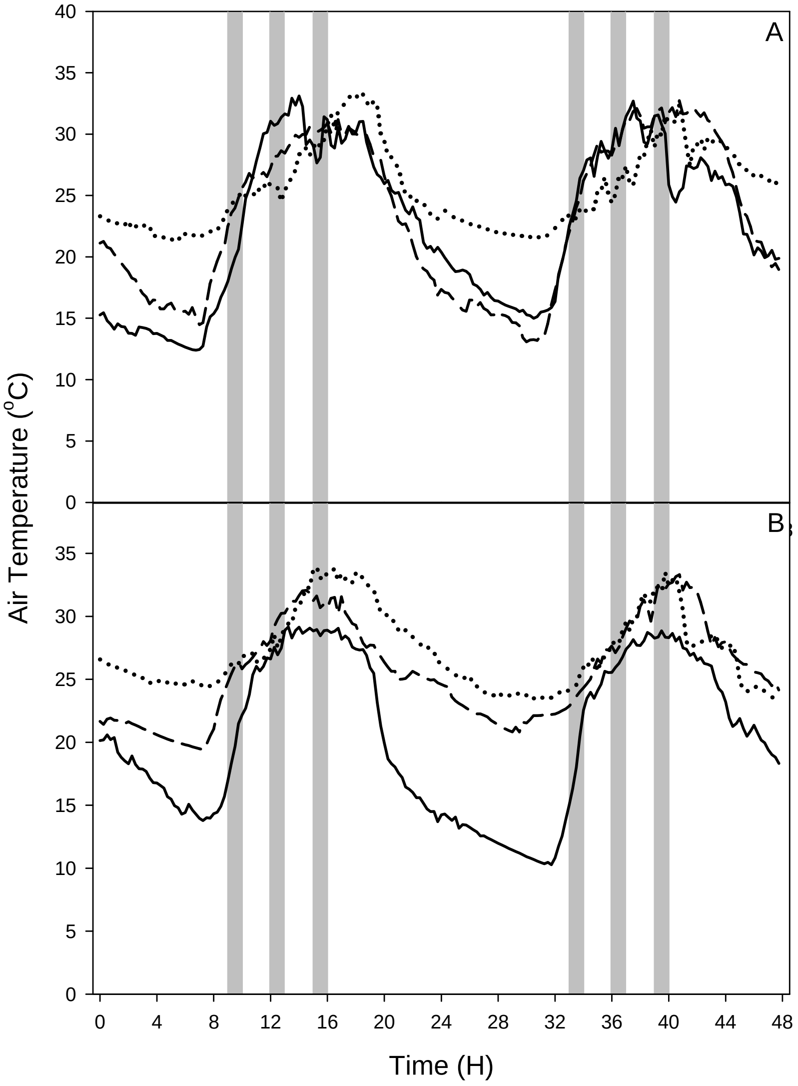

Fig. 2.2. Air temperature (^{o}C) in (A) June and (B) September during the two days in which measurements were made at Blairsville (solid line), Athens (dashed line) and Tifton (dotted line). Gas exchange measurements were conducted three times each day, at the times depicted by the the grey bars.

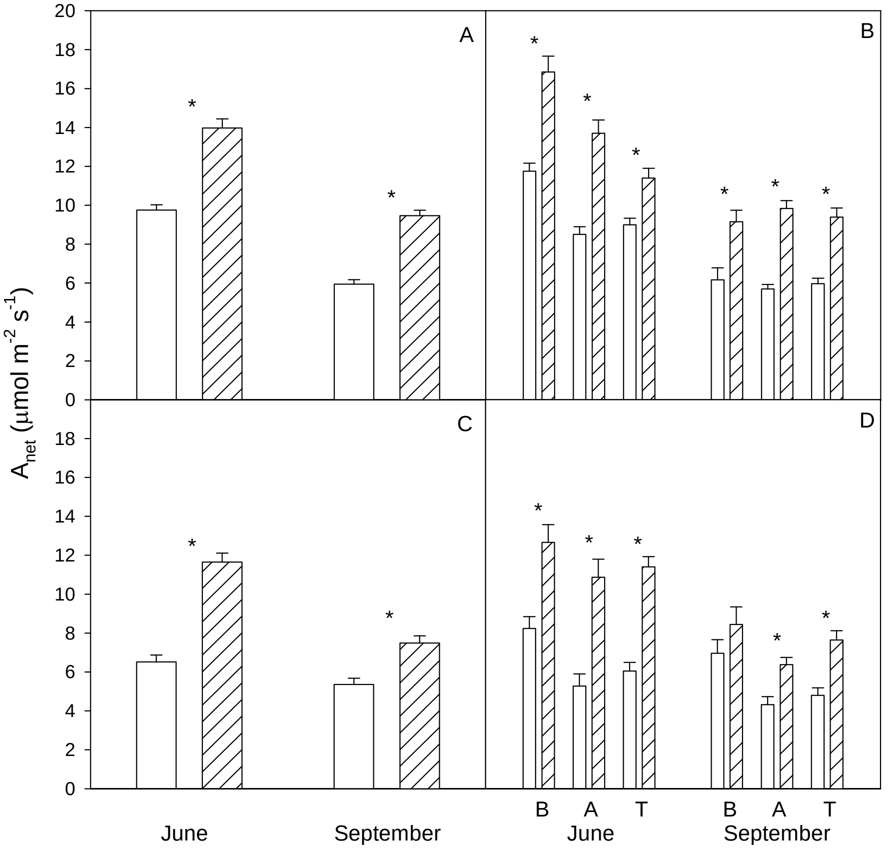

Fig. 2.3. Mean (+ SE) rate of net photosynthesis (A_{net}, µmol m^{-2} s^{-1}) in loblolly pine seedlings grown in ambient [CO_2] (open bars) or elevated [CO_2] (striped bars) in June and September, 2008. Plots A & C display A_{net} averaged over all three sites and both temperature treatments. Plots B & D display A_{net} averaged over both temperature treatments at each site, Blairsville (B), Athens (A) and Tifton (T). Plots A & B display A_{net} in seedlings grown in the high water treatment, while plots C & D display A_{net} in seedlings grown in the low water treatment. Significant difference ($p < 0.05$) between paired bars depicted with asterisk.

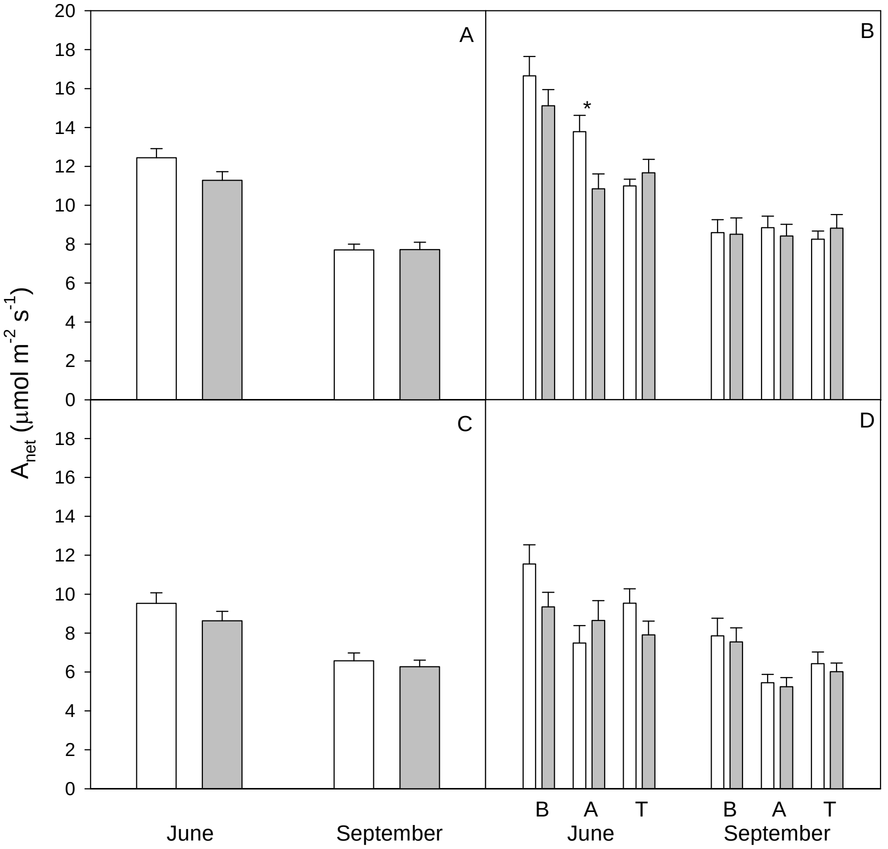

Fig. 2.4. Mean (+ SE) rate of net photosynthesis (A_{net}, µmol m^{-2} s^{-1}) in loblolly pine seedlings grown in ambient temperature (open bars) or elevated temperature (grey bars) in June and September, 2008. Plots A & C display A_{net} averaged over all three sites and both [CO_2] treatments. Plots B & D display A_{net} averaged over both [CO_2] treatments at each site, Blairsville (B), Athens (A) and Tifton (T). Plots A & B display A_{net} in seedlings grown in the high water treatment, while plots C & D display A_{net} in seedlings grown in the low water treatment. Significant difference ($p < 0.05$) between paired bars depicted with asterisk.

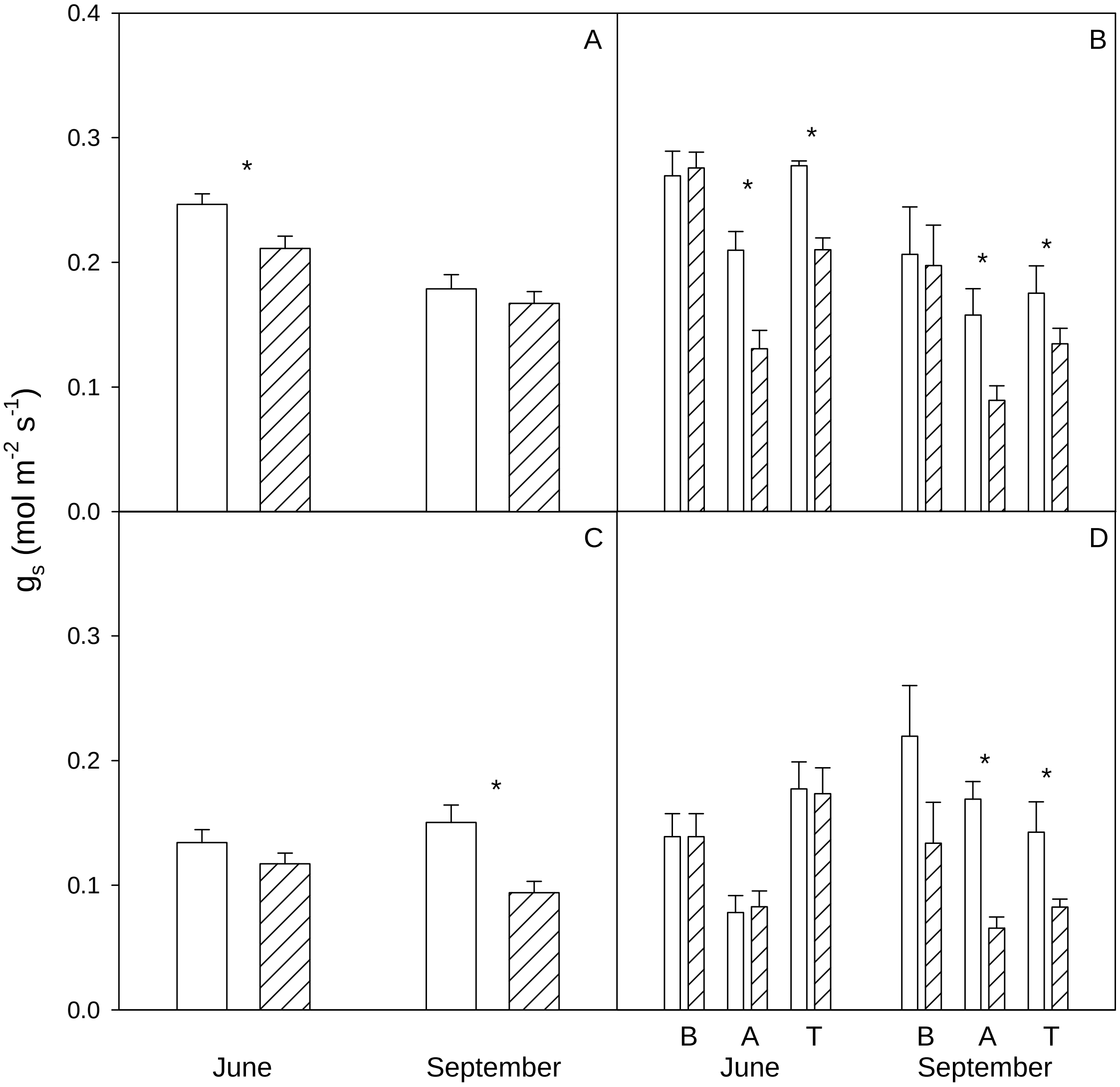

Fig. 2.5. Mean (+ SE) stomatal conductance (g_s, mol m^{-2} s^{-1}) in loblolly pine seedlings grown in ambient [CO_2] (open bars) or elevated [CO_2] (striped bars) in June and September, 2008. Plots A & C display g_s averaged over all three sites and both temperature treatments. Plots B & D display g_s averaged over both temperature treatments at each site, Blairsville (B), Athens (A) and Tifton (T). Plots A & B display g_s in seedlings grown in the high water treatment, while plots C & D display g_s in seedlings grown in the low water treatment. Significant difference ($p < 0.05$) between paired bars depicted with asterisk.

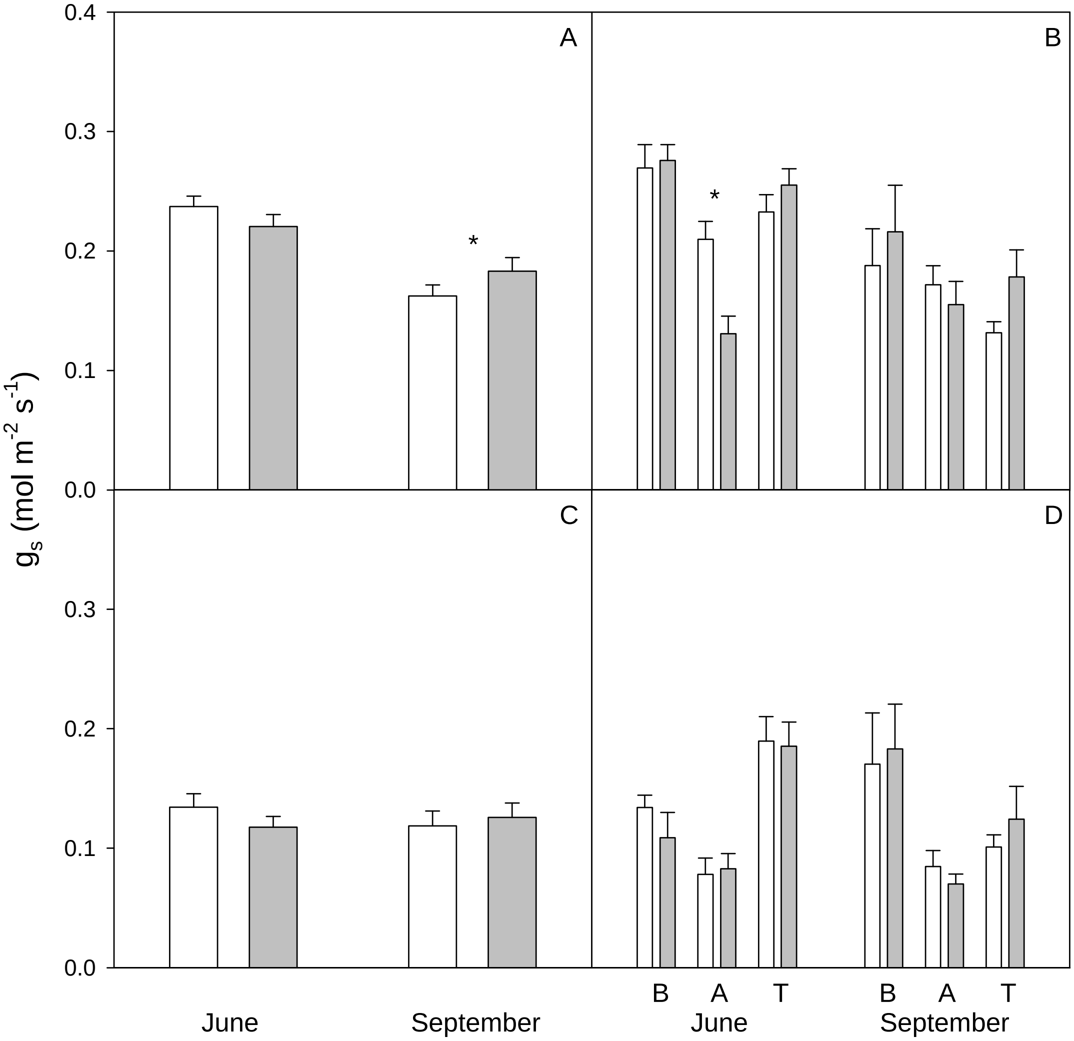

Fig. 2.6. Mean (+ SE) rate of stomatal conductance (g_s, mol m^{-2} s^{-1}) in loblolly pine seedlings grown in ambient temperature (open bars) or elevated temperature (grey bars) in June and September, 2008. Plots A & C display g_s averaged over all three sites and both [CO_2] treatments. Plots B & D display g_s averaged over both [CO_2] treatments at each site, Blairsville (B), Athens (A) and Tifton (T). Plots A & B display g_s in seedlings grown in the high water treatment, while plots C & D display g_s in seedlings grown in the low water treatment. Significant difference ($p < 0.05$) between paired bars depicted with asterisk.

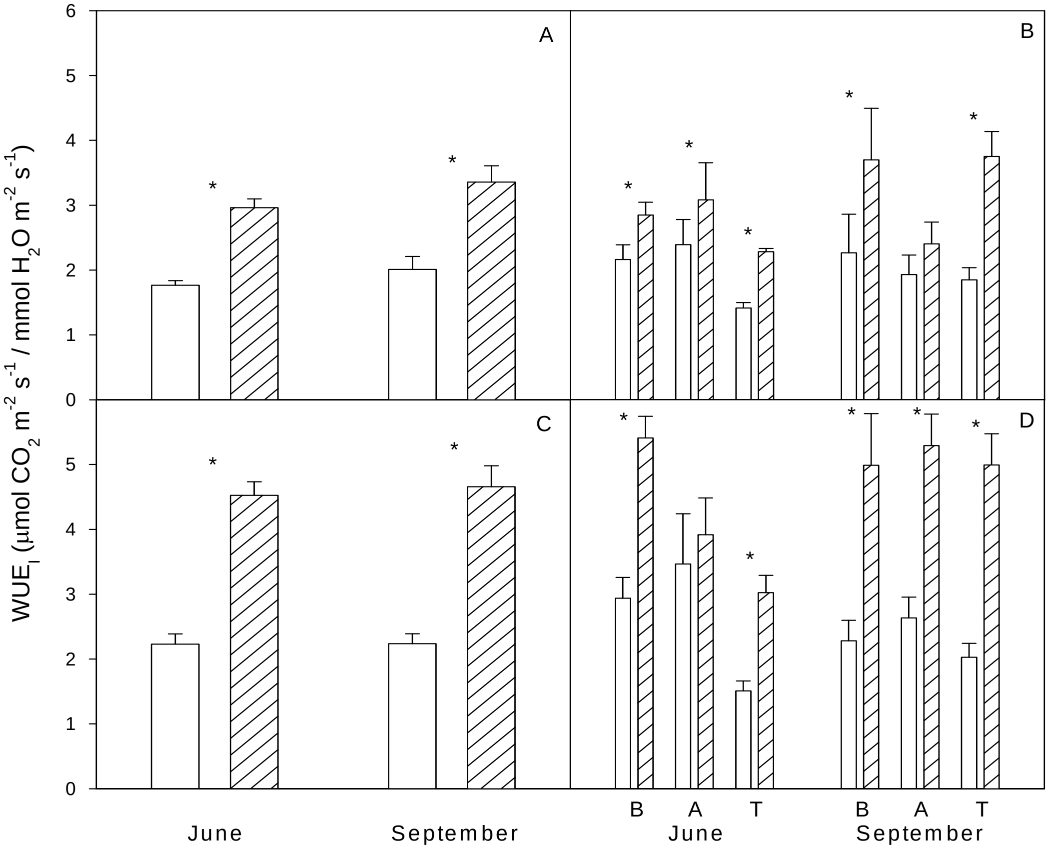

Fig. 2.7. Mean (+ SE) rate of instantaneous water use efficiency (WUE$_I$, µmol CO$_2$ m^{-2} s^{-1} / mol H$_2$O m^{-2} s^{-1}) in loblolly pine seedlings grown in ambient [CO$_2$] (open bars) or elevated [CO$_2$] (striped bars) in June and September, 2008. Plots A & C display WUE$_I$ averaged over all three sites and both temperature treatments. Plots B & D display WUE$_I$ averaged over both temperature treatments at each site, Blairsville (B), Athens (A) and Tifton (T). Plots A & B display WUE$_I$ in seedlings grown in the high water treatment, while plots C & D display WUE$_I$ in seedlings grown in the low water treatment. Significant difference ($p < 0.05$) between paired bars depicted with asterisk.

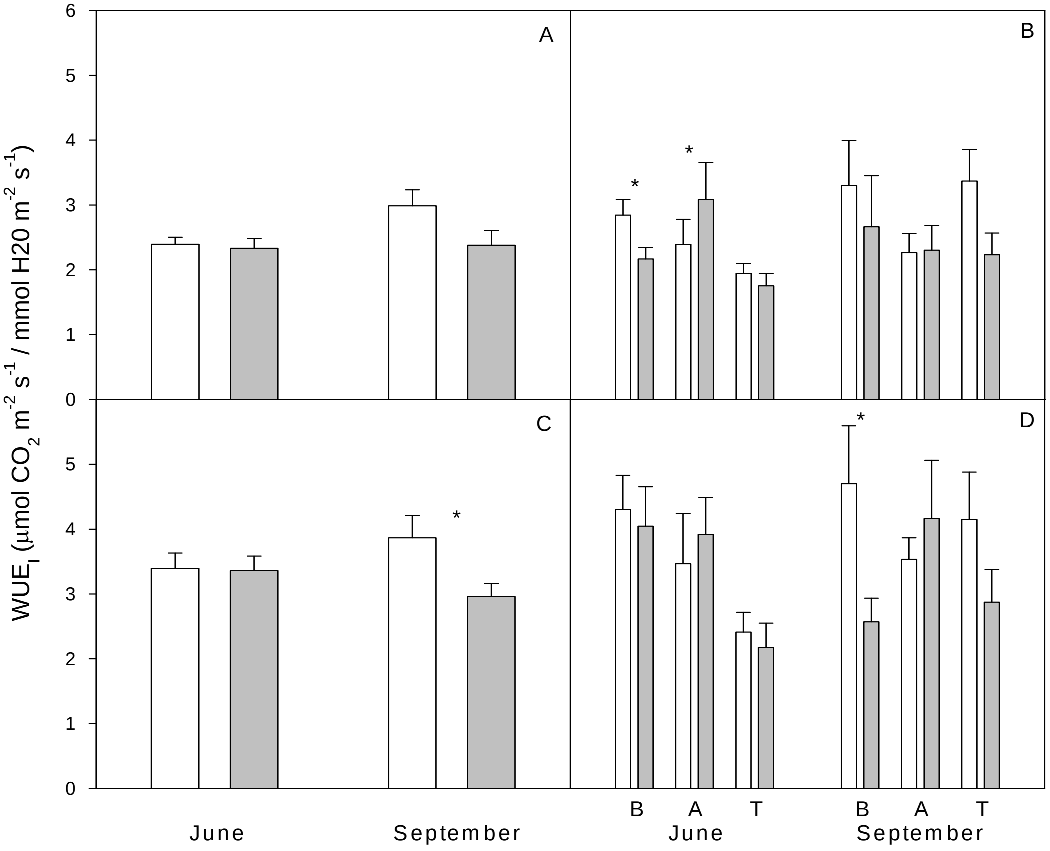

Fig. 2.8. Mean (+ SE) rate of instantaneous water use efficiency (WUE$_I$, µmol CO$_2$ m^{-2} s^{-1} / mol H$_2$O m^{-2} s^{-1}) in loblolly pine seedlings grown in ambient temperature (open bars) or elevated temperature (grey bars) in June and September, 2008. Plots A & C display WUE$_I$ averaged over all three sites and both [CO$_2$] treatments. Plots B & D display WUE$_I$ averaged over both [CO$_2$] treatments at each site, Blairsville (B), Athens (A) and Tifton (T). Plots A & B display WUE$_I$ in seedlings grown in the high water treatment, while plots C & D display WUE$_I$ in seedlings grown in the low water treatment. Significant difference ($p < 0.05$) between paired bars depicted with asterisk.

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CHAPTER 3

EFFECTS OF TEMPERATURE AND [CO_2] ON PHOTOSYNTHESIS, LEAF RESPIRATION AND BIOMASS OF LOBLOLLY PINE SEEDLINGS NEAR THE NORTHERN AND SOUTHERN BORDER OF THE SPECIES RANGE[2]

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Abstract

- We examined the influence of elevated temperature and [CO_2] on photosynthesis (A_{net}), leaf respiration (R_d) and biomass accumulation in loblolly pine seedlings at a northern and southern site in the species range to determine if the response to future climate conditions will significantly differ in two different microclimates within a species distribution.
- Treatment chambers were constructed at both sites to determine the effect of elevated temperature (+2°C) and [CO_2] (700 μmol mol^{-1}), applied singly and in combination, on growth. Four times throughout the growing season A_{net} and R_d temperature response curves were measured and a sample of seedlings was harvested.
- Elevated [CO_2] increased A_{net}, but not biomass. Elevated temperature had no effect on either A_{net} or biomass production. Applied together, elevated [CO_2] and elevated temperature increased biomass production at both the northern and southern site throughout the growing season. Biomass accumulation was strongly correlated to estimated total leaf area; relative growth rate was strongly correlated to thermal temperature sum; both appeared insensitive to site, temperature or [CO_2] treatment.
- Projected increases in [CO_2] and air temperature may boost loblolly pine growth throughout its entire distribution, suggesting that future climatic conditions will not lead to a significant range constriction.

Introduction

Predictions of shifts in species distributions due to climate change are commonly based on climate envelope modeling, which matches the existing climate of a species range with areas of similar conditions in future climate scenarios (Nogues-Bravo 2009). This approach ignores physiological differences among species, such as the degree of respiratory acclimation to prevailing temperature conditions, or the sensitivity of photosynthesis to temperature. However, pertinent physiological information regarding responses to elevated temperature and CO_2 is lacking for many plant species (Hijmans and Graham 2006), especially for climate conditions near the edges of a species range (Wertin et al. 2010). An underlying assumption used to relate climate to species distributions is that growth rates are sensitive to temperature, with sub- and supra-optimal temperatures limiting growth and development at near the northern and southern border of a species range, respectively (in the Northern Hemisphere) (Nedlo *et al.*, 2009). Logically, as air temperatures increase in response to climate change, conditions would be expected to become more favorable for carbon gain and growth in areas with sub-optimal temperatures, and less favorable in areas with supra-optimal temperatures (Pastor & Post, 1988; He *et al.*, 2005). Predictions of future species distribution rely heavily on this assumption (e.g.: Iverson & Prasad, 2001; Iverson & Prasad, 2002; McKenney *et al.*, 2007; Morin *et al.*, 2008; Tang & Beckage, 2010), however with regard to declines in growth in the warmest part of a species range, there is limited empirical evidence supporting it, and the question is open whether high temperatures are actually limiting physiological processes and growth of plants (Woodward, 1987; Bonan & Sirois, 1992).

Elevated [CO_2], anticipated to reach as high as 900 μmol mol^{-1} by the end of the century (IPCC, 2007), is likely to have a positive effect on photosynthesis throughout the range of a species, but the magnitude of the effect may be modified by increased air temperatures and other environmental factors. However, increases in atmospheric [CO_2] and air temperature will occur concurrently and may have varying effects on carbon gain and growth in different portions of a species distribution. The effect of elevated [CO_2] on carbon gain may be greatest in the warmest parts of a species' distribution, since increased CO_2 availability can increase the optimum temperature of photosynthesis (Long, 1991; Sage *et al.*, 2008). Hypothetically, this could result in a relatively larger increase in biomass production in the warmer areas compared to cooler areas in a species range. Temperature acclimation of photosynthesis and respiration can also confer additional resistance to thermal stress. Respiration appears to acclimate to growing temperature in most species (Tjoelker *et al.*, 1999; Atkin & Tjoelker, 2003; Atkin *et al.*, 2005) including loblolly pine (Teskey & Will, 1999), although the degree of acclimation differs among species. However, acclimation of photosynthesis to growing temperature appears to be present in far fewer species (Dillaway & Kruger; Tjoelker *et al.*, 1998; Ow *et al.*, 2008). These characteristics suggest that there may be species-specific responses to the effects of elevated temperature and [CO_2] that will be important for determining future distributions. The physiological characteristics of a species may either alleviate or aggravate effects of thermal stress, substantially modifying its response to climate change and potentially affecting growth differentially across its range.

The objective of this study was to investigate the effect of elevated [CO_2] and temperature on leaf carbon gain (net photosynthesis and respiration) and biomass production of loblolly pine seedlings through one growing season at a warm and cool site within the species' native range. The sites we selected differed in growing season temperature by 4.1°C. We tested two alternative hypotheses: 1) Additional warming (+2°C) will have a greater positive effect on carbon gain and growth at the cooler site than the warmer site, and the combined effect of elevated [CO_2] and elevated temperature will also be substantially greater at the cooler site; or 2) Physiological characteristics of the species, including thermal acclimation of respiration and the effect of elevated [CO_2] on the temperature response of photosynthesis , will alleviate thermal stress at the warmer southern site, resulting in an equal growth enhancement in the combined elevated temperature and [CO_2] treatment at both sites.

Materials and Methods

Experimental Setup

One year old loblolly pine (*Pinus taeda* L.) seedlings were grown at two sites in Georgia, USA, separated by 385 km (Fig. 3.1). The sites were at facilities of the University of Georgia: the Georgia Mountain Research and Education Center at Blairsville, GA (34°87' N, 83° 95' W) (northern site) and the Coastal Plain Experiment Station at Tifton, GA (31°29' N, 83°32' W) (southern site). Mean annual temperature at the two sites over the previous 30 years was 12.6 °C and 18.7 °C, respectively (National Climatic Data Center 2009). The mean growing season temperature at the northern site (15.2^{o}C) is similar to the mean temperature along the northern boundary of the loblolly pine distribution, even at higher latitudes, while the mean growing season temperature at the southern site (21.5°C) is similar to temperatures found throughout the southernmost region of the range. At each site four half-cylindrical treatment chambers measuring 3.6 m long x 3.6 m wide x 2.4 m tall were constructed of wood and PVC pipe and covered with poly-film (6 mil clear GT Performance Film, Green-Tek Inc, Edgerton, Wisconsin, USA) (Boyette & Bilderback, 1996). Chambers were built in an open field at each site, oriented facing north – south and spaced 2.5 m apart to prevent shading. Chambers were randomly assigned one of four treatment combinations: 1) ambient temperature and ambient [CO_2] (380 μmol mol^{-1}), 2) ambient temperature and elevated [CO_2] (700 μmol mol^{-1}), 3) elevated temperature (ambient + 2^{o}C) and ambient [CO_2] and 4) elevated temperature and elevated [CO_2]. To minimize chamber effects, each chamber was constructed to exactly the same dimensions, air within the chambers was thoroughly mixed with an oscillating fan, and seedlings were rotated within the chambers half way through the study.

Air temperature was maintained in each chamber at ambient outside temperature (T_A) or elevated temperature (T_E) (ambient +2^{o}C). Chamber temperature was regulated with a differential thermostat (Model DSD-2, Kera Technologies Inc, Mississauga, Ontario, Canada) in each chamber that controlled air conditioners and electric resistance heaters. Ambient and elevated CO_2 concentrations were maintained at 380μmol mol^{-1} (C_A) or 700μmol mol^{-1} (C_E), respectively. Chamber [CO_2] was measured and regulated with a non-dispersive infrared CO_2 sensor (Model GMT222, Vaisala Inc, Woburn, Massachusetts USA) which controlled a solenoid valve connected to a cylinder of compressed CO_2. Seedlings were watered to saturation 4 times per day with an automated irrigation system and drip emitters (Supertif - PLASTRO, Kibbutz Gvat D. N. Ha'Amakim, Israel). Photosynthetic active radiation (PAR) was measured outdoors and inside one chamber at each site with quantum radiation sensors (Model LI-190SZ, LiCor Biosciences, Lincoln, Nebraska USA).

One-year-old bare root loblolly pine seedlings were planted in early February 2008 in 8 L pots in potting medium (Fafard Nursery Mix, Conrad Fafard Inc, Agawam, Massachussetts, USA). The genetic source of the seedlings was a mixture of open pollinated families from the Georgia Piedmont (Georgia Forestry Commission, Atlanta, Georgia, USA), essentially from a temperature zone in the center of the species range and equidistant, with respect to temperature, from both sites used in this study. Average stem height and diameter of the seedlings at planting were 0.27 m and 3.9 mm, respectively. In each treatment chamber the seedlings were randomly assigned to five blocks, with 8 seedlings in each block. Blocks, and seedlings within blocks, were randomly rotated within each house mid-way through the experiment. Each pot was fertilized with approximately 30 g of 15-9-12 extended release fertilizer (Osmocote Plus #903286, Scotts-Sierra Horticultural Products, Marysville, Ohio USA) in March and August and 4.93 mg of chelated iron (Sprint 138, Becker Underwood Inc, Ames, Iowa USA) in May and August. In May approximately 0.04 ml Imidacloprid was applied topically to the soil in each pot to control pests (Bayer Advanced 12 month tree and shrub insect control; Bayer; Monheim am Rhein, Germany).

Environmental Parameters

Mean air temperature from February to the final harvest (November for the northern site, December for the southern site) followed a similar profile at both sites, with mean air temperature increasing through May and decreasing from mid September until the final measurement harvest. Averaged across the entire experimental period, mean air temperature was 4.1°C warmer at the southern site compared to the northern site (Fig. 3.2). The greatest difference in monthly temperature between the two sites occurred early in the year (6.4°C in February and 6.2°C March), while the smallest difference in temperature occurred in summer (3.7°C July and 3.8°C in August) (Table 3.1). The average daily incoming PAR during the experiment was 37.9 mol m^{-2} day^{-1} at the northern site and 39.4 mol m^{-2} day^{-1} southern site.

Averaged across the entire experiment, air temperature in the elevated temperature treatments (which were initiated at planting) was +1.9 and +2.3°C above the ambient temperature treatments at the northern and southern sites, respectively (Table 3.2). Mean [CO_2]

in the elevated [CO_2] treatments (which were initiated at bud burst) was +254 and +283 µmol mol^{-1} compared to the ambient [CO_2] treatment at the northern and southern sites, respectively. The mean temperature and [CO_2] in each treatment chamber for the month prior to each harvest are shown in Table 3.2.

Measurements of Gas Exchange and Biomass

Four times during the growing season one seedling from each block in each treatment chamber (N = 5) was randomly selected and brought back to the lab in Athens, GA and harvested for total biomass. Seedlings were selected at the northern site on April 15, June 11, August 13, and October 18 and at the southern site on April 17, June 16, August 17 and October 14 (Fig 3.2).

To determine if the treatments had an effect on the temperature response of light saturated net photosynthesis (A_{net}) or foliar dark respiration (R_d), leaf gas exchange was measured at five temperatures in June, August and October. Five seedlings from each treatment were transported to from the sites to the lab, watered to saturation and placed in a walk-in growth chamber (GC 36; Environmental Growth Chambers, Chagrin Falls, OH) overnight. Growth chamber conditions for this period were set at 25°C, 55% relative humidity, 380 µmol mol^{-1} or 700 µmol mol^{-1} CO_2 (depending on growing treatment).

One hour before starting measurements of A_{net}, growth chamber temperature and light were adjusted to 15°C and 500 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR). Measurements of A_{net} were made on one fully developed three-needle fascicle per plant using a LI-6400 Portable Photosynthesis System (Li-Cor Biosciences, Lincoln, NE) with a standard red/blue LED broadleaf cuvette and a CO_2 mixer. Cuvette conditions were set to mimic chamber settings with one exception, PAR was set at 1500 µmol m^{-2} s^{-1}. After measurements were completed, the growth chamber and cuvette temperature was increased by 5°C and allowed to stabilize for one hour before A_{net} was measured again. Measurements of A_{net} were made at 15, 20, 25, 30 and 35°C.

A similar protocol for chamber and cuvette conditions was followed for measurements of R_d, with the exception that measurements were made in the dark using a laboratory constructed cuvette. The cuvette consisted of flat top and bottom pieces made from clear polycarbonate (Lexan, General Electric Co., New York), each measuring 10 cm by 12 cm and fitted around the edge with a closed cell foam gasket. The bottom of the cuvette was equipped with inlet and outlet ports and a small fan to facilitate air mixing. Six fully developed three-needle fascicles were removed from the main stem and clamped between the top and bottom cuvette pieces. The foam gaskets provided a gas-tight seal. Air with a known CO_2 concentration was passed through the cuvette at 0.3 $l\ s^{-1}$ and CO_2 efflux from the foliage was measured using an infrared gas analyzer (IRGA) (LI-7000, Li-Cor Biosciences, Lincoln, NE USA) operated in open configuration using standard procedures (Long & Hallgren, 1985). Measurements of R_d were made at 15, 20, 25, 30 and 35°C.

Measured gas exchange values for both A_{net} and R_d were adjusted to actual leaf area enclosed in the cuvette, which was calculated using the method described by Fites and Teskey (1988). In June and August, needles from the first growth flush were measured. In October, needles from the second flush were measured. Due to the number of samples, measurements of leaf gas exchange were conducted over the course of two consecutive days.

After measurements of gas exchange were completed, seedlings were stored in the dark at 4°C until harvest, which was completed within 2 weeks. Seedlings were separated into shoot and root. Foliage was removed from the stem and branches. Roots were hand washed to remove any potting material. All processed biomass (stem/branch, foliage and root) was dried at 60°C and weighed. A fascicle from each flush was used to determine specific leaf area (SLA). Estimated leaf area for each flush was calculated as the product of foliage mass and SLA, and a estimated total leaf area for each seedling was calculated.

Statistical Analysis

Significant differences in leaf, stem, root and total biomass with treatment were tested with a split-plot design with blocking using Proc Mixed. Fixed effects were site (2 levels), temperature treatment (2 levels), [CO_2] treatment (2 levels), and date (4 levels) with block by chamber (N = 5) as the random factor. Significant differences in A_{net}, R_d and A_{net}/R_d were tested with the fixed effects of site, temperature treatment, [CO_2] treatment, and date with factor measurement temperature (5 levels) by tree (N = 5) as the repeated factor. When interactions occurred we performed tests of simple main effects using the SLICE option in the LSMEANS statement (Schabenberger *et al.*, 2000; Littell *et al.*, 2006).

To determine if biomass allocation patterns shifted with treatment (site, temperature treatment or [CO_2] treatment), values of leaf, stem and root biomass for each seedling were natural log transformed and plotted against the natural log of total biomass for that seedling. Significant differences among the slopes of the 8 treatment combinations were tested using Proc Mixed with estimate statements. The relationship between total biomass and estimated total leaf area were similarly investigated with one exception: data were not log transformed.

Estimated total leaf area was calculated on a tree basis as the sum of the product of specific leaf area and foliar dry weight for each flush. Relative growth rate (RGR) was calculated as the change in natural log of biomass over time. Biomass from sequential harvests, within blocks, was compared and plotted against both the day of year and the hourly temperature sum up to the harvest date. Significant differences in RGR were determined utilizing the same method used for biomass analysis. All statistical analyses were done using SAS 9.2 (SAS Institute Inc., Cary, NC).

Results

Gas Exchange

There was a strong seasonal effect on A_{net} where rates, averaged across all treatments and sites, decreased throughout the growing season with rates in June, August and October of 7.8, 5.6, and 4.8 µmol m^{-2} s^{-1}, respectively (Table 3.2, Fig. 3.2). There was no correlation between light saturated net photosynthesis (A_{net}) and mean air temperature during the month prior to measurements ($P = 0.31$) (Table 3.1). However, A_{net}, averaged across all measurement temperatures, was higher in seedlings grown at the northern site compared to the southern site (June: 9.1 vs. 6.5, July: 7.1 vs. 4.2, October: 4.9 vs. 4.6 µmol m^{-2} s^{-1}) (Table 3.2, Fig. 3.2), where air temperatures were cooler throughout the growing season.

Seedlings grown in elevated [CO_2], averaged across all measurement dates and both temperature treatments, had approximately 32% higher A_{net} than seedlings grown in ambient [CO_2] (Table 3.2, Fig. 3.2). At the northern site, averaged across both temperature treatments and all measurement temperatures, A_{net} was significantly greater in seedlings grown in elevated [CO_2] on all measurement dates (June: 9.96 vs. 8.28, August: 7.44 vs. 6.65, October: 5.96 vs. 3.94 µmol m^{-2} s^{-1}). A similar trend was observed at the southern site (June: 7.63 vs. 5.34, August: 4.57 vs. 3.76, October: 5.96 vs. 3.38 µmol m^{-2} s^{-1},). The elevated temperature treatment did not have a consistent effect on A_{net} (Table 3.2). At the southern site, averaged across all measurement temperatures and both [CO_2] treatments, the elevated temperature treatment did not have a significant effect on A_{net} compared to the ambient temperature treatment on any measurement date (June: 6.5 vs. 6.5, July: 4.2 vs. 4.1, October: 4.5 vs. 4.8, μmol m^{-2} s^{-1}, Fig. 3.2). However, at the northern site, the elevated temperature treatment caused a reduction in A_{net} in June (9.6 vs. 8.6 μmol m^{-2} s^{-1}), increased A_{net} in August (6.4 vs. 7.7 μmol m^{-2} s^{-1}) and had no discernable effect on A_{net} in October (5.1 vs. 4.8 μmol m^{-2} s^{-1}).

The optimum temperature for A_{net}, averaged across all treatments and measurement dates, was similar at the northern and southern sites (26.5 vs. 26.6°C) (Table 3.3), despite a 4.1°C difference in mean growing season temperature. Averaged across both sites, the optimum temperature for A_{net} did not vary during the growing season (June: 26.8°C, August: 26.0°C, October: 26.9°C) (Table 3.2). The elevated [CO_2] treatment significantly increased the optimum temperature for A_{net} at both sites on every measurement date, with one exception: at the southern site in October the elevated [CO_2] treatment had no significant effect (Table 3.3). The shift in the optimum temperature for photosynthesis implies that the difference between A_{net} in the elevated and ambient [CO_2] treatments increased with increasing measurement temperature. The elevated temperature treatment did not have a significant effect on the optimum temperature for A_{net} at any site on any measurement date (Table 3.2).

Averaged across all measurement temperatures, R_d was higher at the northern site compared to the southern site on every measurement date (63% higher in June, 85% in August and 61% in October) (Table 3.2, Fig. 3.3). In contrast to A_{net}, no consistent pattern in mean R_d was observed through the season: averaged across both sites, all treatments and all measurement temperatures, R_d decreased 31% from June to August but increased 13% from August to October. The seasonal fluctuation in R_d did not appear to be driven by site (Table 3.2).

There was no consistent effect of the [CO_2] treatment on R_d across all measurement dates (Table 3.2, Fig. 3.3). In June, R_d tended to be higher in seedlings grown at elevated [CO_2] at both the northern and southern sites (Fig. 3.3). However, in August, R_d was lower in seedlings grown at elevated [CO_2] both sites, while in October R_d was lower in seedlings grown at elevated [CO_2] at the northern site, but higher at the southern site. Averaged across all measurement dates, seedlings grown in the elevated temperature treatment had lower R_d than seedlings grown in the ambient temperature treatment (0.364 vs. 0.391 μmol m^{-2} s^{-1}). Though there was a trend that elevated temperature reduced R_d in all measurement dates and both sites, with one exception, the reduction was only significant in August at the northern site.

While A_{net} was significantly higher at the northern site, R_d was as well. The ratio of A_{net} / R_d was higher at the southern site compared to the northern site when averaged across all measurement temperatures (June: 28.4 vs. 21.5, August: 34.1 vs. 25.3, October: 20.8 vs. 15.5,) (Fig. 3.4). The relationship between A_{net} / R_d was strongly affected by measurement date and elevated [CO_2], but not by elevated temperature. In August, A_{net}/R_d was significantly higher than in June or October, while the ratio of A_{net}/R_d in June was higher than in October. Across all measurement dates, measurement temperatures, and both sites A_{net}/R_d was significantly higher in the elevated [CO_2] treatment (26.8 vs. 21.7), but not the temperature treatment (23.8 vs. 24.8).

Biomass Analysis

The largest effect on seedling biomass was growing location, with seedlings grown at the southern site having more biomass than seedlings at the northern site on every harvest date (Table 3.2, Fig. 3.5). This effect was primarily due to a longer growing season: bud burst occurred two weeks earlier at the southern site, with approximately 50% bud burst achieved on March 14th at the southern site compared to April 1st at the northern site. At the southern site accumulated biomass more rapidly at the beginning of the growing season; at the first harvest, seedlings at the southern site tended to have almost twice the biomass compared to the northern site, though the difference was not significant (7.93 g vs. 4.12 g; $P = 0.84$) (Fig. 3.5). Seedling biomass at the June harvest was 235% greater at the southern site (58.7 g vs. 17.5 g; $P = 0.029$) and 75% greater at the August harvest (166.7 g vs. 95.4 g; $P < 0.002$). At the October harvest biomass was only 31% greater at the southern site compared to the northern site (301.7 g vs. 228.7 g; $P < 0.001$), while by the final harvest biomass was 63% greater at the southern site (440.6 g vs. 289.3 g; $P < 0.001$).

The elevated temperature and elevated [CO_2] treatment (T_E C_E) trended to increasing biomass production at both sites throughout the growing season. At the northern site seedlings grown in the T_E C_E treatment tended to have over 50% more biomass in April and June, though the difference was not significant ($P = 0.65$ & $P = 0.82$, respectively) (Fig. 3.5). In August and October seedlings from the T_E C_E treatment had and approximately 25%, but again the difference was not significant ($P = 0.58$ & $P = 0.19$, respectively). By the end of the growing season, seedlings from the T_E C_E treatment had 48% more biomass compared to seedlings from the T_A C_A treatment ($P < 0.001$). At the southern site in April, August and October seedlings grown in the T_E C_E treatment trended to be larger than seedlings grown in the T_A C_A treatment ($P = 0.954$, $P = 0.35$, & $P = 0.065$, respectively). At the end of the experiment, seedlings grown in the T_E C_E treatment at the southern site seedling biomass were 22% greater than seedlings grown in the T_A C_A treatment ($P < 0.001$).

While elevated [CO_2] and elevated temperature, applied in combination, tended to have a positive effect on biomass production at both sites through the entire growing season, elevated [CO_2] and elevated temperature, applied singly, had no consistent effect on biomass (Table 3.2).

The T_A C_E treatment did not significantly increase biomass production compared to the T_A C_A treatment at the northern site at any harvest date ($P > 0.70$ for all dates) or the southern site at any harvest date ($P > 0.1$ for all dates), with one exception: at the final harvest at the northern site biomass was 20% greater in the T_A C_E treatment ($P = 0.014$). The T_E C_A treatment did not significantly boost biomass accumulation at either site at any measurement date ($P > 0.18$ for all dates), with two exceptions: at the northern site the elevated temperature treatment increased seedling biomass by 34% in October ($P = 0.018$) and 39% at the final harvest ($P < 0.001$).

In general, leaf, stem and root biomass accumulation was affected by elevated temperature and elevated [CO_2], applied singly and in combination, in a similar pattern as total biomass (Table 3.2). This is reflected by only minor shifts in biomass allocation among the three tissues (Table 3.2, Fig.3 6). While leaf, stem and root biomass allocation was significantly affected by site (Table 3.2), there was substantial overlap in allocation patterns between the two sites for all tissues and the difference does not appear biologically relevant. At the southern site, as seedling size increased, seedlings allocated slightly more biomass to foliage and shoots than root compared to the northern site. Leaf, stem and root biomass were not significantly influenced by [CO_2] or temperature treatment.

Across the season, relative growth rate (RGR) was not significantly affected by temperature or [CO_2] treatment ($P > 0.75$); however, there was a significant site effect on RGR. Seedlings at the southern site had a slightly higher RGR than seedlings at the northern site. RGR differed between the sites through the growing season ($P < 0.001$) (Fig. 3.7a). The highest RGR at the southern site occurred in June and reached a rate of approximately 0.030 g day^{-1}, while at the northern site the highest RGR, 0.027 g day^{-1}, occurred in August. When RGR was plotted against hourly temperature sum a strong trend was observed, with RGR at both sites following a similar pattern of variation related to the seasonal change in hourly temperature sum (Fig. 3.7b). There was no significant difference between the northern and southern site in the hourly temperature sum that yielded the largest RGR ($P = 0.56$).

Total biomass accumulation was strongly related to estimated total leaf area (Fig. 3.8), though there was a significant difference in this relationship between sites ($P < 0.001$) with seedlings at the southern site accumulating more biomass with less leaf area. There was significant overlap in the relationship between leaf area and biomass accumulation of seedlings at both sites. There was no temperature or [CO_2] treatment effect on the relationship between leaf area and total biomass at either site ($P > 0.49$, for all).

Discussion

Increasing air temperature is predicted to shift species distribution by making conditions more favorable for growth in sub-optimal temperature regions and less favorable for growth in supra-optimal temperature regions (Pastor & Post, 1988; He *et al.*, 2005). In this study we attempted to verify this hypothesis by growing loblolly pine seedlings at sites near the northern and southern edges of the species range in both ambient and elevated atmospheric conditions. Seedlings grown in elevated temperature and [CO_2] had a higher rate of photosynthesis and accumulated more biomass at both ends of the species range compared to seedlings grown in ambient temperature and [CO_2]. Contrary to models that predict that the distribution of loblolly pine may shrink by as much as 70% (McKenney *et al.*, 2007), these findings indicate that future climate conditions may result in an increase in growth of loblolly pine throughout the species range and suggest that a contraction of the distribution of loblolly pine is unlikely.

Elevated temperature, which we had anticipated would have the largest effect on both photosynthesis and biomass production at the northern and southern edges of the species range, did not have any effect on photosynthesis and no consistent effect on biomass production at either site. While previous studies have demonstrated that long term exposure to elevated temperature (between +1 to +5°C) can increase photosynthesis (Lewis *et al.*, 2001) or suppress photosynthesis (Callaway *et al.*, 1994; Wang *et al.*, 1995; Wayne *et al.*, 1998; Ro *et al.*, 2001), we failed to observe any effect of an increase of +2°C on photosynthesis at either site. This result might be attributed to a shift in the optimum temperature of photosynthesis, which has been demonstrated in tree species (Sage *et al.*, 2008; Gunderson *et al.*, 2010), but we did not observe any consistent shift in the optimum temperature for photosynthesis. Loblolly pine has a wide range of optimum temperature for net photosynthesis (Teskey & Will, 1999); we therefore suggest that the elevated temperature treatment, an increase of +2°C, was well within the optimum range and also was small compared to both the daily and seasonal temperature fluctuations experienced by the seedlings. The elevated temperature treatment may have not been a large enough increase to cause a shift in the intrinsic rate of photosynthesis or the optimum temperature of photosynthesis.

The lack of response of biomass production to elevated temperature is in conflict with previous studies which demonstrated that an increase in growing temperature caused an increase in biomass production of trees grown in sub-optimal temperature environments (Peltola *et al.*, 2002; Danby & Hik, 2007; Penuelas *et al.*, 2007; Hoch & Korner, 2009) or a decrease in production in supra-optimal temperature environments (Norby *et al.*, 2000; Penuelas *et al.*, 2007; Reich & Oleksyn, 2008; Way & Sage, 2008). However, our results are not surprising given that photosynthesis was insensitive to elevated temperature and respiration acclimated to growing temperature at both sites. Our findings suggest that an increase in temperature will not stimulate growth in sub-optimal temperature environments nor suppress growth in supra-optimal temperature environments in loblolly pine and therefore is unlikely to alter the species distribution.

Elevated [CO_2] has been demonstrated to stimulate summertime photosynthesis of loblolly pine saplings by 60-130% (Tissue *et al.*, 1997) and biomass production in loblolly pine trees by approximately 24% (Tissue *et al.*, 1996; DeLucia *et al.*, 1999; Oren *et al.*, 2001). While we did observe a significant and sustained increase in photosynthesis with elevated [CO_2], we failed to observe a consistent effect of elevated [CO_2] on biomass production. Seedlings grown in elevated [CO_2] at both sites tended to have more biomass compared to seedlings grown in ambient [CO_2], though the increase was smaller than previously reported in loblolly pine seedlings, especially given that water and nutrients were not limiting.

Of particular interest in this study was the combined effect of elevated temperature and [CO_2] on growth. Typically, an increase in [CO_2] results in an increase in the optimum temperature for net photosynthesis (Long 2001), suggesting that a combined increase in [CO_2] and temperature would boost carbon fixation. While exposure to elevated [CO_2], regardless of temperature treatment, considerably increased both photosynthesis and the optimum temperature for photosynthesis, there was no significant change in either photosynthesis or the optimum temperature for photosynthesis with exposure to elevated temperature in either [CO_2] treatment. A boost in photosynthesis with an increase in temperature has previously been demonstrated in mature *P. sylvestris* grown in elevated [CO_2] (Wang *et al.*, 1995). While biomass production was not consistently stimulated with exposure to either elevated [CO_2] or elevated temperature in our study, we did observe a synergistic response in biomass production. The combined effect of elevated temperature and [CO_2] significantly boosted growth at both the northern and southern sites, suggesting that in predicted climate conditions loblolly pine growth may be stimulated near both the northern and southern limits of its range, perhaps resulting in expansion of the species distribution. These results agree with studies that report a synergistic response to elevated [CO_2] and elevated temperature in dwarf apple (Ro *et al.*, 2001), black willow (Veteli *et al.*, 2002), and downy birch (Mortensen, 1995).

Both biomass allocation and relative growth rate were insensitive to elevated temperature and elevated [CO_2]. Previous studies have reported that biomass allocation in loblolly pine was insensitive to both [CO_2] (Tissue *et al.*, 1997) and temperature (Nedlo *et al.*, 2009). The insensitivity of allocation to an increase in temperature and [CO_2] we observed at the two sites suggests that changes in climate will not alter loblolly pine seedling growth patterns. Relative growth rate, extrapolated from repeated measurements of seedling height and diameter, was also insensitive to both temperature and [CO_2], though it was strongly influenced by site, primarily due to the longer growing season at the southern site. Of particular interest was the insensitivity of RGR to site, temperature and [CO_2] when plotted against thermal sum. Our findings suggest that loblolly pine growth is strongly influenced by thermal sum, and less sensitive to elevated [CO_2].

Conclusion

Wedid not observe differential responses to elevated [CO_2] and elevated temperature at the northern and southern edges of the loblolly pine range. At both the sites, elevated [CO_2] and temperature, applied in combination, significantly boosted photosynthesis and biomass accumulation above levels observed in seedlings grown in current climate conditions. Our findings suggest that predicted climate conditions are unlikely to reduce the distribution of this species. Loblolly pine has a broad optimum temperature range for photosynthesis and the elevated temperature treatment (+2°C) was small relative to the daily and seasonal temperature range the seedlings experienced. If a larger temperature increase was applied, or if a species has a narrower range of optimal temperature for photosynthesis or is more sensitive to temperature, it is conceivable that future climatic conditions may significantly alter the distribution of a tree species, though we find no evidence to support this notion in our study.

Acknowledgements

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Table 3.1. Average air temperature (T_{MEAN}) and atmospheric [CO_2] concentration ([CO_2]) (SE) in the treatment chambers at the northern and southern sites for the 30 days prior to each seedling harvest. Treatments are: ambient temperature and ambient [CO_2] (T_A C_A), ambient temperature and elevated [CO_2] (T_A C_E), elevated temperature and ambient [CO_2] (T_E C_A) and elevated temperature and elevated [CO_2] (T_E C_E).

Northern Site

		April	June	August	October	Seasonal
T_A C_A	T_{MEAN}	12.9 (0.7)	20.3 (0.6)	23.1 (0.2)	18.3 (0.5)	17.5 (0.3)
	[CO_2]	-	400 (4)	356 (10)	383 (15)	383 (5)
T_A C_E	T_{MEAN}	12.8 (0.7)	20.1 (0.6)	23.0 (0.2)	18.6 (0.5)	17.5 (0.4)
	[CO_2]	-	684 (17)	587 (27)	626 (36)	631 (13)
T_E C_A	T_{MEAN}	14.1 (0.7)	22.4 (0.6)	24.9 (0.2)	22.0 (0.5)	19.2 (0.4)
	[CO_2]	-	403 (4)	362 (11)	375 (14)	386 (4)
T_E C_E	T_{MEAN}	14.2 (0.7)	23.0 (0.6)	25.4 (0.2)	20.6 (0.6)	19.3 (0.4)
	[CO_2]	-	681 (17)	590 (29)	623 (36)	637 (13)

Southern Site

		April	June	August	October	Seasonal
T_A C_A	T_{MEAN}	17.9 (0.6)	26.4 (0.4)	26.5 (0.3)	22.3 (0.3)	21.2 (0.3)
	[CO2]	437 (5)	432 (9)	371 (11)	377 (13)	402 (4)
T_A C_E	T_{MEAN}	18.1 (0.7)	26.9 (0.4)	26.7 (0.3)	22.5 (0.3)	21.5 (0.3)
	[CO2]	714 (29)	685 (29)	623 (29)	591 (31)	655 (10)
T_E C_A	T_{MEAN}	20.5 (0.7)	29.6 (0.4)	28.3 (0.3)	24.5 (0.4)	23.5 (0.4)
	[CO2]	443 (8)	423 (10)	367 (11)	369 (14)	399 (4)
T_E C_E	T_{MEAN}	20.4 (0.7)	29.3 (0.3)	28.7 (0.3)	24.9 (0.3)	23.5 (0.4)
	[CO2]	741 (31)	732 (20)	630 (29)	645 (37)	685 (10)

Table 3.2. Summary of the four way repeated analysis tested for the effects of date, site, temperature treatment and [CO_2] Treatment on various parameters of loblolly pine foliar gas exchange, seedling biomass and biomass allocation.

		Date	Site	Temp	CO2	Date x Site	Date x Temp	Date x CO2	Site x Temp	Site x CO2	Temp x CO2	Date x Site x Temp	Date x Site x CO2	Date x Temp x CO2	Site x Temp x CO2	Date x Site x Temp x CO2
Gas Exchange																
	A_{net}	**0.0001**	**0.0001**	0.953	**0.0001**	**0.0001**	**0.022**	**0.0005**	0.810	0.224	0.292	**0.004**	0.706	**0.005**	**0.005**	**0.011**
	T_{opt}	0.356	0.860	0.638	**0.0001**	0.142	0.930	**0.0309**	0.477	**0.0229**	0.782	0.853	0.990	0.175	0.754	0.911
	R_d	**0.0001**	**0.0001**	**0.028**	0.656	0.542	**0.045**	**0.001**	0.066	**0.008**	0.992	0.642	**0.004**	0.184	**0.001**	0.157
	A_{net} / R_d	**0.0001**	**0.0002**	0.570	**0.005**	0.726	0.393	0.574	0.207	0.310	0.806	0.300	0.365	0.896	**0.011**	0.147
Biomass																
	Total Biomass	**0.0001**	**0.0001**	**0.005**	0.156	**0.0001**	**0.003**	0.156	0.296	0.246	0.287	0.602	0.865	0.721	0.090	0.202
	Leaf Biomass	**0.0001**	**0.0001**	**0.001**	0.271	**0.007**	**0.0005**	0.791	0.276	0.271	0.314	0.770	0.258	0.933	0.076	0.056
	Stem Biomass	**0.0001**	**0.0001**	**0.003**	0.083	**0.0001**	**0.0006**	0.083	0.502	0.193	0.315	0.974	0.434	0.482	**0.033**	0.084
	Root Biomass	**0.0001**	**0.0001**	0.548	0.032	**0.0001**	0.911	**0.040**	0.322	0.724	0.521	0.479	0.872	0.945	0.992	0.990
Allocation																
	Leaf	-	**0.039**	0.116	0.462	-	-	-	0.094	0.100	0.407	-	-	-	0.558	-
	Stem	-	**0.001**	0.364	0.097	-	-	-	0.0188	0.0967	0.541	-	-	-	0.600	-
	Root	-	**0.007**	0.282	0.700	-	-	-	0.454	0.626	0.611	-	-	-	0.632	-

Table 3.3. Optimum temperature for A_{net} (°C) (SE) of loblolly pine seedlings grown in treatment chambers at the northern and southern site. Measurements were conducted in June, August and October 2008 on one year old seedlings. Treatments are: ambient temperature and ambient [CO_2] (T_A C_A), ambient temperature and elevated [CO_2] (T_A C_E), elevated temperature and ambient [CO_2] (T_E C_A) and elevated temperature and elevated [CO_2] (T_E C_E).

Northern Site

	June	August	October
T_A C_A	23.4 (1.4)	21.9 (1.2)	24.7 (0.5)
T_A C_E	31.3 (0.9)	29.8 (1.6)	28.6 (1.8)
T_E C_A	22.9 (0.8)	23.0 (0.8)	22.7 (0.8)
T_E C_E	32.4 (0.9)	29.0 (1.7)	28.9 (1.5)

Southern Site

	June	August	October
T_A C_A	23.4 (0.4)	22.7 (1.1)	26.3 (0.6)
T_A C_E	28.4 (1.5)	29.0 (1.2)	28.1 (0.8)
T_E C_A	22.9 (0.7)	25.0 (0.9)	26.5 (1.4)
T_E C_E	29.9 (0.2)	28.0 (2.8)	29.4 (2.0)

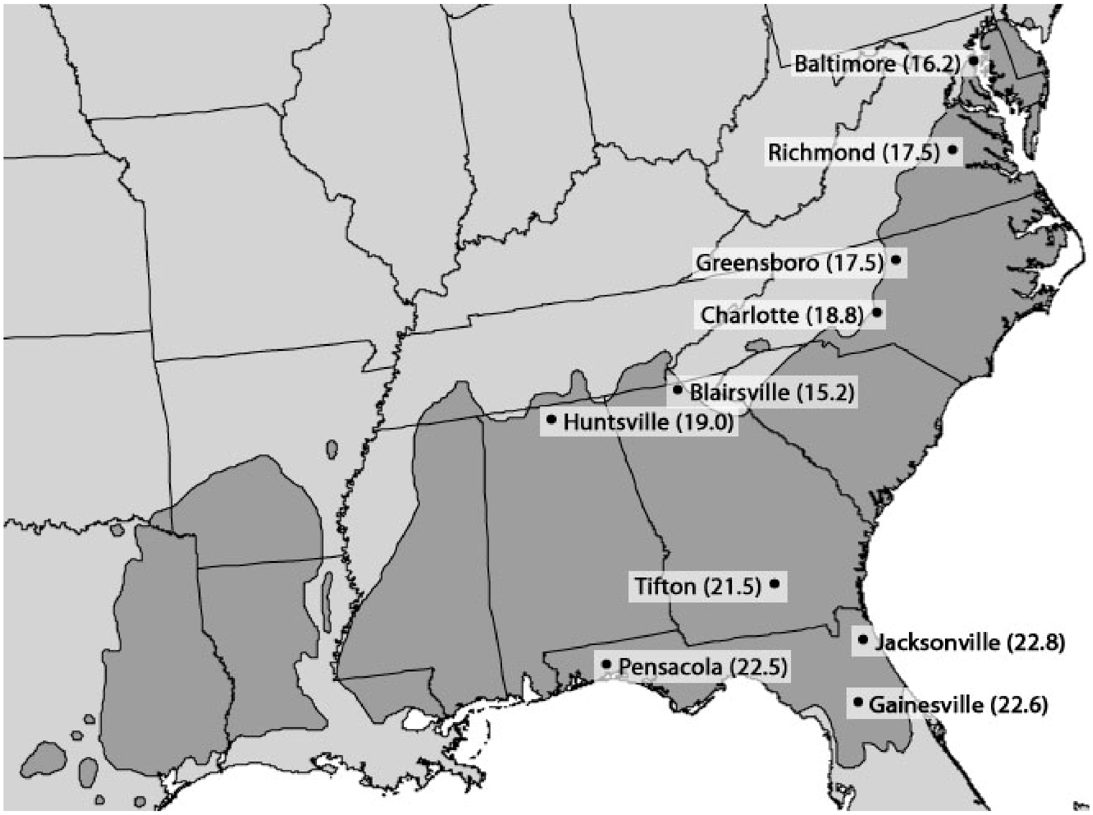

Fig. 3.1. The location of the two study sites: Blairsville and Tifton, GA USA. The native range of loblolly pine is shaded dark grey. Distribution map taken from Little 1971. Included are mean growing season temperature (Feb through October) of the study sites and other locations throughout the species range.

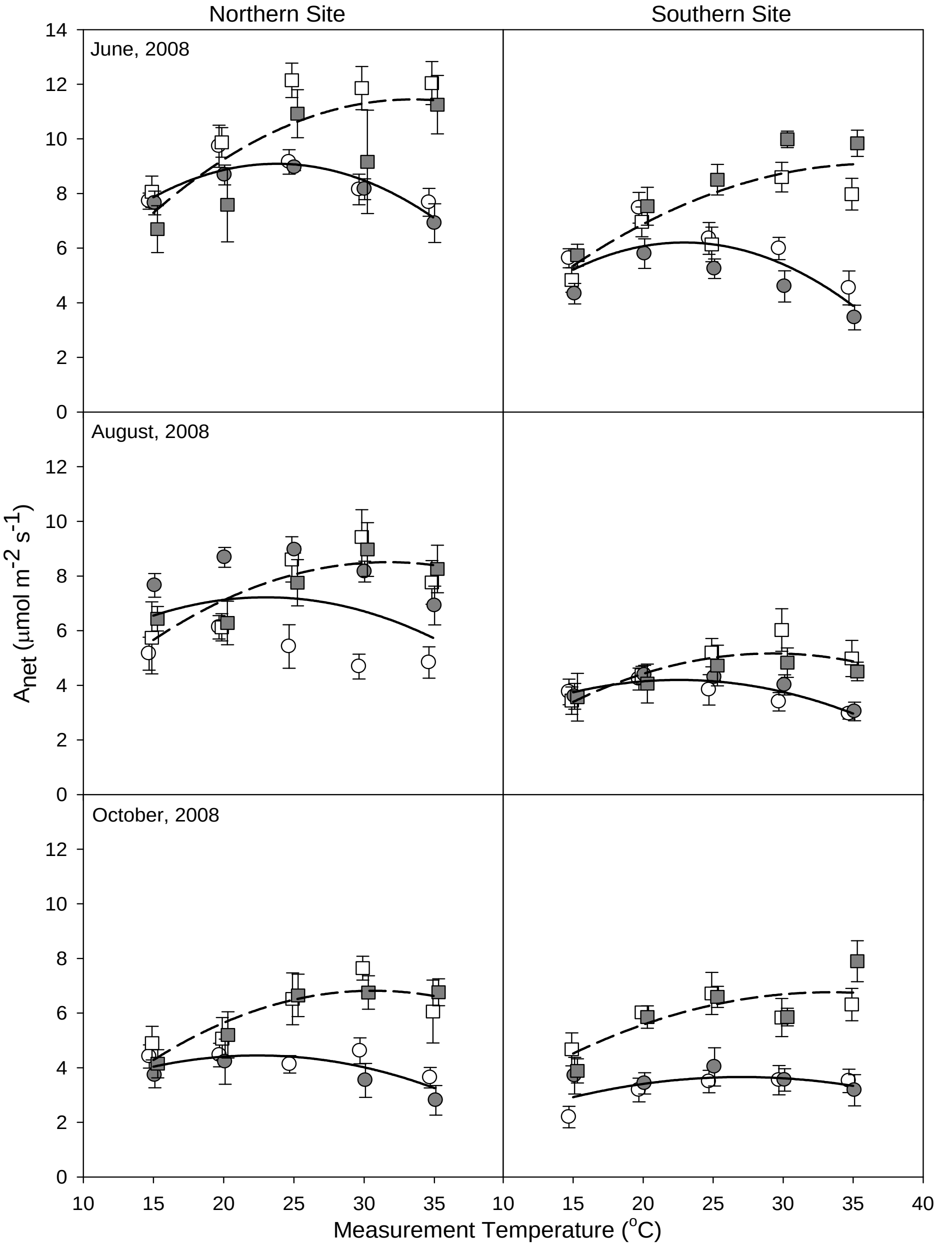

Fig. 3.2. Mean net photosynthesis (A_{net}, µmol m^{-2} s^{-1}) of loblolly pine seedlings grown in treatment chambers at northern and southern sites measured at 5 temperatures three times during the growing season (N = 5). Growth treatment combinations: circles represent ambient temperature, squares represent elevated temperature; open symbols represent ambient [CO_2], filled symbols represent elevated [CO_2]. Error bars represent SE. The solid line is fitted to A_{net} measurements in the ambient [CO_2] treatment, the dashed line is fitted to A_{net} measurements in the elevated [CO_2] treatments.

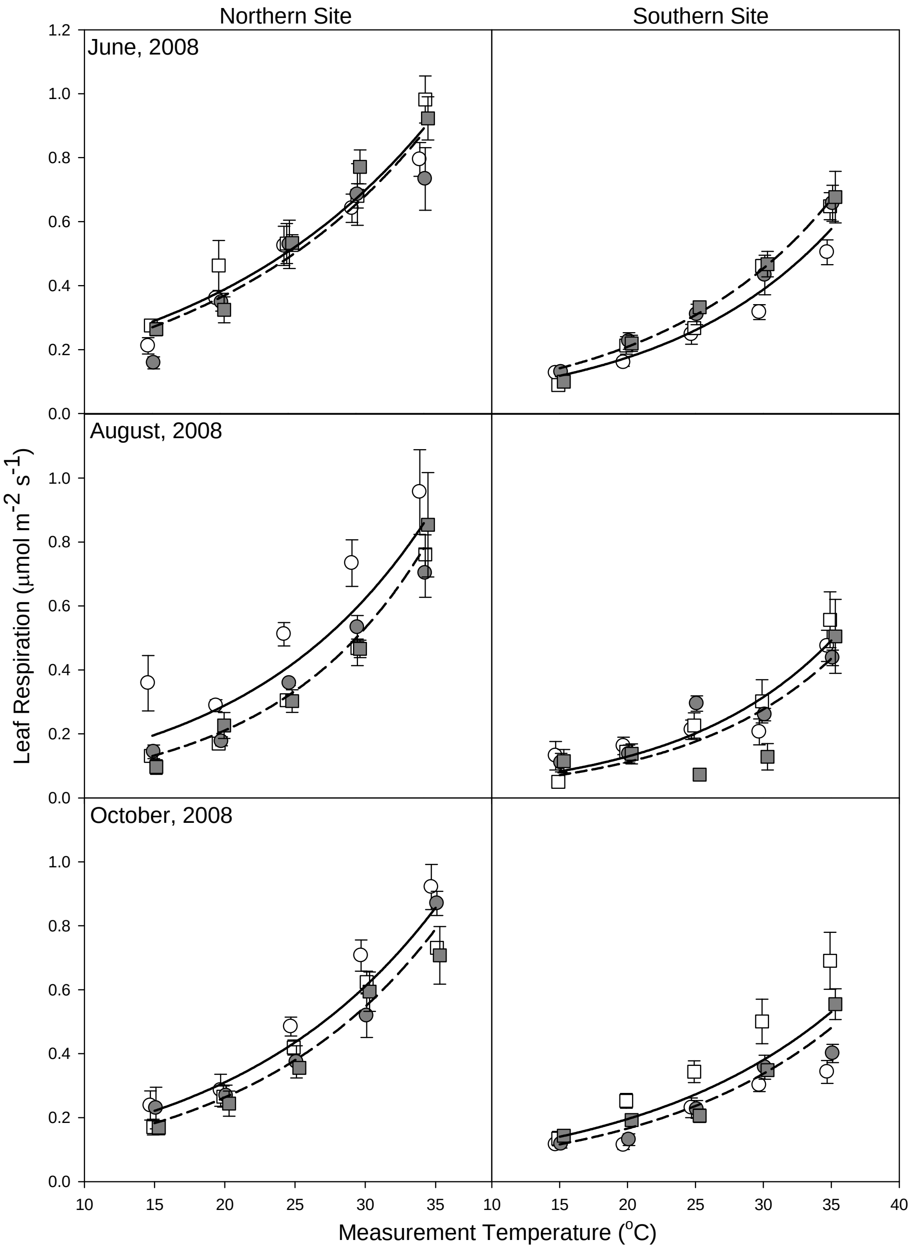

Fig. 3.3. Mean dark respiration (R_d, µmol m^{-2} s^{-1}) of loblolly pine seedlings grown in treatment chambers at northern and southern sites measured at 5 temperatures three times during the growing season (N = 5). Growth treatment combinations: circles represent ambient temperature, squares represent elevated temperature; open symbols represent ambient [CO_2], filled symbols represent elevated [CO_2]. Error bars represent SE. The solid line is fitted to R_d measurementsin the ambient temperature treatment, the dashed line is fitted to R_d measurements in the elevated temperature treatment.

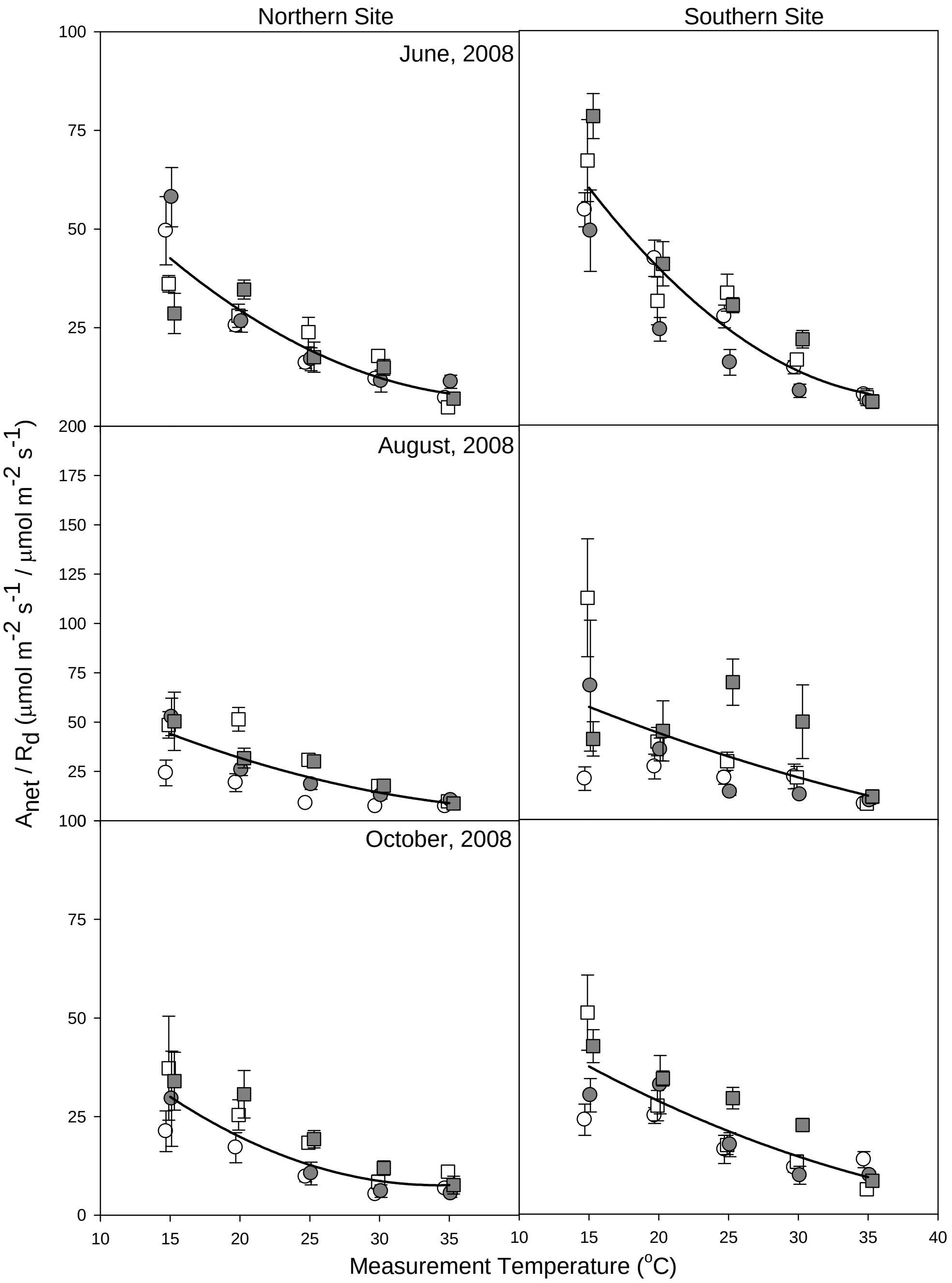

Fig. 3.4. Mean net photosynthesis / dark respiration (A_{net} / R_d, µmol m^{-2} s^{-1} / µmol m^{-2} s^{-1}) of loblolly pine seedlings grown in treatment chambers at northern and southern sites measured at 5 temperatures three times during the growing season (N = 5). Growth treatment combinations: circles represent ambient temperature, squares represent elevated temperature; open symbols represent ambient [CO_2], filled symbols represent elevated [CO_2]. Error bars represent SE. The solid line is fitted to A_{net} / R_d measurements combined across all treatments.

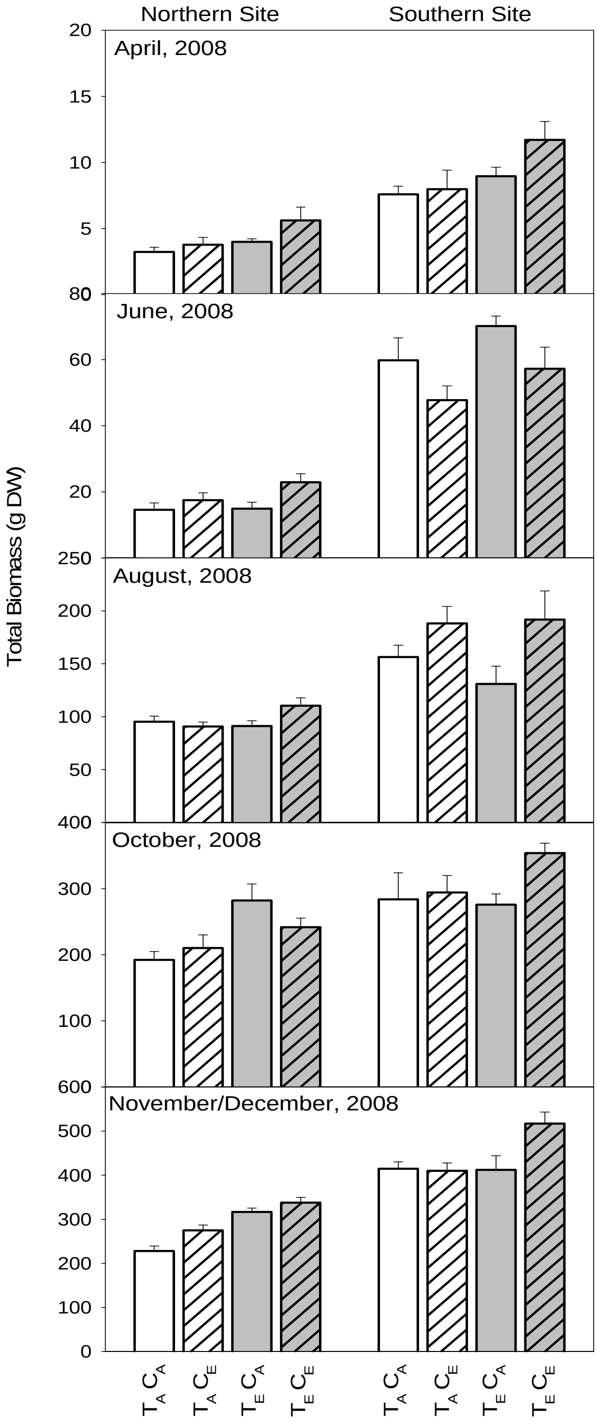

Fig. 3.5. Total biomass (dry weight, g) of loblolly pine seedlings at four harvests during the growing season (N = 5 at each harvest). Seedlings were grown in treatment chambers at northern and southern sites. Treatments were ambient temperature (white bars), elevated temperature (grey bars), ambient [CO_2] (open bars), elevated [CO_2] (striped bars). Error bars represent SE.

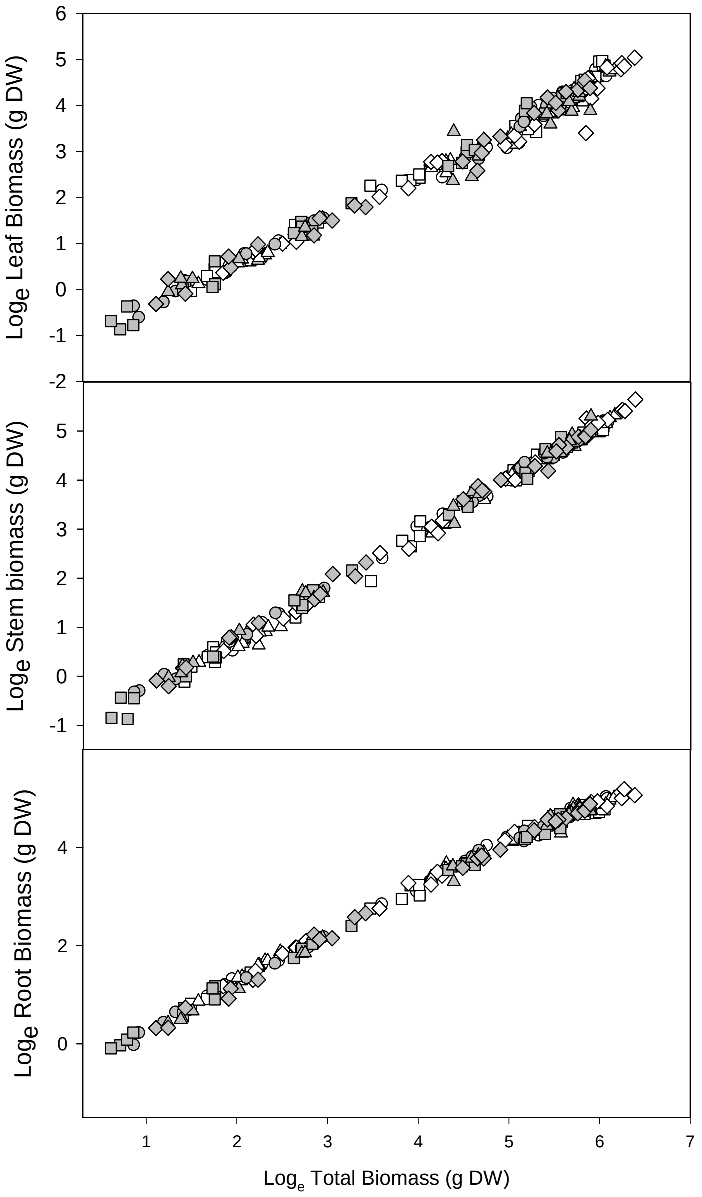

Fig. 3.6. Tissue allocation patterns of loblolly pine seedlings at four harvests during the growing season (N = 5 at each harvest). Seedlings were grown in treatment chambers at northern and southern sites. Treatment combinations were: ○ ambient temperature & ambient [CO_2]; □ ambient temperature & elevated [CO_2]; △ elevated temperature & ambient [CO_2]; ◇ elevated temperature & elevated [CO_2]; open symbols southern site; filled symbols northern site.

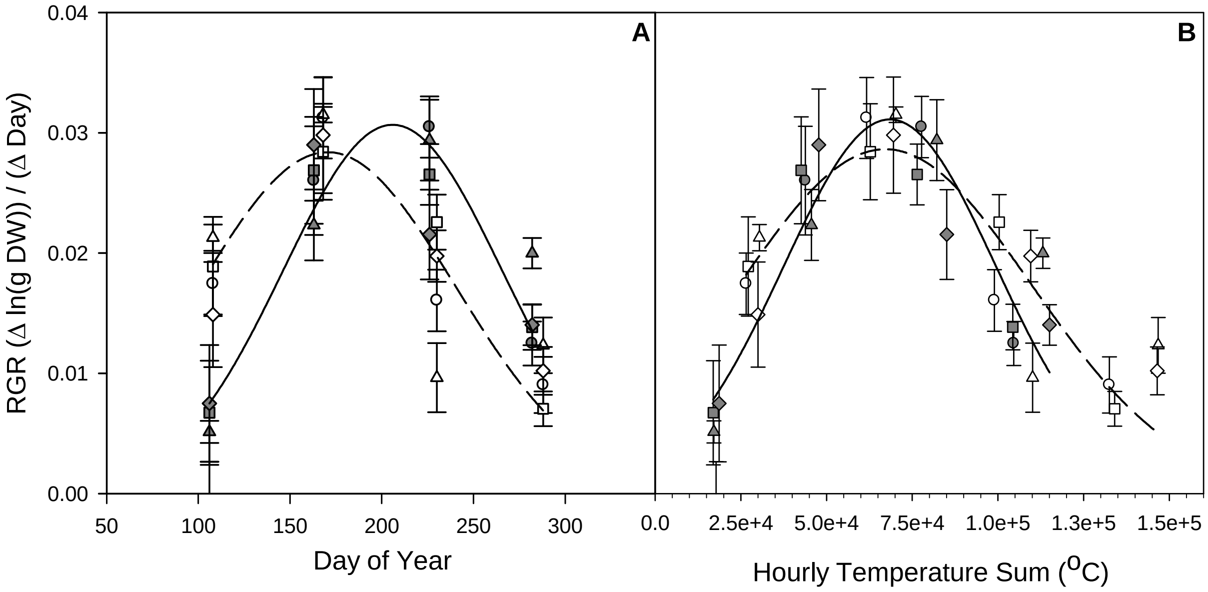

Fig. 3.7. Relative growth rate (RGR), calculated as the change in natural log of biomass over time, of loblolly pine seedlings grown in treatment chambers at northern and southern sites, plotted against A: day of year and B: hourly temperature sum. Treatment combinations were: T_A C_A: ○, T_A C_E: □, T_E C_A: △, and T_E C_E: ◇; open symbols southern site, filled symbols northern site. Dashed line is fit to data from the southern site, solid line is fit to data from the northern site. Error bars represent SE

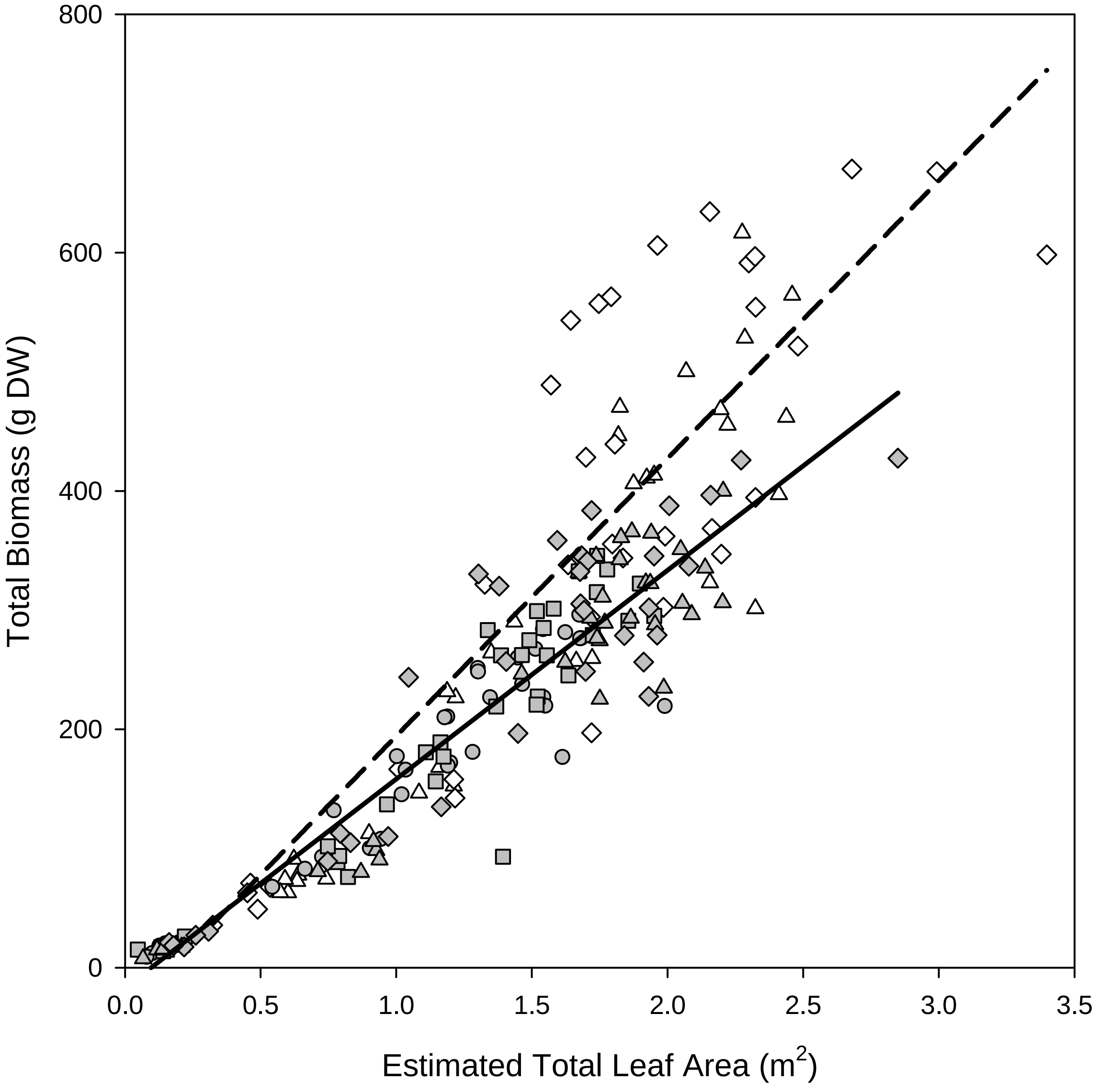

Fig. 3.8. Total biomass (dry weight, g) of loblolly pine seedlings grown at northern and southern sites as a function of estimated leaf area (calculated as a product of specific leaf area and leaf dry mass). Treatments were: T_A C_A: ○, T_A C_E: □, T_E C_A: △, T_E C_E: ◇; open symbols southern site, filled symbols northern site. Dashed line is fit to data from the southern site, solid line is fit to data from the northern site. Error bars represent SE.

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CHAPTER 4

EFFECTS OF PREDICTED AND CURRENT ATMOSPHERIC CONDITIONS AND WATER STRESS ON GROWTH OF *PINUS TAEDA* SEEDLINGS AT SITES NEAR THE NORTHERN AND SOUTHERN EDGE OF THE SPECIES RANGE [3]

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Abstract

1. Predicted future changes in atmospheric conditions, namely increases in atmospheric CO_2 concentrations ($[CO_2]$) and temperature, are expected to alter species distribution. An increase in temperature is expected to boost grown in current sub-optimal temperature environments of a species range, but reduce growth or increase mortality in current supra-optimal temperature environments of a species range. However, elevated $[CO_2]$ may alleviate additional thermal stress.

2. We performed simultaneous experiments at two sites to examine the effect that future climatic conditions may have on growth of loblolly pine seedlings near the northern and southern ends of a tree species distribution.

3. At both sites one year old loblolly pine seedlings were grown in current (ambient temperature and $[CO_2]$) and predicted future atmospheric conditions (ambient +2°C temperature and 700 $\mu mol\ mol^{-1}$ $[CO_2]$). Additionally, high and low soil moisture availability was added to each atmospheric treatment to determine the effect of potential shifts in precipitation.

4. Seedling growth was significantly stimulated by the combined effect of elevated $[CO_2]$ and elevated temperature at both sites. This result suggests that a temperature increase of 2°C, coupled with an increase in $[CO_2]$ (predicted future climate), will create conditions favorable for growth of this species across its N-S range. Reduced soil moisture reversed the beneficial effects of elevated temperature and elevated $[CO_2]$ on growth, indicating that water availability may be the most critical factor determining future productivity.

5. *Synthesis*. Overall, our results did not suggest that a future climate of higher air temperatures coupled with higher $[CO_2]$ will be detrimental to biomass production of this species or force a

contraction of the southern portion of its geographic distribution, but a concurrent reduction in soil moisture availability could.

Introduction

Climate change is expected to have a significant impact on plant species distribution (Thuiller et al., 2008). In the northern hemisphere the entire distribution of many species are expected to shift northward (e.g. Iverson et al., 2008). This prediction is based mainly on the idea that increases in air temperature will be favorable for growth in the northern portion of a species range but unfavorable for growth in the southern portion (e.g.:Xu et al., 2007). Many predictions of future species distributions rely heavily on this concept and while low temperatures are undoubtedly related to the northern distributional limit of a species, it has been argued that high temperatures may not have a similar impact on the southern distributional limit (Woodward, 1987, Bonan and Sirois, 1992). However, there are few direct observations of species responses to temperature near their southern distributional limits. Norby et al. (2000) reported that *Acer saccharum* seedlings planted near the southern border of the species distribution had reduced biomass accumulation with an increase in air temperature (+4°C), suggesting that elevated temperatures during the growing season could impose additional thermal stress in the warmer portion of a species distribution, thereby reducing carbon gain and growth. However, in some species thermal stress may be compensated by respiratory and photosynthetic acclimation to temperature as well as a longer growing season.

Future increases in air temperature will be driven largely by an increase in atmospheric CO_2 concentration ([CO_2]). For plants, the concurrent increase in CO_2 availability may alter the effects of elevated temperature on physiological processes. Elevated [CO_2] may ameliorate the effects of thermal stress by increasing the temperature optimum of photosynthesis (Long, 1991) and by directly boosting carbon fixation, countering potential increases in respiratory losses at higher temperatures. It is therefore possible that the combination of increasing air temperature and [CO_2] may boost growth in both sub- and supra-optimal temperature environments above that currently observed, contrary to some model predictions (Hijmans and Graham, 2006).

An increase in air temperature is also expected to alter precipitation patterns. In the southeastern United States, models predict that the majority of precipitation will occur in shorter, more intense events, resulting in less infiltration and more runoff and decreasing water availability to plants (IPCC 2007: Regional Climate Projections: model AB1). Elevated temperatures may also exacerbate plant water stress by increasing vapor pressure deficit. Plants may compensate for water stress by closing stomata, resulting in reduced photosynthetic capacity and biomass production. In supra-optimal temperature environments the combined effects of thermal stress and water stress may be greater than the ameliorating effects of elevated [CO_2], thermal acclimation, and a potentially longer growing season, thus resulting in a lower growth rate and a competitive disadvantage which may result in significant range constriction in the warmer portions of the species distribution. Thus, the combined effects of thermal stress, water stress and increased CO_2 availability may have a profound, though variable, effect on growth and survival across the range of a species.

In this study we tested the effects of current and simulated future climatic conditions on biomass production of loblolly pine seedlings. The study was conducted simultaneously at two sites, one in the northern (cooler) portion and one in the southern (warmer) portion of the species range. Treatments consisted of elevated air temperature and elevated [CO_2] compared with ambient temperature and [CO_2] in both well-watered and water deficit conditions. We tested two main hypotheses: a) the combination of elevated temperature and elevated [CO_2] will elicit opposite responses in biomass production at the two sites due to the 4°C difference in ambient temperatures at the sites; and b) water stress will significantly reduce biomass production at both sites and in both treatments, though the effect will be less severe at the northern site and in the elevated [CO_2]-elevated temperature treatment at both sites. To our knowledge, no prior experimental study of elevated CO_2 and elevated temperature has contrasted the plant growth response to these climate change factors at different locations in a species' range.

Materials and methods

Experimental Setup

One year old loblolly pine (*Pinus taeda* L.) seedlings were grown at two sites, separated by 385 km, in Georgia USA (Fig. 4.1). The sites were located at facilities of the University of Georgia: the Georgia Mountain Research and Education Center at Blairsville, GA (34°87' N, 83° 95' W) and the Coastal Plain Experiment Station at Tifton, GA (31°29' N, 83°32' W). The mean growing season temperature at Blairsville (15.2°C) is similar to the mean temperature along the northern boundary of the loblolly pine distribution, even at higher latitudes. Tifton is located in the southern portion of the species range but not at the range limit. However, it is among the warmest locations in the loblolly pine range, with mean growing season temperatures similar to locations closer to the southern limit. During the experiment the difference in mean ambient temperature between the northern site and the southern site was 4.1°C.

Seedlings were grown in two treatment chambers at each site. Treatments consisted of ambient temperature and ambient [CO_2] (380 μmol mol^{-1}) (T_A C_A) or elevated temperature and elevated [CO_2] (T_E C_E). Within each treatment chamber seedlings were divided into 10 blocks and each block was randomly assigned to either a high or low water treatment. The treatment chambers were half-cylinder shaped measuring 3.6 m long x 3.6 m wide x 2.4 m high. Chamber frames were constructed of wood and PVC pipe and were covered with poly-film (6 mil clear GT Performance Film, Green-Tek Inc, Edgerton, Wisconsin, USA) (Boyette and Bilderback, 1996). Chambers were oriented facing south and placed 2.5 m apart. To minimize chamber effects, each chamber was constructed to exactly the same dimensions, air within the chambers was thoroughly mixed with an oscillating fan, and seedlings were rotated within the chambers half way through the study.

In each chamber, a differential thermostat (Model DSD-2, Kera Technologies Inc, Mississauga, Ontario, Canada) controlled an air conditioner and electric resistance heater to continuously maintain the treatment target temperatures at ambient (measured outside the chambers) or ambient +2°C. Chamber [CO_2] was measured and controlled with a non-dispersive infrared CO_2 sensor (Model GMT222, Vaisala Inc, Woburn, Massachusetts USA) and a solenoid valve connected to a cylinder of compressed CO_2. The ambient and elevated CO_2 target concentrations were 380 and 700 μmol mol^{-1}, respectively. Seedlings were watered with an automated irrigation system and drip emitters (Supertif - PLASTRO, Kibbutz Gvat D. N. Ha'Amakim, Israel). Water treatments were applied beginning in May at both sites. Seedlings in the high water treatment were watered to saturation 4 times per day; seedlings in the low water treatment were also watered 4 times per day but given 25% of the amount of water received in the high water treatment. Photosynthetic active radiation (PAR) was measured outdoors and inside one chamber at each site with quantum radiation sensors (Model LI-190SZ, LiCor Biosciences, Lincoln, Nebraska USA). All sensor data (temperature, [CO_2], PAR) were averaged and recorded every 10 min with a datalogger (23X, Campbell Scientific, Logan, UT, USA).

One-year-old bare-root loblolly pine seedlings (Georgia Forestry Commission, Atlanta, Georgia, USA) were planted in February 2008 in 8 L pots in potting medium (Fafard Nursery Mix, Conrad Fafard Inc, Agawam, Massachussetts, USA). The seed source was a mixture of open pollinated families from the Georgia Piedmont. Average seedling height and stem diameter at base at planting were 0.27 m and 3.9 mm, respectively. Each pot was fertilized with approximately 30 g of 15-9-12 extended release fertilizer (Osmocote Plus #903286, Scotts-Sierra Horticultural Products, Marysville, Ohio USA) in March and August and 4.93 mg of chelated iron (Sprint 138, Becker Underwood Inc, Ames, Iowa USA) in May and August. In May approximately 0.04 ml Imidacloprid (Bayer Advanced 12 Month Tree and Shrub Insect Control, Bayer, Monheim am Rhein, Germany) was applied topically to the soil in each pot to control insect pests.

Environmental Conditions

Mean ambient temperature during the experiment, from implementation of the temperature treatments (February 15) until final harvest (November 6 at the northern site, December 1 at the southern site), was 16.7°C at Blairsville (the northern site) and 20.8°C at Tifton (the southern site). At both sites seasonal mean daily air temperature followed a similar trend, generally increasing until approximately day 160 and declining after day 260. The greatest temperature differences between the two sites occurred early in the season (February & March ~6.5°C), while at during the middle of the growing season the temperature difference between the two sites was the smallest (July & August: ~3.6°C).

At the northern site, mean temperatures during the experiment in the ambient and elevated temperature treatments were 17.5 and 19.3°C, respectively (Δ1.8°C). At the southern site, mean temperatures in those treatments were 21.2 and 23.5°C (Δ 2.3°C). Mean daily [CO_2] in the ambient and elevated CO_2 treatments were 383 and 637 μmol mol^{-1}, respectively, (Δ 254 μmol mol^{-1}) at the northern site and 402 and 685 μmol mol^{-1} (Δ 283μmol mol^{-1}) at the southern site. Average incoming photosynthetic active radiation (PAR) from February to December was 37.1 mol m^{-2} day^{-1} at both sites.

Growth Measurements

Height (H) and stem diameter at base (D_{base}) of each seedling were measured at planting and 3 times during the growing season using a meter stick and a digital caliper (ABSOLUTE Digimatic 500-196-20, Mitutoyo USA, Aurora, IL, USA). At the northern site, measurements were made on February 12, June 11, September 3 and November 6, 2008. At the southern site, measurements were made on February 14, June 6, September 9 and December 1, 2008. Seedlings were harvested at the end of the growing season (northern site: November 6; southern site: December 1). Harvested seedlings were separated into flushes, the length of each flush was recorded and a single fascicle from each flush was used to determine specific leaf area (SLA). Each flush was separated into foliage and stem/branch woody tissue and dried at 60°C for at least a week and weighed. Estimated total leaf area (LA_{tot}) for each flush was calculated as the product of foliage mass and SLA. Roots were hand washed to remove all potting material, dried at 60°C and weighed.

Statistical Analysis

Treatment differences in foliage, stem/branch, root and total biomass as well as final number of flushes, average flush length, estimated total leaf area, average leaf area per flush and average SLA at final harvest were tested with a split-plot ANOVA. (Proc Mixed, SAS, Cary, NC, USA). Fixed effects were site, and temperature, [CO_2] and water treatments with blocking as a random effect. Differences with treatment in height and diameter at base across time were analyzed using a repeated measures analysis with the fixed effects of site, temperature treatment, [CO_2] treatment, water treatment, measurement period (4 levels) and the block by tree effect being repeated. When interactions occurred we performed tests of simple main effects using the SLICE option in the LSMEANS statement (Schabenberger et al., 2000, Littell et al., 2006).

To determine if biomass allocation patterns shifted with treatment (site, temperature, [CO_2], water), values of leaf, stem and root biomass for each seedling were natural log transformed and plotted against the natural log of total biomass for that seedling. Differences among the slopes of the 16 treatment combinations were determined by ANOVA (Proc Mixed, SAS 9.2, SAS Institute, Cary, NC, USA).

Results

Height and Diameter

In February, just after planting, seedling H and D_{base} were the same both between the northern and southern site ($P = 0.95$ and $P = 0.55$, respectively) and between the elevated temperature and elevated [CO_2] treatment (T_E C_E; predicted future climate conditions) and the ambient temperature and ambient [CO_2] treatment (T_A C_A; current climate conditions) within each site ($P > 0.46$ for all). Early in the season, growth in H and D_{base} were greater at the southern site so that by mid-June (the second measurement), seedlings at the southern site were significantly taller and had a significantly larger D_{base} compared to seedlings at the northern site ($P < 0.001$) (Table 4.1). Between June and September, the rate of growth increased substantially at the northern site; however, seedlings were still taller and had larger D_{base} at the southern site in September ($P < 0.001$ and $P < 0.001$, respectively). At both sites, from early September (the third measurement) until the end of the growing season, the rate of height growth decreased while diameter growth continued at near the mid-season rate. Averaged across both the T_A C_A and T_E C_E treatments, the water treatments had no effect on seedling size through mid-June ($P = 0.36$ and $P = 0.34$, respectively), but by September H and D_{base} were significantly reduced at both sites in the low water treatment compared to the high water treatment ($P < 0.001$ in all instances) and the effect of water stress on H and D_{base} continued until the end of the growing season.

From the February planting until mid-June, seedling size was not significantly affected by T_E C_E treatment compared with the T_A C_A treatment (Table 4.1). By early September seedling height was significantly greater in the T_E C_E treatment compared with the T_A C_A treatment in all instances but one: in the low water treatment at the northern site there was no significant difference ($P = 0.53$). The effect of the climate treatments on diameter growth differed from their effect on height growth; by September, D_{base} was significantly greater in the T_E C_E treatment compared with the T_A C_A treatment at the northern site but not at the southern site, regardless of water treatment. The significant increases in H and D_{base} in the T_E C_E treatment carried through until the final measurements at the end of the growing season.

Biomass

The two strongest determinants of final biomass in this study were site and water treatment. At the end of the growing season, averaged across all treatments, seedlings grown at the southern site had 58% more biomass (356.4 g) than seedlings grown at the northern site (231.9 g; $P < 0.001$). There were two site differences that likely had strong effects on this outcome: bud burst occurred two weeks earlier at the southern site compared to the northern site

(50% bud burst on March 15 versus April 3, respectively) and daytime temperature reached 20°C at the southern site one month earlier than the northern site. The low water treatment reduced final biomass at both sites and to a similar degree. Averaged across the T_A C_A and T_E C_E treatments, the low water treatment reduced biomass by 38% at the northern site and 40% at the southern site ($P < 0.001$). Total biomass at the southern site in low water was not significantly different from total biomass at the northern site in high water ($P = 0.158$).

Seedlings grown in the T_E C_E treatment had significantly more total biomass at both sites and in both the high and low water treatment, compared to the T_A C_A treatment (Fig. 4.2). In the high water treatment, seedlings grown in the T_E C_E treatment had 48% more biomass at the northern site (337.7 vs. 228.0 g; $P < 0.001$) and 22% more biomass at the southern site (517.1 vs. 423.0 g; $P < 0.001$), compared to seedlings grown in the T_A C_A treatment. In the low water treatment, seedlings grown in the T_E C_E treatment had 29% more biomass at the northern site (190.8 vs. 148.1 g; $P = 0.0515$) and 23% more biomass at the southern site (312.7 vs. 255.7 g; $P = 0.004$) than those grown in the T_A C_A treatment. A comparison of potential future climate conditions (T_E C_E W_L) against current conditions (T_A C_A W_H) showed that seedling biomass was reduced at both sites (northern: 190.8 vs. 228.0 g, $P = 0.085$; southern: 312.7 vs. 423.0 g, $P < 0.001$).

The T_E C_E treatment effect on component biomass followed a trend similar to its effect on total biomass. At both sites and in both water treatments, seedlings grown in the T_E C_E treatment had more leaf (Fig. 4.2b), stem (Fig. 4.2c) and root (Fig. 4.2d) tissue than seedlings grown in the T_A C_A treatment, though the effect was not always significant. Leaf biomass of seedlings grown in the T_E C_E treatment was greater in both the high and low water treatment at the northern site (35% in high water, $P < 0.001$; 20% in low water, $P = 0.0931$) and southern site (22% in high water, $P = 0.003$; 16% in low water, $P = 0.029$) compared to seedlings grown in the T_A C_A treatment in the respective water treatments. Stem biomass of seedlings grown in the T_E C_E treatment was greater in both the high and low water treatment at the northern site (42% in high water, $P < 0.001$; 35% in low water, $P = 0.0519$) and southern site (15% in high water, $P = 0.008$; 20% in low water, $P = 0.021$) compared to seedlings grown in the T_A C_A treatment. Root biomass of seedlings grown in the T_E C_E treatment was greater in both the high and low water treatment at the northern site (67% in high water, $P < 0.001$; 34% in low water, $P = 0.146$) and southern site (33% in high water, $P < 0.001$; 30% in low water, $P = 0.006$) compared to seedlings grown in the T_A C_A treatment.

Biomass allocation between leaf, stem and root tissue was not significantly affected by any factor in our study. Leaf biomass allocation (Fig. 4.3a) was not affected by site ($P = 0.08$), nor by temperature / [CO_2] treatment at either the northern or southern site ($P = 0.71$ and $P = 0.75$ respectively). The water treatments also had no effect on biomass allocation at either site ($P = 0.41$) in either the T_A C_A (Northern: $P = 0.94$; Southern: $P = 0.51$) or the T_E C_E treatment (Northern: $P = 0.30$; Southern: $P = 0.96$). Stem (Fig. 4.3b) and root (Fig. 4.3c) allocation followed a similar trend as leaf biomass allocation and were not significantly affected by any combination of site, temperature / [CO_2] treatment or water treatment.

Morphological Parameters

The number of growth flushes during the 2008 growing season was significantly affected by site ($P < 0.0001$) and water ($P < 0.0001$). Seedlings at the southern site had, on average, 1.4 more flushes than seedlings at the northern site (5.8 vs. 4.4), while seedlings in the high water treatment had, on average, one more flush than seedlings in the low water treatment (5.6 vs. 4.6) (Table 4.2). The T_E C_E treatment had an inconsistent affect on number of flushes. At the northern site, in the high water treatment, seedlings grown in the T_E C_E treatment had approximately 0.5 more flushes than seedlings grown in ambient conditions ($P = 0.033$). At the southern site, in the low water treatment, a similar trend was observed, with seedlings grown in the T_E C_E treatment having on average 0.8 more flushes more than seedlings grown in the T_A C_A treatment ($P < 0.001$). The T_E C_E treatment did not significantly affect flush number in the low water treatment at the northern site ($P = 0.894$) or in the high water treatment at the southern site ($P = 0.096$).

Specific leaf area was not affected by site, treatment or treatment combination (all $P > 0.10$) (Table 4.2). Estimated total leaf area at the end of the growing season was significantly affected by site ($P = 0.003$) and water treatment ($P < 0.001$). Seedlings grown at the southern site had 41% more leaf area compared to the northern site. Seedling leaf area was 88% greater at the northern site and 67% greater at the southern in the high water treatment compared to the low water treatment. There was no significant difference in estimated total leaf area between seedlings grown in the T_E C_E treatment compared to those grown in the T_A C_A treatment.

Discussion

An increase in air temperature is predicted to lead to shifts in species distribution by increasing growth in sub-optimal temperature environments and reducing growth in supra-optimal temperature environments near the borders of a species range (Xu et al., 2007). In this study, elevated temperature and elevated [CO_2] (applied in combination to mimic future climatic conditions) had a positive effect on biomass accumulation of loblolly pine seedlings grown at sites located near both the northern and southern edge of the eastern distribution. The increase in biomass with an increase in air temperature and [CO_2] at the northern site supports our hypothesis and agrees with previous studies; however, our findings at the southern site, namely that elevated temperature applied in combination with elevated [CO_2] did not negatively affect growth, is in contradiction with both our hypothesis and with assumptions most models rely upon to predict shifts in species distribution.

While an increase in growing temperature has been demonstrated to increase seedling biomass accumulation, e.g. in *Fagus sylvatica* (Overdieck et al., 2007), *Eucalyptus grandis* (Thomas et al., 2007), and *Picea asperata* and *Abies faxoniana* (Yin et al., 2008), this effect may be especially pronounced at or near the cold-limited border of the species distribution (Danby and Hik, 2007, Penuelas et al., 2007, Reich and Oleksyn, 2008, Hoch and Korner, 2009, Wieser et al., 2009). In contrast, previous studies have shown that increasing air temperature at the southern limit of a species distribution can cause a decrease in biomass production. For example, *Acer saccharum* seedlings, planted near the southern limit of the species distribution, had reduced biomass accumulation with an increase in air temperature (+4°C) in both ambient and elevated [CO_2] (Norby et al., 2000). Reich and Oleksyn (2008) reported that an increase in air temperature (+1 to +4°C) caused a significant decrease in height growth and an increase in mortality of *Pinus sylvestris* at the southern limit of its European distribution. In our study, the unexpected lack of sensitivity to an increase in air temperature at the southern site demonstrates that a 2°C increase in air temperature was not great enough to induce thermal stress in this species, even in the warmest part of its natural range. It should be noted that seedlings in every treatment were taller and accrued more biomass at the southern site compared to their counterparts at the northern site. Even seedlings grown in the most stressed treatment (elevated temperature, low soil moisture) at the southern site were as large as seedlings grown in ambient conditions at the northern site, further demonstrating a lack of thermal stress. Our results may indicate that the southern border of the loblolly pine distribution is not limited by high temperatures, but is likely defined by other competitive factors (Schultz, 1997), and suggest that predicted climatic conditions may result in an expansion of its distribution both in the cooler and hotter limits of the range.

Exposure to elevated [CO_2] (approximately double ambient [CO_2]) has been shown to increase biomass production in loblolly pine seedlings by as much as 40% (Tschaplinski et al., 1993, Sullivan and Teramura, 1994) to 55% (Tissue et al., 1996). This response in loblolly pine has been observed with both high and low soil moisture availability (approximately 30 & 25% stimulation, respectively) (Friend et al., 2000). Of greater interest is the similar response of biomass production at both sites to the combined treatment of elevated [CO_2] and elevated temperature. We hypothesized that in a sub-optimal temperature environment, seedlings would benefit from both elevated temperature and [CO_2], while in a supra-optimal environment, elevated [CO_2] might mitigate stress induced by elevated temperature. Overall, at the northern site, the seedlings responded as anticipated, with the combined effects of elevated [CO_2] and elevated temperature acting to boost biomass production. However, sequential height and diameter measurements showed that the combined treatment had little or no effect on growth early in the season when temperatures were coolest. Later in the season, both height and diameter growth were greater in the T_E C_E treatment compared to the T_A C_A treatment. At the southern site, biomass production was significantly stimulated by elevated temperature and elevated [CO_2] applied in combination. The increase in biomass production, while unexpected, may be attributed to an increase in the optimum temperature for photosynthesis resulting from elevated [CO_2] (Long, 1991); thus, in supra-optimal temperature environments, elevated [CO_2] may not only neutralize potential thermal stress, but also significantly stimulate biomass production. Our findings suggest that future climatic conditions, in which atmospheric [CO_2] may reach 900 μmol mol^{-1} and air temperature may increase by up to 3°C (IPCC, 2007), may significantly stimulate loblolly pine production across the species distribution.

Models predict shifts in species distributions, with elevated [CO_2] and elevated temperature expanding distributions in current sub-optimal temperature regions and shrinking distributions in current supra-optimal temperature regions. Two such models show conflicting scenarios for loblolly pine. McKenney *et al.* (2007) predict a future loss of habitable area for loblolly pine of 57 to 79%, while Iverson *et al*. (2008) predict a future gain of habitable area of 25 to 86%. Our findings support the prediction of an increase in habitable range, but also suggest that more direct evidence is needed to validate modeling work and better elucidate how tree species will respond to future climate conditions. The response of growth to warming temperature will likely differ among species (Iverson and Prasad, 2002). For example, temperate species like loblolly pine with wide thermal ranges may respond positively to warming throughout their distributions, with possible range expansion northward, while boreal species with narrower thermal tolerances may respond negatively to warming except at the northern or high-altitude limits of their ranges, resulting in severe range contractions. Evidence of these varying effects is shown by comparing the response to warming in our study with the results of Way & Sage (2008) who found that elevated temperature negatively affected photosynthesis, growth and survival of black spruce (*Picea mariana*) seedlings throughout the majority of their distribution.

Perhaps the greatest future stress on tree growth will be a decrease in available soil moisture. Changes in mean air temperature are expected to alter precipitation patterns, which may lead to fewer, more intense rain events that result in less infiltration, more runoff and subsequently less available water for plants. While the typical response to decreased water availability is a decrease in biomass production, this response may be confounded by an increase in air temperature and [CO_2]. In our study, the increase in biomass production in the T_E C_E treatment was greater in the high water treatment compared to the low water treatment. This result suggests that elevated [CO_2] was less effective in boosting biomass production in the low water treatment, or that elevated temperature exacerbated the stress associated with low soil moisture. An increase in [CO_2] may counteract water stress by decreasing transpiration and mitigating water loss (Guak et al., 1998 , Poorter and Perez-Soba, 2001, Centritto et al., 2002), but a concurrent increase in air temperature, especially in supra-optimal temperature environments, may significantly exacerbate the impact of water stress on biomass production (Adams et al., 2009). These findings suggest that the decline in available soil moisture predicted to occur with changing atmospheric conditions will substantially reduce any boost in biomass production resulting from predicted increases in air temperature and [CO_2]. Since loblolly pine naturally grows over a large thermal range (Schultz, 1997), it was not entirely unexpected that simulated future atmospheric conditions caused increased growth, even at the southern site. However, the partial negation of this effect in low soil moisture was more surprising, given that the species grows on a wide range of soils from moist to relatively dry (Harlow et al., 1991).

Conclusions

Predicted changes in climate conditions are expected to affect tree growth, though the response to a changing environment may differ across the range of a species. We are not aware of any other study that has investigated the response of seedling biomass production to a concurrent increase in temperature and [CO_2] applied in combination with high and low soil moisture availability simultaneously at a northern and southern site within a species distribution. We found that seedling growth responded to the combination of elevated temperature and elevated [CO_2] consistently across a large spatial and thermal range (345 km north to south, 4.1°C difference in mean air temperature during the growing season). At both the northern and southern sites, biomass production was significantly boosted in seedlings exposed to elevated temperature and elevated [CO_2], set to mimic predicted future climatic conditions, compared to seedlings grown in ambient atmospheric conditions. While this response was conservative in both high and low soil moisture environments, the low water treatment significantly reduced biomass production at both sites and partially negated the positive effect of elevated temperature and [CO_2], suggesting that if precipitation decreases, biomass production may be negatively affected regardless of expected increases in temperature and [CO_2]. Contrary to species distribution models which have predicted a dramatic reduction in the distribution of loblolly pine and other tree species, we do not anticipate that the southern limit of loblolly pine will contract as a result of elevated temperature. However, if soil moisture availability decreases concurrently with increases in temperature and [CO_2], we expect biomass production across the species range to decrease, and a potential contraction of the species distribution may occur.

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Table 4.1. Mean (SE) height (m) and stem diameter at base (mm) of one year old loblolly pine seedlings, grown at a northern and southern site in the species range, measured in June, September and at the end of the 2008 growing season (November 6 at the northern site and December 1 at the southern site). Treatments were: ambient temperature and [CO_2] (T_A C_A) or elevated temperature and [CO_2] (T_E C_E); grown in high water (W_H), or low water (W_L). Asterisk (*) denotes a significant difference ($P < 0.05$) between seedlings grown in current (T_A C_A) and potential future (T_E C_E) climate conditions within a water treatment.

Height

Site	Treatment	June	September	Final
North	T_A C_A W_H	0.552 (0.03)	1.207 (0.04)	1.325 (0.05)
	T_E C_E W_H	0.608 (0.03)	1.392 (0.02) *	1.549 (0.03) *
	T_A C_A W_L	0.510 (0.02)	0.995 (0.03)	1.039 (0.03)
	T_E C_E W_L	0.59 (0.02)	0.956 (0.07)	1.069 (0.05)
South	T_A C_A W_H	0.952 (0.07)	1.395 (0.04)	1.482 (0.05)
	T_E C_E W_H	0.856 (0.05)	1.488 (0.04) *	1.643 (0.03) *
	T_A C_A W_L	0.838 (0.06)	1.161 (0.03)	1.168 (0.03)
	T_E C_E W_L	0.923 (0.03)	1.266 (0.04) *	1.299 (0.04) *

Diameter

Site	Treatment	June	September	Final
North	T_A C_A W_H	8.6 (0.1)	17.4 (0.3)	22.8 (0.5)
	T_E C_E W_H	9.7 (0.7)	21.4 (0.6) *	24.5 (0.6) *
	T_A C_A W_L	7.7 (0.3)	14.8 (0.5)	19.1 (0.5)
	T_E C_E W_L	8.6 (0.4)	16.4 (0.4) *	21.0 (0.9) *
South	T_A C_A W_H	13.6 (0.7)	23.5 (0.3)	31.9 (0.5)
	T_E C_E W_H	12.3 (0.5)	23.9 (0.7)	32.4 (1.0)
	T_A C_A W_L	11.4 (0.6)	19.5 (0.4)	24.8 (0.5)
	T_E C_E W_L	13.9 (1.0)	19.7 (0.6)	25.8 (1.0)

Table 4.2. Mean (SE) number of flushes, average specific leaf area (cm^2 g^{-1}) and estimated total leaf area (m^2) of one year old loblolly pine seedlings grown for one season (2008) at a northern and a southern site in the species range. Treatments were: ambient temperature and [CO$_2$] (T_A C_A) or elevated temperature and [CO$_2$] (T_E C_E); grown in high water (W_H), or low water (W_L). Asterisk (*) denotes a significant difference ($P < 0.05$) between seedlings grown in current (T_A C_A) and potential future (T_E C_E) climate conditions. Comparisons only within a site and within a water treatment.

Treatment	Number of flushes	SLA (cm^2 g^{-1})	Estimated total leaf area (m^2)
Northern site			
T_A C_A W_H	4.7 (0.1)	1.60 (0.06)	1.39 (0.07)
T_E C_E W_H	5.2 (0.2) *	1.53 (0.04) ns	1.77 (0.09) ns
T_A C_A W_L	3.9 (0.1)	1.54 (0.06)	0.88 (0.07)
T_E C_E W_L	3.9 (0.2) ns	1.47 (0.05) ns	0.87 (0.09) ns
Southern site			
T_A C_A W_H	6.1 (0.2)	1.91 (0.53)	2.34 (0.51)
T_E C_E W_H	6.5 (0.2) ns	1.37 (0.03) ns	2.04 (0.14) ns
T_A C_A W_L	4.8 (0.2)	1.37 (0.02)	1.21 (0.04)
T_E C_E W_L	5.6 (0.2) *	1.36 (0.03) ns	1.42 (0.09) ns

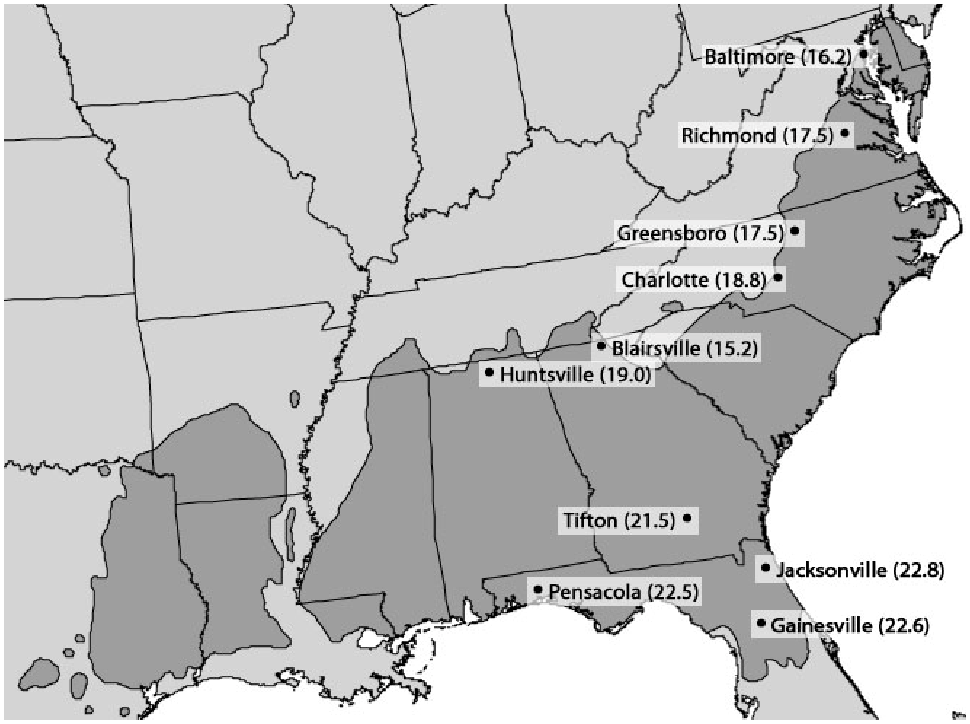

Fig. 4.1. The location of the two study sites: Blairsville and Tifton, GA USA. The native range of loblolly pine is shaded dark grey. Distribution map from Little (1971). Numbers indicate mean growing season temperatures (calculated a mean monthly temperature from February through November) for various sites throughout the range of loblolly pine.

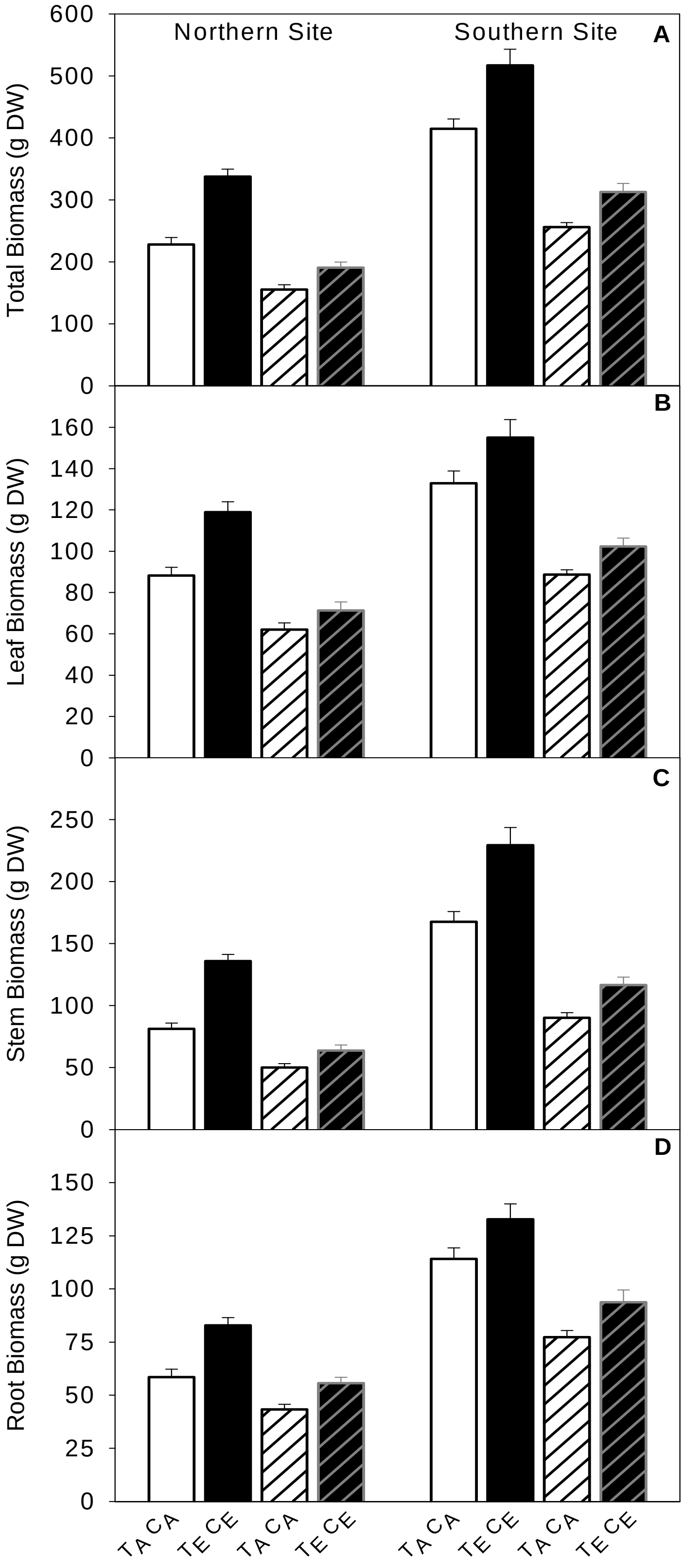

Fig 4.2. Total biomass accumulation (dry weight, g) of loblolly pine seedlings at the end of one growing season (2008). Seedlings were grown at the northern site (Blairsville, GA) and southern site (Tifton, GA). Treatment combinations were: ambient temperature and ambient [CO_2] (T_A C_A, white bars); elevated temperature and elevated [CO_2] (T_E C_E, black bars); and high water (no stripes) or low water (stripes). Error bars represent 1 SE.

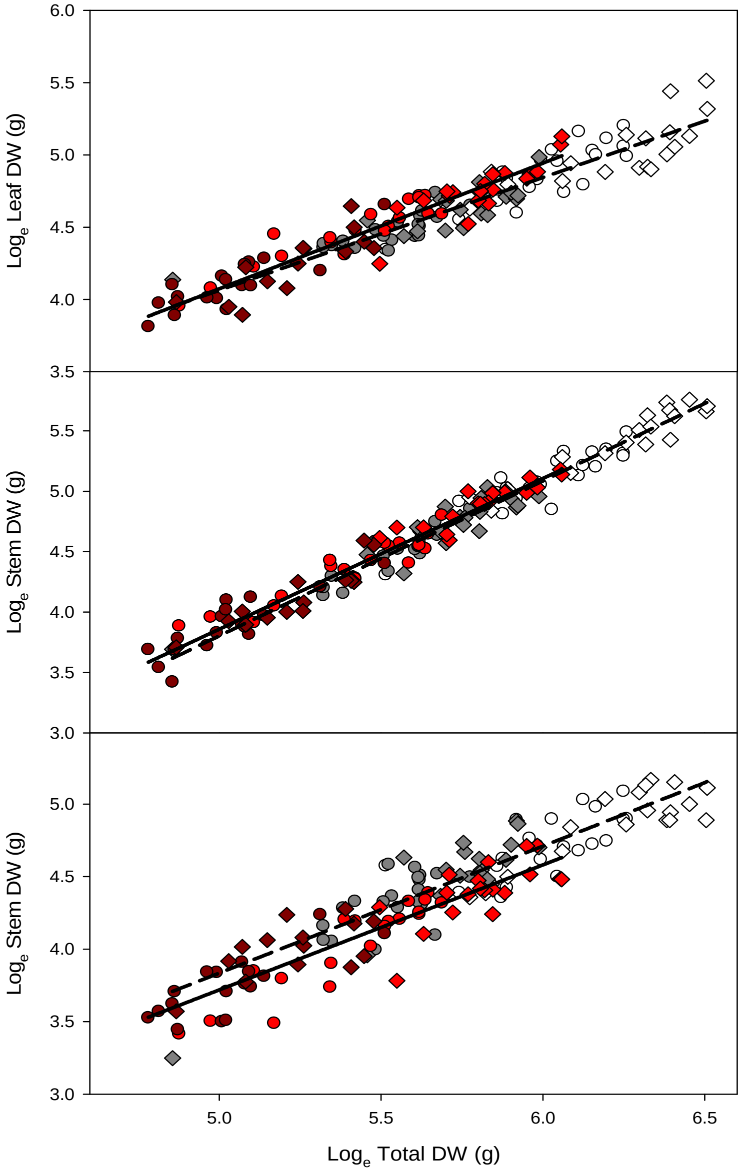

Fig. 4.3. Biomass allocation patterns of leaf, stem and root tissue in loblolly pine seedlings grown in 2008 at the northern site (red symbols; Blairsville, GA) and southern site (white symbols; Tifton, GA) in ambient temperature and ambient [CO_2] (○) or elevated temperature and elevated [CO_2] (◇). Seedlings grown in high water (light symbols) or low water (dark symbols) treatments. Solid line is regression of northern site (all treatments), dashed line is regression of southern site (all treatments).

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CHAPTER 5

THE EFFECTS OF ELEVATED TEMPERATURE ON NORTHERN RED OAK GROWTH AND PHOTOSYNTHETIC PERFORMANCE AT A SITE IN ITS SOUTHERN DISTRIBUTION[4]

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Abstract

Increases in air temperature are likely to affect tree physiological processes and growth, with the greatest impacts expected to occur near the edges of a species range. To determine the effect that future temperature and atmospheric CO_2 conditions may have on northern red oak (*Quercus rubra* L.) seedlings at the southern edge of its range, seedlings were grown in four treatments: ambient temperature and ambient [CO_2] (T_A C_A), ambient temperature and elevated [CO_2] (650 μmol mol) (T_A C_E), ambient temperature +3°C and elevated [CO_2] (T_{+3} C_E) and ambient temperature + 6°C and elevated [CO_2] (T_{+6} C_E). Leaf gas exchange, leaf photosynthetic capacity (i.e., V_{cmax}, J_{max} and TPU), seedling height, diameter and biomass were measured during one growing season.

Throughout the growing season elevated [CO_2] significantly boosted photosynthesis, which resulted in seedlings having greater height, diameter and biomass. As the growing season progressed and mean daily ambient air temperature increased, the elevated temperature treatments significantly reduced the effect of elevated [CO_2] on photosynthesis, height, diameter, and biomass accumulation. The response was temperature dependent, with the T_{+6} C_E treatment reducing growth to a greater extent than the T_{+3} C_E treatment. Foliar health and photosynthetic capacity were not significantly affected in either elevated temperature treatment and no mortality occurred, even in the T_{+6} C_E treatment. Interestingly, foliar respiration did not acclimate to growing temperature, although photosynthesis did. Despite the reductions in growth imposed by the T_{+6} C_E treatment, photosynthesis and seedling total biomass were not drastically lower compared to the T_A C_A treatment. Our findings indicate that elevated temperatures may counteract increases in photosynthesis and growth produced by elevated [CO_2]. However we find little evidence that the combined effect of elevated [CO_2] and increased air temperatures,

even as great as a +6°C increase in mean growing season temperature, will have a significant impact on growth near the southern distributional limit of northern red oak.

Introduction

Increasing air temperature, caused by rising atmospheric CO_2 concentration ($[CO_2]$), is predicted to significantly affect tree photosynthesis and biomass production, with the largest impact expected to occur at the coolest and warmest portions of a species range (He *et al.* 2005; Thuiller *et al.* 2008), where sub- and supra-optimal temperatures, respectively, are believed to limit carbon fixation and biomass production (Xu *et al.* 2007). It is often assumed that an increase in air temperature will positively affect growth in sub-optimal temperature environments and negatively affect growth in supra-optimal temperature environments (Penuelas *et al.* 2007), and if the temperature increases are large enough, species distributions will be altered (Tang & Beckage 2010). In areas with sub-optimal temperatures, an increase in temperature could have a positive impact on growth by raising air temperature closer to the optimum for photosynthesis (Kirschbaum 2000) and increasing the length of the growing season (Koca *et al.* 2006; Bronson *et al.* 2009). In areas with supra-optimal temperatures, physiological processes are already considered stressed to some degree by temperature, so an increase in growing season air temperature could further reduce photosynthesis (Doughty & Goulden 2008), increase respiration and perhaps damage foliage, leading to additional reductions in biomass production.

Previous studies on the effect of elevated temperature on tree growth, applied alone or in combination with elevated $[CO_2]$, have typically reported a stimulation of growth with an increase in temperature. For example, *Betula pendula* seedlings accrued more biomass and were taller when grown in elevated temperature (+3°C) in both ambient and elevated [CO_2] (Kuokkanen *et al.* 2004). Stem diameter of mature *Pinus sylvestris* trees was stimulated by exposure to elevated temperature (+2 to 6°C) in both ambient and elevated [CO_2] (Peltola *et al.* 2002). Similarly, *Picea asperata* and *Pinus tabulaeformis* seedlings accumulated more biomass in elevated temperature (~2.5°C), compared to seedlings grown in ambient temperature, when grown in both high and low fertilization regimes (Zhao & Liu 2009). Yin et al. (2008) reported that biomass production of *Picea asperata* and *Abies faxonian* trees was increased with even a small increase in temperature (+0.5°C). However, some studies have reported a lack of any effect of elevated temperature on tree growth (Kostiainen et al. 2009, Rasmussen et al 2002).

At the southern edge of a species range species, where the greatest thermal stress likely occurs, an increase in temperature should result in a suppression of photosynthesis and biomass production. A negative effect of increased temperature on biomass production near the warmest edge of a species range has been demonstrated in a few studies. Elevated temperature (+4°C) reduced biomass production of *Acer saccharum* seedlings planted near the southern border of the species distribution and grown in both ambient and elevated [CO_2], though the reduction was greatest in ambient [CO_2] (Norby *et al.* 2000). An increase in air temperature (+1 to +4°C) at the southern limit of the distribution of *Pinus sylvestris* was correlated with a significant decrease in height growth and an increase in mortality (Reich & Oleksyn 2008). However, several studies have suggested that warm temperatures *per se* do not define a species border (Woodward 1987; Bonan & Sirois 1992). An increase in temperature, even in supra-optimal environments, may not necessarily have a negative effect on photosynthesis or biomass production. Many tree species exhibit acclimation of respiration to temperature (Atkin & Tjoelker 2003) and some demonstrate at least partial acclimation of photosynthesis to temperature (Sage & Kubien 2007). Additionally, elevated temperatures may result in a longer growing season, providing additional time to accumulate carbon. Increases in air temperature will be driven partly by an increase in [CO_2] availability, and a doubling of [CO_2] is predicted to shift the optimal temperature for photosynthesis by up to +5°C (Long 1991). This suggests that a concurrent increase in [CO_2] and temperature may synergistically boost photosynthesis by both reducing photorespiration and subjecting foliage to temperatures which may be more optimal for photosynthesis.

Understanding how a species responds to elevated temperature at the southern end of its range will help us predict future distributions. Most models, including envelope models, process-based models and dynamic models, have suggested that the majority of tree species in North America will see an expansion of the northern distribution and a contraction of the southern distribution (Iverson & Prasad 2001, 2002; McKenney *et al.* 2007; Morin *et al.* 2008; Tang & Beckage 2010). Overpeck et al. (1991) predicted a northward shift in the range of eastern North America plant species of between 100 to 500 kilometers per century, demonstrating both the uncertainty in predicting the response of species to changing climates and the potential variability in species response to changes in climate. However, a better understanding of how physiological processes respond to elevated temperature and [CO2] is likely to reduce this uncertainty. For example, a model by Xu et al. (2007) predicts that elevated [CO_2] may mitigate the negative effects of elevated temperature on growth by raising the optimal temperature of photosynthesis, and therefore species ranges may not be significantly altered by increasing temperature. With the exception of Norby et al. (2000), which estimated biomass production, we are unaware of any studies that have actually investigated the effect of elevated temperature and [CO_2] on photosynthesis and biomass production at the current southern border of a species range to verify any of these model predictions.

The objective of this study was to determine if an increase in air temperature of +3°C or +6°C, combined with an increase in [CO_2], would affect carbon fixation and biomass production at the southern portion of a species range. Northern red oak seedlings were grown near the southern edge of the species range in four treatment chambers which were maintained for one growing season at: ambient temperature and ambient [CO_2] (T_A C_A), ambient temperature and elevated [CO_2] (700 μmol mol^{-1}) (T_A C_E), ambient temperature +3°C and elevated [CO_2] (T_{+3} C_E) and ambient temperature +6°C and elevated [CO_2] (T_{+6} C_E). Measurements of photosynthesis (A_{net}), leaf respiration (R_d) and fluorescence were conducted throughout the growing season and seedling biomass at the end of the growing season was obtained. We tested two main hypothesizes: Elevated [CO_2] will increase photosynthesis and biomass production, but elevated temperature will reduce both photosynthesis and production. The highest temperature treatment (+6°C) will reduce both fixation and accumulation to levels significantly lower than occur in current atmospheric conditions.

Materials and methods

Experimental Setup

Two year old northern red oak (*Quercus rubra* L.) seedlings were grown in four treatment chambers located at the University of Georgia Whitehall Experimental Forest in Athens, GA (33°57’ N, 83°19’ W, elevation 230 m) for one growing season. The experimental site is located near the southern edge of the species range (Fig. 5.1). Half-cylinder domed treatment chambers measuring 3.6 m long x 3.6 m wide x 2.4 m high were constructed of wood and PVC pipe and covered with poly-film (6 mil clear GT Performance Film, Green-Tek Inc, Edgerton, Wisconsin, USA) (Boyette & Bilderback 1996). The chambers were constructed in a large open field, oriented facing south and placed side-by-side 2.5 m apart. To minimize chamber effects, each chamber was constructed to exactly the same dimensions, air within the chambers was thoroughly mixed with an oscillating fan, and seedlings were rotated within chambers several times throughout the study. In each chamber, a differential thermostat (Model DSD-2, Kera Technologies Inc, Mississauga, Ontario, Canada) controlled an air conditioner and an electric resistance heater to continuously maintain the targeted treatment temperatures. Chamber [CO_2] was measured and controlled with a non-dispersive infrared CO_2 sensor (Model GMT222, Vaisala Inc, Woburn, Massachusetts USA) and a solenoid valve connected to a cylinder of compressed CO_2. The ambient and elevated CO_2 concentrations were targeted at 380 and 700 μmol mol^{-1}, respectively. Air was circulated in each chamber with an oscillating fan to promote uniform [CO_2] dispersion and a similar temperature profile throughout the treatment chamber. Seedlings were watered to saturation four times a day with an automated irrigation system and drip emitters (Supertif - PLASTRO, Kibbutz Gvat D. N. Ha'Amakim, Israel). Photosynthetically active radiation (PAR) was measured outdoors and inside one chamber with quantum radiation sensors (Model LI-190SZ, LiCor Biosciences, Lincoln, Nebraska USA). All sensor data (temperature, [CO_2], and PAR) were recorded with a datalogger (23X, Campbell Scientific, Logan, UT, USA). The treatment combinations, which were randomly assigned to chambers, consisted of 1) ambient temperature and ambient [CO_2] (380 μmol mol^{-1}) (T_A C_A), 2) ambient temperature and elevated [CO_2] (700 μmol mol^{-1}) (T_A C_E), 3) ambient air temperature +3°C and elevated [CO_2] (T_{+3} C_A) and 4) ambient air temperature +6°C and elevated [CO_2] (T_{+6} C_E).

Two-year-old bare-root northern red oak seedlings were planted in March 3, 2009 in 12 L pots in potting medium (Fafard Nursery Mix, Conrad Fafard Inc, Agawam, Massachussetts, USA). The seed source was from wild stands collected from the South Carolina Piedmont, which has a very similar climate to the Georgia Piedmont where the study was conducted (South Carolina Forestry Commission, Taylor Nursery, Trenton, South Carolina, USA). Average stem height and diameter of the seedlings at planting were 0.47 m and 54 mm, respectively. A total of 25 seedlings were randomly assigned to each treatment chamber. After one month of growth the smallest and largest seedlings were removed, leaving a total of 13 seedlings in each treatment. Seedlings were fertilized with approximately 30 g of 15-9-12 extended release fertilizer (Osmocote Plus #903286, Scotts-Sierra Horticultural Products, Marysville, Ohio USA) on April 26 and July 28, 2009. On May 28, 2009 approximately 0.04 ml Imidacloprid was applied topically to the soil in each pot to control insect pests (Bayer Advanced 12 Month Tree and Shrub Insect Control; Bayer; Monheim am Rhein, Germany).

Environmental Conditions

Mean ambient temperature during the experiment, from implementation of the temperature treatments (May 1, 2009) until final harvest (September 29, 2009), was 25.1°C (Table 5.1). Air temperature followed a typical pattern through the growing season. Air temperatures generally increased until approximately day 160 (June 9) and slowly decreased after day 225 (August 13) (Fig. 5.2). Air temperature inside each treatment chamber closely followed the diurnal pattern of ambient air temperature (Fig 5.3). Averaged across the experiment, mean air temperature in the ambient temperature chambers was 24.5°C, while the mean temperature for the elevated temperature treatments was 27.8°C and 30.5C for the +3°C and +6°C treatments. The elevated [CO_2] treatments were also initiated on May 1, 2009. Mean daily atmospheric [CO_2] in the chambers was maintained near 400 µmol mol^{-1} in the ambient treatment and near 682 µmol mol^{-1} in the elevated treatments (Table 5.1). The average daily incoming PAR from March to September was 28.5 mol m^{-2} day^{-1}.

Gas Exchange

Temperature response curves of light saturated photosynthesis (A_{net}) and leaf respiration (R_{d}) were made three times throughout the growing season: June 1, July 20 and September 8 (Fig. 5.2). At each measurement date four seedlings were randomly selected from each treatment chamber and transported to two growth chambers (GC 36; Environmental Growth Chambers, Chargin Falls, OH). Initial growth chamber conditions were maintained at 20°C, 55% relative humidity and either 500 or 0 µmol m^{-2} s^{-1} PAR. In June and September seedlings from the elevated [CO_2] treatments were assigned to a lit chamber and seedlings from the ambient [CO_2] treatment was assigned to a darkened chamber and allowed to acclimate to chamber conditions for at least one hour prior to measurements. In August, seedlings were assigned to the opposite chambers. Measurements of A_{net} and R_{d} were made at 20, 27, 34 and 41°C using a LI-6400 Portable Photosynthesis System (Li-Cor Biosciences, Lincoln, NE) with a standard red/blue LED broadleaf cuvette and a CO_2 mixer. All measurements were made on fully expanded leaves. In June measurements were made on the first flush only, while in July and September measurements were made on the first flush and the most recently fully formed flush (typically second or third flush). The cuvette conditions for the LI-6400 were 1500 µmol m^{-2} s^{-1} PAR for A_{net} or 0 µmol m^{-2} s^{-1} PAR for R_{d}, air temperature and [CO_2] equal to the growth chamber: 20, 27, 34 or 41°C and 380 or 700 µmol mol^{-1}, respectively. After measurements of A_{net} and R_{d} at a specific temperature were completed the air temperature of the growth chamber was raised and seedlings were allowed to acclimate to the new temperature for approximately 45 minutes. When the temperature response curves were completed, seedlings were switched from lit to darkened chambers, and vice versa and the opposite measurements were conducted. The temperature response curves of A_{net} were fitted with a polynomial function ($y = a + b*x + c*x^2$), and the value for the optimum temperature of A_{net} was taken as the temperature corresponding to maximum net photosynthesis. The temperature response curves of R_d were fitted with an Arrhenius function ($y = a * \exp^{(b*x)}$).

In addition, on June 3, July 22 and September 10 five random seedlings from each treatment, excluding seedlings used for the most recent temperature response curves, were transported to environmentally controlled growth chambers, which were maintained at 25°C, 55% relative humidity and 500 μmol m^{-2} s^{-1} PAR, and allowed to acclimate for one hour. After acclimation the response of A_{net} to intercellular [CO_2] (C_i) was measured over a range of seven external [CO_2] (C_a), from 30 to 1000 μmol mol^{-1} using a LI-6400. The environmental conditions in the leaf cuvette were 1500 μmol m^{-2} s^{-1} PAR, 25°C and approximately 50% relative humidity. The A/Ci curves were fitted using an A/Ci curve fitting program (Version 1.1, Sharkey et al. 2007) from which estimates of the maximum rate of carboxylation (V_{cmax}), rate of electron flow at saturating light (J_{max}) and triose phosphate use limitation (TPU) were obtained.

Fluorescence

Dark-acclimated leaf fluorescence (F_v/F_m) was measured on fully formed and visually healthy leaves of each flush for five randomly selected seedlings from each treatment on June 8, July 27, and September 3, 2009 (Fig. 5.2). On the following day, between 12:00 noon and 3 PM, light acclimated steady-state fluorescence (F_s) made at 1500 μmol m^{-2} s^{-1} PAR, the basic fluorescence after induction (F_o') and maximal fluorescence during a saturating light flash (F_m') was measured with a portable photosynthesis system fitted with a fluorescence chamber (Model

LI-6400, LiCor Biosciences, Lincoln, NE). Values for the effective quantum yield of PSII, a measurement of the fraction of absorbed photons that are used for photochemistry in a light acclimated leaf (Φ_{PSII}), and photochemical, an indication of the proportion of PSII reaction centers that are open (qP), were calculated according to (Maxwell & Johnson 2000).

Growth Measurements

Height (*H*) and stem diameter measured at the base (D_{base}) of each seedling was measured five times throughout the growing season using a meter stick and a digital caliper (ABSOLUTE Digimatic 500-196-20, Mitutoyo USA, Aurora, IL, USA). Measurements were made on April 24, May 19, June 8, July 30 and September 3, 2009 (Fig. 5.2). On September 30, the destructive harvest of all seedlings was initiated, and was completed in three days. At harvest all foliage was removed from the seedlings, *H* and D_{base} were measured and total leaf area was measured using a Li-3000 (LiCor Biosciences, Lincoln, Nebraska USA). Roots were hand washed to remove all potting material. Foliage, stem and root biomass was dried at 60°C for one week and weighed.

Statistical Analysis

Temperature response curves of A_{net} and R_d made on the first flush of foliage in June, July and September were analyzed using mixed-model repeated-measures analysis with treatment (4 levels) and date (N = 3) as the fixed effects and measurement temperature by tree (4 levels) as the fixed repeated effect. In July and September measurements of A_{net} and R_d made on the top whorl of foliage were analyzed using mixed-model repeated-measures analysis with treatment (4 levels) as the fixed effects and measurement temperature by tree (4 levels) as the fixed repeated effect. Physiological parameters V_{cmax}, J_{max}, and TPU conducted on the first flush at all measurement dates were analyzed using mixed-model analysis with treatment (four levels) and date (3 levels) as the fixed effects. As well, measurements of V_{cmax}, J_{max}, and TPU conducted in September on all flushes were analyzed using mixed-model analysis with treatment (four levels) and flush (2 to 3 levels) as the fixed effects. Measurements of F_v/F_m, ΦPS_{II} and qP from all flushes and all measurement dates were analyzed using a one way ANOVA with treatment (N = 4) as the fixed effect.

Differences in height and diameter among treatments across time were analyzed using a repeated-measures analysis with the fixed effects of treatment (4 levels) and measurement date (7 dates) and tree effect being repeated. The effect of temperature and [CO_2] treatment combinations on total biomass accumulation and final leaf area were tested using a one way ANOVA. All statistical analyses were done using SAS 9.2 (SAS Institute Inc., Cary, NC).

Results

Gas exchange and fluorescence

Measurements of A_{net} conducted on the foliage from the first flush displayed a strong seasonal trend with A_{net} decreasing through the growing season, regardless of treatment (Fig. 5.4). Averaged across all treatments and measurement temperatures, A_{net} decreased by 23% from June to July (8.98 vs. 6.92 µmol m^{-2} s^{-1}, $P = 0.07$) and 28% from July to September (6.92 vs. 4.99 µmol m^{-2} s^{-1}, $P = 0.094$). At all measurement dates A_{net} of foliage from the first flush, averaged across all measurement temperatures, was higher in seedlings from the elevated [CO_2] treatments, regardless of the temperature treatment, than seedlings from the T_A C_A treatment (June $P = 0.084$; July $P = 0.75$; & September $P = 0.49$). The elevated temperature treatments did not have an effect on A_{net} ($P > 0.43$). Unlike A_{net}, g_s did not vary significantly across the growing season (June: 0.0467 mol mol^{-1}, July: 0.0681 mol mol^{-1}, September: 0.0556 mol mol^{-1}, $P = 0.17$), nor was g_s affected by any treatment at any measurement dates ($P > 0.13$ for all).

In July and September measurements of A_{net} were also conducted on a fully developed leaf from the top whorl of the most recent flush. In July, elevated [CO_2] significantly boosted A_{net}, while elevated temperature reduced this boost. Averaged over all measurement temperatures, A_{net} was 90% higher in seedlings from the T_A C_E treatment compared to the T_A C_A treatment (14.6 vs. 7.7 μmol m^{-2} s^{-1}; $P = 0.018$). While A_{net} did not differ significantly among the other three treatments ($P > 0.6$), there was a strong trend with increasing temperature further reducing A_{net}. Seedlings from the T_{+3} C_E treatment had a higher rate of A_{net} (9.2 μmol m^{-2} s^{-1}) compared to seedlings from the T_{+6} C_E treatment (7.8 μmol m^{-2} s^{-1}). Averaged across all measurement temperatures, g_s, measured in July on a leaf from the top whorl, was significantly greater in seedlings grown in the T_A C_E treatment (0.201 μmol m^{-2} s^{-1}) than any of the other three treatments ($P < 0.03$, for all), which did not differ significantly (T_A C_A: 0.0107, T_{+3} C_E: 0.0086, T_{+6} C_E: 0.0924 μmol m^{-2} s^{-1}, $P > 0.51$). In September, while not significant, A_{net} of seedlings from the T_{+6} C_E treatment was lower than any other treatment ($P < 0.34$). Interestingly the effect of elevated [CO_2] also appeared to diminish: A_{net} of seedlings grown in the T_A C_E and T_{+3} C_E treatments was not significantly greater than seedlings grown in the T_A C_A treatment ($P = 0.56$ & $P = 0.65$, respectively). Averaged across all measurement temperatures, g_s, measured on a leaf from the top whorl, was significantly lower in seedlings from all elevated [CO_2] treatments compared to the T_A C_A treatment (0.403 μmol m^{-2} s^{-1}) ($P < 0.03$ for all), and the response was insensitive to temperature treatment (T_A C_E: 0.221, T_{+3} C_E: 0.191, T_{+6} C_E: 0.157 μmol m^{-2} s^{-1}, $P > 0.35$).

The optimal temperature (T_{opt}) for A_{net}, when averaged across all measurement dates and flushes, was significantly affected by the treatments ($P = 0.007$). Seedlings from the T_A C_E

treatment had a significantly higher T_{opt} for A_{net} than seedlings from the T_A C_A treatment (25.1 vs. 22.1°C ($P = 0.03$). The T_{+6} C_E treatment further shifted the T_{opt} for A_{net} (27.0°C), however this effect was not apparent the T_{+3} C_E treatment (24.5°C).

While A_{net} was influenced by elevated [CO_2] and temperature, R_d was not affected by either growing condition or date (Fig. 5.5). Across the entire growing season, R_d measured on foliage from the first flush was not significantly different at any date ($P = 0.74$); nor was R_d, when averaged across all dates, significantly affected by any treatment ($P = 0.99$) (Fig. 5.5). When measured on the top whorl in July and September, R_d was insensitive treatment as well ($P = 0.80$). These results indicate that R_d was unaffected by elevated [CO_2] and did not acclimate to growing temperature.

Measurements in June of V_{cmax}, J_{max}, and TPU made on the first flush did not change significantly across the four treatments ($P > 0.13$, for all) (Table 5.2). Measurements were repeated in July and September on the first flush and suggest that aging had a small effect on all parameters, but the effect appeared independent of treatment, with seedlings from the T_{+3} C_E and T_{+6} C_E treatment responding to cumulative seasonal stress in a similar manner to seedlings from the T_A C_A treatment. In September measurements were made on all flushes and V_{cmax}, J_{max} and TPU were unaffected by treatments, with one exception: the T_{+6} C_E treatment caused a reduction in J_{max} ($P < 0.006$) and TPU ($P < 0.020$) (Table 5.3).

Averaged across all flushes, dark acclimated fluorescence (F_v/F_m) was not affected by any treatment at any date ($P = 0.45$) (Fig. 5.6a). There was a seasonal trend ($P < 0.001$), where F_v/F_m was lower in June (0.78) than July (0.82) or September (0.80). However, for all measurements made across the entire season and in all treatments F_v/F_m was consistently higher than 0.75, which is typically considered the threshold for healthy foliage. Measurements of both the photochemical quenching (Fig. 5.6b) and the effective quantum yield of PSII (Φ_{PSII}) (Fig. 5.6c), when averaged across all flushes, were not significantly affected by treatment at any date ($P = 0.189$ and $P = 0.42$, respectively). Measurements of qP and Φ_{PSII} did not vary across the growing season ($P = 0.96$ and $P = 0.41$, respectively).

Heights and Diameters leading up to final biomass

Both seedling height (*H*) and diameter measured at the base of the seedling (D_{base}) followed a similar trend (Fig. 5.7a). From March 3 through measurements made on June 8, *H* was not significantly affected by treatment on any measurement date ($P > 0.09$). For measurements made on July 30 and September 9, *H* was significantly greater in the T_A C_E treatment than any other treatments ($P < 0.016$ & $P < 0.003$, respectively). There was no difference in *H* between the other three treatments at either measurement day ($P > 0.34$ & $P > 0.37$, respectively) suggesting that elevated temperature negated the boost that elevated [CO_2] had on *H* growth. At the harvest in September *H* was greatest in seedlings from the T_A C_E treatment (1.28 m, $P < 0.005$ for all), and did not vary among the other three treatments (T_A C_A: 0.99 m; T_{+3} C_E: 0.90 m; T_{+6} C_E: 1.04 m; $P > 0.10$ for all).

From planting, March 3, through July 30, D_{base} was not affected by any treatment at any measurement date ($P > 0.15$) (Fig. 5.7b). By September 3, D_{base} was significantly larger in seedlings from the T_A C_E treatment than any other treatments ($P < 0.006$ for all) and there was no significant difference in D_{base} among the other three treatments ($P > 0.2$ for all). At the harvest, September 30, seedlings from the T_A C_E treatment had significantly larger D_{base} compared to the other treatments (17.9 mm, $P < 0.04$, for all), while seedlings from the T_{+6} C_E treatment had significantly smaller D_{base} compared to the other treatments (14.6 mm, $P < 0.01$ for all). There was no significant difference in D_{base} between the T_A C_A and T_{+3} C_E treatment at the final harvest (16.4 & 16.6 mm respectively, $P = 0.69$).

Seedling total biomass at the end of the growing season was affected by treatments. Elevated [CO_2] only boosted biomass production in the T_A C_E treatment (Fig. 5.8). Seedlings grown in the T_A C_E treatment had the greatest total biomass accumulation (263.6 g), 38% greater than seedlings from the T_A C_A treatment (191.4 g, $P < 0.001$). Biomass of seedlings grown in the T_{+3} C_E was not significantly different from that of the T_A C_A treatment (206.4 & 191.4, $P = 0.21$), and seedlings grown in the T_{+3} C_E treatment had significantly less biomass than seedlings in the T_A C_E treatment ($P < 0.004$). Seedlings from the highest temperature treatment, T_{+6} C_E, accumulated the smallest amount of biomass (169.5 g), and were smaller than seedlings from all the other elevated [CO_2] treatments ($P < 0.009$), but not different from seedlings in the T_A C_A treatment ($P = 0.144$).

Total leaf area was greater in seedlings from the T_A C_E treatment (4293 cm^2) than seedlings from the T_A C_A treatment (2982 cm^2, $P = 0.015$). Seedlings from the T_{+3} C_E and T_{+6} C_E treatments had less leaf area than seedlings from the T_A C_E treatment, though the decrease was not significant (T_{+3} C_E: 3276 g, $P = .06$; T_{+6} C_E: 3505 g, $P = 0.1431$). There was no significant difference in leaf area among the T_A C_A, T_{+3} C_E and T_{+6} C_E treatments ($P > 0.34$).

Discussion

Changing climatic conditions are expected to affect tree physiological processes, growth and subsequently species distribution, with the largest impact expected to occur at the warmest and coolest portion of a species range (He *et al.* 2005; Thuiller *et al.* 2008). At the warmest portion of the range temperatures are presumed to be supra-optimal for growth, so a further increase in temperature is expected to have a negative effect on photosynthesis and biomass production (Kirschbaum 2000). Our findings do not support the hypothesis that projected temperature increases in the warmest part of a species range will significantly reduce growth, if as expected, those increases are accompanied by an increase in atmospheric [CO_2]. Elevated temperature reduced the boost that elevated [CO_2] provided photosynthesis and growth, but seedlings grown in the T_{+3} C_E treatment still had a higher rate of A_{net} and produced as much biomass as seedlings grown in current atmospheric conditions. The T_{+6} C_E treatment negatively impacted growth, with seedlings having the lowest A_{net} and accruing less biomass than seedlings grown in current atmospheric conditions, but biomass accumulation was still equivalent to the T_A C_A treatment and no seedling mortality was noted, nor was damage to photosynthetic capacity observed. It is also unlikely that an increase in mean air temperature will be as great as +6°C in this region in the next century (IPCC 2007). These findings suggest that, for at least some tree species, a modest increase in temperature may reduce the positive effect that elevated [CO_2] has on A_{net} and biomass accumulation at the southern portion of a species distribution, though it appears unlikely that even a substantial increase in temperature will lead to substantial growth reductions or large scale mortality, provided that the plants have an adequate supply of water.

Predictive models have suggested that changes in climatic conditions will lead to a shift in the optimum latitude of growth by hundreds of kilometers (e.g.: Iverson & Prasad 2002; Hamann & Wang 2006; McKenney *et al.* 2007; Tang & Beckage 2010) resulting in a significant shift in species distributions. However, Xu et al. (2007) suggest that elevated [CO_2], by raising the T_{opt} of A_{net}, may reduce the impact higher growing season temperatures will have on species distributions. Our findings are in agreement with Xu et al. (2007): In this study T_{opt} of A_{net}

appeared to acclimate to both growing temperature and elevated [CO_2] and elevated [CO_2] appeared to mitigate the effect of elevated temperature.

As expected, elevated [CO_2] alone significantly boosted A_{net} throughout growing season, which resulted in an increase in H and D_{base} growth during the second half of the growing season and significantly boosted biomass production. These findings are in agreement with previous studies which have documented an average increase of 30 to 60% in A_{net} (Ceulemans & Mousseau 1994) and an average increase of 28% in biomass accumulation (Curtis & Wang 1998; Ainsworth & Long 2005) with long term exposure to elevated [CO_2] (typically doubled over ambient). The effect of elevated temperature on growth in our study, when present, negated the positive effect elevated [CO_2] had on both photosynthesis and biomass production. Other studies have observed a negative effect of increasing temperature on A_{net} (e.g.: Callaway *et al.* 1994; Wang *et al.* 1995; Ro *et al.* 2001) and biomass production (e.g.: Wayne *et al.* 1998; Way & Sage 2008), though none of these studies were conducted at temperatures similar to those of this study. Despite the reduction in A_{net} with elevated temperature, the optimal temperature for A_{net} shifted upwards with both the elevated [CO_2] and elevated temperature treatments, which has been reported in other tree species (Kirschbaum 2005; Sage & Kubien 2007), and suggests that without acclimation, A_{net} in the elevated temperature treatments may have been even lower and biomass production further reduced.

Throughout the experiment foliar respiration (R_d) did not acclimate to growing temperature. Both short and long term acclimation have been clearly demonstrated in numerous tree species (Atkin & Tjoelker 2003), including northern red oak which has been reported to rapidly and reversibly acclimate to changes in temperature (Bolstad *et al.* 2003). We are unsure of why we did not observe acclimation of R_d to treatments, especially when A_{net} demonstrated plasticity. However, it should be pointed out that Bolstad et al. (2003) grew seedlings under constant day/night temperatures, while our seedling were exposed to daily changes in temperature and within day fluctuations in temperature which were often much larger than 6°C (our largest temperature treatment). The concurrent decrease in A_{net} with increasing growth temperature and apparent lack of acclimation of respiration appears to largely explain the temperature treatment dependent reduction in H, D_{base} and biomass production.

Another interesting finding was the lack of apparent damage to photosynthetic capacity, as measured by fluorescence and A/C_i curves. Darbahet al. (2010) demonstrated that exposure to high temperatures can significantly reduce photosynthetic potential, though the reduction was dependent both upon the amount of heat stress and if a tree emits isoprene to cope with thermal stress. We had expected that a continuous +6°C increase in temperature during the growing season would have stressed the plants to a level where foliage damage would be significant, and while we did observe a small treatment effect on V_{cmax} and J_{max}, the effect was only observed at the last measurement period, and fluorescence measurements did not suggest any temperature induced damage to either the photochemical efficiency of PSII or the effective quantum yield of PSII. However, oak trees can emit substantial amounts of isoprene and it is possible that elevated [CO_2] or isoprene emissions may have protected the photosynthetic apparatus (Haldimann & Feller 2004; Darbah *et al.* 2010).

Typically, long term exposure to elevated [CO_2] has been demonstrated to reduce stomatal conductance (Saxe *et al.* 1998), though throughout the majority of our study stomatal conductance was not affected by elevated [CO_2]. While this finding is unusual, it is not unprecedented (Uddling *et al.* 2009). The lack of response of stomatal conductance to elevated [CO_2] may have been beneficial by allowing more evaporative cooling in the elevated temperature treatments, which may have occurred, since in this study all seedlings were well watered, so water stressed induced stomatal closure was likely not a factor.

Conclusion

While elevated [CO_2] significantly stimulated carbon fixation and biomass accumulation, elevated temperature mitigated this stimulation. However, the effect of elevated temperature on growth was smaller than we expected, with an increase in air temperature of +6°C only reducing A_{net} and biomass production to levels comparable to those observed in current atmospheric conditions. These findings suggest that a moderate increase in temperature will unlikely result in a significant reduction in productivity or a shift in the southern distribution of northern red oak. Of note, in this particular tree species respiration failed to acclimate to growing temperature, which may explain the decreased biomass accumulation with increased growing temperature treatment. If a tree species is capable of temperature acclimation of respiration the effect of a +6°C increase would likely be even less than what we observed.

Table 5.1. Mean (SE) air temperature (°C) and atmospheric [CO_2] (µmol mol^{-1}) in the four treatment chambers during the 2009 experimental period (March 5 through September 30). Treatments were: ambient temperature and ambient [CO_2] (T_A C_A), ambient temperature and elevated [CO_2] (T_E C_A), ambient temperature +3°C and elevated [CO_2] (T_{+3} C_E), and ambient temperature +6°C and elevated [CO_2] (T_{+6} C_E).

Treatment	Temperature	[CO2]
T_A C_A	24.2 (0.2)	399.8 (2.3)
T_A C_E	24.7 (0.2)	708.4 (5.0)
T_{+3} C_E	27.8 (0.2)	669.0 (11.5)
T_{+6} C_E	30.5 (0.2)	670.4 (7.1)

Table 5.2. Measurements of V_{cmax}, J_{max} and TPU (µmol m^{-2} s^{-1}) (as calculated from A/C$_i$ curves) made on the first fully developed flush of northern red oak seedlings in June (Date 1), July (Date 2) and September (Date 3). Treatments were: ambient temperature and ambient [CO$_2$] (T$_A$ C$_A$), ambient temperature and elevated [CO$_2$] (T$_E$ C$_A$), ambient temperature +3°C and elevated [CO$_2$] (T$_{+3}$ C$_E$), and ambient temperature +6°C and elevated [CO$_2$] (T$_{+6}$ C$_E$). Significant difference of a parameter within a treatment is denoted by letters ($P < 0.05$).

Trt	Date	Vcmax	Jmax	TPU
T$_A$ C$_A$	1	49.4 (9.3) ^{a,b}	69.6 (7.6) ^{a}	4.7 (0.2) ^{a}
	2	57.6 (8.0) ^{a}	72.8 (7.0) ^{a}	4.8 (0.4) ^{a}
	3	31.0 (13.1) ^{b}	52.3 (12.7) ^{a}	3.3 (0.8) ^{b}
T$_A$ C$_E$	1	57.0 (6.9) ^{a}	79.8 (5.9) ^{a}	5.3 (0.4) ^{a}
	2	58.6 (6.9) ^{a}	73.0 (4.3) ^{a}	4.8 (0.4) ^{a}
	3	8.6 (2.7) ^{b}	16.4 (3.7) ^{b}	1.2 (0.3) b
T$_{+3}$ C$_E$	1	48.3 (11.9) ^{a,b}	66.8 (9.4) ^{a}	4.2 (0.7) ^{a,b}
	2	29.6 (4.4) ^{a}	42.8 (12.4) ^{b}	3.2 (0.4) ^{a}
	3	69.6 (6.5) ^{b}	87.8 (4.7) ^{a}	5.5 (0.2) ^{b}
T$_{+6}$ C$_E$	1	46.0 (5.7) ^{a}	64.8 (5.2) ^{a}	4.3 (0.3) ^{a}
	2	44.6 (5.1) ^{a}	58.8 (1.8) ^{a}	4.0 (0.2) ^{a}
	3	39.6 (7.3) ^{a}	52.4 (8.9) ^{a}	3.6 (0.5) ^{a}

Table 5.3. Measurements of V_{cmax}, J_{max} and TPU (µmol m^{-2} s^{-1}) (as calculated from A/C$_i$ curves) in September 2009 on all fully developed flushes of northern red oak seedlings. Treatments were: ambient temperature and ambient [CO$_2$] (T_A C_A), ambient temperature and elevated [CO$_2$] (T_E C_A), ambient temperature +3°C and elevated [CO$_2$] (T_{+3} C_E), and ambient temperature +6°C and elevated [CO$_2$] (T_{+6} C_E). Significant difference of a parameter across treatments is denoted by letters ($P < 0.05$).

Treatment	Flush	V_{cmax}	J_{max}	TPU
T_A C_A	1	31.0 (13.1)	52.3 (12.7)	3.3 (0.8)
	2	63.3 (5.2)	98.8 (5.4)	7.0 (0.6)
	3	83.4 (9.7) ^{a}	115.2 (7.4) ^{a}	8.1 (0.4) ^{a}
T_A C_E	1	8.6 (2.7)	16.4 (3.7)	1.2 (0.3)
	2	42.2 (14.7)	51.2 (12.4)	3.4 (0.9)
	3	88.2 (15.1) ^{a}	107.0 (5.9) ^{a,b}	7.8 (0.4) ^{a}
T_{+3} C_E	1	69.6 (6.5)	87.8 (4.7)	5.5 (0.2)
	2	87.6 (8.6) ^{a}	109.0 (5.7) ^{a,b}	7.8 (0.4) ^{a}
T_{+6} C_E	1	39.6 (7.3)	52.4 (8.9)	3.6 (0.5)
	2	70.0 (4.1) ^{a}	83.8 (3.8) ^{b}	5.7 (0.2) ^{b}

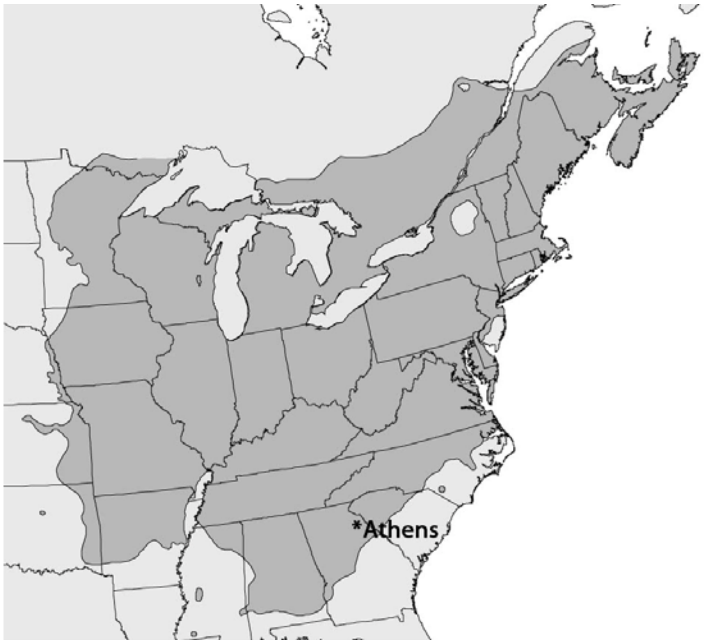

Fig. 5.1. The location of the study site: Athens, GA USA. The native range of northern red oak is shaded dark grey. Distribution map from Little (1971).

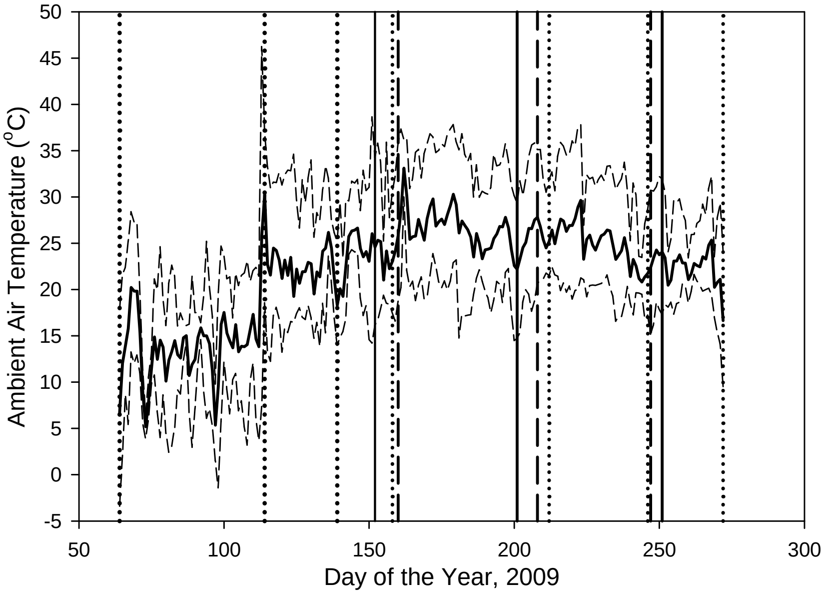

Fig 5.2. Daily mean (bold line), minimum (dashed) and maximum (dashed) ambient temperature for the experimental period (March through September). Vertical lines represent when measurements of seedling height and diameter (dotted), leaf gas exchange (solid) and leaf fluorescence (dashed) were conducted.

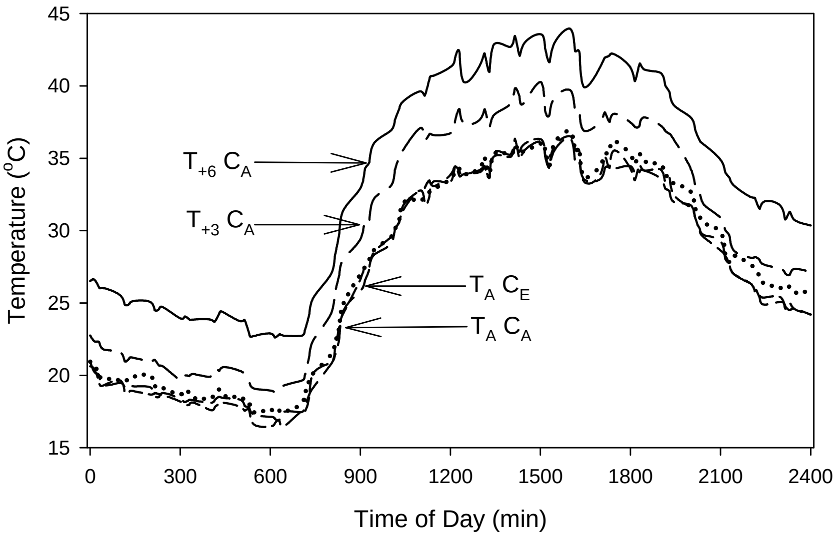

Fig 5.3. Air temperature measured inside treatment chambers of a representative day during the growing season (July 1, 2009). Treatments are ambient temperature and ambient [CO_2] (short dashed line), ambient temperature and elevated [CO_2] (short dashed line), ambient temperature +3C and elevated [CO_2] (long dashed line) and ambient temperature +6 and elevated [CO_2] (solid line). Outside ambient air temperature represented by dotted line.

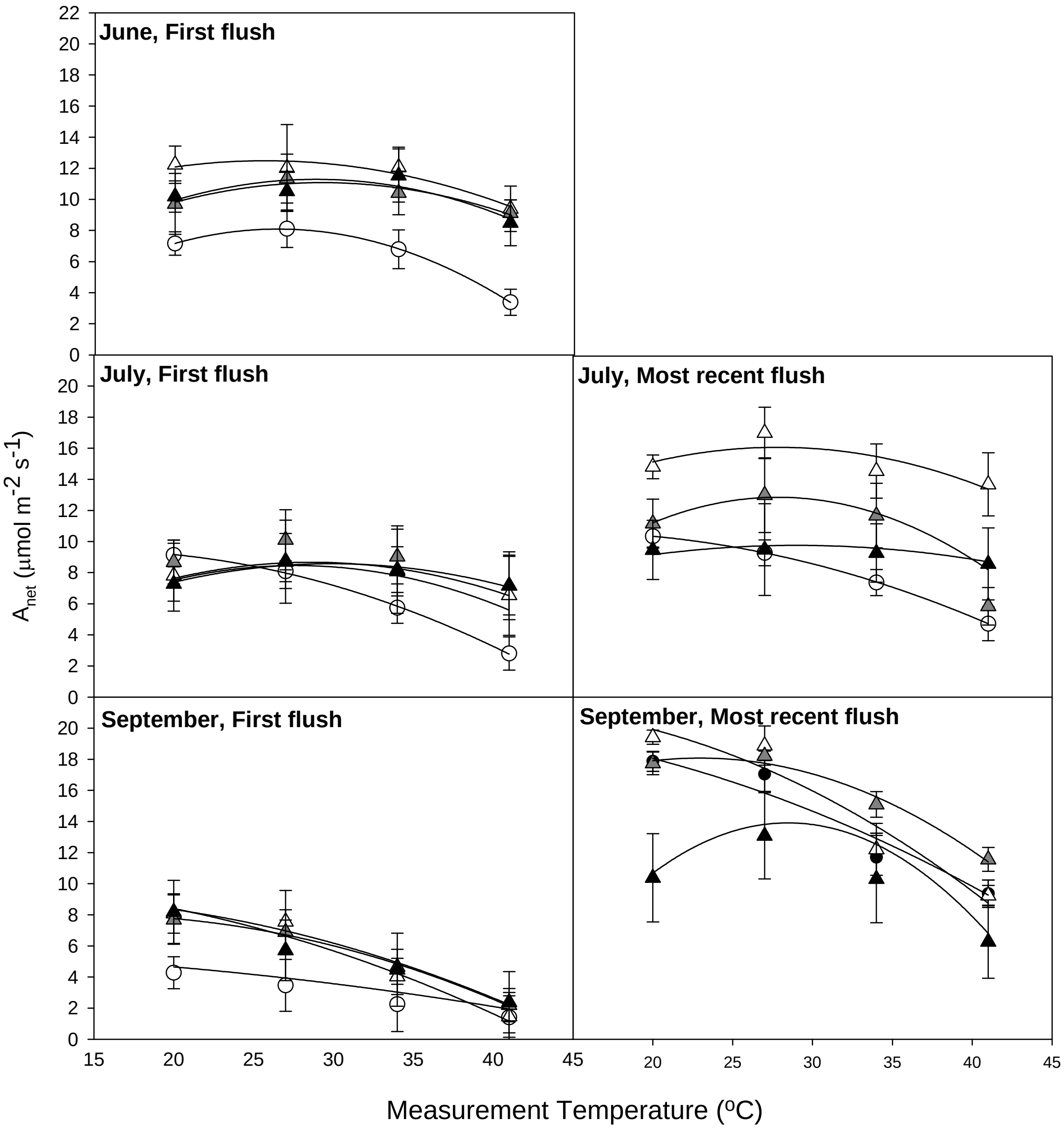

Fig. 5.4. Mean light saturated net photosynthesis (A_{net}) (µmol m^{-2} s^{-1}) of northern red oak seedlings. Measurements were made on a fully developed leaf from the first flush (**A**, **B**, **C**) and a fully developed leaf from the top whorl (**D**, **E**) in June (**A**), July (**B**, **D**) and September (**C**, **E**). Growing treatments are: ○ ambient temperature and ambient [CO_2]; △ ambient temperature and elevated [CO_2]; ▲ ambient temperature + 3°C and elevated [CO_2]; ▲ ambient temperature +6°C and elevated [CO_2]. Error bars represent 1 SEM.

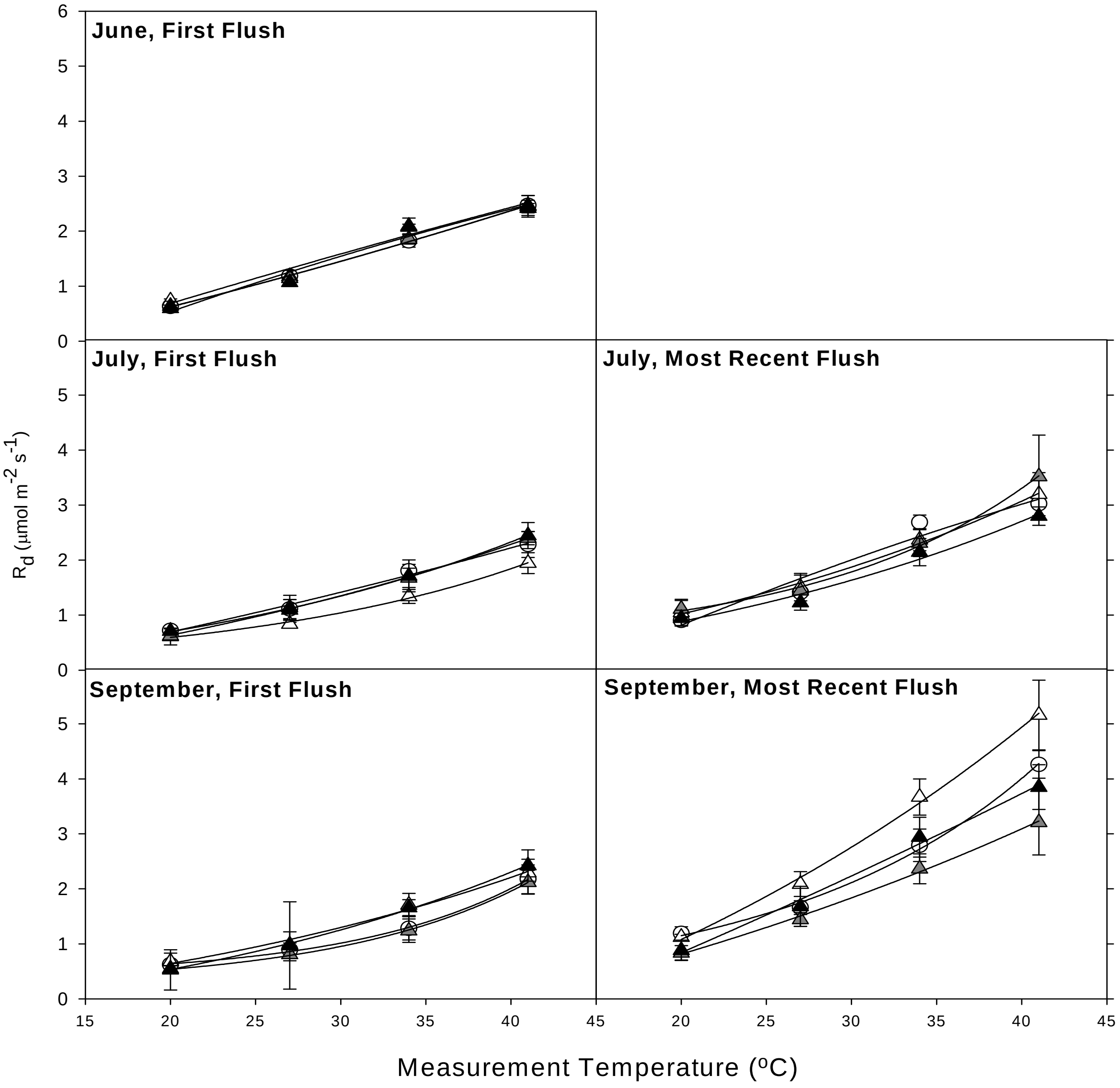

Fig. 5.5. Mean leaf dark respiration (R_d) (µmol m^{-2} s^{-1}) of northern red oak seedlings. Measurements were made on a fully developed leaf from the first flush (**A**, **B**, **C**) and a fully developed leaf from the top whorl (**D**, **E**) in June (**A**), July (**B**, **D**) and September (**C**, **E**). Growing treatments are: ○ ambient temperature and ambient [CO_2]; △ ambient temperature and elevated [CO_2]; ▲ ambient temperature + 3°C and elevated [CO_2]; ▲ ambient temperature +6°C and elevated [CO_2]. Error bars represent 1 SEM.

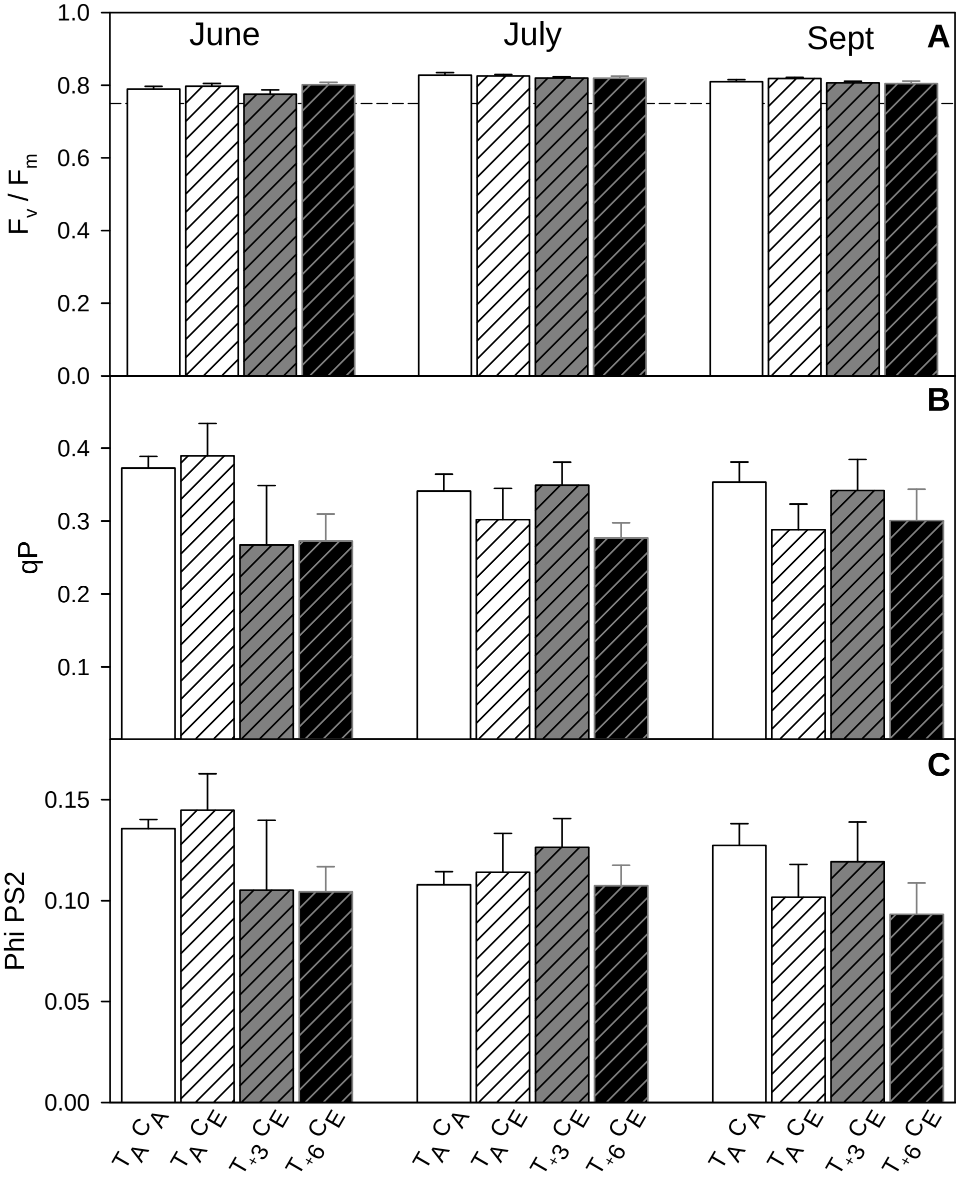

Fig. 5.6. (A) Mean dark-acclimated leaf fluorescence (F_v/F_m), **(B)** photochemical quenching (qP) and **(C)** effective quantum yield of PSII (Φ_{PSII}) of northern red oak seedlings. Measurements were made on a leaf from each flush in June, July and September 2009. Treatments are: ambient temperature and ambient [CO_2] (T_A C_A); ambient temperature and elevated [CO_2] (T_A C_E); ambient temperature + 3°C and elevated [CO_2] (T_{+3} C_E); ambient temperature +6°C and elevated [CO_2] (T_{+6} C_E). Error bars represent 1 SEM.

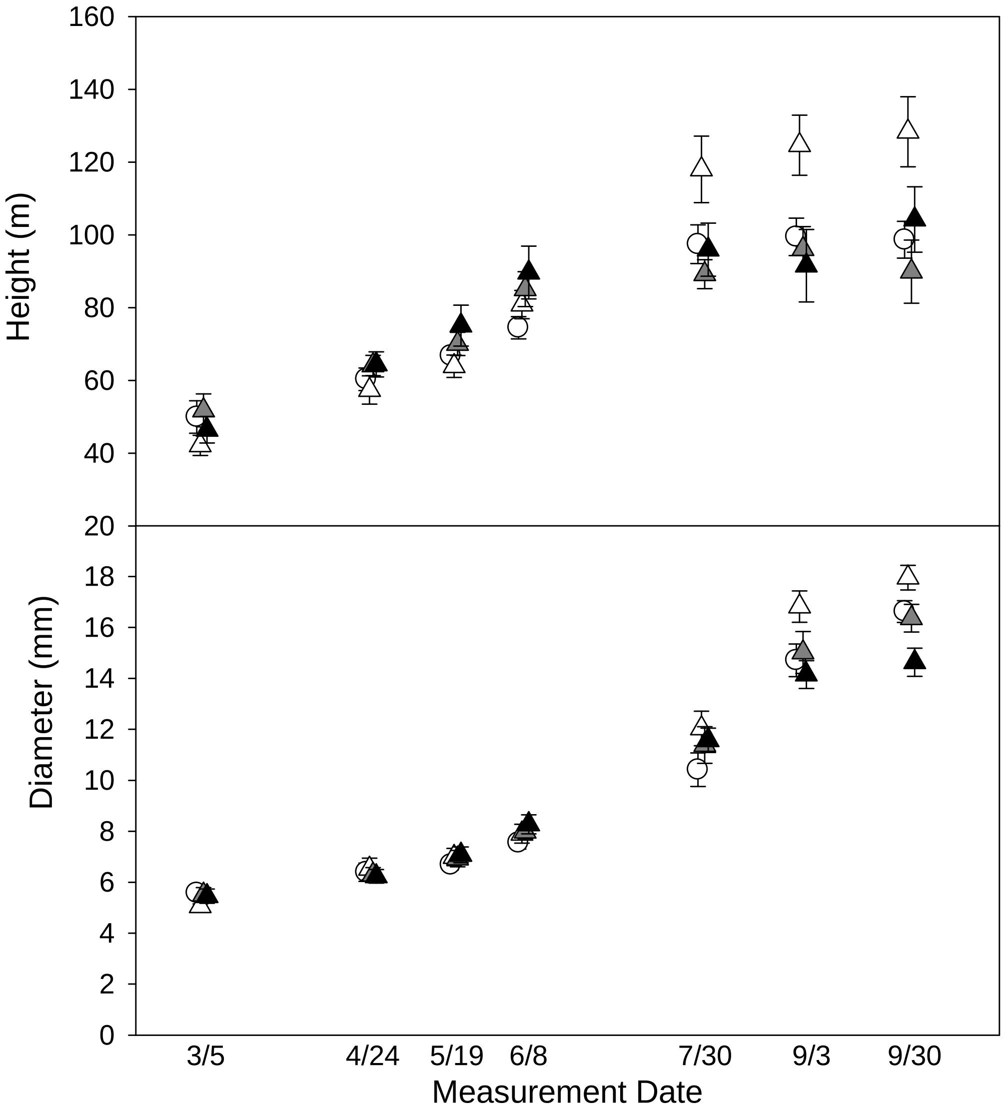

Fig. 5.7. (A) Height (m) and **(B)** diameter (mm) measured at the base of northern red oak seedlings. Measurements were made throughout the growing season in 2009. Treatments are: ambient temperature and ambient [CO_2]: ○; ambient temperature and elevated [CO_2]: △; ambient temperature + 3°C and elevated [CO_2]: ▲; ambient temperature +6°C and elevated [CO_2]: ▲. Error bars represent 1 SEM.

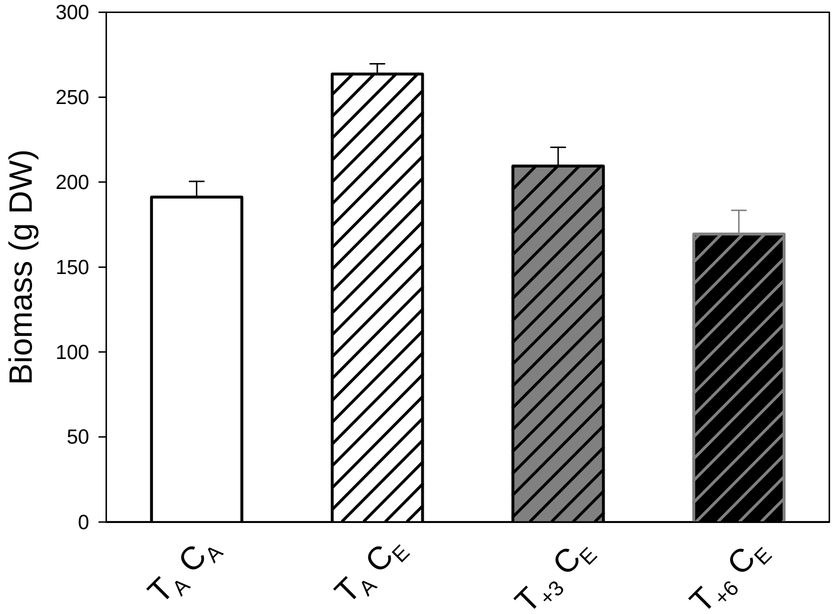

Fig. 5.8. Total biomass (dry weight, g) of northern red oak seedlings at the end of a growing season. Seedlings were grown in treatment chambers and the treatments were: ambient temperature and ambient [CO_2] (T_A C_A); ambient temperature and elevated [CO_2] (T_A C_E); ambient temperature + 3°C and elevated [CO_2] (T_{+3} C_E); ambient temperature +6°C and elevated [CO_2] (T_{+6} C_E). Error bars represent 1 SEM.

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CHAPTER 6

CONCLUSIONS

Summary of Previous Chapters

Two studies were conducted to investigate the effect of elevated temperature and [CO_2] on tree growth and physiology, and subsequently the potential for a shift in species distribution with a change in climate conditions. In the first study loblolly pine seedlings were grown at three sites that were located near the Northern edge, in the central region and near the Southern edge of the species range. Seedlings were grown in elevated and ambient temperature, elevated and ambient [CO_2] and high and low soil moisture availability. Foliar gas exchange was measured *in situ* and under environmental controlled conditions throughout the growing season. Seedling total biomass was obtained throughout the growing season and at the end of one complete growing season. Based on the findings from the loblolly pine study, northern red oak seedlings were grown at a site near the Southern edge of the species range in elevated temperature and [CO_2] to determine if a broad leaf deciduous tree responded in a similar manner as a conifer evergreen, and to determine how large an increase in temperature was necessary to negatively affect growth.

In general, no negative effect of elevated temperature on loblolly pine net photosynthesis or growth was observed at either end of the species range throughout the growing season. Measurements of *in situ* net photosynthesis suggested that predicted increases in [CO_2] will significantly increase fixation, while an expected 2–3 °C increase in air temperature will have little or no effect on net photosynthesis throughout the native range of loblolly pine. This suggests that an increase in air temperature of 2°C will not mitigate the [CO_2]-induced increase in net photosynthesis in the warmer portions of the loblolly pine range, nor will net photosynthesis benefit from an increase in temperature the cooler portions of the range. Decreased precipitation, predicted to occur in concert with increased temperature, will likely cause a significant reduction in net photosynthesis under current [CO_2]. However, an increase in [CO_2] may at least partly mitigate reductions in net photosynthesis if precipitation decreases in the future.

A similar result was observed in biomass accumulation. Seedling growth responded to the combination of elevated temperature and elevated [CO_2] consistently across a large spatial and thermal range. At both the northern and southern sites, biomass production was significantly increased in seedlings exposed to elevated temperature and elevated [CO_2], compared to seedlings grown in ambient conditions. The low water treatment significantly reduced biomass production at both sites and partially negated the positive effect of elevated temperature and [CO_2], suggesting that if precipitation decreases, biomass production may be negatively affected regardless of expected increases in temperature and [CO_2].

The findings of the loblolly pine study suggest that, contrary to species distribution models which have predicted a dramatic reduction in the distribution of loblolly pine and other tree species (McKenney *et al.* 2007), there is no evidence supporting the notion that the southern limit of loblolly pine will contract as a result of elevated temperature. The lack of any effect of an increase in air temperature may be due to several factors. Loblolly pine has a broad optimal temperature range for net photosynthesis (Teskey *et al.* 1987). Other species with a narrower optimal temperature range for net photosynthesis may respond differently to increases in temperature. An increase of 2°C was small relative to the daily and seasonal temperature range the seedlings experienced. Acclimation of net photosynthesis and respiration to an increase in growing temperature may have occurred, though we were unable to elucidate them.

While loblolly pine growth was insensitive to an increase in temperature at the southern edge of the range, northern red oak growth was reduced by elevated temperature. Elevated [CO_2] significantly stimulated carbon fixation and biomass accumulation in northern red oak, though elevated temperature mitigated this stimulation. However, the effect of elevated temperature on growth was smaller than we expected. Net photosynthesis and biomass accumulation in trees exposed to elevated [CO_2] and elevated temperature (+6°C) were similar to values of trees grown in ambient temperature Our findings suggest that a moderate increase in temperature, *per se*, will unlikely drive a reduction in productivity or a shift in the southern distribution of northern red oak. It is possible that the mitigating effect of elevated temperature may decrease northern red oak seedlings ability to compete in future climate conditions. Changes in climate are predicted to alter species interactions, which may lead to shifts in species distributions (Tylianakis *et al*. 2008). Of note, in this particular tree species respiration failed to acclimate to growing temperature, which may partially explain the decreased biomass accumulation with increased growing temperature treatment. If a tree species is capable of temperature acclimation of respiration, the effect of a +6°C increase would likely be less than what we observed.

Loblolly pine and northern red oak responded differently to elevated temperature in the southern portion of the species range: loblolly pine growth was stimulated by elevated temperature and [CO_2] across the species distribution while northern red oak growth was reduced by elevated temperature at the southern edge of the species range. Van der Putten *et al*. (2004) suggest that species are likely to respond differently to changes in climate. While the findings of the loblolly pine study were in conflict with the original hypothesis (in supra-optimal temperature environments growth is expected to be reduced by an increase in temperature), the findings of the northern red oak study support this hypothesis. It is possible that elevated [CO_2] may negate temperature induced suppression of net photosynthesis (Long 1991; Xu *et al.* 2007), though the interaction is dependent upon the increase in temperature and [CO_2] and the thermal sensitivity of a species. In the second study, elevated [CO_2] did appear to negate thermally induced reductions in net photosynthesis and growth of northern red oak seedlings, however this mitigation was dependent upon the amount temperature increased. The variable response of net photosynthesis, and subsequently growth, to temperature across the species range is believed to be a driving force that may lead to a shift in species distribution (He *et al.* 2005). Numerous models have suggested that the range of a substantial number of trees in North America will shift northward with changes in climate conditions (e.g.: Iverson & Prasad 2001; Hamann & Wang 2006; Prasad *et al.* 2006; McKenney *et al.* 2007; Morin *et al.* 2008; Tang & Beckage 2010). It is important to note that growing season temperature, which was tested in these studies, is not the only determinate of a species range. Factors such as precipitation, winter time minimum temperature, herbivory pressure and competition are likely to be equally important in regulating growth. However, climate on a continent scale is a strong determinate of species occurrence (Field *et al.* 2009). The differing response of loblolly pine and northern red oak to elevated temperature near the southern edge of their distribution suggests that it is unwise to model ecosystem response to climate change as species are apt to respond differently. The different findings of the two studies suggest that species specific verification of the response to elevated temperature and [CO_2] near the northern and southern edges of their distributions is required before predictions of range shifts with changes in climate can be estimated.

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