

Response of *Quercus phellos* L. Seedlings to Increased Root-zone Temperature and Simulated Herbivory

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ABSTRACT

Quercus phellos increased mean foliage production ($F_{(2,33)} = 58.56, P < 0.0001$) three leaves per plant in response to increasing root-zone temperature and eight leaves per plant in response to a concomitant increase in herbivory ($F_{(2,23)} = 33.62, P < 0.0001$) at the expense of the root system. A decrease in shoot biomass ($F_{(2,33)} = 88.47, P < 0.0001$) and stomatal conductance, in response to increasing temperature ($F_{(2,33)} = 85.8, P < 0.0001$) and herbivory ($F_{(2,33)} = 443.8, P < 0.0001$), translated into a decrease in transpiration rate ($F_{(2,33)} = 60.06, P < 0.0001$) and a concomitant decrease in net photosynthesis in response to temperature ($F_{(2,33)} = 170.07, P < 0.0001$) and herbivory ($F_{(2,33)} = 259.85, P < 0.0001$) while internal C concentration increased in response to temperature ($F_{(2,33)} = 4.39, P = 0.015$) and herbivory ($F_{(2,33)} = 26.19, P < 0.0001$). The findings of this study imply this wetland tree species will experience losses across its range in response to global warming, and the associated increase in insect herbivores, due allocating resources away from the root system and decreases in photosynthesis.

Keywords: global warming, internal carbon, net photosynthesis, stomatal conductance, transpiration, wetlands

Abbreviations: Ci, Internal carbon; E, transpiration rate; Eh, oxidation-reduction potential; gs, stomatal conductance; PAR, photosynthetically active radiation; Pn, net photosynthesis

INTRODUCTION

We investigated the effects of global warming and herbivory on willow oak (*Quercus phellos*). *Q. phellos* is one of the most common canopy layer trees of the bottomland hardwood forests of the southeastern U.S. (Lockhart *et al.* 2010). Bottomland hardwood forests are highly productive ecosystems with well documented ecological and economic values where frequent disturbances result in a high biodiversity. Changes in hydrology (Franklin *et al.* 2009) have wide-ranging ecological impacts on these systems, including changes to the insect herbivore populations.

Global warming, and the associated increase in insect herbivores, could affect both the productivity and diversity of bottomland hardwood forests (Gosling *et al.* 2011). Today's temperature increases are occurring too rapidly for some trees species to respond (Gugger *et al.* 2010), impacting the future distribution of species (Thuiller *et al.* 2005) as well as species survival (Thomas *et al.* 2004).

The behavioral response of herbivores to climatic change is critical in determining the persistence of plant species (Brodie *et al.* 2011). While herbivory generally has a negative impact, plants have a continuum of responses to herbivory (Hjalten *et al.* 1993) ranging from no effect (e.g., Karban and Courtney 1987) to overcompensation (Cargill and Jeffries 1984). Individual plant species should express a large variation in magnitude and type of response to herbivory (Hjalten *et al.* 1993) due to seasonality (Bryant *et al.* 1991), resource availability (Maschinski and Whitham 1989), intensity and frequency of herbivory (e.g., Kulman 1971), or stress (Oosterheld and McNaughton 1991). Long-lived, slow-growing plants have less ability to regenerate lost tissue compared to short-lived but fast-growing species (Garnier 1991).

Many plants can adapt photosynthesis and respiration to changing temperature (Lambers *et al.* 1998). In temperate latitudes the effects on photosynthetic processes is probably

positive (Saxe *et al.* 2001) due to photosynthetic pigments increasing in response to temperature increases (Ormrod *et al.* 1999). However, if temperatures exceed the maximum for protein functioning photosynthesis is inhibited (Larcher 1994) due to the sensitivity of Photosystem II (Berry and Bjorkman 1980). While higher temperatures can also stimulate photorespiration, thus reducing net photosynthesis (Long 1991), different species respond differently to increased soil temperature (Graves and Aiello 1997).

The major force in moving moisture from the terrestrial system back to the atmosphere is transpiration and leaf herbivory can substantially alter transpiration affecting plant reproduction and growth (Cunningham *et al.* 2009). Defoliation also reduces the area available for photosynthesis and reduces net photosynthesis (Pn) in the remaining leaf tissues (Meyer *et al.* 2001). Decreases in Pn can also result from cessation of root growth through feed-back inhibition due to carbohydrate buildup (Pezeshki and DeLaune 1998) which can translate into decline in total biomass (Brown and Pezeshki 2000).

To determine the effects of climate change on bottomland hardwood forest woody species we tested the hypotheses that *Q. phellos*, one of the most common canopy level trees of these southeastern wetland forests, will respond to increasing herbivory, and temperature, by 1) increasing new foliage, 2) shifting resources towards the shoots, 3) decreasing transpiration, 4) increasing net photosynthesis, 5) decreasing stomatal conductance, 6) increasing internal C, and 7) having an interactive effect.

MATERIALS AND METHODS

Plant materials

Willow oak (*Q. phellos*) seeds were collected along the Coldwater River in Coldwater, MS, USA, stored at approximately 4°C for 30 days, then spread over a 2:3 sand:soil mixture and lightly covering

with oak leaves. First year plants were transplanted to 10.5 cm h × 7 cm diameter plastic pots, allowed to stabilize for two weeks, than randomly assigned to treatments in a 3 × 3 factorial design. Treatments were applied simultaneously in order to test for interactive, as well as separate, effects.

Experimental procedures

The potted first year plants were grown under soil temperatures of 25°C, 23°C, and 21°C (ambient temperature) with temperature maintained by a thermostatically controlled heat pad. Pots were placed directly on the pad to achieve 25°C, raised 1 cm above the pad for 23°C, no heat was applied for ambient soil temperature.

Plants were exposed to an 18 hour photoperiod of photosynthetically active radiation (PAR) averaging 800-1000 μmol photons/m²/sec in the laboratory at the University of Memphis campus.

There were 12 plants per temperature treatment. All plants were well-watered and fertilized monthly using Peters Professional 20-20-20. Soil redox (Eh) was measured biweekly to monitor soil moisture conditions using one platinum-tipped electrode per pot, four pots randomly selected per treatment. The electrodes were inserted in soil to a depth of 3 cm, and the data recorded using a Model 250 A ORION millivolt redox meter and a calomel reference electrode (Thermo Orion, Beverly, MA, USA). Oxygen begins to disappear from the soil at an approximate Eh value of +350 mV (DeLaune and Pezeshki 1991).

Herbivory was simulated using a hole punch to remove leaf tissue from newly developed foliage without severing major veins. No tissue was removed for 0% leaf herbivory, 10% of the total leaf tissue/plant was removed for 10% herbivory, and 30% leaf tissue was removed for 30% herbivory.

On day 74 the total number of leaves was recorded for each plant for determination of resource allocation. The first mature leaf at the apex of the shoot was used to measure net photosynthesis (P_n) and transpiration (E) using a portable gas exchange analyzer (Model CIRAS 2, PP Systems, Haverville, MA, USA). The following day, plants were harvested and divided into roots and shoots. Resource allocation was determined by measuring shoot and root biomass after drying at 70°C to a constant weight and the final weights recorded. Root/shoot ratio was calculated as the ratio of dry root weight to dry shoot weight.

Data analysis

A completely randomized design was employed. Significant differences between treatments were tested with a two-way ANOVA and Scheffe's *post hoc* multiple range test was used to examine all pair-wise group differences. Differences were considered significant at $P \leq 0.05$. All statistical analyses were made using SPSS 14.0.

RESULTS

Q. phellos foliage increased in response to increased soil temperature ($F_{(2,33)} = 58.56$, $P < 0.0001$) and increased herbivory ($F_{(2,33)} = 33.62$, $P < 0.0001$) (Fig. 1) resulting in a shift in resource allocation to the shoots (Fig. 2) in response to both temperature ($F_{(2,33)} = 9.35$, $P = 0.0002$) and herbivory ($F_{(2,33)} = 67.1$, $P < 0.0001$). However, shoot biomass decreased in response to herbivory ($F_{(2,33)} = 88.47$, $P < 0.0001$) (Fig. 3).

Transpiration rate decreased in response to herbivory ($F_{(2,33)} = 60.06$, $P < 0.0001$) (Fig. 4) while net photosynthesis decreased in response to both temperature ($F_{(2,33)} = 170.07$, $P < 0.0001$) and herbivory ($F_{(2,33)} = 259.85$, $P < 0.0001$) (Fig. 5) with a concomitant decrease in stomatal conductance in response to temperature ($F_{(2,33)} = 85.8$, $P < 0.0001$) and herbivory ($F_{(2,33)} = 443.8$, $P < 0.0001$) (Fig. 6) resulting in increased internal C in response to temperature ($F_{(2,33)} = 4.39$, $P = 0.015$) and herbivory ($F_{(2,33)} = 26.19$, $P < 0.0001$) (Fig. 7).

Temperature and herbivory were interactive for resource allocation ($F_{(2,33)} = 11.28$, $P < 0.0001$) (Fig. 2) and for stomatal conductance ($F_{(2,33)} = 17.64$, $P < 0.0001$) (Fig. 6).

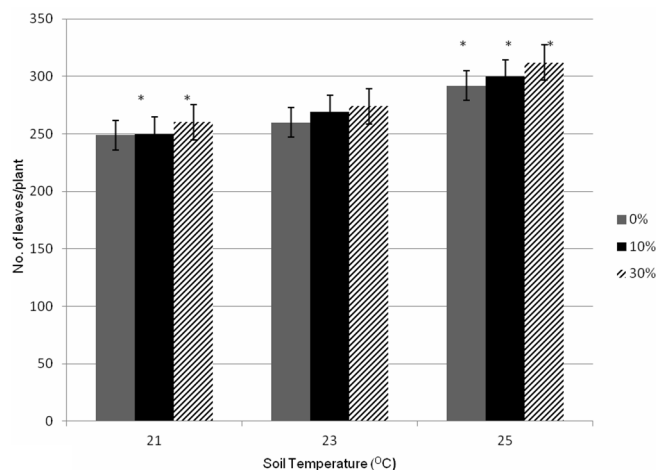


Fig. 1 Effects of enhanced soil temperature and increased simulated herbivory on number of leaves/*Q. phellos* seedlings. Increase in the number of leaves/plant in response to simulated leaf herbivory was only significant at 30% defoliation ($F_{(2,33)} = 33.62$, $P < 0.0001$) across all temperature regimes. While there was a corresponding increase in leaves/plant across the temperature regimes when compared to the control ($F_{(2,33)} = 58.56$, $P < 0.0001$), there was no temperature-herbivory interactions. Significance is indicated by * and values represent +/- standard error (SE).

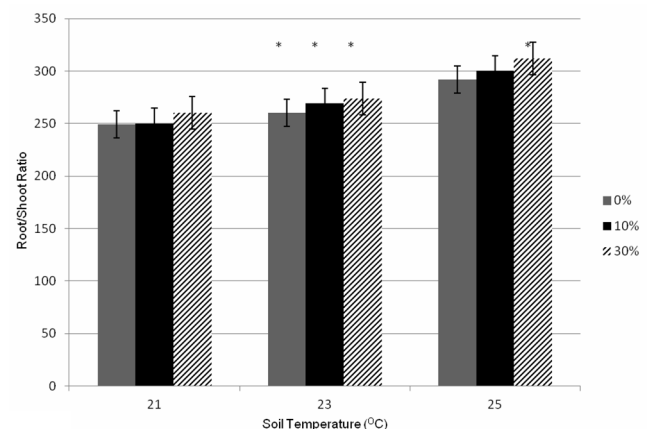


Fig. 2 *Q. phellos* responded to increased herbivory ($F_{(2,33)} = 67.1$, $P < 0.0001$) and increasing root-zone temperature ($F_{(2,33)} = 9.35$, $P = 0.0002$) by shifting the allocation of resources to the shoots. Significance is indicated by * and values represent +/- standard error (SE).

DISCUSSION

While *Q. phellos* responded to herbivory with an overcompensatory increase in foliage irrespective of temperature, the root-zone temperature response was significant only at 25°C (Fig. 1). The response to herbivory, and increased root-zone temperature, was a shift in the allocation of resources to the shoots indicating that leaf loss due to herbivory was compensated for at the expense of the root system.

There was a positive interaction between root-zone temperature and herbivory resulting in a greater shift in resources than expected if responses were additive (Fig. 2). This increase in foliage, and the subsequent shift in resources, may not accurately reflect the response of older *Quercus* individuals, as younger trees, such as used in this study, exhibit greater response to defoliation than mature individuals (Boege 2005). However, if the resultant shift in resource allocation by *Q. phellos* in response to herbivory and to increased root-zone temperature is a long-term strategy, the ultimate survival of this wetland species may be in question because fewer resources to the roots results in decreasing root growth, ultimately resulting in a decrease in both nutrient and water uptake for the plant.

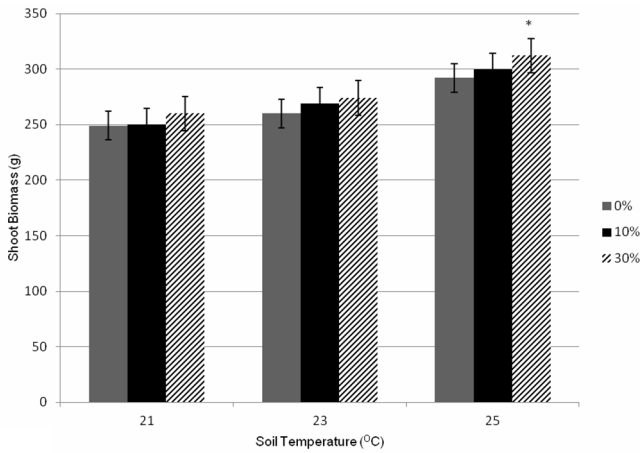


Fig. 3 *Q. phellos* decreased shoot biomass in response to herbivory ($F_{(2,33)} = 88.47, P < 0.0001$). Significance is indicated by * and values represent +/- standard error (SE).

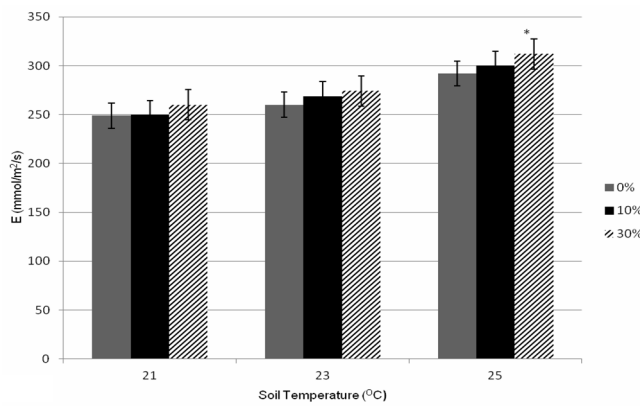


Fig. 4 Transpiration rate (E) decreased in *Q. phellos* plants ($F_{(2,33)} = 60.06, P < 0.0001$) in response to herbivory. Significance is indicated by * and values represent +/- standard error (SE).

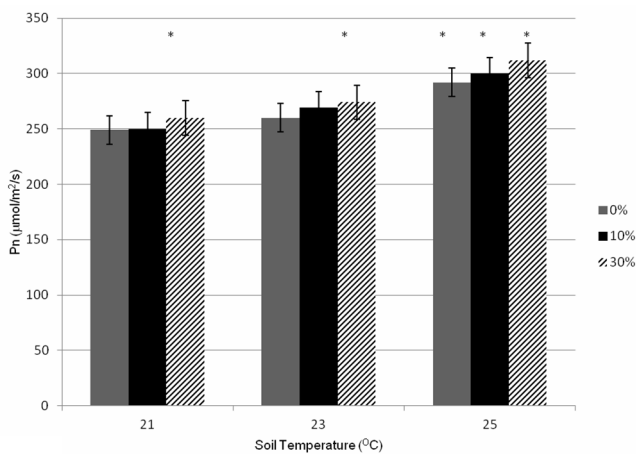


Fig. 5 Net photosynthesis (P_n) in *Q. phellos* plants decreased in response to increased soil temperature ($F_{(2,33)} = 170.07, P < 0.0001$) and in response to herbivory ($F_{(2,33)} = 259.85, P < 0.0001$). Significance is indicated by * and values represent +/- standard error (SE).

The overcompensatory foliage increase, measured as an increase in number of leaves (Fig. 1) was offset by smaller leaf size, indicated by an overall decrease in shoot biomass decrease in response to increasing herbivory (Fig. 3). The combination of increasing temperature and increasing herbivory resulted in smaller overall plants due to tissue loss and decreases in foliage growth.

Herbivory had no effect on transpiration rates (E) however, there was a trend for (E) to decrease in *Q. phellos*

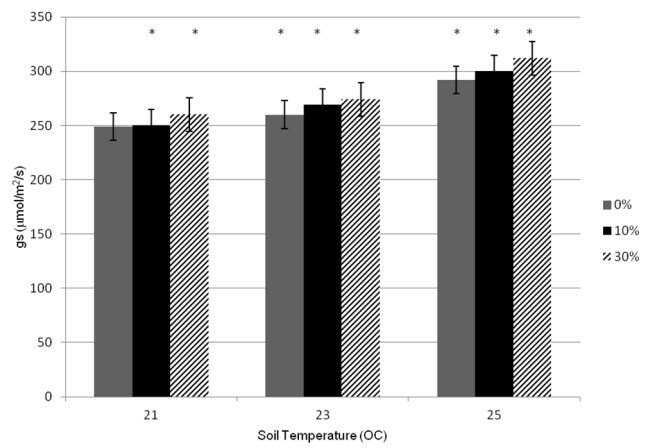


Fig. 6 *Q. phellos* decreased stomatal conductance (gs) in response to both herbivory ($F_{(2,33)} = 443.8, P < 0.0001$) and root-zone temperature ($F_{(2,33)} = 85.8, P < 0.0001$) increases with an positive interaction effect at 25°C and 30% herbivory ($F_{(2,33)} = 17.64, P < 0.0001$). Significance is indicated by * and values represent +/- standard error (SE).

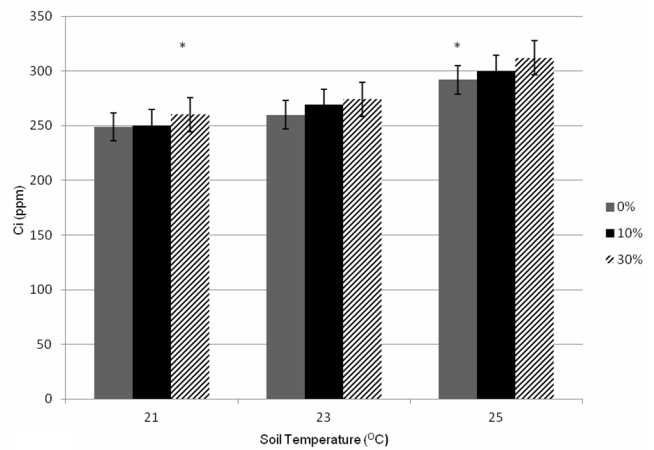


Fig. 7 *Q. phellos* increased internal C concentrations (C_i) in response to herbivory ($F_{(2,33)} = 26.19, P < 0.0001$) and temperature ($F_{(2,33)} = 4.39, P = 0.15$). Significance is indicated by * and values represent +/- standard error (SE).

in response to increasing root-zone temperature with significance only found at 25°C (Fig. 4) unlike a compensatory transpiration rate reported by Quentin *et al.* (2011) in response to 45% defoliation resulted in compensatory transpiration. As the *Q. phellos* trees were maintained in a well-watered soil condition this decrease in E was not a reflection of negative soil water conditions and is more likely a result of shifting physiological demands related to foliage production overcompensation. However, a warmer rhizosphere, and the increased water demand generated by increased foliage development, can result in stomatal regulation and decreased E.

The response of *Q. phellos* to a root-zone temperature of 25°C and herbivory at 30% was a decrease in Pn (Fig. 5) due to decrease in stomatal conductance (gs) (Fig. 6) which would inhibit both E and gas exchange. Temperature and herbivory were also interactive. However, decreased Pn can also result from metabolic (non-stomatal) inhibition (Pezeshki 1993). The decrease in Pn also helps to explain the increase in internal C concentration (C_i) in response to high levels of herbivory (30%) and high soil temperature (25°C) (Fig. 7).

In conclusion, *Q. phellos* responded to increasing soil temperature by increasing foliage, which helps to explain the shift in resources to the shoot system, and a decrease in transpiration and a concomitant decrease in Pn rates. In response to herbivory *Q. phellos* exhibits a foliage decrease followed by a significant increase in foliage as herbivory

levels increase, once again shifting resources to the shoot system with declines in Pn and transpiration rates. It appears *Q. phellos* has a threshold response to increased soil temperature and increased defoliation which limits stomatal functioning leading to declining Pn and E indicating possible negative consequences for this species if global temperatures increase.

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