

Full Length Research Paper

Root and leaf changes in *Salix nigra* cuttings in response to increasing soil temperature

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***Salix nigra*, black willow, is used for wetland restoration and streambank stabilization and like other wetland species has the ability to develop aerenchyma tissue to avoid root anoxia. Root aerenchyma development could be affected if the temperature of the rhizosphere increases as a result of climatic change. Root porosity (used as an indication of aerenchyma tissue) of *S. nigra* cuttings grown under three soil temperature regimes was found to increase in response to increases in temperature ($F_{2,40} = 56.57$; $p < 0.0001$). There was also an increase in internal C (carbon) concentration ($F_{2,27} = 27.36$; $P < 0.0001$) and a corresponding decrease in stomatal conductance ($F_{2,27} = 3.728$; $P = 0.037$), transpiration rate ($F_{2,27} = 5.084$; $P = 0.013$), and net photosynthesis ($F_{2,27} = 14.07$; $P < 0.0001$) in response to increased soil temperature. The present research demonstrated that the stress response of *S. nigra* to increased soil temperature is similar to its response to anoxic conditions.**

Key words: Aerenchyma, global warming, *Salix nigra*, remediation, rhizosphere, riparian systems.

INTRODUCTION

Salix nigra Marsh. (Black willow) is a ubiquitous member of bottomland hardwood forests and floodplains of the southeastern United States. Due to its ease of propagation and rapid growth, not only is *S. nigra* a commercial important species (Lyra et al., 2006), its cuttings are used in wetland and riparian remediation and restoration projects (Schaff et al., 2003).

S. nigra, as with many wetland plant species, possesses several physiological and morphological adaptations, such as adventitious rooting (Li et al., 2006) and the development of aerenchyma tissue (Visser et al., 1996), that allow it to tolerate a variety of moisture regimes. Aerenchyma, a parenchyma tissue having a large volume of intercellular space, forms air passages within the cortex of roots and stems to facilitate the transport of O₂ from shoots to roots (Green, 2010), thus alleviating O₂-deficiency within roots (Pezeshki, 1991, 1993). This O₂ transport function of aerenchyma tissue is especially critical under anoxic soil conditions (Colmer,

2003) such as found in wetlands. Development of aerenchyma tissue can also be induced by other stresses, such as soil compaction (He et al., 1996), nutrient deficiency (Postma and Lynch, 2011a), and UV-B and Cu exposure (Baud and Pezeshki, 2011). In *S. nigra*, as with many wetland species, aerenchyma development is constitutive (Li et al., 2006), that is, developing in the absence of environmental stimuli.

Anthropogenic climatic change has been documented on every continent (Du et al., 2011; Hoegh-Guldberg, 2005; Walther et al., 2005) and has already affected biota (Chambers et al., 2011; Memmott et al., 2007). The major climatic constituents determining plant distribution and growth are temperature and precipitation (Aber et al., 2001). In the past, trees were able to adjust to climatic warming (Jacobson and Dieffenbacher-Krall, 1995); however, the rapid rate of temperature increase seen in the last few decades (Hansen et al., 2006) has led to changes in both species distribution and abundance (Hughes, 2000). Evidence indicates that climatic change is likely to continue over the next 50 to 100 years (IPCC, 1996) with temperature increase especially pronounced in the northern latitudes (Hulme et al., 1999).

The effect of increased temperature on photosynthetic processes at temperate latitudes is probably positive

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(Saxe et al., 2001) due to increases in photosynthetic pigments as temperature increases (Ormrod et al., 1999), more beneficial, due to avoidance of photoinhibition with a resultant increase in net photosynthesis, then similar day-time temperature increases (Germino and Smith, 1999). However, different species respond differently to warming (Sun and Sweet, 1996), including increases in soil temperature (Graves and Aiello, 1997).

Increased soil temperature could result in a decline in O₂ availability to the roots and thus inhibit root growth. Declines in root growth have been shown to limit photosynthesis via feedback inhibition (Pezeshki and Santos, 1998). Likewise, enhanced soil temperature may increase photorespiration, resulting in increased leaf temperature which effects enzyme functioning in photosystem II. Likewise, an increase in stomatal resistance would shift the [O₂] to [CO₂] ratio making O₂ more attractive to RuBP which enhances photorespiration. Therefore, increased soil temperature, as a result of global warming, could initiate a stress response in wetland tree species that induces a cascade of reactions leading to increased aerenchyma development.

We tested the hypotheses that *S. nigra* responses to increases in soil temperature by 1) increasing aerenchyma tissue formation, measured as increases in root porosity, 2) shifting the root: shoot ratio in favor of the roots, 3) increasing internal C concentrations, 4) decreasing net photosynthesis, 5) decreasing transpiration, and 6) decreasing stomatal conductance.

MATERIALS AND METHODS

Plant materials

S. nigra clones were collected along the Coldwater River in Coldwater, MS, USA, trimmed to cuttings 9.5 cm in length with a basal diameter of 7 mm, and had all existing branches removed to conform to common planting practices.

Experimental procedures

S. nigra cuttings were grown in a 2:3 sand:soil mixture utilizing 10.5 cm height × 7 cm diameter plastic pots. The potted cutting were randomly divided into thirds and grown under soil temperatures of 25, 23, or 18°C (ambient temperature). Temperature was maintained by a thermostatically controlled heat pad with the pots either placed directly on the pad for 25°C or elevated 1 cm above the pad for 23°C. No heat was applied for maintaining ambient soil temperature; however, data showed a consistent temperature of 18°C.

The cuttings were exposed to an 18 hour photoperiod of photosynthetic photon flux density (PPFD) averaging 800 - 1000 μ moles photons/m²/sec: conducted in the laboratory equipped with supplemental light, illuminated by four 400 W high pressure sodium and four 400 W metal halide lamps in water-cooled ballasts, providing 800 – 1000 μmols m⁻² s⁻¹ PPFD at the leaf canopy level.

There were 12 replicates per temperature treatment. All cuttings

were well-watered and were fertilized bi-weekly using Peters Professional 20-20-20 at 5 g/L. 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 were recorded using a Model 250 A ORION millivolt redox meter and a calomel reference electrode (Thermo Orion, Beverly, MA, USA).

On day 64, net photosynthesis (P_n), stomatal conductance (g_s), internal CO₂ (C_i), and transpiration (E) were measured using a portable gas exchange analyzer (Model CIRAS 2, PP Systems, Haverville, MA, USA). On day 65, the cuttings were harvested and divided into roots and shoots to determine resource allocation. Changes in aerenchyma was measured as changes in root porosity (POR), the gas space volume as a percentage of the total root volume, utilizing the method described by Li et al. (2006). Shoots and roots were then dried at 70°C to a constant weight and the final weights were 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 one-way ANOVA with differences at or less than p > 0.05 were considered significant.

RESULTS AND DISCUSSION

S. nigra responded to an increase in soil temperature by increasing aerenchyma tissue (measured as POR) (F_{2,40} = 56.57; P < 0.0001) from 26 to 40% for a 5°C increase and to 46% for a 7°C increase (Figure 1), indicating a stress response similar to that reported by Baud and Pezeshki (2011) for *S. nigra* in response to UV-B and Cu exposure. This plasticity and rapid response (65 days) to increased temperature at the rhizosphere level, by increasing aerenchyma development within the roots, allowed an increased flow of oxygen from shoots to roots. The risk of root damage is further reduced through radial oxygen loss to the rhizosphere (Jackson and Armstrong, 1999). Unlike some species that correlate an increase in POR with a shift in resources allocation (Postma and Lynch, 2011b), the response of *S. nigra* to increased aerenchyma development did not include a re-allocation of resources.

Net photosynthesis (P_n) decreased (F_{2,27} = 14.07; P < 0.0001) in *S. nigra* in response to increases in root-zone temperature (Figure 2), possibly a result related to excessive internal temperatures adversely affect protein functioning (Berry and Bjorkman, 1980). The increase in C_i (F_{2,27} = 27.36; P < 0.0001) (Figure 3) found in *S. nigra* is a result of excessive temperature within the leaf tissue leading to increased photorespiration. Increase in internal temperature is a result of a decrease in E (F_{2,27} = 5.084; P = 0.013) (Figure 4), itself the result of the plant responding to stress conditions by closing the stomata. A partial closing of stomata was indicated by decreased g_s

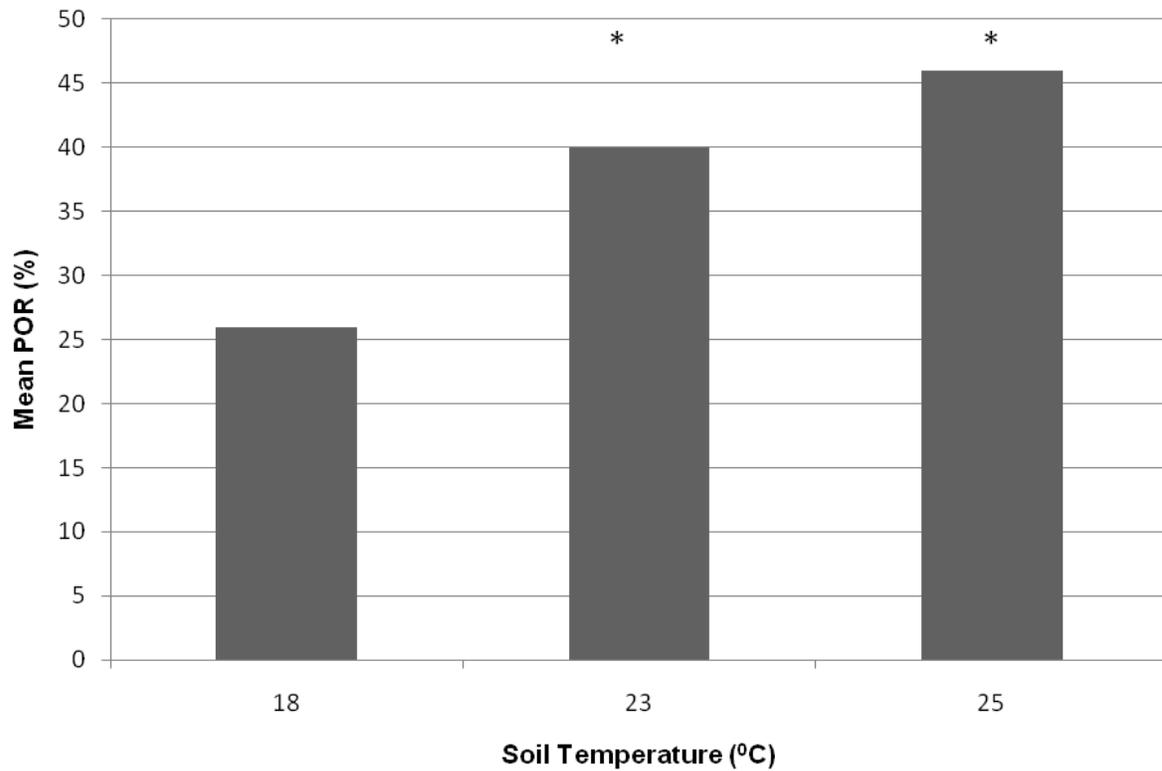


Figure 1. *S. nigra* responded to increased soil temperature by increasing aerenchyma tissue development, measured as POR ($F_{2,40} = 56.57$; $P < 0.0001$).

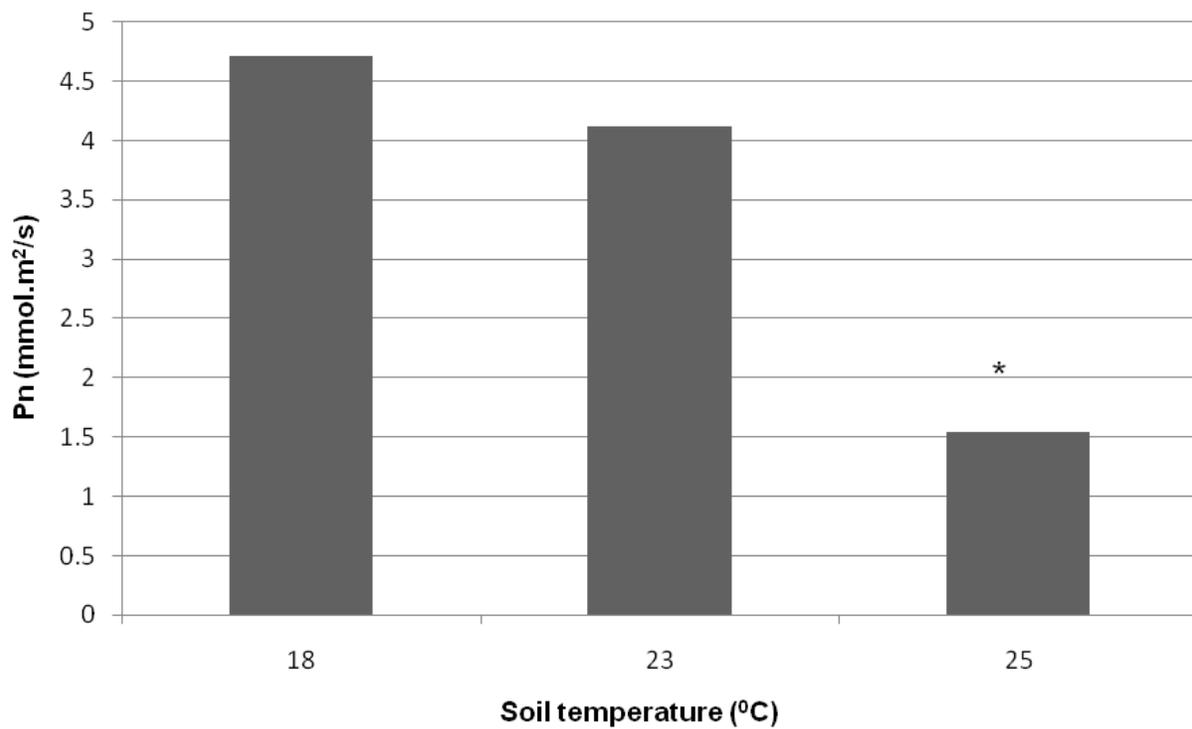


Figure 2. Net photosynthesis (Pn) decreased ($F_{2,27} = 14.07$; $P < 0.0001$) in *S. nigra* in response to increasing soil temperature.

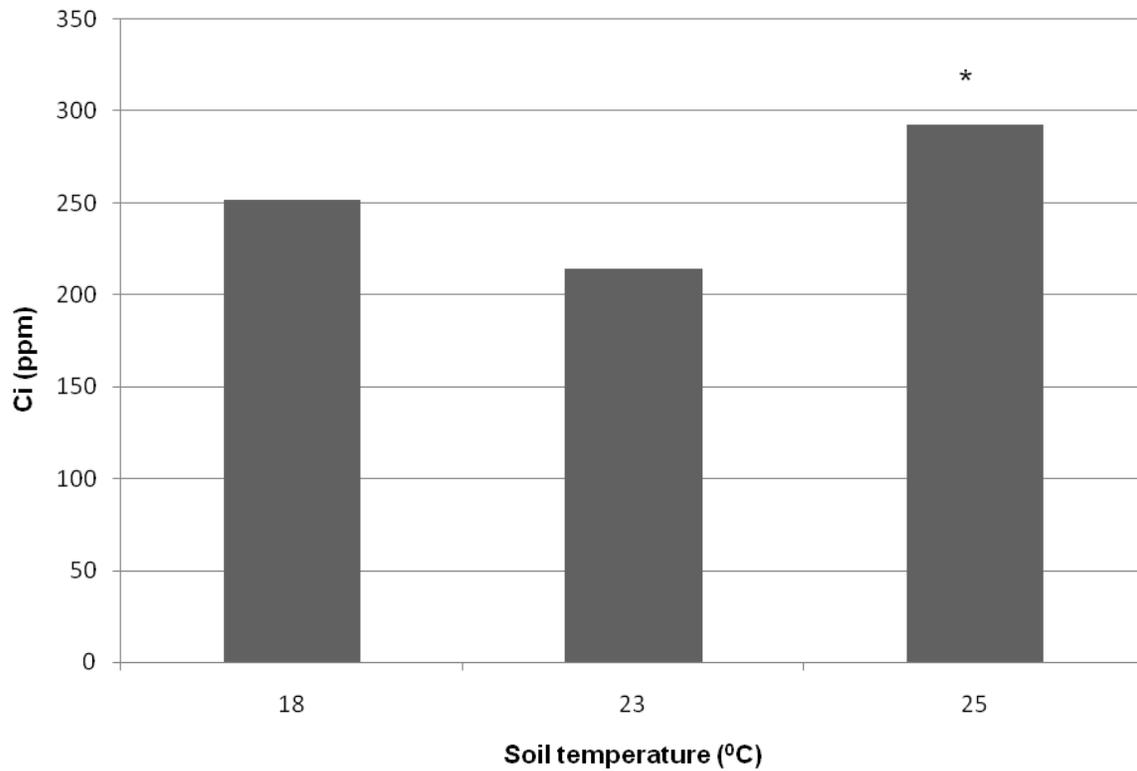


Figure 3. Internal C concentration (Ci) increased ($F_{2,27}= 27.36$; $P < 0.0001$) in *S. nigra* response to increased soil temperature.

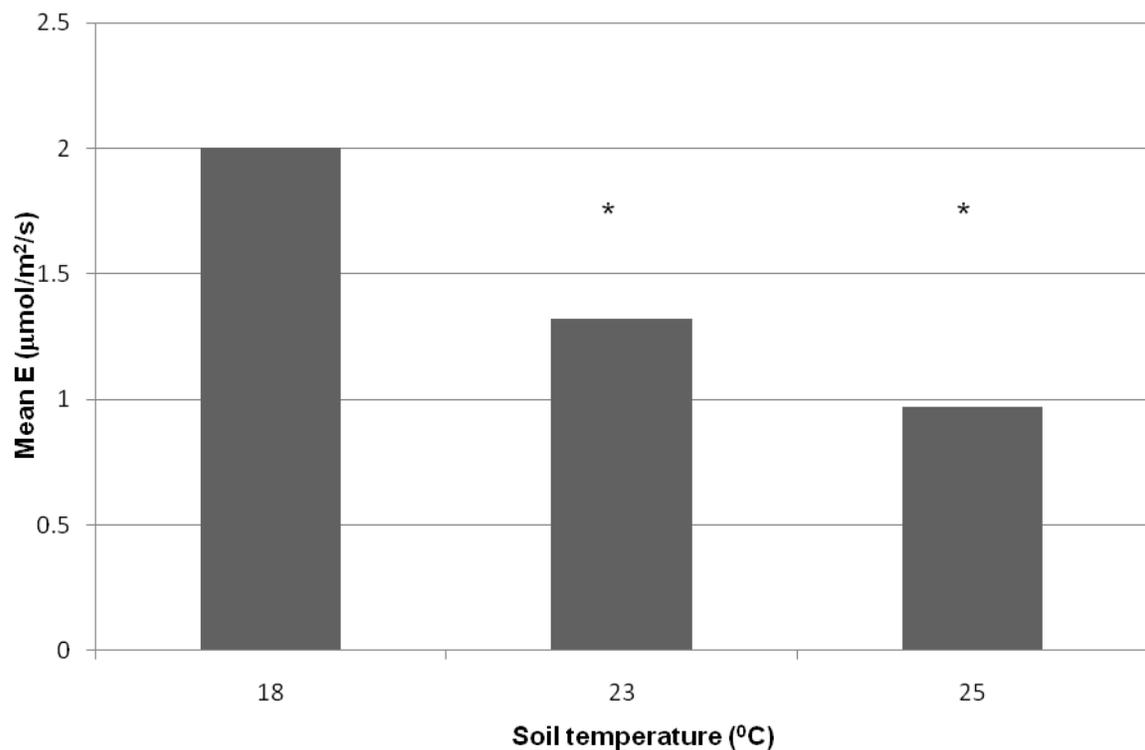


Figure 4. Transpiration rate (E) decreased ($F_{2,27}= 5.084$; $P = 0.013$) in *S. nigra* in response to increased soil temperatures.

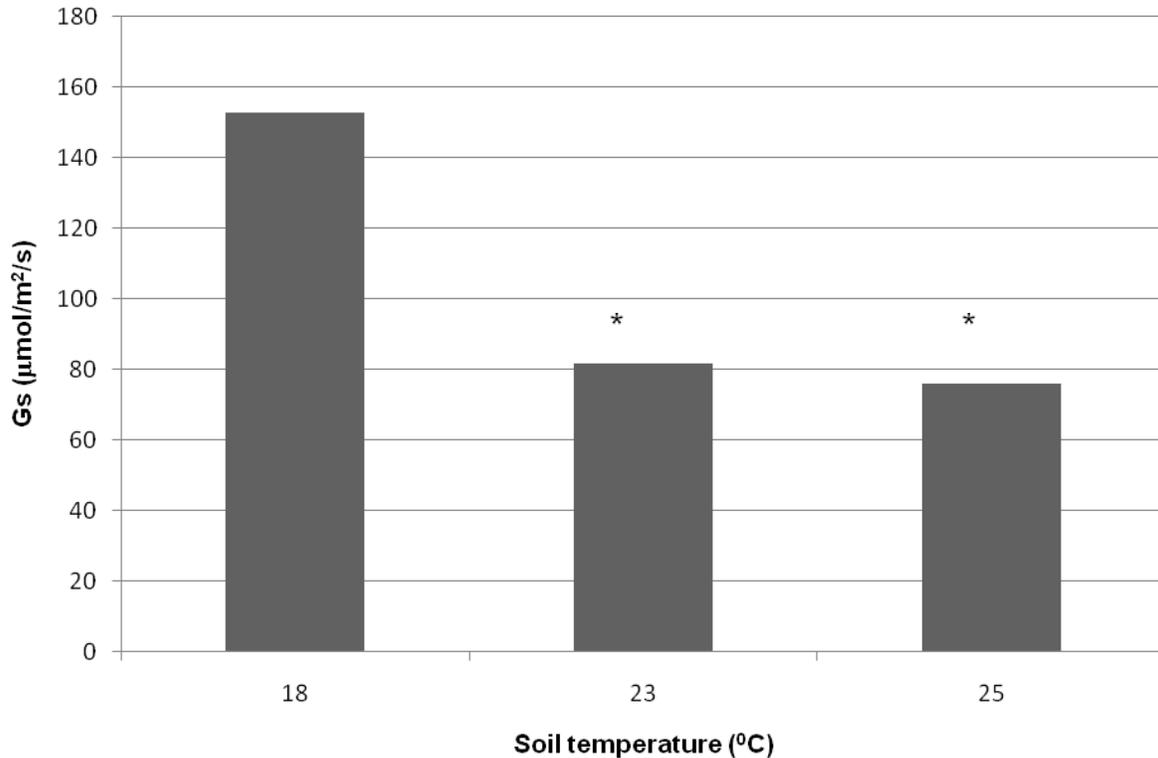


Figure 5. Stomatal conductance (gs) decreased ($F_{2,27} = 3.728$; $P = 0.037$) in *S. nigra* in response to increased soil temperature.

($F_{2,27} = 3.728$; $P = 0.037$) (Figure 5). Therefore, the increase in C_i with the concomitant decrease in E and g_s would explain the decrease in P_n . These responses to the stress placed upon the adventitious roots by increased soil temperatures are similar to responses found for anoxic soil conditions, although soil redox measurements remained above + 400 mV, indicating anoxic conditions were not present. Increased soil temperature leads to a decline in O_2 availability to the roots. The response to this potential anoxic condition involves increasing aerenchyma tissue to enhance the availability of O_2 to the roots and decreasing g_s to inhibit water loss. As g_s decreases E decreases leading to increased C_i which ultimately leads to a decrease in P_n through feedback inhibition.

The response of *S. nigra* to elevated soil temperature is a preview of possible limitations global warming places upon woody wetland species. Over time, the decrease in P_n can lead to a decrease in fitness and possibly the survival of *S. nigra* as a wetland species.

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