

Physiological responses of black willow (*Salix nigra*) cuttings to a range of soil moisture regimes

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Abstract

We quantified the physiological responses of black willow to four soil moisture regimes: no flooding (control, C), continuous flooding (CF), periodic flooding (PF), and periodic drought (PD). Stomatal limitation was one of the factors that led to the reduced photosynthetic capacity in CF cuttings. Under PD, stomatal closure, decreased leaf chlorophyll content, and increased dark fluorescence yield contributed to photosynthetic decline. CF cuttings accumulated the lowest shoot biomass while the final height and root growth were most adversely affected by PD. PF cuttings tended to allocate more photoassimilates to root growth than to shoots.

Additional key words: chlorophyll *a* fluorescence; drought; flooding; growth; photosynthesis; stomatal conductance.

Introduction

Black willow is commonly found in floodplains and bottomland hardwood forests of the southeastern United States (Mitsch and Gosselink 1993). It is an excellent species for erosion control because it is propagated easily from cuttings, rapidly produces adventitious roots, grows quickly, and provides soil stabilization (Schaff *et al.* 2003). However, many projects in the southeastern US have reported low survival rates, less than 6 % in some cases (Wolfe 1992). The exact causes of the poor performance are still obscure. Previous studies indicate that poor performance is associated with plant locations either too high or too low relative to the groundwater table and with soils with relatively high silt-clay content (Pezeshki *et al.* 1998a, Shields *et al.* 1998, Schaff *et al.* 2003). Controlled studies have demonstrated adverse impacts due to excessive soil moisture (flooding) and soil moisture deficits (drought) (Pezeshki *et al.* 1998b).

Black willow is subjected to dynamic hydrologic conditions in riparian systems. Depending on the slope and depth to base flow, black willow may be exposed to continuous flooding, periodic flooding, and periodic drought. Net photosynthetic rates (P_N) also demonstrated the sensitivity of willow cuttings to these two conditions (Pezeshki *et al.* 1998b). However, information regarding the mechanisms underlying such photosynthetic respon-

ses is scant. Therefore, data are needed to determine the major contributing factors to the photosynthetic declines in black willow in response to soil flooding and drought. Such data are critical to our understanding of the causes of the poor performance of this species in some stream corridor restoration projects and may also help understand the mechanisms involved in plant responses to flooding and drought.

Decreases of photosynthesis due to flooding and water deficit have both been reported for many species (*e.g.* Gimenez *et al.* 1992, Pezeshki 2001, Elcan and Pezeshki 2002, Fortini *et al.* 2003). However, the response mechanisms involved have not been clearly identified. Literature has indicated that the decline of photosynthetic capacity may be attributed to both stomatal and non-stomatal limitations (Pezeshki 2001, Panković *et al.* 1999).

Chlorophyll (Chl) fluorescence can provide insights into the ability of a plant to tolerate those environmental stresses and into the extent to which those stresses have damaged the photosynthetic apparatus (Fracheboud *et al.* 1999, Maxwell and Johnson 2000). Therefore, damages to the photosynthetic machinery may impose an additional non-stomatal limitation to the photosynthesis under flooding or water deficit (Souza *et al.* 2004).

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This experiment was designed to examine the photosynthetic and growth responses of black willow under flooding and drought and to determine the factors that are responsible for such responses. We hypothesized that both stomatal limitation and non-stomatal limitation could contribute to the decreased carbon uptake and thus

Materials and methods

Plants: Black willow cuttings were collected from a localized population on the Loosahatchie River in western Tennessee, USA in March 2002 while plants were dormant. Each cutting was ~0.60 cm in diameter at the base and 38 cm in length. All existing branches were removed from each cutting to conform to common planting practices.

Experimental procedures: Prior to planting, the cuttings were kept in 4 °C refrigerator with the bottom half remained moist. Cuttings were planted on April 20 in a greenhouse. Pots 20 cm high and 20 cm in diameter were filled with two parts sand and one part soil (v/v). Holes were drilled on the side to allow control of soil moisture regimes. One single cutting was planted in each pot with 1/3 of the cutting below ground and 2/3 above ground. The study was conducted in an air-conditioned greenhouse with an average daily low temperature of 27 °C and average daily high temperature of 40 °C. Natural light in the greenhouse supplied a photon flux density (PPFD) of 500–700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the plant canopy during sunny days. Plants were maintained under well-watered and well-drained conditions prior to the treatment initiation. Cuttings were fertilized with 50 cm³ of 20-20-20 *Peters Fertilizer* mixed with tap water at 1.25 kg m⁻³ on June 12 and June 20. The mean total biomass (root+shoot, excluding the original cutting) per plant was 3.42±0.86 g and mean height was 30.50±1.82 cm when the treatments were initiated on July 3. Plants grew under each treatment for 56 d after treatment initiation.

Four treatments were used to test the responses of willow cuttings to soil moisture regimes. The treatments represented a relatively wide range of soil moisture that could be expected in the fields during the growing seasons. These treatments were (1) control (C): cuttings were watered daily and allowed to drain freely; (2) CF: cuttings were flooded to 5 cm above the soil surface throughout the experiment; (3) PF: plants were inundated to 5 cm above the soil surface for one week then drained for one week—this treatment cycled through the study period for a total of four flooded periods and four drained periods; and (4) PD: water was withheld until the soil water potential dropped from about -0.02 MPa to between about -0.05 and -0.60 MPa, which occurred on days 7, 21, 34, 42, and 56. The experiment followed a completely randomized design. Each treatment consisted of a total of 15 replicate cuttings.

the growth reduction of black willow cuttings under continuous flooding (CF), periodic flooding (PF), and periodic drought (PD). Stomatal limitation is symbolized by decreased stomatal conductance (g_s) and non-stomatal limitations may include decreased Chl content and photo-damage.

Soil measurements: Soil redox potential (Eh) was monitored at 10 cm below the soil surface using platinum-tipped electrodes, a model 250 A *ORION* millivolt redox meter, and a calomel reference electrode (*Thermo Orion*, Beverly, MA, USA) as described in detail in Patrick and DeLaune (1977). Measurements were replicated six times per treatment per measurement day for C, CF, and PF treatments on days 7, 14, 21, 28, 35, 42, and 49. An Eh value of +350 mV represented the approximate level at which oxygen begins to disappear from the soil. Well-aerated condition was represented by +400 to +700 mV, while reduced condition may be as low as -300 mV (DeLaune and Pezeshki 1991). Soil water potential was monitored using a model *KS-D1* soil moisture tester (*Delmhorst*, Towaco, NJ, USA) everyday for C and PD treatments. Six gypsum soil blocks per treatment were placed at depth of 10 cm in soil (one per pot). Meter readings were interpreted in terms of soil moisture tension and converted to MPa.

Photosynthetic responses of C, CF, and PF plants were determined on days 7, 14, 21, 28, 35, 42, and 49 and of C and PD plants on days 7, 21, 34, 42, and 56 (before water was added to PD each time). All photosynthetic measurements were done between 09:00 and 12:00. P_N and g_s were measured on five sample leaves per treatment (one leaf per plant) per measurement day on the third fully-developed leaf from the tallest shoot apex using a portable gas exchange analyzer (*CIRAS 1*, *PP Systems*, Haverville, MA, USA). Immediately after the measurement of gas exchange, leaf Chl content was recorded on the same leaves using a *CCM-200* meter (*Opti-Sciences*, Tyngsboro, MA, USA).

Leaf Chl fluorescence was measured with the model *OS-100* Modulated Fluorometer (*Opti-Sciences*, Tyngsboro, MA, USA) on the same leaves that were used for P_N , g_s , and Chl determination. Three records were made as follows: (1) at predawn, dark fluorescence yield (F_0), (2) at predawn, the efficiency of excitation capture of open PS2 (F_v/F_m): $F_v/F_m = (F_m - F_0)/F_m$, where F_m is the maximal fluorescence obtained with a 0.8 s saturation flash, and (3) under ambient irradiance in the morning, yield of energy conversion (Y): $Y = (F_{sm} - F_s)/F_{sm}$, where F_{sm} is the maximal fluorescence obtained with a 0.8 s saturation flash under steady state conditions, and F_s is the steady state fluorescence yield under ambient irradiance.

Plant growth: Plant height was measured at the conclusion of the experiment (day 56) for all studied plants. Then each cutting was separated into aboveground and belowground portions. The aboveground biomass was further divided into shoot (branches and leaves) and aboveground cutting. Belowground biomass was separated into root and belowground cutting. All biomass components were dried at 70 °C to a constant dry mass (DM).

Statistics: This experiment was replicated once in 2003 using similar experimental procedures. Two-way

ANOVA (*SPSS 11.5*) with soil moisture regime and day of measurement was used to test the differences in means for photosynthetic responses including P_N , g_s , Chl content, F_0 , F_v/F_m , and Y between C, CF, and PF as well as between C and PD groups. One-way ANOVA (*SPSS 11.5*) was used to test the differences in height, root biomass, shoot biomass, and root/shoot ratio across all four soil moisture treatments. Tukey procedure was used to examine all pair-wise group differences. Differences were considered significant at $p < 0.05$.

Results

Soil measurements: Soil Eh in C remained above +407 mV for the duration of the experiment, indicating sufficient oxygen in this treatment. But in CF the soil Eh was mildly reduced (+296 to +18 mV). In PF treatment, soil Eh cycled between reduced and oxic in response to

flooding and draining (Fig. 1, *top*). Soil water potential remained around -0.02 MPa in C group. Water was withheld in the PD treatment until the soil water potential dropped to between around -0.5 and -0.6 MPa, which were on days 7, 21, 34, 42, and 56 (Fig. 1, *bottom*) and

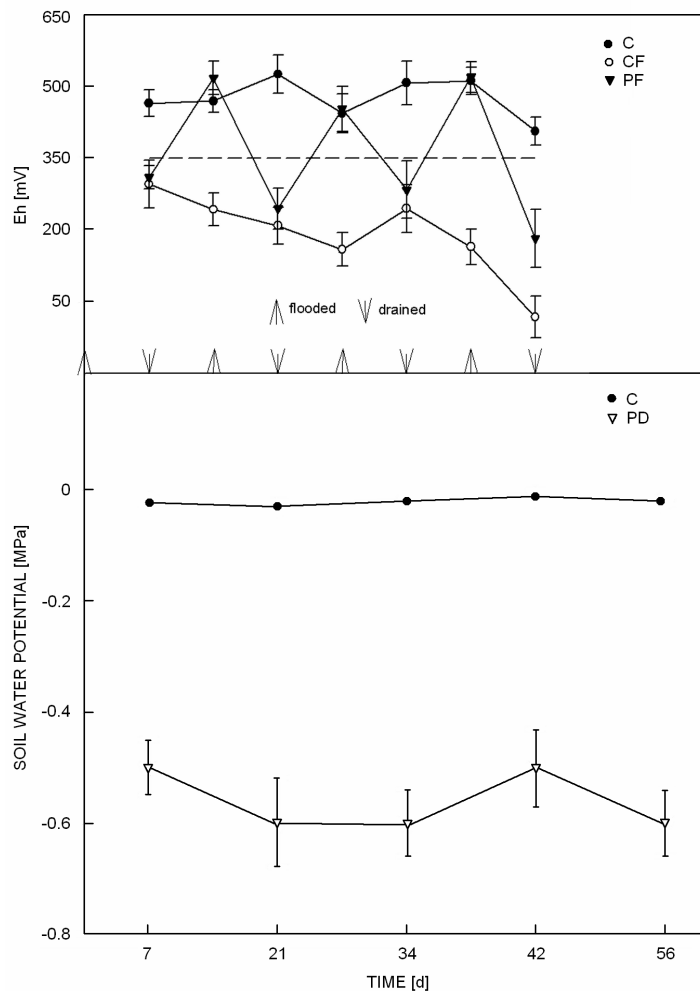


Fig. 1. Time-course of soil redox potential (Eh) (*top*) and soil water potential (*bottom*) recorded for control (C), continuous flooding (CF), periodic flooding (PF), and periodic drought (PD) treatments. The dashed line at +350 mV represents the approximate level at which oxygen begins to disappear from the soil (DeLaune and Pezeshki 1991). Each value is the mean for six replications \pm SE. The arrows symbolize the initiation of flooding and draining for the PF treatment.

indicated low water content in the soil. Soil water potential recovered to about -0.02 MPa after water was added.

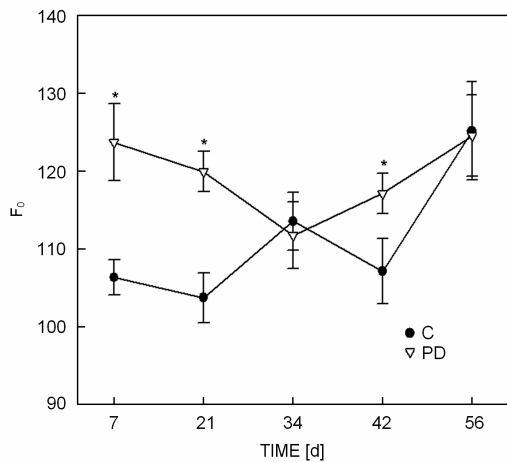


Fig. 2. Time-course of F_0 for black willow cuttings in control (C) and periodic drought (PD) plants. Means of five replications \pm SE. *, significant difference between PD and C on that sampling day.

Photosynthesis: In C, CF, and PF treatments, there was no significant interaction between soil moisture regime and day of measurement for P_N ($p=0.941$), g_s ($p=0.260$), Chl content ($p=0.453$), or Chl fluorescence parameters (F_0 , $p=0.590$; F_v/F_m , $p=0.857$; Y , $p=0.910$). Therefore, the main effect of soil moisture on photosynthetic function was tested. Only P_N ($p=0.027$) and g_s ($p=0.035$) showed differences among these three soil moisture regimes. In CF cuttings, P_N and g_s were 22 and 28 % lower than those in C.

There was no significant interaction between soil moisture treatment and day for P_N ($p=0.701$), g_s ($p=0.188$), and Chl content ($p=0.896$) under C and PD. These three parameters exhibited similar response patterns with PD treatment being lower than C by 32, 55, and 15 %, respectively ($p=0.046$, $p=0.016$, $p=0.025$, respectively). For Chl fluorescence parameters, only the interaction between treatment and day was detected for F_0 ($p=0.003$). Increases of F_0 were observed in PD group on most measurement days (days 7, 21, and 42; Fig. 2). There was no interaction between soil moisture treatment and day of measurement for F_v/F_m ($p=0.415$) and Y

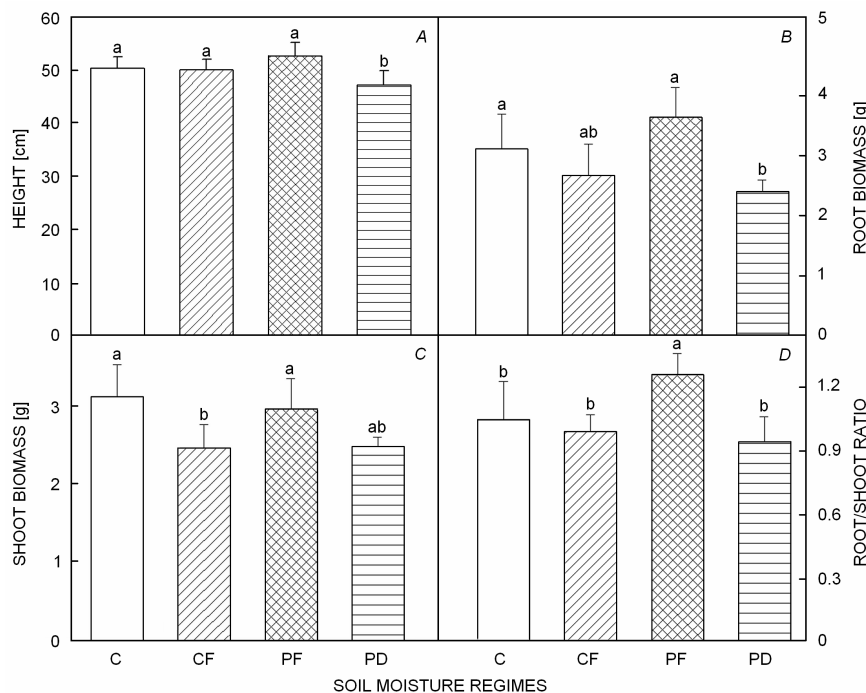


Fig. 3. Height (A), root biomass (B), shoot biomass (C), and shoot/root ratio (D) for black willow cuttings in four soil moisture regimes: control (C), continuous flooding (CF), periodic flooding (PF), and periodic drought (PD). Means for 15 replications \pm SE. Significant differences are shown across treatments using different letters.

($p=0.833$). In addition, PD did not have any effects on these two parameters ($p=0.739$ and $p=0.256$, respectively).

Plant growth: Final height was not significantly different across treatments except in PD that led to the lowest final height of 47.2 cm ($p=0.039$, Fig. 3). The root and shoot

biomass showed differences across treatments ($p=0.022$, $p=0.036$, respectively). Cuttings in C and PF groups accumulated the greatest root and shoot biomass. The lowest root biomass (2.38 g per plant) was recorded under PD and lowest shoot biomass (2.46 g per plant) under CF. The root/shoot ratio in PF was 1.26, which was higher than other treatments ($p=0.020$, Fig. 3).

Discussion

Black willow cuttings exhibited decreased P_N in both CF and PD treatments, confirming an earlier study which reported the sensitivity of this species to flooding as well as drought (Pezeshki *et al.* 1998b). This finding may partially explain the poor performance of black willow cuttings at some restoration sites (Wolfe 1992, Shields *et al.* 1995), particularly at high and low elevations relative to the stream (Watson *et al.* 1997). However, we did not find the photosynthetic decline in PF cuttings reported by Pezeshki *et al.* (1998b). The reason is that the range of soil Eh in the flooding cycle was milder in present study than the previous one.

Reduced plant photosynthetic capacity in response to soil flooding is common among many species. Mielke *et al.* (2003) reported that between 14 and 63 d after flooding, P_N in *Genipa americana* seedlings was only 52.9 % of control. Several mangrove seedlings also showed a significant decrease in P_N in response to root anoxia (Youssef and Saenger 1998). The photosynthetic decline has been attributed to stomatal closure and metabolic (non-stomatal) inhibition (Pezeshki 1993). The present study indicated that g_s corresponded to the P_N responses in C, CF, and PF cuttings. Therefore, stomatal limitation was one of the factors that contributed to the observed reduction in P_N of black willow in response to CF. Mielke *et al.* (2003) also concluded that stomatal limitation of photosynthesis was the main cause of reduced carbon uptake and plant growth in flooded *G. americana* seedlings.

Likewise, stomatal regulation of photosynthesis due to water deficit has been well documented. By decreasing available internal CO_2 concentration, stomata closure is dominant and leads to the decline in P_N (Chaves 1991, Cornic 2000, Llusià and Peñuelas 2000). The decreased g_s along with reduced P_N under PD condition were noted in our study. In addition, decreased Chl content was observed under this treatment, which also likely contributed to the decline in P_N . Increased drought stress severity caused large decreases in P_N and leaf Chl content in *Eragrostis curvula* cv. Ermelo (Colom and Vazzana 2003). Meanwhile, enhanced F_0 on many of the measurement days was recorded (Fig. 2) for plants in PD treatment, which suggested a down regulation of photochemistry.

We did not find any differences in F_v/F_m among treatments. Therefore, F_m was also influenced by drought stress in a similar manner to F_0 as was reported in other species (Li *et al.* 2004). The present study revealed the similar response patterns between g_s , leaf Chl content, F_0 , and P_N . This suggested that under drought, whereas stomatal closure played a role in limiting photosynthesis, non-stomatal limitation such as decreased Chl content and increased F_0 was also present.

Wetland plants growing under flood tend to have small and shallow root system (Pezeshki 2001). However, at the end of this experiment, root biomass in PD was lower than other treatments (Fig. 3). Together with final height (Fig. 3), the low root growth in PD suggests that black willow is more sensitive to drought than to flooding. This partially explains why black willow naturally occurs in floodplains and riparian zones (Mitsch and Gosselink 1993).

Black willow cuttings under CF accumulated the lowest shoot biomass (Fig. 3). Decreased shoot biomass increment in response to soil flooding was found for some woody species, including those in bottomland forests (Pezeshki and DeLaune 1998). Brown and Pezeshki (2000) observed that shoot DM was significantly inhibited in flooded Chinese privet, *Ligustrum sinense*.

The biomass partitioning data indicated that PF cuttings allocated more photoassimilate to root growth than to shoots as compared to cuttings in other treatments (Fig. 3). This might be because the root growth of the PF cuttings had the chance to recover during the draining periods and may have overcompensated.

Our study indicated that photosynthesis and growth of black willow cuttings in response to both reduced soil condition and moisture deficits were limited by stomatal closure. Non-stomatal factors such as decreased Chl content and increased F_0 also were involved in such responses under PD. These findings further help explain the poor performance of this species at some restoration sites that are characterized by periodic drought at higher elevations and low soil Eh conditions at the bank toe during the growing seasons. Our results also suggest that the soil moisture condition should be taken into consideration before the restoration project is undertaken.

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