Research paper

Response Variations of *Alnus subcordata* (L.), *Populus deltoides* (Bartr. ex Marsh.), and *Taxodium distichum* (L.) Seedlings to Flooding Stress

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【 Summary 】

Alnus subcordata is a native species distributed along bottomlands of Hyrcanian forests of northern Iran. In the last decade, this species along with the exotic species *Populus deltoides* and *Taxodium distichum*, has been widely used also for afforestation of bottomland areas. However, the relative flooding tolerance of these 3 species and their potential mechanisms for coping with flooding conditions are unknown to the present. Thus, in this study, variations in growth and morphophysiological responses to flooding of these species' seedlings were investigated during a 120-d outdoor experiment. Seedlings were subjected to 3 fixed treatments of 1) unflooded, 2) flooded to 3 cm in depth, and 3) flooded to 15 cm in depth, and their survival, growth, and some metabolic parameters were measured at the end of the experiment. Survival in seedlings of these 3 species was very high, but the root length, biomass accumulation, and chlorophyll content were reduced by flooding. Diameter growth in *T. distichum* increased with flooding, while it was negatively affected in the other 2 species. The leaf area, specific leaf area, and height growth were reduced in *A. subcordata* and *P. deltoides* by flooding, while no significant effect on these parameters was observed in *T. distichum*. Flooding also induced the formation of hypertrophied lenticels, and adventitious roots in all 3 species. But, proline concentrations of roots and leaves of all species did not vary with flooding. Generally, our results clearly indicated that the 3 species assayed in this experiment could be used for afforestation of riparian and floodplain regions of Hyrcanian forests, since survival percentages with 120 d of flooding were very high (> 90%).

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研究報告

檁木、楊樹及落羽杉苗木在淹水環境下生長變異之研究

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摘　要

檁木(*Alnus subcordata*)是分佈於伊朗北部Hyrcanian河灘森林的本土樹種。在過去數十年間，此樹種與引進的楊樹(*Populus deltoides*)及落羽杉(*Taxodium distichum*)廣泛地被用來作為河灘低漥地的造林樹種。然而此三種對淹水的容忍度以及其因應洪水的潛在機制目前仍不明瞭。基於此，本研究針對此三樹種的幼苗進行為期120天的戶外試驗，以探討在不同淹水情況下其生長及型態生理的變異情形。所採取的處理計有：1.不淹水(對照)；2.淹水至3 cm深；3.淹水至15 cm深等三種，並在試驗結束後進行存活率、生長情形與相關的代謝參數量測。結果顯示，三種苗木在淹水環境下的存活率均相當高，但是其根系長度、累積生物量以及葉綠素含量則有減少或降低的現象。落羽杉幼苗的徑生長量在淹水情況下有增加的現象，其他兩種苗木則呈現相反的情形。淹水會使檁木及楊樹的葉面積、比葉面積以及高生長呈現降低的現象，但對落羽杉卻無顯著的影響。此外淹水還會誘發此三種幼苗形成肥大的皮孔及不定根，但根和葉的脯氨酸濃度則不受影響。由於本試驗所選定的三種樹種在淹水120天後的存活率均相當高(>90%)，因此研究結果清楚地證實此三種樹種可作為 Hyrcanian 林地濱水帶和河灘地的造林樹種。

關鍵詞：不定根、造林、淹水容忍度、葉面積、脯氨酸、存活率。

INTRODUCTION

Continuous or temporary flooding of forest ecosystems, which occurs commonly as a result of overflowing of rivers, storms, construction of dams, and overirrigation, leads to soil anaerobiosis and consequent negative ecological effects. Roots of plants need to have a constant supply of O_2 to provide the aerial parts with adequate amounts of water, mineral nutrients, and certain hormonal growth regulators, among other compounds. When a soil is flooded, the water occupies the previously gas-filled pores, and gas exchange between the soil and air is restricted to molecular diffusion in the water of the soil (Kozlowski and Pallardy 1997). Such diffusion, however, is very slow, and the supply of O_2 to roots is limited. Flooded trees are injured much more by stagnant water (which contains less O_2) than by moving water, as moving water has a higher oxygen concentration due to the permanent mixture of deep and shallow water layers (Glenz et al. 2006). In fact, even the most flood-tolerant species (e.g., *Taxodium distichum*) may be injured by standing water (Kozlowski and Pallardy 1997). Flooding is an unavoidable environmental stress for seedlings in the plains and riparian areas and may affect normal plant functioning (Jackson and Colmer 2005). Flooding of soils during the growing season may affect woody plants

at all stages of development. Typical responses of unadapted plants to flooding include inhibition of seed germination, decreases in survival and growth, massive losses of plant biomass, changes in plant morphology and anatomy, suppression of vegetative and reproductive growth, and often death (Kozlowski 1997, Shiono et al. 2008).

Inundation of soil adversely affects shoot growth of many species by inhibiting the formation and expansion of leaves, reducing internode elongation, and inducing chlorosis, leaf senescence, and abscission (Kozlowski 1997, Glenz et al. 2006). Flooding may increase or decrease the diameter growth of woody plants, is also followed by death and decay of roots, and is often associated with reduced water and nutrient uptake and metabolism (Dat et al. 2004). Responses of seedlings to flooding reflect species' habitats and growth patterns (Sakio 2005).

Factors that influence the flooding tolerance of plants include both biotic factors (e.g., species, morphological and physiological adaptations, and development stage) (Kozlowski 1997, Bailey-Serres and Voesenek 2008) and abiotic factors (e.g., the duration, depth, timing, and frequency of flooding) (Kozlowski 1997, Glenz et al. 2006). Most plants of flooded areas have adapted to flooding in various ecological and physiological ways (Pezeshki 2001). Mechanisms related to mediating flooding tolerance include avoidance strategies (e.g., formation of lenticels, aerenchyma, and adventitious roots) (Kozlowski 1997, Dat et al. 2004, Glenz et al. 2006), escape strategies (e.g., enhanced shoot growth; Parolin 2002), physiological resistance strategies (e.g., reduction in stomata conductance and photosynthesis as well as root hydraulic conductivity; Parent et al. 2008), and decreases in chlorophyll and protein levels (Kozlowski 2002, Yordanova et al. 2007). Ac-

cumulation of some biochemical components, including sugars, amino acids, and alcohols, in organs under environmental stresses is an important feature of tolerant plants to this condition (Morgan 1984). Proline is an amino acid known as the most important osmolyte that accumulates under environmental stress such as drought and salt stress (Roger 2003). In spite of this, so far, accurate knowledge of the impacts of flooding on proline accumulation is not available, although García-Sánchez et al. (2007) reported that proline accumulation increased in roots under flooding but did not differ in leaves.

Flooding is a severe stress to trees, resulting in reduced species richness (Parolin and Wittmann 2010); furthermore, a correct hydrologic regime is an important factor in seedling survival (Conner et al. 1986).

Species of the Salicaceae, Betulaceae, and Taxodiaceae are utilized for riparian restoration projects (Cao and Conner 1999, Karrenberg et al. 2002, Francis et al. 2005). Alnus subcordata (L.) or Caucasian alder is a native, pioneer, hardwood, and fast-growing tree species widely distributed in the Middle East especially temperate areas of Iran and the Caucasian Mountains. Likewise, in the Hyrcanian forest of northern Iran, which is one of the most ancient and unique forest communities in the world with 80 tree and 50 shrub species in about 2×10^6 ha (Marvi-Mohajer 2007), *A. subcordata* is frequently found as a native species which grows on colouvial soils. This species is also found in wetlands, along streams and rivers, and at the bottom of valleys and plains (Sabeti 2006). *Taxodium distichum* (L.) is known to be tolerant to flooding stress (Pezeshki and DeLaune 1998). This species is native to the US (Florida) and has been introduced in Iran for plain region afforestation of Hyrcanian forests. *Populus deltoides* (Bartr.) Marsh is native to

North America (Florida, Texas, and Canada) and has been widely cultivated in Iran. The fast growth of these 3 species fulfills the extensive demand for wood for poles, pulp, and fuel in Iran.

Plant growth in floodplains is often subject to physical disturbances and physiological water stress during floods (Higa et al. 2011). Thus, detailed scientific information about the most tolerant species for afforestation projects in floodplains is necessary to be successful, whereas suitable well-known species should be chosen. Although the flooding tolerance of some species including *T. distichum* and *P. deltoides* was demonstrated by previous research under several environmental conditions (Kozlowski 1997, Elcan and Pezeshki 2002, Kozlowski and Pallardy 2002), detailed knowledge of these species and particularly for *A. subcordata* under Hyrcanian forest conditions of Iran is not available. The main objective of this study was to determine the flooding tolerance of *A. subcordata*, *P. deltoids*, and *T. distichum* for afforestation of floodplains of Hyrcanian forests and the influence of the water level on the responses of these plants to flooding. In order to achieve this aim, we studied the effects of flooding on survival, growth variables, leaf and root proline contents, and chlorophyll concentrations of these species.

MATERIALS AND METHODS

Plant material and experimental design

In this experiment, 1-yr-old seedlings of *A. subcordata*, *P. deltoides*, and *T. distichum* were used, which were prepared at the Koloudeh nursery, situated approximately 12 km from the city of Amol, Mazandaran, Iran (36°34'N, 52°17'E, 76 m in elevation).

In May 2010, 144 uniform 1-yr-old plants were transplanted into 22 × 23 × 23-cm pots, previously filled with a homogeneous soil mixture taken from the Koloudeh nursery (sand: clay-loam of 1: 5 by volume, pH 8.4) with no added fertilizer. After 20 d, the pots were transported to the Natural Resources campus, located at Tarbiat Modares Univ. in the city of Noor, Mazandran Province (36°35'N, 52°02'E, -25 m in elevation) to be used in the experiment, which was carried out at an open-air site. The experiment was conducted in a completely randomized 3 × 3 factorial design, with 3 flood treatments (control, and flooded to depths of 3 and 15 cm above the soil surface), 3 species (*P. deltoides*, *A. subcordata*, and *T. distichum*), 4 replicates for each combination of species and treatment, and 4 pots per replicate.

Plants were randomly assigned to one of the following 3 treatments: (1) control (C) which consisted of well-watered and drained plants to maintain a soil water content of plants at field capacity; (2) flooded to a level of 3 cm above the soil surface (F1), and (3) flooded to a level of 15 cm above the soil surface (F2). A concrete basin (2.5 × 3.5 m) covered with plastic was used to apply the 15-cm flooding treatment. Flood treatments were maintained for 120 d until the end of the experiment on 15 October 2010. In reality, the flood treatments were based on a range of flood depths observed on the floodplains of the Hyrcanian forest. Water was periodically added whenever the water level decreased due to evapotranspiration. Mean daily minimum and maximum air temperatures and cumulative precipitation during the 120 d of experiment were 19 and 40 ℃, and 71 mm, respectively.

Measurement of growth and morphological parameters

The height, diameter, and survival of all plants were recorded at the beginning and end

of the experiment. Height was measured on the main stem, from the base to the apex. Diameter was measured using a digital calipers at the base of the main stem. Differences in height and diameter between days 1 and 120 were used to estimate growth. Leaves were collected from each plant, scanned, and analyzed with an UTHSCSA Image Tool analysis system (Univ. of Texas Health Science Center, San Antonio, TX, USA) to estimate the leaf area. Throughout the experiment, any visible effects of flooding stress on the root, stem, and leaf morphology (e.g., hypertrophied lenticels, aerenchyma, and adventitious roots) were recorded.

Biomass and allometric parameters

At the end of the experiment, plants (4 randomly selected plants from all treatments) were carefully removed from the pots and separated into root, stem, and leaf components. Stems were cut at the base of the plant. Roots were washed to remove the soil and divided into original and adventitious roots. Root nodules containing nitrogen-fixing bacteria were visually assessed during harvest. All materials were dried in the oven at $70^{\circ}\mathrm{C}$ for 48 h and weighed to calculate the root, stem, and leaf biomass. From these measurements, the root: shoot ratio (root biomass divided by shoot biomass) and specific leaf area (leaf area divided by leaf biomass, $\mathrm{cm}^2\ \mathrm{g}^{-1}$) were calculated.

Free proline and chlorophyll content determinations

At the end of the experiment on day 120, leaf samples of 4 selected plants per treatment were also used to determine the chlorophyll content (mg g^{-1} of fresh weight, FW) following Arnon's (1949) procedure. Fresh leaves (0.5 g) were triturated in 80% acetone. The absorbance of the extracts was measured on

a spectrophotometer at 645 and 663 nm, and concentrations of chlorophyll were calculated using the appropriate equations. Free proline contents of leaves and roots of plant were determined according to the protocol described by Bates et al. (1973). Proline was extracted from liquid nitrogen-frozen tissue by homogenizing 0.5 g of fresh leaves in 10 ml of a 3% aqueous solution of sulfosalicilic acid at $25^{\circ}\mathrm{C}$. The homogenate was filtered through Whatman no. 2 filter paper (Whatman company, Kent, UK), and 2.0 ml of the filtrate was reacted with 2.0 ml glacial acetic acid and 2.0 ml acidic ninhydrine in a test tube for 1 h in a water bath at $95^{\circ}\mathrm{C}$. The reaction mixture was cooled in an ice bath. Then, 4 ml of toluene was added to the reaction mixture and mixed vigorously with a test tube magnetic stirrer for 20 s. The toluene layer at the top (a pinkish-red color) was collected with a pipette. The absorbency of the toluene layer was read at 520 nm using a spectrophotometer with toluene as a blank. A standard curve was produced with 0 to 30 µg ml-1 L-proline (Sigma, St. Louis, MO, USA) dissolved in a 3% aqueous solution of sulfosalicilic acid. The curve was then used to determine the proline levels of the samples on an FW basis.

Data analysis

All measured variables were subjected to a 2-way analysis of variance (ANOVA) with 2 main effects (flooding and species treatments). Assumptions of normality and equality of variances were checked for all variables with Shapiro-Wilk's and Levene tests, respectively. Differences between means were analyzed by the least significant difference (LSD) multiple-range test ($p < 0.05$). When significant statistical interactions between species and flood treatments were observed, means of significant treatment effects were separated by a standard LSD test. All statistical analyses

were conducted using the statistical software package SPSS 17 for Windows (SPSS, Chicago, IL, USA).

RESULTS

Growth and morphological parameters

The survival percentage of seedlings was > 90% in all treatments, and there were no substantial differences among species (data not shown). The net height growth of seedlings of *P. deltoides* was significantly reduced in the F2 treatment, whereas it was not affected by the F1 treatment (Fig. 1a). In *A. subcordata*, this variable was significantly reduced by both flooding treatments of F1 and F2 (Fig. 1a). Flooding did not change the net height growth of seedlings of *T. distichum* (Fig. 1a). The net diameter growth of seedlings of *P. deltoides* and *A. subcordata* was significantly and progressively reduced with an increasing flooding depth, while in seedlings of *T. distichum*, it significantly increased with an increased flooding depth (Fig. 1b). Leaves of flooded seedlings became yellow and were shed during the flooding period, although these symptoms were more severe in *P. deltoides* and *A. subcordata* than in *T. distichum* (data not shown).

Leaf areas of *P. deltoides* and *A. subcordata* were significantly greater in control than in flooded treatments, where there were no significant differences between F1 and F2 treatments (Fig. 1c). Flooding, however, did not change the leaf area of *T. distichum* seedlings (Fig. 1c). Leaf areas of *A. subcordata*, *P. deltoids*, and *T. distichum* seedlings were reduced by approximately 85, 70, and 20% with flooding treatments, respectively. For all species, the root length was significantly higher in the control treatment than in F1 and F2 treatments, and no significant difference was found between F1 and F2 treatments (Fig. 1d).

Biomass and allometric parameters

Growth variables including leaf, stem, root and total biomass values were significantly reduced by flooding (Fig. 2a-d). The leaf biomass of flooded seedlings was significantly reduced in all species (Fig. 2a). Stem biomass was considerably reduced in *A. subcordata* in both flooded treatments, while only F2 treatment reduced this parameter in *T. distichum* and *P. deltoides*, and this reduction was less than that in *A. subcordata* (Fig. 2b). Total root biomass and original root biomass in flooded seedlings were significantly lower than those of unflooded seedlings in all 3 species (Fig. 2c, e). For all species, mean values of total biomass were significantly greater for the control treatment than for the flooded F1 and F2 treatments, but differences between flooded treatments were not significant (Fig. 2f). Mean values of total biomass in *A. subcordata*, *P. deltoides*, and *T. distichum* seedlings decreased by approximately 75, 35, and 30% by flooding treatments, respectively. Production of adventitious roots occurred in flooded seedlings of all species. Adventitious root biomass increased with an increasing water level in all species (Fig. 2f).

Specific leaf areas of *P. deltoides* and *A. subcordata* were significantly greater in the control than in F1 and F2 plants while no significant effects were observed in *T. distichum* (Fig. 3a). The root: shoot ratio of *P. deltoides* was significantly higher in the control than for F1 and F2 plants while in *A. subcordata*, the root: shoot ratio was significantly lower in the control than in F1 and F2 plants. In *T. distichum*, the root: shoot ratio did not significantly differ among the control, F1, and F2 treatments (Fig. 3b).

Free proline and chlorophyll contents

Proline concentrations in leaves and roots were not significantly affected by flood-
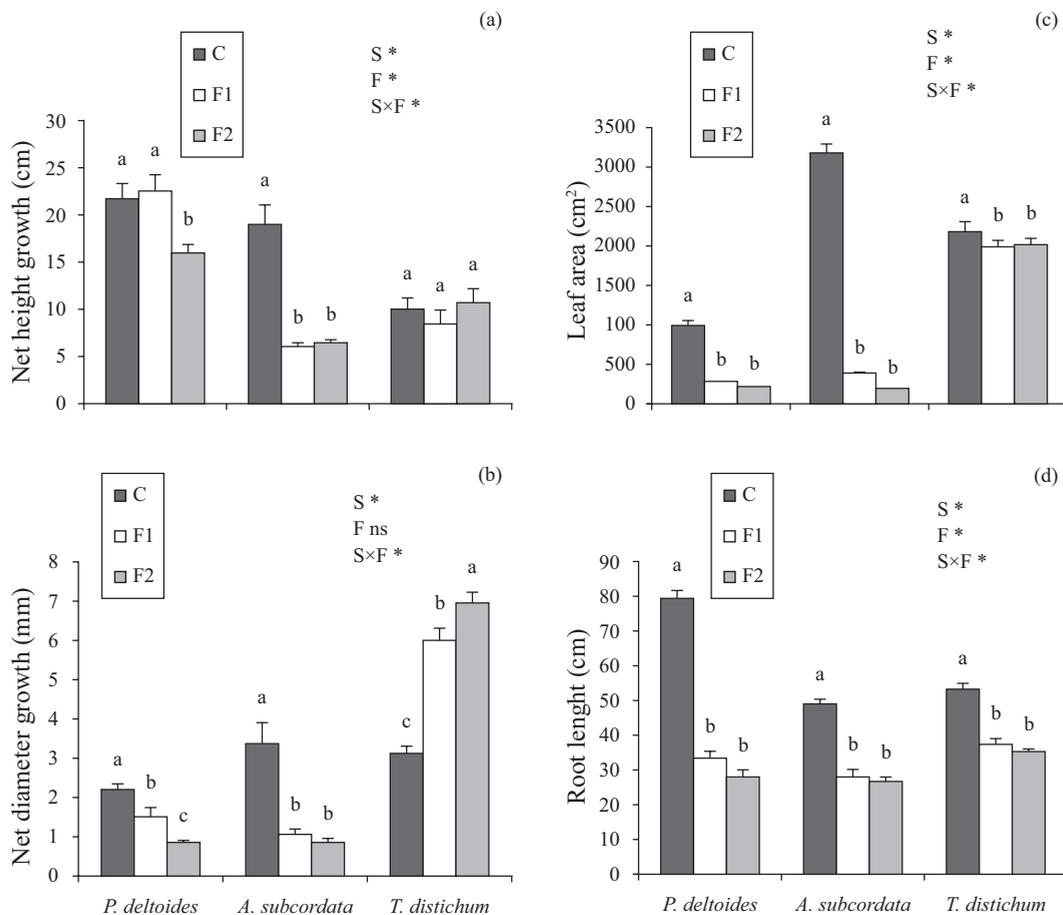
Fig. 1. Interaction effects of species and flooding on comparison of means of net height growth (a), net diameter growth (b), leaf area (c), and root length (d) of *Alnus subcordata*, *Populus deltoides*, and *Taxodium distichum* seedlings after the 120-d experiment. C, F1, and F2 indicate control, flooded to 3 cm in depth, and flooded to 15 cm in depth, respectively. Bars labeled with different letters significantly differ (*p* < 0.05). S, F, and S×F indicate the effects of species, flooding treatment, and their interaction. * Significant difference at the 0.05 level; ns, no significant difference.

ing in any species (Fig. 4a, b). However, chlorophyll contents of all species were lower in flooding treatments than in controls (Fig. 4c). In *A. subcordata*, a significant difference in chlorophyll contents between F1 and F2 plants was observed, while in *P. deltoides* and *T. distichum*, there were no significant differences between the 2 flooding treatments.

Visible morphological variations

Visible morphological changes in responses to flooding were also observed. Within 20 d of initiation of flooding, many adventitious roots appeared on submerged portions of the stems of *A. subcordata*, and also on original roots of most of the flooded seedlings of all 3 species. Hypertrophied lenticels had developed on submerged stems of
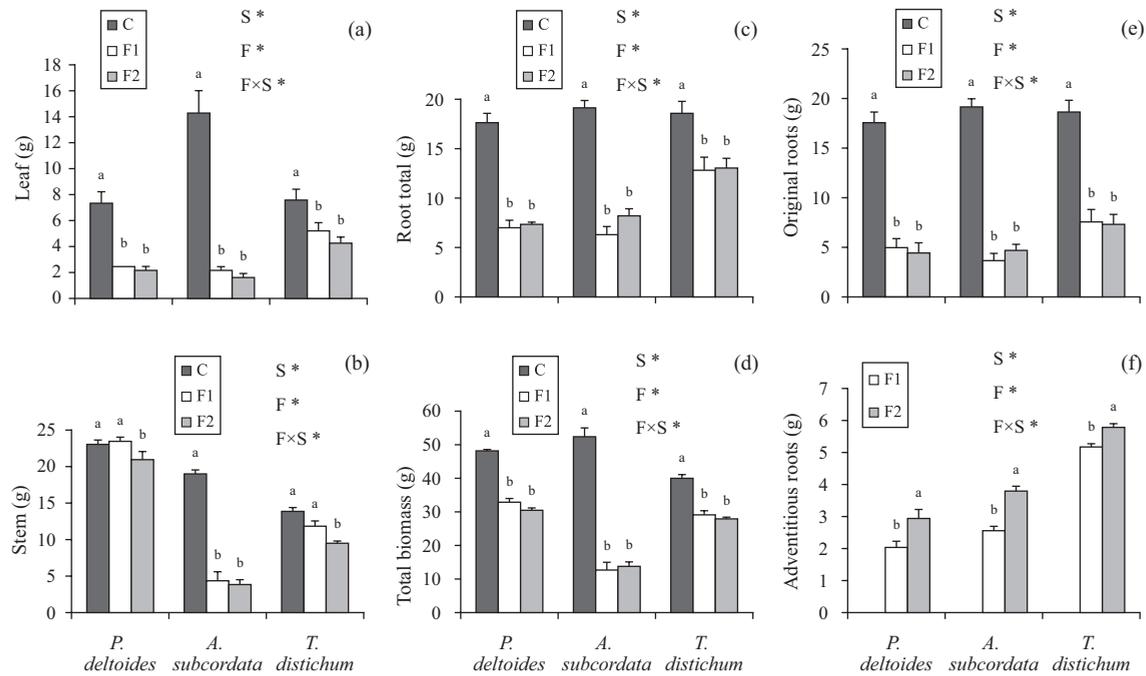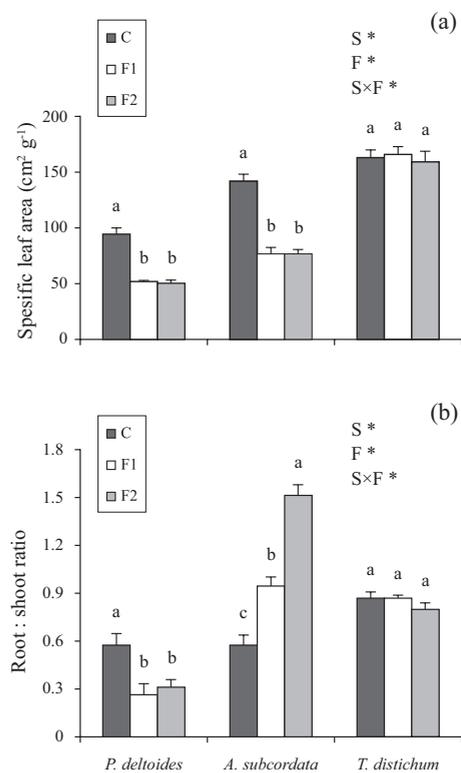
Fig. 2. Interaction effects of species and flooding on comparison of means of the biomass of leaves (a), stems (b), total roots (c), total biomass (d), original roots (e), and adventitious roots (f) of *Alnus subcordata*, *Populus deltoides*, and *Taxodium distichum* seedlings after the 120-d experiment. C, F1, and F2 indicate control, flooded to 3 cm in depth, and flooded to 15 cm in depth, respectively. Bars labeled with different letters significantly differ (*p* < 0.05). S, F, and S×F indicate the effects of species, flooding treatment, and their interaction. * Significant difference at the 0.05 level; ns, no significant difference.

P. deltoides and *A. subcordata* within 15 d of flooding (data not shown).

DISCUSSION AND CONCLUSIONS

Flood tolerance varies greatly with plant species and genotype, rootstock, age of the plant, time and duration of flooding, and conditions of the floodwater (Kozlowski and Pallardy 1997, Glenz et al. 2006), and also according to criteria on which tolerance is based. In this study, flooding for 120 d induced several growth responses in *P. deltoides*, *A. subcordata*, and *T. distichum* that were similar to those reported in the literature, including the formation of hypertrophied

lenticels, aerenchyma, and adventitious roots as well as reductions in growth (Pezeshki 2001, Kozlowski and Pallardy 2002, Dat et al. 2004, Parent et al. 2008). However, in our experiment, flooding during 120 d did not considerably affect the survival of seedlings of these 3 species. It seems that morphological and physiological adaptations in the 3 species maintained high survival percentages. But our data indicated that when using height growth as a criterion of flooding tolerance, *T. distichum* showed greater flooding tolerance than the other species. Indeed, in a riparian situation, one of the conditions necessary for regeneration of species is the ability of individuals to increase in height fast enough
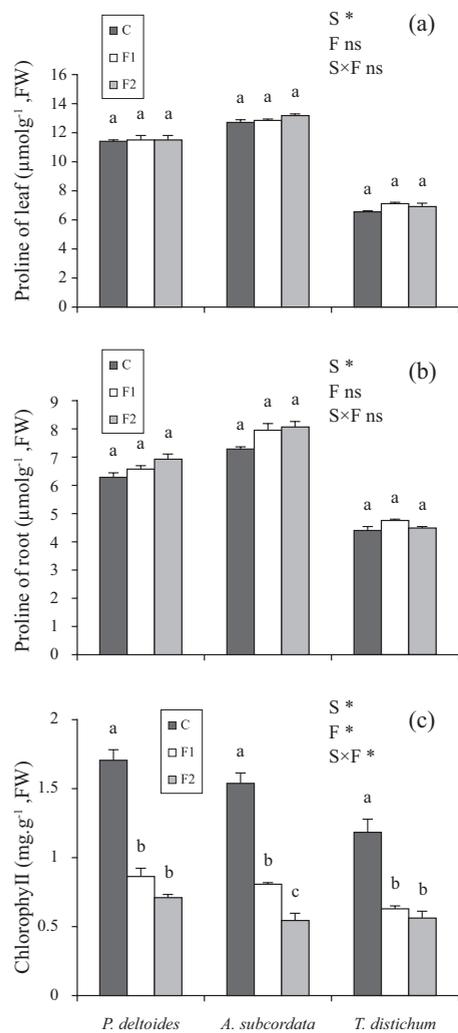
Fig. 3. Interaction effects of species and flooding on comparison of means of the specific leaf area (a) and root: shoot ratio (b) of *Alnus subcordata*, *Populus deltoides*, and *Taxodium distichum* seedlings after the 120-d experiment. C, F1, and F2 indicate control, flooded to 3 cm in depth, and flooded to 15 cm in depth, respectively. Bars labeled with different letters significantly differ ($p < 0.05$). S, F, and S×F indicate the effects of species, flooding treatment, and their interaction. * Significant difference at the 0.05 level; ns, no significant difference.

to survive the flood rise (Day et al. 2006). Pezeshki (1990) and Day et al. (2006) reported that flooding did not reduce the height growth of flooding-tolerant species of *T. distichum* and *Salix nigra* seedlings. In fact, in response to flooding, *T. distichum* seedlings showed an increase in the stem diameter growth, while *P. deltoides* and *A. subcor-*

data species showed reductions in the stem diameter growth, supporting the idea that *T. distichum* may be the most flooding-tolerant species. An increase in diameter growth was observed for *T. distichum*, *Quercus robur*, and *Fraxinus excelsior*; this phenomenon was often observed in flood-tolerant species, as they produced more intercellular spaces and lower-density cells, thus facilitating oxygen transport (Yamamoto 1992, Glenz et al. 2006). These intercellular spaces are called "aerenchyma" which connect across organs to enable oxygen movement from shoots to roots (Kozlowski and Pallardy 2002, Li et al. 2006, Shiono et al. 2008). Aerenchyma tissues were described in *T. distichum* (Pezeshki 1991), *F. excelsior* (Frye and Grosse 1992) and *S. pentandra* (Glens et al. 2006).

Reductions in the leaf area in *A. subcordata* and *P. deltoides* were considerable, while flooding did not affect the leaf area of *T. distichum*. Reductions in leaf area and the number of leaves are common responses to flooding stress that were reported for many tree species. For example, decreases in leaf area by flooding were reported for *T. distichum*, *P. deltoides*, *Q. nuttallii*, and *Q. michauxii* (Cao and Conner 1999, Anderson and Pezeshki 2001, Gong et al. 2007). Generally, a reduction in shoot growth by flooding occurs due to a variety of factors including anaerobic respiration of roots and disruption in the translocation of root metabolites (Pezeshki 1994).

The more flood-tolerant species have various ways to survive and recover from flooding (Pezeshki 2001). Among these mechanisms, changes in biomass allocation between organs are included. This is a typical strategy against environmental stresses (Visser et al. 2000, Van Eck et al. 2004, Yin et al. 2009). Reduced biomass accumulation in response to flooding is a common response observed in many wetland species (Pezeshki

Fig. 4. Interaction effects of species and flooding on comparison of means of the proline of leaves (a), proline of roots (b), and chlorophyll contents (c) of *Alnus subcordata*, *Populus deltoides*, and *Taxodium distichum* seedlings after the 120-d experiment. C, F1, and F2 indicate control, flooded to 3 cm in depth, and flooded to 15 cm in depth, respectively. Bars labeled with different letters significantly differ (*p* < 0.05). S, F, and S×F indicate the effects of species, flooding treatment, and their interaction. * Significant difference at the 0.05 level; ns, no significant difference.

2001). In the present study, the root: shoot ratio was significantly reduced by flooding in *P. deltoides*, but it increased in *A. subcordata*. Thus there was not a clear relationship between biomass allocation and flooding tolerance. On the other hand, it seems that the negative effects of flooding on biomass and growth of *A. subcordata* were more severe than those of the other 2 species. A reduction in *A. subcordata* biomass as a consequence of flooding was largely the result of decreased shoot growth, root decay, and inhibition of root initiation, growth, and length, whereas in the other 2 species, these were largely the result of root decay and decreased root growth.

In this study, after mortality of the original roots occurred induced by flooding, adventitious roots were produced on the original root system and also on the submerged portions of stems in *A. subcordata*, which was described as an adaptive mechanism against flooding. According to Polomski and Kuhn (1998), the dieback of the original root system due to flooding may favor the development of younger roots, as observed for *Salix* spp., *Populus* spp., and *Alnus* spp. (Glens et al. 2006). Root mortality under soil anaerobiosis is followed by initiation and growth of adventitious roots (Kozlowski and Pallardy 2002). Examples of species that produce adventitious roots in response to flooding include *A. glutinosa*, *A. rubra*, *A. japonica*, *T. distichum*, *Populus nigra*, and *P. deltoides* (Kozlowski 1997, Pezeshki 2001, Kozlowski and Pallardy 2002, Iwanaga and Yamamoto 2008). Morphological adaptations, such as hypertrophied lenticels, aerenchyma tissues, and adventitious roots, increase the uptake of O_2 by aerial tissues and promote its transport into the root system (Glens et al. 2006). In the current study, both *P. deltoides* and *A. subcordata* produced hypertrophied lenticels, which may have assisted in the exchange of dissolved

gases and release of toxic compounds which could have boosted survival under flooding conditions, although these mechanisms were not enough to avoid reductions in growth variables as in the case of *T. distichum*.

Alders (*Alnus* spp.) are actinorhizal trees and shrubs with the capacity to fix atmospheric dinitrogen through symbiosis with actinomycetes of the genus *Frankia* (Furlow 1979). Flooding stress causes valuable bacteria to die (Kaelke and Dawson 2003), so we frequently observed nodules containing nitrogen-fixing bacteria in the control, with no nodules on the roots of flooded seedlings.

As expected, chlorophyll contents in leaf tissues of seedlings were reduced in all 3 species under flooding. The reduction in chlorophyll content caused by flooding may be due to either slow synthesis or fast breakdown of chlorophyll pigments (Ashraf 2003). However, we observed no significant changes in free proline accumulations after applying the flooding stress. This observation differs from the findings of Garcia-Sanchez et al. (2007), who reported no significant effects of flooding on proline levels in leaves but significantly increases in root of *Citrus sinensis* and *C. resnhi*. Other authors such as Yordanova et al. (2007) observed that flooding increased proline accumulation in leaves of *Zea mays*.

Finally, it should be pointed out that the lack of substantial differences between our F1 and F2 treatments may have been due to the fact that once the soil is flooded, the depth has little significance until a substantial part of the plant foliage is covered. Our water level of 15 cm (below the limit of the lower foliage of the seedlings) was probably insufficient to observe more differences with the 3-cm treatment, besides a higher adventitious root biomass and associated root: shoot ratios, and a lower chlorophyll content.

Flooding is recognized as a major threat to the survival and establishment of plant species in areas prone to flooding. Indeed, flooding conditions would cause oxygen deficiency in rhizosphere roots, and nutrient uptake would decrease. As a result, morphophysiological traits changed, and plant growth and biomass were reduced. In summary, our research demonstrated that these 3 species have high resistance to flooding stress, although *T. distichum* exhibited greater flooding tolerance than the other 2 species, and *P. deltoides* was more tolerant than *A. subcordata*. Thus, under natural periodic flooding, which in rainy seasons can lead to multiple floods in plain regions and riparian areas, these species seem capable of surviving without long-term damage, and can adapt to flooding by growth rate and biomass losses and morphophysiological adjustments, such as the production of adventitious roots and hypertrophied lenticels.

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