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Effects of submergence on growth and survival of saplings of three wetland trees differing in adaptive mechanisms for flood tolerance

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Abstract

Aim of study: Withstanding total submergence and reaeration following submergence is essential for the survival and establishment of wetland species. We focused on “LOES–low oxygen escape syndrome” and “LOQS–low oxygen quiescence syndrome” and compared tolerances to total submergence among wetland woody species differing in morphological adaptation to soil flooding.

Area of study, materials and methods: This study examined the survival of 2-year-old saplings of *Taxodium distichum* and *Metasequoia glyptostroboides* (LOQS species), and *Alnus japonica* (LOES species), during and after total submergence. Saplings were completely submerged, then de-submerged to determine trends in survival and growth.

Main results: The *M. glyptostroboides* and *A. japonica* saplings could not survive prolonged submergence for more than 8 weeks, whereas saplings of *T. distichum* survived for over 2 years. Submerged saplings of all species showed no significant growth or modifications in morphology and anatomy under water, such as shoot elongation, adventitious root formation, and/or aerenchyma development. All *T. distichum* saplings that were de-submerged in the second year had the same pattern of shoot growth regardless of differences in timing and seasonality of de-submergence. Wood formation in *T. distichum* saplings ceased during submergence and resumed after de-submergence in spring and summer, but not in autumn.

Research highlights: *T. distichum* saplings, which survived longer submergence periods than *A. japonica* and *M. glyptostroboides*, had physiological characteristics, such as suspension of growth and metabolism, which allowed survival of protracted total submergence (at least 2 years) when saplings were immersed during the dormant stage before leaf flushing.

Keywords: *Alnus japonica*; *Metasequoia glyptostroboides*; survival rates; *Taxodium distichum*; total submergence.

Abbreviations: LOES, low oxygen escape syndrome; LOQS, low oxygen quiescence syndrome.

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Introduction

Soil flooding and submergence occur temporarily or continuously in floodplains, wetlands, and marshes, where is one of the major factors determining plant growth and ecosystem component. Total submergence or simple submergence refers to deep flooding over the top shoot or leaf. The energy deficiency accompanying oxygen limitation is more severe under submergence than flooding condition. Thus, mesophytic plants exposed to oxygen limitation would mitigate energy deficiency by getting bypass to air from under water

to recover aerobic respiration, or to suspend growth and metabolic activity to reserve energy (Setter & Laureles, 1996; Voesenek *et al.*, 2006; Colmer & Pederson, 2008). Bailey-Serres & Voesenek (2008) categorized submergence tolerance into two classes by the presence or absence of rapid shoot adaptations beneath the water. The Low Oxygen Quiescence Syndrome (LOQS) is characterized by a lack of a shoot extension and a deceleration of nonessential metabolism to conserve metabolites that promote survival until floodwaters recede. The Low Oxygen Escape Syndrome (LOES) is characterized by rapid shoot growth that

positions some plant parts (mostly young leaves) above the water to enable increased gas diffusion into the plant body. These two adaptation categories differ mainly in the plasticity of growth and metabolism.

The LOES generally occurs in herbaceous species (Colmer & Voesenek, 2009). Woody plants have relatively slower growth than herbaceous species, and therefore employ the LOQS. In Amazon floodplain forests, where the river water level rises for several months per year, woody trees commonly have no shoot elongation during submergence (Parolin, 2009) but are able to maintain photosynthetic activity under water for several months (Waldhoff *et al.*, 2002). Submergence is routine for wetland woody species growing in riparian and coastal areas, especially when plants are in the juvenile stage. The degree and duration of submergence varies by soil and hydrological conditions. To date, however, limited information still exists on the flood-tolerance abilities of woody species in temperate regions.

In this investigation, we performed a comparative total submergence experiment on three woody wetland species that differ in flood tolerance and growth characteristics during flooding. We examined changes in shoot and stem growth in response to total submergence in early spring and de-submergence in various seasons using two deciduous conifer species and one broadleaf species, *Taxodium distichum*, *Metasequoia glyptostroboides*, and *Alnus japonica*, respectively. *A. japonica* responds to soil flooding: plants underwater develop numerous adventitious root and lenticels with abundant aerenchyma, and shoots are formed rapidly during submergence. In contrast, *T. distichum* and *M. glyptostroboides* have LOQS characteristics, *viz.*, slow root development, reduced shoot formation, and few hypertrophied lenticels. We compared patterns of survival and growth during total submergence among these woody riparian tree species aiming to detect differences in flood tolerance.

Rapid exposure to air when floodwaters recede may cause physiological difficulties because free oxygen radical production is promoted under these conditions. These radicals may lead to severe membrane damage and cell death (Colmer & Voesenek, 2009). Gibbs & Greenway (2003) proposed a criterion for anoxia tolerance in plant tissues based on the ability to survive a period of anoxia and to recover physiological and metabolic processes after reaeration. We postulated that *T. distichum* and *M. glyptostroboides* (LOQS species) would have greater survival capability than *A. japonica* under total submergence. We measured shoot growth responses after de-submergence and determined the effects of prolonged submergence in each of the three experimental species.

Materials and methods

Plant Materials and Experimental Conditions

Plant materials and habitats

Taxodium distichum is widely distributed in the Mississippi basin, from southern Illinois to the Gulf Coast of the United States (Middleton, 2000; 2005). It forms wetland forests, even in permanently flooded areas (Dicke & Toliver, 1990). Buttress stems and knee roots develop in this species over decades under flooding conditions, but adventitious roots and elongated shoots are absent.

Metasequoia glyptostroboides prefers fertile, moist soil conditions (Momohara, 2005), and its natural forests occur only in small numbers in Modaoxi, Sichuan, China. For *M. glyptostroboides*, information on flood tolerance is still limited. Previous observations (Inoue, 1998) on the growth response of *M. glyptostroboides* to flooding revealed that this species also forms stem buttresses, but the development is faster than in *T. distichum* (Moriwaki, 2000).

Alnus japonica often forms swamp forests in cool-temperate regions of Asia (Fujita, 2002). The species has morphological adaptations for submergence, such as flooding-adapted root development and rapid shoot formation in response to soil flooding (Shinshoh, 1985). The species rapidly forms numerous hypertrophied lenticels and adventitious roots, together with aerenchyma, in response to flooding, all of which combine to mitigate oxygen deficiency (Grosse *et al.*, 1993).

Experiment 1

Two-year-old *M. glyptostroboides*, *A. japonica*, and *T. distichum* saplings were used in submergence Expt. 1. Potted saplings were obtained from Takemoto-en Co. Ltd. (Tottori, Japan). The plastic pots in which plants grew contained planting medium (1:3, granite sand:bark); they were 10 cm in diameter and 9 cm tall. At the end of March 2000, saplings were completely submerged in tap water contained in a plastic container (114 cm upper diameter, 102 cm lower diameter, 116 cm tall).

A. japonica saplings were submerged on March 17, while *M. glyptostroboides* and *T. distichum* saplings were submerged on March 27, 2000. The different start times resulted from difficulties in experiment preparation. Submergence treatments were initiated before sapling bud break. Forty-two saplings of each species were

submerged in the plastic containers without further aeration and without a change of water. The saplings of each species were divided evenly between two submergence containers. Average stem diameters 3 cm above ground level and heights of the saplings were 6.3 ± 0.2 mm (mean \pm SE) and 71.1 ± 0.3 cm, respectively, for *M. glyptostroboides*, 3.4 ± 0.1 mm and 50.9 ± 0.5 cm for *A. japonica*, and 7.1 ± 0.1 mm and 69.8 ± 0.5 cm for *T. distichum*. The distances between the water surfaces and sapling shoots below were ~ 30 cm.

Each container was held outdoors, and water levels were maintained by the addition of tap water as needed. Using a portable DO meter (D-24; Horiba Ltd., Fukushima, Japan), we measured dissolved oxygen (DO) concentration and temperature of the water at three container depths: upper (5 cm below the water level), middle (near the shoot apex), and bottom (near the soil surface). Measurements were made every 2 weeks during the experimental period. Water temperature and DO concentration in the water in which saplings were submerged did not differ significantly among depths (Fig. 1). Until the end of June, water temperature remained below 20 °C, but then gradually increased thereafter to 29.5 °C during July and August. A rapid temperature decrease occurred after August through the end of the experiment. DO levels decreased slowly in the first 40 days of the experiment. During June and July, DO values were slightly lower in the deepest water than in the upper and middle levels.

Drainage treatments were performed in the 4th (4W), 8th (8W), 12th (12W), and 16th weeks (16W) of submergence for *M. glyptostroboides* and *A. japonica*, and in the 6th (6W), 12th (12W), 18th (18W), and 24th weeks (24W) of submergence for *T. distichum* (Fig. 2).

Each treatment was replicated sevenfold (seven separate saplings). The saplings used as replicate experimental units were chosen randomly and drained on each drainage day. These de-submerged saplings were grown outdoors under flooded conditions (with water levels 3 cm above the soil surfaces); growth and survival were measured on the saplings held under these conditions. Seven previously unsubmerged saplings of each species were also flooded and grown as controls.

Experiment 2

The submergence treatment in Expt. 2 was applied from March 2002 to July 2005. Two-year-old potted saplings of *T. distichum* were obtained from Takemotoen Co. Ltd. on March 20, 2002, and 100 of the saplings were submerged completely in tap water in plastic containers (as in Expt. 1). The submergence containers were held outdoors for 2 years without aeration and without a change of water. The water levels were maintained by adding tap water as needed. The initial distance between the water surface and the shoots of submerged saplings below was ~ 50 cm.

Submerged saplings were randomly chosen and removed from their containers in the 104th (May 20), 112th (July 24), and 120th (August 26) weeks in 2004, and in the 148th (April 19), 156th (June 15), and 164th (August 24) weeks in 2005 (Fig. 3). Eight and 10 saplings were de-submerged in 2004 and 2005, respectively. Average stem diameters 3 cm above ground level and heights of the saplings were 13.0 ± 0.1 mm (mean \pm SE) and 88.5 ± 0.1 cm, respectively. De-submerged saplings were flooded to a depth 3 cm above the soil

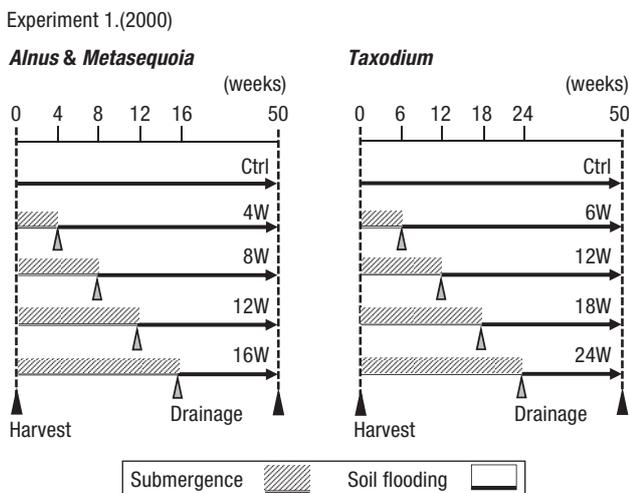


Figure 1. Time schedule of Experiment 1. White and black arrows indicate the drainage and harvest, respectively. Gray zone means period of submergence treatment.

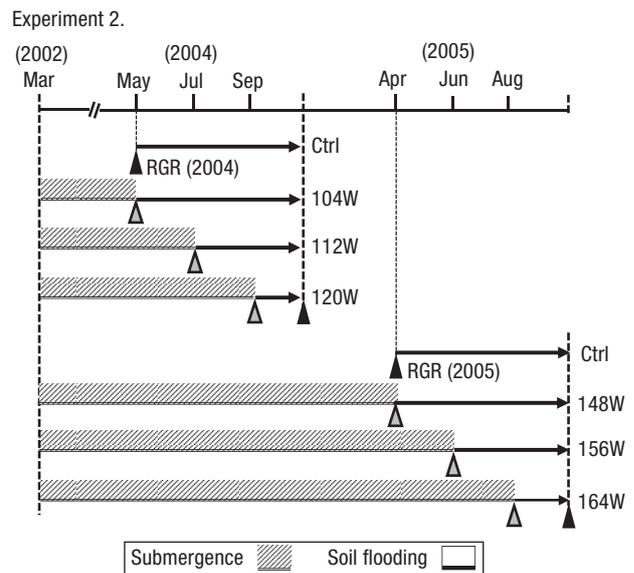


Figure 2. Time schedule of Experiment 2.

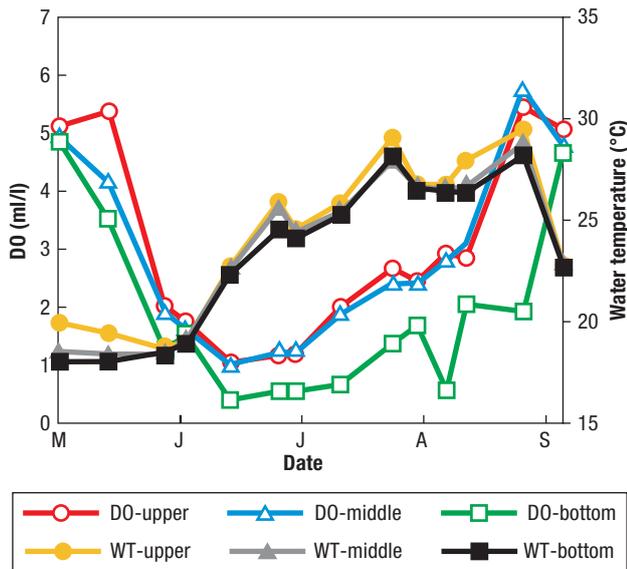


Figure 3. Dissolved oxygen and temperature of submerging water at upper (●, ○), middle (▲, △), and bottom (■, □) of container in Experiment 1.

surface through the end of each growing season. Control saplings, which were not subjected to submergence, were held in a nursery for the duration of the submergence treatments. When treatment saplings were de-submerged and flooded, we also flooded the controls.

Measurements

Survival rate, and shoot and stem growth before and after de-submergence

After draining saplings in Expts. 1 and 2, we measured shoot and stem growth every 2–3 weeks through the experimental period. We did not know in advance whether de-submerged saplings would live through the end of the experiment. Therefore, these saplings were maintained under flooded soil conditions until the end of the growing season. Immediately prior to leaf fall, all saplings were harvested and dissected in order to obtain the dry weights of each plant organ. Dry weight was determined as indicated above. The survival rate for each treatment was measured as the proportion of live saplings at the end of the experimental term among all drained saplings.

Anatomical observation

In Expt. 2, we collected the stem portions of all saplings 3 cm above the soil surface, fixed them in FAA (formalin–acetic acid–alcohol) solution, and sectioned

them transversely through needle-marked portions. We used the pin-marking method of Wolter (1968) immediately after drainage to identify the amount of xylem produced during the recovery period. A small rust-resistant insect-mounting needle was horizontally inserted through the bark and cambial zones of stems, 3 cm above the soil surface, and withdrawn after a few seconds. The pin mark was made at the time of de-submergence. The resulting injury to the dividing and differentiating cells caused formation of abnormal cambial derivatives that could be later identified microscopically. This allowed us to discriminate xylem produced before submergence from that produced after drainage. Using light microscopy, we measured xylem growth, the number of libriform wood fibers, and bark thickness in each section. At harvest time, we collected stem segments from five saplings. When the numbers of live saplings were inadequate, we used all live saplings (Fig. 4). We made five measurements on each sapling and used the mean values in our data analyses. We obtained the mean moisture content of stem portions above and below the marked section of each sapling to estimate the dry weights of stem parts included in the anatomical observations.

Statistical Analysis

We applied Dixon's test to measurements made in Expts. 1 and 2 to obtain outlier data. For Expt. 1, we used an analysis of variance (ANOVA) to examine the effects of treatments on height and diameter growth, and biomass of each species. All data were processed with SPSS statistical analysis software (ver. 18; SPSS, Inc., Chicago, IL, USA) or R 3.02 software (CRAN: the Comprehensive R Archive Network at <http://cran.r->

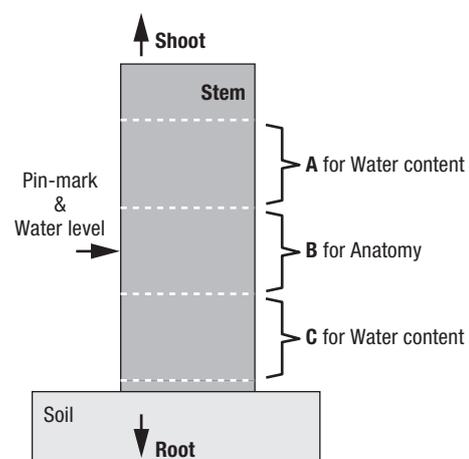


Figure 4. Schematic chart of stem sampling for anatomical observation in Experiment 2. Arrow indicates position of pin-mark and water level.

project.org/). To determine whether submergence treatment affected seedling survival of each species in Expt. 1, we constructed survival curves using the Kaplan–Meier method. This method treated each seedling as a replicate and the survival curves were constructed from data indicating whether individuals were alive or dead on each monitoring date. In Expt. 2, we constructed survival curves for each year. After constructing survival curves, we used the log-rank test to assess differences in the survival rate among treatments.

To identify significant differences in biomass and growth among treatments, we subjected data for each species to ANOVA. We used log- or reciprocal transformations to normalize distributions, as required. When significant differences were found, we applied Scheffe's multiple comparisons *F*-tests to identify significant pairwise differences among treatments. When no significant effects were identified by ANOVA, we applied Kruskal–Wallis tests. When comparisons were to be made between only two groups each comprising three live saplings of the species in question, we used Student's *t*-tests to identify significant differences among treatment mean values. We used two-way ANOVA to explore data on anatomical characteristics collected during the de-submerged season in Expt. 2. When a significant interaction occurred between submergence and season terms, we applied Scheffe's *F*-test to identify significant pairwise differences among treat-

ments in each year. When the interaction term was not significant, we used a *post hoc* test (Scheffe's *F*-test, $p < 0.05$) for each factor.

Results

Survival rates

The survival rates of submerged saplings differed among species in Expt. 1 (Table 1). In *M. glyptostroboides* and *A. japonica*, the survival rate in treatment 16W was 0%. The survival rate of *M. glyptostroboides* in treatment 4W (drained on April 24) was 100%. In treatment 8W (drained on May 22), the survival rate of saplings with green shoots was 100% immediately after drainage, 71.4% in the first 14 days of the recovery period, and 14.3% after 14 days. In treatments 12W (drained on June 19) and 16W (drained on July 17), shoots and stems of all saplings had decayed before drainage.

In *A. japonica* saplings subjected to treatment 12W (drained on June 8), the apical shoot of two of the seven saplings had decayed before drainage. The proportion of saplings with colored leaves in treatment 12W decreased from 85.7% on the day of drainage to 28.6% after 6 weeks; the final survival rate was 14.3% at

Table 1. Growth, biomass and shoot:root (T/R) ratios of *M. glyptostroboides*, *A. japonica* and *T. distichum* saplings in Experiment 1.

	Survival (%)	Shoot growth (cm)	Stem growth (mm)	Biomass (g)				T/R ratios (g:g)
				Whole plants	Leaves	Stems	Roots	
<i>M. glyptostroboides</i>								
US	100	22.2±3.8	6.9±1.0	25.0±4.3	4.1±0.8	9.6±0.6	11.3±2.0	1.3±0.1
S 4W	100	25.8±4.1 n.s.	5.0±0.3 n.s.	25.3±2.0 n.s.	3.6±0.3 n.s.	8.9±0.6 n.s.	12.9±1.3 n.s.	1.0±0.1 *
8W	14	–	–	–	–	–	–	–
12W	0	–	–	–	–	–	–	–
16W	0	–	–	–	–	–	–	–
<i>A. japonica</i>								
US	100	28.4±4.8 a	9.6±1.0 a	19.7±7.1 a	3.1±0.5 a	6.0±1.0 a	10.6±3.0 a	1.2±0.2 a
S 4W	100	28.3±0.9 a	8.7±0.6 a	13.9±2.2 a	2.0±0.1 a	4.8±0.9 a	7.8±1.4 a	0.8±0.1 a
8W	100	21.3±2.2 a	6.1±1.2 a	12.5±3.1 a	1.9±0.4 a	3.5±1.0 a	5.1±1.8 a	1.5±0.2 a
12W	14	–	–	–	–	–	–	–
16W	0	–	–	–	–	–	–	–
<i>T. distichum</i>								
US	100	9.4±1.7 ab	2.7±0.6 a	12.0±2.5 ab	1.1±0.2 a	5.3±1.0 a	5.7±1.3 a	1.2±0.1 ab
S 6W	100	9.0±1.2 ab	2.1±0.3 ab	13.5±1.8 a	0.9±0.1 a	5.6±0.6 a	5.5±0.6 a	1.1±0.1 a
12W	100	9.3±2.1 ab	2.0±0.2 b	11.3±1.4 ab	1.1±0.1 a	5.1±0.7 a	4.9±0.6 ab	1.3±0.1 ab
18W	100	11.3±2.0 a	1.0±0.1 bc	9.2±0.5 ab	1.1±0.1 a	4.7±0.1 a	3.4±0.4 ab	1.7±0.1 b
24W	100	2.5±0.6 b	0.2±0.1 c	5.6±0.9 b	0.3±0.1 b	3.0±0.5 a	1.9±0.2 b	1.5±0.1 ab

Means were given with standard errors. Each value is the mean of three to seven measurements of living saplings. Means followed by different letter in *A. japonica* and *T. distichum* indicate the existence of significant differences by Scheffes *F*-test ($p < 0.05$). Asterisks in *M. glyptostroboides* indicate the existence of significant differences by Student's *t*-test ; *, $p < 0.05$; **, $p < 0.01$. Abbreviations: US, unsubmergence; S, submergence.

harvest. In treatment 16W (drained on July 6), the shoots of all *A. japonica* saplings had decayed. Sapling survival rates in treatment 8W differed significantly from the controls for both *A. japonica* ($\chi^2 = 13$, $p = 0.0003$) and *M. glyptostroboides* ($\chi^2 = 14.5$, $p = 0.0003$). The survival rates were therefore not significantly different between these two species.

T. distichum survived total submergence for 24 weeks in Expt. 1 and for 2–3 years in Expt. 2. After 2 years of submergence, survival rates ranged from 75% to 87.5%. After 3 years of submergence, survival rates gradually declined to 10% in August. Survival rates in *T. distichum* did not differ significantly among treatments (Kaplan–Meier procedure; $p = 1.0$) in Expt. 1. In the third year of the submergence treatment, the survival rates of de-submerged and unsubmerged saplings were not significantly different (Kaplan–Meier procedure; $p = 0.52$), but they were in the fourth year (Kaplan–Meier procedure; $p = 0.008$). Survival rates did not differ significantly between treatment 148W and controls ($\chi^2 = 13$; $p = 0.92$), but were significantly different between the controls and treatments 156W ($\chi^2 = 14.1$; $p = 0.0001$) and 164W ($\chi^2 = 17.8$; $p < 0.0001$).

Effects of submergence on growth after drainage

Experiment 1

Growth rates (height and diameter) of all surviving saplings of *M. glyptostroboides* and *A. japonica* in the treatments were similar to those of unsubmerged individuals; no significant differences were observed

among treatments (Table 1). Shoots of *M. glyptostroboides* saplings rarely grew (<2 cm) in treatment 8W; those of *A. japonica* rarely grew in treatment 12W. Rapid shoot development occurred in *T. distichum* saplings submerged for 18 weeks, even in those drained in September. The relative extension rates of shoots (cm day⁻¹) before the beginning of the growth plateau phase (reached after 71 days in *A. japonica*, 98 days in *M. glyptostroboides*, and 57 days in *T. distichum*) did not differ significantly ($p < 0.05$) among treatments in any of the species. Stem growth and biomass increment tracked a declining trend with decreasing duration of time for recovery (Table 1). Biomass of plant parts other than stems decreased in treatment 24W. Biomass of whole plants, leaves, and roots did not decrease significantly with duration of submergence in treatments 6W, 12W, and 18W (Table 1).

Experiment 2

Shoot and stem growth in *T. distichum* saplings changed little underwater. Although several buds formed on the twigs and branches of saplings that had been submerged for 2 years, no leaf expansion took place underwater. Dieback occurred in some apical shoots during submergence; the rate of dieback accelerated after drainage.

Remaining buds on saplings that had been submerged for 3 years continued to develop, although dieback occurred and continued after drainage, as indicated above. Shoot development and diameter growth was observed in saplings that had been submerged for 2 years (Table 2). Shoots of drained saplings elongated rapidly, as in Expt. 1. Shoot growth

Table 2. Growth in height and diameter, and biomass of *T. distichum* saplings in Experiment 2.

	Survival (%)	Shoot growth (cm)	Stem growth (mm)	Biomass (g)			T/R ratios (g:g)	
				Whole plants	Leaves	Stems		Roots
2004								
US	100	6.7±0.7 b	3.6±0.5 a	41.2±0.9 a	2.7±0.5 a	16.4±0.3 a	22.7±0.4 a	0.87±0.01 b
S 104W	88	11.3±0.7 a	2.3±0.2 ab	20.3±1.8 bc	1.5±0.1 b	11.2±1.3 ab	10.9±2.4 b	1.19±0.07 ab
112W	88	12.5±0.9 a	1.7±0.3 b	23.7±2.8 b	1.5±0.1 b	12.5±1.4 ab	9.5±1.3 b	1.50±0.04 a
120W	75	4.2±0.5 b	0.0±0.3 c	15.4±3.9 c	0.2±0.1 c	7.0±0.6 b	8.1±2.4 b	1.07±0.19 ab
2005								
US	100	7.8±1.0 b	4.0±0.4 a	53.7±3.4 a	4.4±0.4 a	19.8±1.4 a	29.6±2.3 a	0.83±0.06 b
S 148W	25	24.8±1.7 a	1.5±0.8 b	27.5±4.1 b	4.7±1.1 a	11.2±1.9 b	11.7±1.2 b	1.55±0.14 a
156W	70	25.7±2.1 a	4.3±0.3 a	35.3±2.3 b	4.6±0.2 a	11.6±1.3 b	19.1±1.3 b	0.89±0.03 b
164W	10							

Abbreviations: US, unsubmergence; S, submergence. Each value is the means of more than three measurements in living saplings, given with standard errors. Means followed by different letter indicate the existence of significant differences by Scheffé's F-test ($p < 0.05$).

rates of unsubmerged saplings were lower than those of submerged saplings, but the biomass of plant parts and whole plants were higher in unsubmerged plants. Shoot elongation rates in treatments 104W and 112W were significantly different from their respective controls. Growth and biomass were significantly different between treatments 148W and 156W, as in 2004, but the shoot/root (T/R) ratio was not significantly different between these treatments (Table 2). High mortality precluded comparisons with treatment 164W.

Submergence decreased not only the diameter increment, but also the cell counts during the recovery period in treatments 112W and 120W (Table 3). In treatment 120W, we observed xylem formation and cell division in only one sapling (Fig. 5); other saplings did not form any xylem after drainage. Cell numbers and sizes were similar in xylem formed before and after drainage (Fig. 5).

Discussion

Many factors, including climatic, topographic, and geographic features, affect the duration and timing of flooding and the submergence regime, and consequently, plant recruitment and establishment. Most species growing in low-lying and more frequently flooded habitats are tolerant to flooding, although susceptibility to low-oxygen stress can vary with plant age and stage of development (Kozłowski *et al.*, 1991; Kozłowski, 1997). All three species examined are

dominant species in riparian forests in their respective ranges and are considered to have high tolerance to flooding. In the present study, we demonstrated that the effects of total submergence differed among these three species (Tables 1 and 2). The survival rate in all species decreased gradually with the prolongation of total submergence treatment. Saplings of *M. glyptostroboides* and *A. japonica* could not survive total submergence for 3 months. In contrast, *T. distichum* survived total submergence for more than 2 years, and a high survival rate and dramatic growth were found at week 120 (drained in August in 2004), and at weeks 112 and 156 (drained in July in 2004 and 2005, respectively; Table 2). Hence, even within wetland woody species, wide variations in the survival rate were observed during submergence. *T. distichum*, a species without rapid growth adaptation (LOQS), had greatest total submergence tolerance among the species we tested. This outcome was consistent with the resource allocation strategies of the LOQS; rapid growth would have depleted energy reserves essential for surviving protracted submergence. However, none of the species responded rapidly through shoot elongation or root development when beneath the water regardless of their growth characteristics under the flooding conditions tested. Therefore, among the species, only *T. distichum* had physiological traits (other than the LOQS) that were adequate for surviving submergence.

In Amazon floodplain forest species, new leaf expansion, photosynthetic activity, and wood production occur underwater during the submergence season (Waldhoff *et al.*, 2002; da Fonseca *et al.*, 2009). Although direct comparisons between anatomical data obtained in this study and those for Amazonia are not possible because measurements were taken from tall

Table 3. Anatomical characteristics of *T. distichum* saplings after drainage in Experiment 2.

		Rind width (mm)	Cell no.	Cell size (μm)
2004				
104W	US	1.58±0.26 ab	51±5 ab	32.7±1.0 ab
	S	1.16±0.12 bc	41±4 bc	28.2±1.2 ab
112W	US	2.33±0.31 a	72±8 a	32.0±0.8 a
	S	0.65±0.14 cd	24±4 c	27.5±1.0 ab
120W	US	0.65±0.02 cd	22±1 c	23.5±0.6 b
	S	0.02±0.02 d	1±1 d	3.5±3.5 c
2005				
148W	US	1.61±0.14	52±5	31.1±1.2
	S	1.49±0.09	46±3	30.5±1.4
156W	US	2.36±0.29 n.s.	70±7 n.s.	33.3±1.3 n.s.
	S	1.96±0.22	58±5	34.5±0.8
164W	US	1.78±0.26	55±7	31.9±0.9
	S	–	–	–

Means were given with standard errors. Each value is the mean of five measurements: US, unsubmerged; S, submerged. Means followed by different letters indicate the existence of significant differences by Tukey HSD-test. In 2005, Kruskal-Wallis test was used: n.s., not significant.

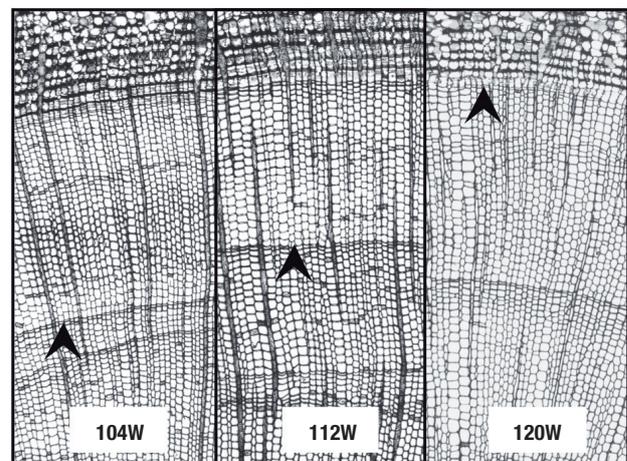


Figure 5. Tracheid production after de-submergence in *T. distichum* saplings submerged over 2 years in Experiment 2. Arrows indicate de-submergence points.

flooded trees in the Amazonian floodplain (Schöngart *et al.*, 2005; da Fonseca *et al.*, 2009), note that deep flooding in the Amazon also caused the cessation of wood production, although root respiration and reproduction (flowering and fruiting) were maintained. Similarly, *T. distichum* stopped wood production during submergence, but leaf bud development proceeded on submerged twigs and stems, suggesting that cambium activity had been suspended to conserve energy.

Leaf desiccation of tree saplings after de-submergence is a major factor in submergence intolerance (Setter *et al.*, 2010) due to inhibition of leaf photosynthetic activity, which would constrain the formation of new organs adapted to the de-submerged conditions. In the present study, all submergence treatments for each species were conducted before leaf expansion, and the leaves remained unexpanded until de-submergence. Hence, environmental differences on the various drainage dates should not have affected energy production and allocation underwater or under ambient conditions for these species that had no modifications for survival underwater. Shoot growth in *T. distichum* after de-submergence did not consistently decrease with increasing submergence period. Shoot growth was not inhibited by leaf desiccation during summer in any of the years (Table 2). This observation was consistent with previous studies reporting that no leaf damage occurs in *T. distichum* saplings that had been de-submerged for short periods.

Conclusions

T. distichum was more tolerant to total submergence than *M. glyptostroboides* and *A. japonica*. Nevertheless, all species had similar growth responses underwater. The underlying physiological and anatomical characteristics causing the difference in tolerance could include efficient aerobic and anaerobic respiration, carbon dioxide assimilation, and increased access to external and internal carbon dioxide and oxygen. Further investigation is needed to elucidate the mechanisms of submergence tolerance in these wetland species, including analysis of the relationship between the amount of reserve carbohydrates and their metabolic conservation at various plant ages.

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References

- Bailey-Serres J, Voesenek L. 2008. Flooding stress: acclimations and genetic diversity. *Annu Rev Plant Biol* 59, 313-319. <http://dx.doi.org/10.1146/annurev.arplant.59.032607.092752>
- Colmer TD, Pedersen O. 2008. Oxygen dynamics in submerged rice (*Oryza sativa*). *New Phytol* 178, 326-334. <http://dx.doi.org/10.1111/j.1469-8137.2007.02364.x>
- Colmer TD, Voesenek LACJ. 2009. Flooding tolerance: suites of plant traits in variable environments. *Funct Plant Biol* 36, 665-681. <http://dx.doi.org/10.1071/FP09144>
- da Fonseca SF, Pirdade MTF, Schöngart J. 2009. Wood growth of *Tabebuia barbata* (E. Mey.) Sandwith (Bignoniaceae) and *Vatairea guianensis* Aubl. (Fabaceae) in Central Amazonian black-water (igapó) and white-water (várzea) floodplain forests. *Trees* 23, 127-134. <http://dx.doi.org/10.1007/s00468-008-0261-4>
- Dicke SG, Toliver JR. 1990. Growth and development of bald cypress/water-tupelo stands under continuous versus seasonal flooding. *For Ecol Manage* 33/34, 523-530.
- Fujita H. 2002. Wetland forests. In: *Ecology of riparian forests* (Sakio H, Yamamoto F, eds). Tokyo University Press, Tokyo, Japan. pp. 98-137. [in Japanese].
- Gibbs J, Greenway H. 2003. Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. *Funct Plant Biol* 30, 1-47. <http://dx.doi.org/10.1071/PP98095>
- Grosse W, Schulte A, Fujita H. 1993. Pressurized transport in two Japanese alder species in relation to their habitats. *Ecol Res* 8, 151-158. <http://dx.doi.org/10.1007/BF02348527>
- Inoue K. 1998. Comparative study on flood-tolerance and the growth characteristics under flooding between *Taxodium distichum* and *Metasequoia glyptostroboides*. Bachelor's Thesis, Tottori University, Tottori. [in Japanese].
- Kozłowski TT. 1997. Responses of woody plants to flooding and salinity. *Tree Physiol Monograph* No.1.
- Kozłowski TT, Kramer PJ, Pallardy SG. 1991. Soil aeration, compaction, and flooding. In: *The physiological ecology of woody plants* (Kozłowski TT, Kramer PJ, Pallardy SG, eds). Academic Press, Tokyo, Japan. pp. 303-337. <http://dx.doi.org/10.1016/B978-0-12-424160-2.50012-4>
- Middleton B. 2000. Hydrochory, seed banks, and regeneration dynamics along the landscape boundaries of a forested wetland. *Plant Ecol* 146, 167-181. <http://dx.doi.org/10.1023/A:1009871404477>
- Middleton B. 2005. Primary production in an impounded baldcypress swamp (*Taxodium distichum*) at the northern limit of the range. *Wetlands Ecol Manage* 13, 15-24. <http://dx.doi.org/10.1007/s11273-003-5024-9>
- Momohara A. 2005. Palaeoecology and history of *Metasequoia* in Japan, with reference to extinction and survival in East Asia. In: *Topics in geobiology 22, The geobiology and ecology of Metasequoia* (Lepage B, Williams C, Yang H, eds). Springer, Norwell, MA, Netherlands. pp. 115-136.
- Moriwaki Y. 2000. Growth and physiological characteristics of *Taxodium distichum* saplings under flooding condition. Bachelor's thesis, Tottori University, Tottori. [in Japanese].
- Parolin P. 2009. Submergence in darkness: adaptation to prolonged submergence by woody species of the Amazon

- nian floodplains. *Ann Bot* 103, 359-376. <http://dx.doi.org/10.1093/aob/mcn216>
- Setter TL, Laureles EV. 1996. The beneficial effect of reduced elongation growth on submergence tolerance of rice. *J Exp Bot* 47, 1551-1559. <http://dx.doi.org/10.1093/jxb/47.10.1551>
- Setter TL, Bhakashut P, Greenway H. 2010. Desiccation of leaves after de-submergence is one cause for intolerance to complete submergence of the rice cultivar IR 42. *Funct Plant Biol* 37, 1096-1104. <http://dx.doi.org/10.1071/FP10025>
- Schöngart J, FPiedade MT, Wittmann F, Junk WJ, Worbes M. 2005. Wood growth patterns of *Macrobium acaciifolium* (Benth.) Benth. (Fabaceae) in Amazonian black-water and white-water floodplain forests. *Oecologia* 145, 454-461. <http://dx.doi.org/10.1007/s00442-005-0147-8>
- Shinshoh H. 1985 Alder forests in Kushiro Shitsugen marshes. *North For* 37, 92-97. [in Japanese].
- Voeselek LACJ, Colmer TD, Pierik R, Millenaar FF, Peeters AJM. 2006. How plants cope with complete submergence. *New Phytol* 170, 213-226. <http://dx.doi.org/10.1111/j.1469-8137.2006.01692.x>
- Waldhoff D, Furch B, Junk WJ. 2002. Fluorescence parameters, chlorophyll concentration, and anatomical features as indicators for flood adaptation of an abundant tree species in Central Amazonia: *Symmeria panicuata*. *Environ Exp Bot* 48, 225-235. [http://dx.doi.org/10.1016/S0098-8472\(02\)00037-0](http://dx.doi.org/10.1016/S0098-8472(02)00037-0)
- Wolter KE. 1968. A new method for making xylem growth. *Forest Science* 14, 102-104.