

Long- and short-term flooding effects on survival and sink–source relationships of swamp-adapted tree species

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Summary About 95% of swamp tupelo (*Nyssa sylvatica* var. *biflora* (Walt.) Sarg.) and sweetgum (*Liquidambar styraciflua* L.) seedlings survived continuous root flooding for more than two years, whereas none of the swamp chestnut oak (*Quercus michauxii* Nutt.) and cherrybark oak (*Q. falcata* var. *pagodifolia* Ell.) seedlings survived one year of flooding. Death of oak seedlings occurred in phases associated with periods of major vegetative growth, e.g., after bud burst in spring, after summer stem elongation, and during the winter deciduous stage, suggesting that stored reserves and sources were inadequate to maintain the seedlings when vegetative sinks were forming. Additional evidence that flooding induced a source deficiency in oak was that leaves of flooded oak were 65 to 75% smaller than leaves of nonflooded oak. Flooded swamp tupelo seedlings had a normal leaf size and patchy stomatal opening compared with nonflooded seedlings.

Flooding caused increases in alcohol dehydrogenase (ADH) specific activity in taproot cambial tissues and increases in starch concentrations of swamp tupelo seedlings that were reversed when seedlings were removed from flooding. Flooding had little effect on soluble sugar concentrations in swamp tupelo or sweetgum. In the long-term flood-dry-flood treatment, in which all species had survivors, upper canopy leaf photosynthetic rates were higher in all species during the dry period than in nonflooded controls, whereas their starch and soluble sugars concentrations were similar to those of nonflooded controls. Based on seedling survival and the sink–source relationships, the order of flood tolerance was: swamp tupelo > sweetgum > swamp chestnut oak > cherrybark oak.

Keywords: alcohol dehydrogenase, cherrybark oak, flood tolerance, *Liquidambar styraciflua*, *Nyssa sylvatica* var. *biflora*, patchy stomata, photosynthesis, *Quercus falcata* var. *pagodifolia*, *Quercus michauxii*, sinks, sources, starch, sucrose, swamp chestnut oak, swamp tupelo, sweetgum.

Introduction

An extensive literature exists on anaerobic and hypoxic stress in plants, particularly stress caused by root flooding (Hook 1984, Kozłowski 1984, Sachs and Ho 1986, Kennedy et al. 1992, Perata and Alpi 1993, Andrews et al. 1994, Armstrong et al. 1994, Ricard et al. 1994). Some plants, e.g., maize (*Zea mays* L.), cannot survive even a few days of flooding, whereas other plants, e.g., baldcypress (*Taxodium distichum* (L.) Rich. var. *distichum*) and swamp tupelo (*Nyssa sylvatica* var. *biflora* (Walt.) Sarg.) grow well under flooded conditions. On some sites, these plants may be partially submersed for several months or longer and experience almost continuous root anaerobiosis (Hook 1984, Kennedy et al. 1992, Armstrong et al. 1994).

Tree leaf photosynthesis also responds in a species-specific manner to root flooding. For example, photosynthesis of cottonwood (*Populus deltoides* Battr. ex Marsh.) was reduced by about 50% after 7 days of flooding, but recovered within 7 days after 28 days of flooding (Regehr et al. 1975). In contrast, flooding of green ash (*Fraxinus pennsylvanica* Marsh.) and baldcypress did not cause significant decreases in photosynthesis despite a 50% decrease in stomatal conductance (Pezeshki and Chambers 1986). Low tolerance to anaerobic stress has been related to decreases in the partitioning of carbon to flooded organs (Vartapetian et al. 1977). However, high sucrose and low starch concentrations were found in flooded alfalfa (*Medicago sativa* L.) roots which led to the suggestion that flooding reduces the ability of roots to utilize photosynthates in growth (Barta 1987, Castonguay et al. 1993).

Anaerobic and hypoxic stress is known to stop the normal synthesis of cellular proteins and to induce (within approximately 5 h) the synthesis of about 20 anaerobic polypeptides (ANPs) (Sach and Ho 1986, Neuman and Smit 1993); one of which was identified as alcohol dehydrogenase (ADH) (Hage-

man and Flesher 1960). Most of the identified ANPs are glycolytic enzymes (Andrews et al. 1994) including sucrose synthase (Ricard et al. 1991), which can be used as an indicator of sink strength (Sung et al. 1993).

Although the anaerobic biochemistry of various herbaceous plants is partially understood, little is known about the anaerobic biochemistry and physiology of trees. We have examined how swamp-adapted trees change their sink-source relationship in response to flooding. Here we report the survival, growth, and sink-source traits of four swamp-adapted tree species when seedlings were flooded either for 4 to 6 months or continually for over 2 years.

Materials and methods

Plant materials and flooding treatments

The long-term experiment was conducted at the Santee Experiment Forest, Berkeley County, South Carolina in the Santee Hydroedaphytron from February 1993 through February 1995. The hydroedaphytron is comprised of 12 concrete tanks (1.8 × 1.8 × 1.8 m, inside dimension) (Harms 1973). The soil profile in each tank comprised a 15-cm layer of gravel on the bottom, followed by a 100-cm layer of coarse sand, a 46-cm layer of sandy loam subsoil from a Goldsboro series, and a final 15-cm layer of loam surface soil from a Meggett series. Goldsboro is an Aquic Paleudult and the Meggett is a Typic Albaqualf. Both soils commonly occur in the lower Atlantic coastal plain.

Locally collected seeds of swamp tupelo (*Nyssa sylvatica* var. *biflora* (Walt.) Sarg.), sweetgum (*Liquidambar styraciflua* L.), swamp chestnut oak (*Quercus michauxii* Nutt.), and cherrybark oak (*Q. falcata* var. *pagodifolia* Ell.) were germinated and grown for 5 months. In July 1992, 25 seedlings of each species were transplanted in randomized groups to each soil tank and grown under well-drained soil conditions until February 1993 when the flooding treatments started.

Three common wetland growth conditions were compared in the treatments: (1) a pond-type treatment (CF) where seedlings were continuously flooded with stagnant water to a depth of 15 cm above the soil surface for the duration of the study; (2) a swamp bottomland-type treatment (FDF) where seedlings were flooded with water constantly moving at 15 cm above the soil surface, followed by drying to a water table at 15 cm below the soil surface. The dry cycle of the flood-dry-flood treatment started in June and the flood cycle started in November, except in 1993 when flooding started in February; and (3) an upland-type treatment (NF) where the water table was maintained at 30 cm below the soil surface. Periodically, seedling height, root collar diameter (RCD), survival, and visible morphological changes were recorded. Survival was assessed by checking for green leaves and bark peeling. Periodically, root and shoot tissues were sampled, immediately frozen in liquid N₂, transported to Athens, and stored at -80 °C until analyzed for carbohydrates or enzymatic activity.

Short-term (9 week) experiments with swamp tupelo were conducted in a greenhouse at the University of Georgia. Seedlings were grown without supplemental lights and maximum photosynthetic active radiation (PAR) was about 1600 μmol

m⁻² s⁻¹. Day/night temperatures were 25/20 °C. Seedlings about 20 cm tall were placed in 64-l tubs lined with plastic sheets and flooded with tap water to the soil surface for 3 weeks followed by a 3-week dry period and a second 3-week flooding period (short-term FDF). A set of nonflooded control seedlings was maintained for each short-term experiment (short-term NF).

Enzyme assays and analysis of nonstructural carbohydrates

Composite samples of five seedlings were used for both the enzyme assays and the nonstructural carbohydrate analyses. Cambial tissues of stems and taproots were obtained by peeling the bark from either the stem or taproot, rapidly scraping the vascular cambial tissues and immediately freezing them in liquid N₂. Soluble proteins were extracted and desalted as described by Sung et al. (1993). The following enzymes were assayed at 25 °C from each extract: sucrose synthase, acid invertase, neutral invertase, pyrophosphate-dependent phosphofructokinase (both forward and reverse reactions), ATP-dependent phosphofructokinase, UDPglucopyrophosphorylase, fructose 1,6-bisphosphate aldolase, and glucose 6-phosphate dehydrogenase following the procedures described by Xu et al. (1989). Pyruvate decarboxylase and alcohol dehydrogenase were assayed as described by Kimmerer (1987).

Tissues for nonstructural carbohydrate analysis were collected from taproot segments 2 cm beneath the soil surface and from stem segments 2 cm above the soil surface. One g of tissue was extracted for ethanol-soluble sugars (glucose, fructose, and sucrose) and ethanol-insoluble starch following the modified procedures described by Angelov et al. (1993).

Leaf photosynthesis, chlorophyll analysis, and ¹⁴C₂ fixation

A portable, closed-system LI-6200 CO₂ analyzer (Li-Cor, Inc., Lincoln, NE) with a 4-l chamber was used to measure intact, attached leaf photosynthetic rates of upper-canopy leaves. Measurements were made between 1000 and 1200 h at 1100–1600 μmol m⁻² s⁻¹, 25–30 °C, 55–65% relative humidity, and 330–350 ppm CO₂. Chlorophyll concentrations were determined from composite leaf discs of all leaves from five seedlings (Angelov et al. 1993). The products of ¹⁴C₂-photosynthesis were determined in a mature attached leaf (number 12 from the top) from swamp tupelo after 21 days of flooding. The whole leaf was enclosed in a 0.8-dm³ Plexiglas chamber. About 40 μCi of ¹⁴CO₂ (56 mCi mmol⁻¹) was injected to give an initial CO₂ concentration of 360 μl l⁻¹ in the chamber. Chamber temperature was between 28–30 °C and PAR was 800–900 μmol m⁻² s⁻¹. After a 1- or 2-min ¹⁴CO₂ pulse, the leaf was immediately frozen in liquid N₂, powdered in liquid N₂, extracted with 80% boiling ethanol (v/v), and the ¹⁴C-labeled products were chromatographed as described by Angelov et al. (1993). Values presented are an average of two replications.

Seedling morphology and anatomy

For the anatomical studies, samples were processed and fixed as described by Angelov et al. (1993) and examined with a Philips 505 Scanning Electron Microscope at the University of

Georgia Microscopy Laboratory.

Results

Long-term survival and growth

The four swamp tree species exhibited different responses to long-term flooding (Figure 1). Swamp tupelo and sweetgum survived 2 years of continuous flooding (CF) with less than 5% mortality, whereas the CF treatment was lethal for cherrybark and swamp chestnut oaks within 1 year (Figure 1a). Mortality of oak seedlings occurred in phases associated with periods of major vegetative growth: after spring bud break and stem elongation, after summer stem elongation, and in the winter deciduous stage before spring bud break (Figure 1a), which suggests that death occurred because stored reserves and current sources were inadequate to maintain the plants and support new vegetative sinks.

After 2 years in the flood-dry-flood (FDF) treatment, survival of swamp tupelo, sweetgum, and swamp chestnut oak was 90–100%, whereas survival of cherrybark oak was only 49% (Figure 1). All species grew in the FDF treatment and the growth of swamp tupelo exceeded that of the NF controls, whereas the growth of cherrybark oak seedlings was less than that of the NF controls (Table 1).

Sink studies

Long-term flooding and ADH activity of swamp tupelo and sweetgum In response to flooding for 5 and 8 months, taproot cambial ADH specific activity of swamp tupelo seedlings increased 3.3- and 2-fold, respectively, and stem cambial ADH

specific activity increased 1.7- and 3-fold, respectively, compared with the values for NF seedlings (Table 2). Flood-induced stem cambial ADH specific activity decreased to the pre-flood value in 6 weeks after drying. Flood-induced root cambial ADH activity was not completely reversible and roots of FDF seedlings had 75 and 30% higher ADH specific activities than roots of NF seedlings 6 weeks and 4 months after the termination of flooding, respectively (Table 2).

The NF sweetgum seedlings had a similar amount of ADH

Table 1. Height and root collar diameter (RCD) of swamp-adapted tree species after two growing seasons in the hydroedaphytions. All flooding treatments began in February 1993 when the seedlings were about 1 year old. For the flood-dry-flood treatment, drying began in early June and lasted until early November and flooding resumed from November 1993 until early June 1994 followed by a second cycle of drying and flooding. The data were collected in November 1994.

Species	Treatment	Height (cm)	RCD (mm)
Swamp tupelo	CF ¹	210 ± 20	32 ± 3
	FDF	269 ± 34	26 ± 5
	NF	204 ± 33	20 ± 3
Sweetgum	CF	86 ± 26	9 ± 2
	FDF	120 ± 14	14 ± 2
	NF	115 ± 23	12 ± 3
Cherrybark oak	CF	Dead	—
	FDF	60 ± 18	6 ± 2
	NF	83 ± 28	7 ± 2
Swamp chestnut oak	CF	Dead	—
	FDF	103 ± 25	10 ± 3
	NF	95 ± 26	10 ± 1

¹ CF = continuous flooding; FDF = flood-dry-flood; NF = nonflooded control.

Table 2. Effect of long-term flooding on the specific activity of alcohol dehydrogenase (ADH) ($\text{nmol mg}^{-1} \text{min}^{-1}$) of stem and taproot cambial tissues of swamp tupelo and sweetgum seedlings. A composite sample of five seedlings were used for each enzyme assay.

Treatment	Swamp tupelo		Sweetgum			
			Stem ¹		Root	Stem ¹
	July	Oct	July	Oct	July	July
CF ²	811	425	208	169	— ³	331
FDF	429	274	89	76	— ³	135
NF	243	212	122	57	225	120

¹ Stem cambial tissue was obtained from stem sections 15 cm above the soil surface.

² CF = continuous flooding; FDF = flood-dry-flood; NF = nonflooded control. For seedlings in the FDF treatment, July 1993 samples were collected 6 weeks after lowering the water level to 15 cm below the soil surface and October 1993 samples were collected 4 weeks before the start of the second winter flooding.

³ The bark could not be separated from the cambial tissue in these seedlings.

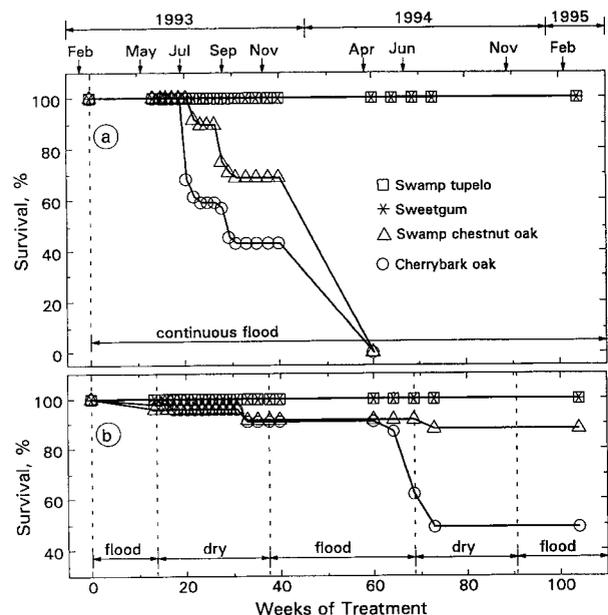


Figure 1. Effects of long-term flooding on survival of four swamp-adapted tree species: (a) continuous flood treatment and (b) flood-dry-flood treatment in the hydroedaphytion. Arrows indicate the first day of the month.

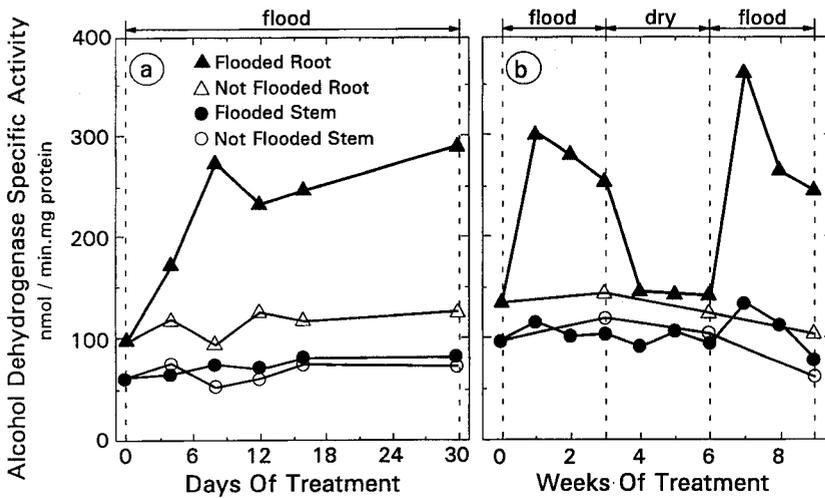


Figure 2. Effects of short-term continuous flooding (a) and flood-dry-flood treatment (b) on specific activity of alcohol dehydrogenase in stem and taproot cambial tissues of swamp tupelo seedlings. A composite sample of five seedlings was used for each enzyme assay.

specific activity as the NF swamp tupelo seedlings (Table 2). By July, stem cambial ADH specific activity in CF sweetgum seedlings was almost 3-fold higher than in NF sweetgum seedlings. The flood-induced stem cambial ADH activity was almost completely reversible (see values for FDF sweetgum seedlings in Table 2). Alcohol dehydrogenase was not assayed in taproot cambial tissue of sweetgum because the taproots were deformed and the bark and cambium could not be separated. None of the flooding treatments affected the activities of sucrolytic and glycolytic enzymes in swamp tupelo and sweetgum (Sung et al. 1989).

Short-term flooding and enzymatic activities in swamp tupelo

All the sucrolytic and glycolytic enzymes assayed were present in root and stem cambial tissue extracts (data not shown). The specific activities of these enzymes, except for ADH, were not influenced by flooding. Flooding caused an almost 3-fold increase in root ADH specific activity of swamp tupelo seed-

lings within 10 days (Figure 2a). The flood-induced root ADH specific activity increase declined to the value in NF roots within one week of drying (Figure 2b). Stem cambial ADH specific activity was not influenced by the short-term flooding treatment (Figure 2).

Partitioning of nonstructural carbohydrates Seedling starch

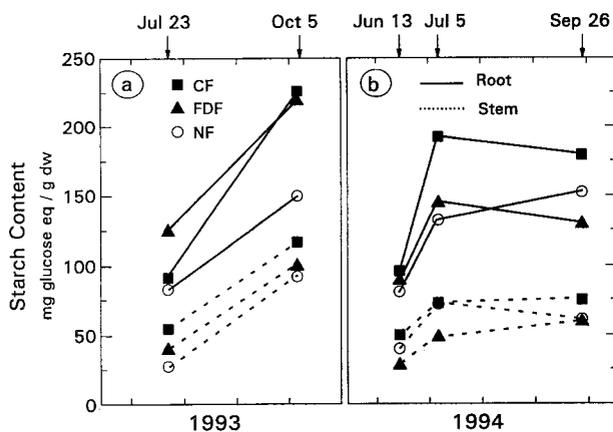


Figure 3. Effects of long-term flooding on starch concentrations of swamp tupelo seedling taproots and stems during 1993 and 1994. A composite sample of five seedlings was used for each carbohydrate analysis. Abbreviations: NF = nonflooded control, CF = continuous flood treatment, and FDF = flood-dry-flood treatment.

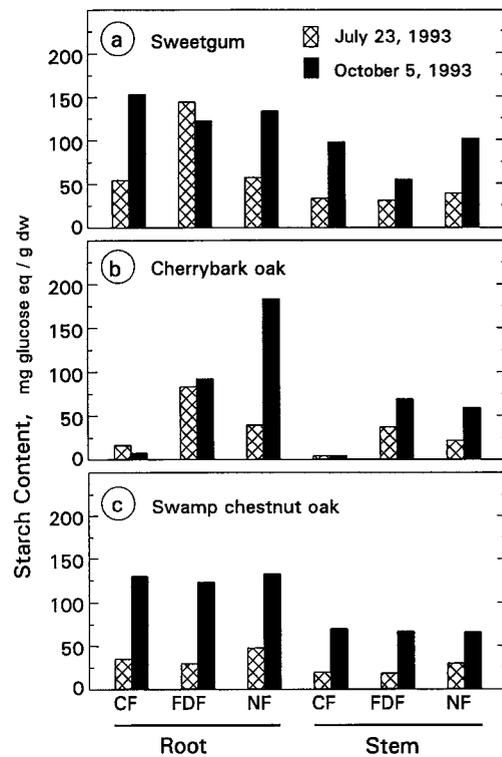


Figure 4. Effects of long-term flooding on starch concentration of (a) sweetgum, (b) cherrybark oak, and (c) swamp chestnut oak in the months of July and October of 1993. A composite sample of five seedlings was used for each carbohydrate analysis. Abbreviations: NF = nonflooded control, CF = continuous flood treatment, and FDF = flood-dry-flood treatment.

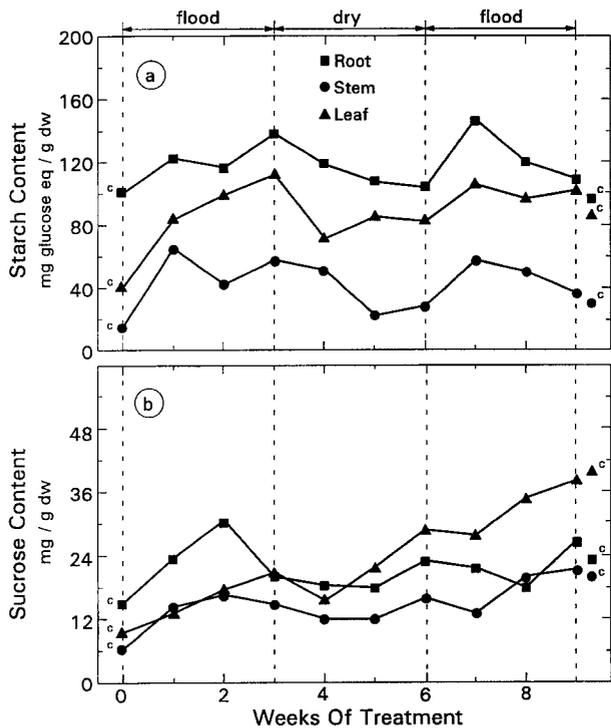


Figure 5. Effects of a short-term flood-dry-flood treatment on (a) starch concentration and (b) sucrose concentration in roots, stems and leaves of swamp tupelo seedlings. The letter "c" beside a symbol indicates that the value was obtained from nonflooded control seedlings. A composite sample of five seedlings was used for each carbohydrate analysis.

concentrations were higher than the sucrose, glucose and fructose concentrations combined (data not shown) and taproots had higher starch concentrations than stems (Figures 3 and 4).

A seasonal increase in starch concentration from summer to fall was observed in NF seedlings of all four species (Figures 3a and 4). During the growing season, flooding increased the starch concentration of swamp tupelo seedling taproots, but it had no effect on total stem starch concentration, although the portion of the stem that was submerged in water swelled and had a greater starch concentration than the portion of the stem above the water surface (159 versus 117 mg glucose eq g_{dw}^{-1}). Flooding had no effect on the starch concentrations of sweetgum and swamp chestnut oak seedlings (Figures 4a and 4c). The CF treatment decreased the starch and sucrose concentrations of cherrybark oak seedlings by 94% (Figure 4b) and 70% (from 40.5 to 11.8 mg g_{dw}^{-1}), respectively.

In the short-term FDF study, starch concentrations of taproots, leaves, and stems of swamp tupelo increased within 1 week of the first flooding period and decreased within 1 week of drying (Figure 5a). During the second flooding period, starch concentrations initially increased and then decreased to control values. The short-term FDF treatment had no effect on the concentrations of sucrose (Figure 5b), glucose or fructose (data not shown).

Source studies

Flooding, photosynthesis and leaf size Despite leaf chlorosis and patchy stomatal opening (see Figure 7), photosynthetic rates in upper-canopy leaves of CF swamp tupelo seedling were higher than in upper-canopy leaves of NF controls (Figure 6a). In contrast, the CF treatment decreased photosynthesis in sweetgum and caused the leaves to turn dark red (Figure 6c). After flooded seedlings were drained, leaf photosynthetic rates in all four species were about the same as in the corresponding NF control seedlings (Figure 6). For both oaks, leaves formed during the dry period had 3- to 4-fold greater leaf area than leaves formed during flooding and these leaves were used for

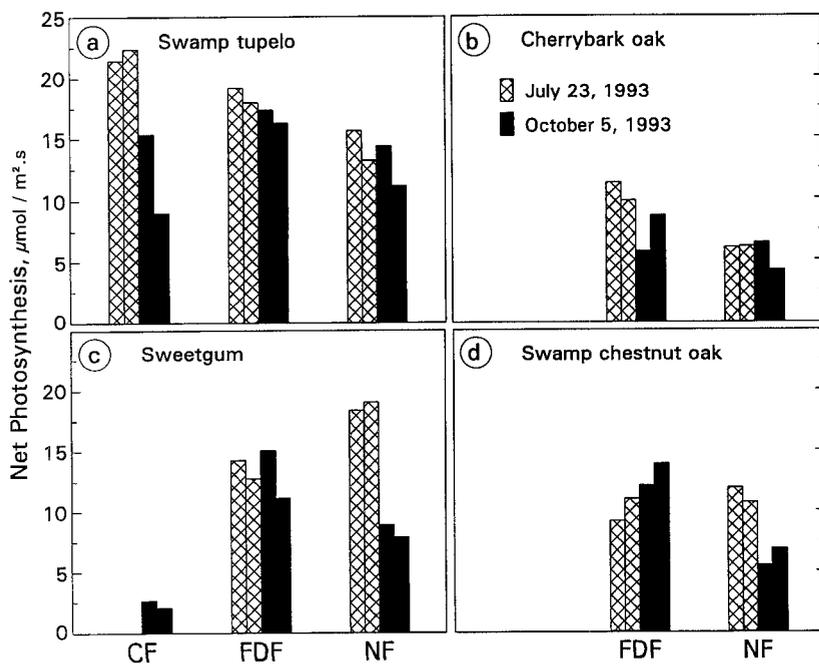


Figure 6. Effects of long-term flooding on net photosynthesis of four swamp-adapted tree species. For each species, two upper-canopy mature leaves were measured per treatment and plotted separately. Abbreviations: NF = nonflooded control, CF = continuous flood treatment, and FDF = flood-dry-flood treatment.

the photosynthesis measurements of FDF seedlings presented in Figure 6. The CF treatment had no effect on the leaf area of swamp tupelo and sweetgum seedlings.

Flooding, chlorophyll concentration and ^{14}C -labeled photosynthates in swamp tupelo During the 9-week study, total leaf chlorophyll concentrations of NF tupelo seedlings decreased from 1.89 to 1.56 mg $\text{g}_{\text{fw}}^{-1}$. One week after flooding, total chlorophyll concentrations had decreased to 1.44 mg $\text{g}_{\text{fw}}^{-1}$ and they continued to decrease throughout the flooding treatment to reach 0.98 mg $\text{g}_{\text{fw}}^{-1}$ after 9 weeks.

After a 2-min pulse, a similar amount of $^{14}\text{CO}_2$ was fixed by leaves of nonflooded seedlings and seedlings flooded for 21 days (Table 3), but larger amounts of sucrose and starch were labeled in leaves of flooded plants than in leaves of control plants. The amino acids and organic acid in the flooded seedlings contained less radioactive carbon than in the controls, indicating an enhanced diversion of ^{14}C into starch and sucrose (Table 3). For example, ^{14}C -labeling of malate in flooded seedlings was only about 10% of that in NF control seedlings. Flooding had no effect on ^{14}C -labeling of the other compounds measured (Table 3).

Morphological and anatomical responses to flooding

Continuous flooding caused several morphological changes. These were most evident in swamp tupelo and included formation of lenticels on stems, both above and below the flood water level, and development of adventitious roots from lenticels below the flood water level. Lateral roots that developed after flooding were more succulent, with less branching, and with more space between aerenchyma cells than lateral roots of NF seedlings. Stomata on the lower surface of CF seedling leaves exhibited a nonhomogeneous patchy opening (Figure 7a) compared with NF seedling leaves (Figure 7b). About 63% of the stomata were open in the CF leaves, whereas almost 100% of the stomata were open in the NF leaves. Furthermore, the pore sizes of the open stomata in CF leaves were much less homo-

Table 3. Percent of total ^{14}C fixed in ethanol-soluble and ethanol-insoluble compounds after upper-canopy mature attached leaves of nonflooded control and flooded swamp tupelo seedlings were pulsed for 2 min with $^{14}\text{CO}_2$. The seedlings had been flooded for 21 days. Total $^{14}\text{CO}_2$ fixed by control and flooded leaves was 381×10^3 and 366×10^3 dpm $\text{cm}^{-2} \text{min}^{-1}$, respectively.

Labeled compounds	Control	Flooded
<i>Ethanol-soluble products</i>		
3-Phosphoglyceric acid	5.6	9.9
Sugar phosphates	9.5	8.3
Sucrose	23.4	30.4
Aspartate	11.9	5.4
Malate	3.2	0.2
Alanine	2.2	2.8
Glycine + Serine	20.8	12.5
Unknown	2.5	1.4
<i>Ethanol-insoluble products</i>		
Starch	20.7	29.0

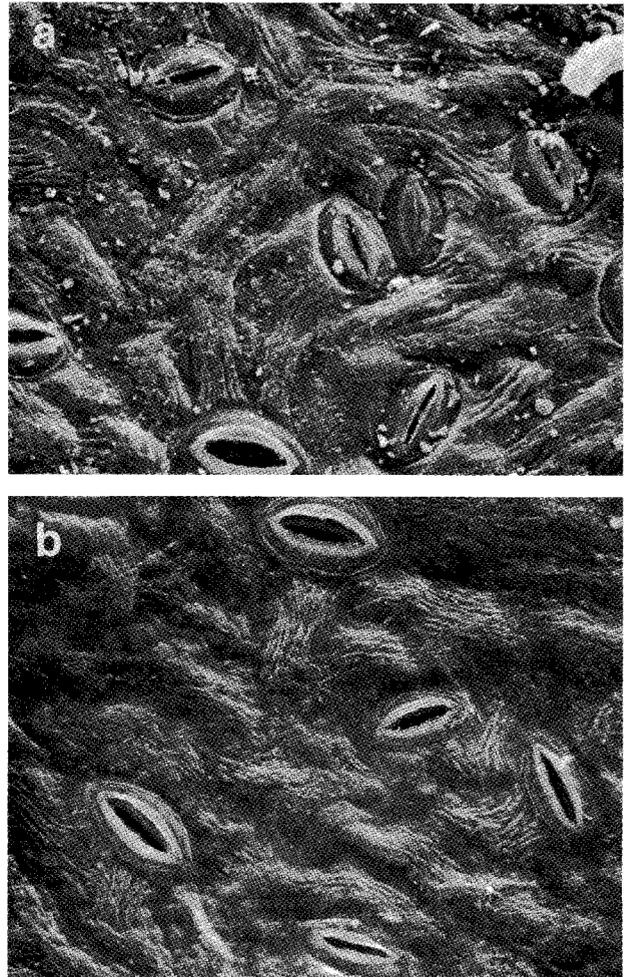


Figure 7. Scanning electron microscopy of lower leaf surface of swamp tupelo seedlings in (a) the long-term continuously flooded treatment and (b) the nonflooded control treatment.

geneous than in NF leaves (Figure 7a versus 7b).

Lenticels formed on sweetgum stems, above and below the flood water level, and adventitious roots emerged from some of the lenticels below the water level. The unique morphological response of sweetgum to flooding was a proliferation of a fibrous hairy root mass around the stem 1–3 cm below the floodwater level. Belowground, roots of the CF sweetgum seedlings were deformed and the bark could not be separated from the cambium. After 2 years of continuous flooding, some sweetgum roots disintegrated and broke off; nevertheless, about 95% of the sweetgum seedlings were still alive in February 1995. Neither lenticels nor new roots were observed in either oak species. Oak leaves formed during flooding periods were chlorotic and 60 to 75% smaller than leaves formed during dry periods.

Discussion

Survival and growth during flooding

The long-term survival data confirmed the flood tolerance

rankings of the four wetland tree species (see Hook 1984). Under continuously flooded conditions, swamp tupelo can live and grow for up to 2 years, sweetgum can tolerate 1 year of flooding, and swamp chestnut can tolerate up to 6 months. Cherrybark oak tolerates less than 6 months of continuous flooding and cannot tolerate flooding for two consecutive winter seasons (Figure 1). In the FDF regime, which closely mimicks the conditions found in wetlands in the southern USA, all four swamp species grew, though cherrybark oak began to die during the second year (Figure 1, Table 1).

Sink studies

Although most published work on ADH activity in flooded trees has focused on enzymatic activity of new root tips (e.g., Kimmerer 1987), we examined ADH activity in vascular cambial tissues because these tissues are indicative of organ and tree growth and its physiological status (Sung et al. 1993). Alcohol dehydrogenase activity in the taproot cambial tissues of swamp tupelo seedlings increased 3-fold within 10 days of flooding (Figure 2a) and remained higher than in NF roots for 8 months (Table 2). This flood-induced increase in ADH activity was reversed when the seedlings were placed under well-drained conditions (Figure 2b, Table 2). Burdick and Mendelssohn (1990) reported a similar reversibility of flood-induced root ADH activity in *Spartina*. Unlike taproot cambial ADH, stem cambial ADH activity did not respond to 4 weeks or less of flooding (Figure 2). Although ADH is the only enzyme showing consistent changes in response to short- and long-term flooding or drying in swamp tupelo and sweetgum seedlings, the physiological role of flood-induced ADH activity is unclear (Perata and Alpi 1993, Ricard et al. 1994). All plant species that have been examined exhibit increased ADH activity in response to flooding, but only some of these species are flood tolerant.

More starch accumulated in leaves of flooded swamp tupelo seedlings than in nonflooded seedlings (Figure 5 and Table 3). Similar results have been reported for flooded conifer seedlings (Topa and Cheeseman 1992) and alfalfa (Castonguay et al. 1993). Several studies have shown that flooding causes a reduction of starch concentrations in roots (Castonguay et al. 1993, Armstrong et al. 1994). However, we found that both short- and long-term flooding of swamp tupelo seedlings resulted in increased concentrations of starch (Figures 3 and 5a) in stems and taproots, whereas the concentrations of soluble sugars remained unchanged (Figure 5b). We postulate that flooded swamp tupelo seedlings have normal glycolytic enzyme activities to maintain intermediary metabolism and produce ATP, increased ADH activity for recycling NAD^+ , and increased starch reserves for use in sink growth and respiration.

Source studies

Reports on net photosynthesis in flooded tree species are contradictory. A few days of flooding decreased photosynthesis 77 and 88%, respectively, in sweetgum (Pezeshki and Chambers 1985a) and cherrybark oak (Pezeshki and Chambers 1985b), whereas a 21-day flooding period had no effect

on photosynthesis of green ash or baldcypress (Pezeshki and Chamber 1986). Pezeshki (1994) reported that the photosynthetic rate of baldcypress decreased 40% within 48 h of flooding but that the rate returned to that of unflooded control trees after 28 days of flooding. We found that when swamp chestnut oak and cherrybark oak seedlings were removed from flooded conditions they formed leaves that were greater in area and had higher photosynthetic rates than leaves formed during the flood treatment or leaves formed on the NF control seedlings (Figures 6b and 6d). Similarly, leaves formed after sweetgum and swamp tupelo seedlings were removed from flooded conditions had equal or greater photosynthetic rates than leaves of nonflooded controls (Figures 6a and 6c). Therefore, provided that leaf development of flooded seedlings can take place during a dry summer period, all four species can survive winter flooding (Figures 1 and 6, Table 1).

Morphology and anatomy

The flood-induced structural changes in swamp tupelo were similar to those described by Hook et al. (1971) and Hook and Brown (1973), who postulated that the changes supplied O_2 to the flooded tissues. We found that sweetgum also underwent morphological and structural changes in response to flooding. These changes included lenticel formation, the emergence of adventitious roots from lenticels, the production of a fibrous hairy root mass on the submerged stem, and the disintegration of the below-ground roots. The large increase in fibrous root surface area on the sweetgum stem likely enabled this species to survive continuous flooding after the taproot began to disintegrate.

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References

- Andrews, D.L., D.M. MacAlpine, J.R. Johnson, P.M. Kelley, B.G. Cobb and M.C. Drew. 1994. Differential induction of mRNAs for the glycolytic and ethanolic fermentative pathways by hypoxia and anoxia in maize seedlings. *Plant Physiol.* 106:1575–1582.
- Angelov, M.N., J. Sun, G.T. Byrd, R.H. Brown and C.C. Black. 1993. Novel characteristics of cassava, *Manihot esculenta* Crantz, a reputed C_3 - C_4 intermediate photosynthesis species. *Photosyn. Res.* 38:61–72.
- Armstrong, W., R. Brändle and M.B. Jackson. 1994. Mechanisms of flood tolerance in plants. *Acta Bot. Neerl.* 43:307–358.
- Barta, A.L. 1987. Supply and partitioning of assimilates to roots of *Medicago sativa* L. and *Lotus corniculatus* L. under anoxia. *Plant Cell Environ.* 10:151–156.
- Burdick, D.M. and I.A. Mendelssohn. 1990. Relationship between anatomical and metabolic responses to soil waterlogging in the coastal grass *Spartina patens*. *J. Exp. Bot.* 41: 223–228.
- Castonguay, Y., P. Nadeau and R. Simard. 1993. Effects of flooding on carbohydrate and ABA levels in roots and shoots of alfalfa. *Plant Cell Environ.* 16:695–702.

- Hageman, R.H. and D. Fleisher. 1960. The effect of an anaerobic environment on the activity of alcohol dehydrogenase and other enzymes of corn seedlings. *Arch. Biochem. Biophys.* 87:203–209.
- Harms, W.R. 1973. Some effects of soil type and water regime on growth of tupelo seedlings. *Ecology* 54:188–193.
- Hook, D.D. 1984. Waterlogging tolerance of lowland tree species of the South. *South. J. Appl. For.* 8:136–148.
- Hook, D.D. and C.L. Brown. 1973. Root adaptations and relative flood tolerance of five hardwood species. *For. Sci.* 19:225–229.
- Hook, D.D., C.L. Brown and P.P. Kormanik. 1971. Inductive flood tolerance in swamp tupelo (*Nyssa sylvatica* var. *biflora* (Walt.) Sarg.). *J. Exp. Bot.* 22:78–89.
- Kennedy, R.A., M.E. Rumpho and T.C. Fox. 1992. Anaerobic metabolism in plants. *Plant Physiol.* 100:1–6.
- Kimmerer, T.W. 1987. Alcohol dehydrogenase and pyruvate decarboxylase activity in leaves and roots of eastern cottonwood (*Populus deltoides* Bartr.) and soybean (*Glycine max* L.). *Plant Physiol.* 84:1210–1213.
- Kozlowski, T.T. 1984. Responses of woody plants to flooding. In *Flooding and Plant Growth*. Ed. T.T. Kozlowski, Academic Press, New York, pp 129–163.
- Neuman, D.S. and B.A. Smit. 1993. Root hypoxia-induced changes in the pattern of translatable mRNAs in poplar leaves. *J. Exp. Bot.* 44:1781–1786.
- Perata, P. and A. Alpi. 1993. Plant responses to anaerobiosis. *Plant Sci.* 93:1–17.
- Pezeshki, S.R. 1994. Responses of baldcypress (*Taxodium distichum*) seedlings to hypoxia: leaf protein content, ribulose-1,5-bisphosphate carboxylase/oxygenase activity and photosynthesis. *Photosynthetica* 30:59–68.
- Pezeshki, S.R. and J.L. Chambers. 1985a. Stomatal and photosynthetic response of sweetgum (*Liquidambar styraciflua*) to flooding. *Can. J. For. Res.* 15:371–375.
- Pezeshki, S.R. and J.L. Chambers. 1985b. Responses of cherrybark oak seedlings to short-term flooding. *For. Sci.* 31:760–771.
- Pezeshki, S.R. and J.L. Chambers. 1986. Variation in flood-induced stomatal and photosynthetic responses of three bottomland tree species. *For. Sci.* 4:914–923.
- Regehr, D.L., F.A. Bazzaz and W.R. Boggess. 1975. Photosynthesis, transpiration and leaf conductance of *Populus deltoides* in relation to flooding and drought. *Photosynthetica* 9:52–61.
- Ricard, B., J. Rivoal, A. Spiteri and A. Pradet. 1991. Anaerobic stress induces the transcription and translation of sucrose synthase in rice. *Plant Physiol.* 95:669–674.
- Ricard, B., I. Couée, P. Raymond, P.H. Saglio, V. Saint-Ges and A. Pradet. 1994. Plant metabolism under hypoxia and anoxia. *Plant Physiol. Biochem.* 32:1–10.
- Sachs, M.M. and T.D. Ho. 1986. Alteration of gene expression during environmental stress in plants. *Annu. Rev. Plant Physiol.* 37:363–376.
- Sung, S.S., P.P. Kormanik, D.-P. Xu and C.C. Black. 1989. Sucrose metabolic pathways in sweetgum and pecan seedlings. *Tree Physiol.* 5:39–52.
- Sung, S.S., P.P. Kormanik and C.C. Black. 1993. Vascular cambial sucrose metabolism and growth in loblolly pine (*Pinus taeda* L.) in relation to transplanting stress. *Tree Physiol.* 12:243–258.
- Topa, M.A. and J.M. Cheeseman. 1992. Carbon and phosphorus partitioning in *Pinus serotina* seedlings growing under hypoxic and low-phosphorus conditions. *Tree Physiol.* 10:195–207.
- Vartapetian, B.B., I.N. Andreeva, B.I. Koslova and L.P. Agapova. 1977. Mitochondrial ultrastructure in roots of mesophyte and hydrophyte at anoxia and after feeding glucose. *Protoplasma* 91:243–256.
- Xu, D.-P., S.S. Sung and C.C. Black. 1989. Sucrose metabolism in lima bean seeds. *Plant Physiol.* 89:1106–1116.