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Inductive Flood Tolerance in Swamp Tupelo (*Nyssa sylvatica* var. *biflora* (Walt.) Sarg.)

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ABSTRACT

Under flooding or anoxia the newly initiated roots of swamp tupelo produce ethanol and lactic acid and oxidize their rhizosphere. Unflooded roots produce less ethanol than flood roots and do not oxidize their rhizosphere. Oxygen enters the stem via the lenticels and appears to be diffused or transported via the cortex or phloem. Flood roots have less suberization in the epidermis in the terminal 2-cm section and casparian strips were less evident than in the same sections in unflooded roots. Swamp tupelo roots tolerated 10 per cent CO_2 without adverse effects but 31 per cent CO_2 reduced the initiation of new roots, rate of O_2 uptake, and transpiration rate. Tolerance to high CO_2 around the root seemed to be related to the oxidation of the rhizosphere by new roots. The combined adaptations of accelerated anaerobic respiration in the absence of O_2, oxidation of the rhizosphere, and CO_2 tolerance of new roots appear to be sufficient conditions to account for flood tolerance in this species.

INTRODUCTION

Flood tolerance in higher plants is generally attributed to one of two adaptive mechanisms: (*a*) diffusion of O_2 from the atmosphere to the roots via the stem, or (*b*) anaerobic respiration in the roots.

Van Raalte (1940) and Barber, Ebert, and Evans (1962) found that O_2 diffused from the roots of rice into an anaerobic surrounding, and Armstrong (1964, 1968) obtained similar results from the roots of several bog species and from two woody genera, *Salix* and *Myrica*. Oxidizing activity has also been found around the roots of *Salix atrocinerea* (Leyton and Rousseau, 1958) and *Betula pubescens* (Huikari, 1954). In contrast, accelerated or sustained rates of anaerobic respiration have been shown to occur in the rhizomes of *Nuphar advenum* (Laing, 1940), and the roots of rice (Taylor, 1942), *Senecio aquaticus*, *Phalaris arundinacea*, *Ranunculus flammula*, *Juncus effusus* (Crawford, 1966, 1967; Crawford and McManmon, 1968), and *Nyssa sylvatica* var. *biflora* (Walt.) (Sarg.) (Hook and Brown, 1967) under low O_2 concentrations or in the absence of O_2.

A basic assumption is that either of the above mechanisms permit roots to grow in an environment devoid of O_2 and therefore, is sufficient to account for flood

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WITH ONE PLATE IN THE TEXT

tolerance in a species. This assumption appears to be inadequate for several reasons. First, O_2 is transported through the stems to the roots of onion, pea, lettuce, beetroot, leek, buckwheat, turnip, barley, rye grass, carrot, cabbage (Greenwood, 1967), and corn (Jensen, Letey, and Stolzy, 1964), yet none of these species are tolerant to flooding. Therefore, transport of O_2 to roots may be a necessary, but apparently not a sufficient condition for flood tolerance in plants. Second, the energy yield per glucose molecule from anaerobic respiration is about one-tenth that of the aerobic pathway, yet many flood-tolerant species make the best growth with their roots and lower stem flooded, for example rice (International Rice Research Institute, 1964) and swamp and water tupelos (Applequist, 1960; Dickson, Hosner, and Hosley, 1965). Third, rice gives greater yields under partially submerged than under well-drained conditions and appears to have both adaptive mechanisms (Van Raalte, 1940; Barber *et al.*, 1962; and Taylor, 1942).

In addition to the effects on aeration, flooding results in the accumulation of CO_2 and other gases as well as carbonates and bicarbonates of iron, manganese, and aluminium ions in the soil water (Robinson, 1930; and International Rice Research Institute, 1964). Species differences in tolerance to these factors could also influence their flood tolerances.

We report the effects of aerated and non-aerated root environments on the induction of new roots and their adaptations to anaerobic respiration, gas transport, and tolerance to CO_2 in swamp tupelo seedlings.

GENERAL METHODS

Swamp tupelo was used as the primary test species because of its flood tolerance. It is seldom found on sites that are not inundated most of the growing season, and experimental evidence shows that it grows as well in flooded as in well-drained, moist soils (Applequist, 1960). However, its growth is reduced by about 50 per cent by flooding in stagnant as compared with moving water (Hook, 1968).

Newly germinated seedlings were transferred from sand flats to (*a*) drained or (*b*) flooded pots in the greenhouse, or (*c*) aerated hydroponic solutions until new roots developed, when they were placed in a closed liquid-culture system in a growth chamber.

A 1:1 mixture of sand and ground peat moss was used in the greenhouse pot studies. Every third day the plants were watered to saturation in drained pots, and to a flood depth of 4 to 5 cm in the flooded pots. Day length was extended to 18 h by supplemental fluorescent and incandescent lights, and minimum temperature was maintained at 22 °C.

The liquid culture system consisted of eight culture bottles connected by thick-wall vacuum tubing to a gas cylinder via a gas manifold (Plate 1). The rate of gas flow was regulated by a screw clamp between bottle and manifold. Stems of the seedlings were sealed airtight in split rubber stoppers by silicon rubber (RTV-11). The tops of seedlings were exposed to atmospheric conditions and the roots to selected gas mixtures, passed through the culture bottle by an inlet and outlet glass tube. A small glass vial containing water was attached to the

outlet side of the culture bottle to monitor gas flow when the culture bottle was covered light-tight with aluminium foil. In addition, the vial acted as a valve by permitting the gas to escape but preventing air from entering the bottle.

After treatment for a variable number of days, roots were analysed in three groups of experiments: (1) anaerobic respiration studies, (2) O_2 transport studies, and (3) CO_2 tolerance studies.

ANAEROBIC RESPIRATION STUDIES

Methods

Swamp tupelo seedlings were grown in the liquid-culture system with their roots in distilled water gassed with atmospheric air or N_2 (prepurified and passed through alkaline pyrogallol). Seedlings were also grown in flooded or drained pots in the greenhouse. After 9 and 22 days in liquid culture and 31 days in the flooded pots, the seedlings were removed from each treatment and their roots were surface sterilized by holding in 1·0 per cent formaldehyde—0·5 per cent soap for 20 s and rinsed three times in sterilized distilled water. The terminal 1-cm segment of lateral roots was cut off and macerated with a glass rod in preweighed test-tubes with 1·0 ml of hot distilled water. After centrifuging for 5 min at 1000 *g* the extracts were analysed for lactic acid by the colorimetric method of Barker and Summerson (1941), and for ethyl alcohol by the colorimetric-enzymatic technique described by Teller (1958) and Brink, Bonnichsen, and Theorell (1954). (Reagents for this assay are sold by Worthington Biochemical as Determatube C-ALC.) The macerate was dried at 70 °C for 24 h to determine oven-dry weight.

The distilled water media from the two bottles in which the roots had grown separately in the air and N_2 series were concentrated in a rotary flash evaporator, extracted by standard acid extraction methods, esterified with ethanol, and chromatographed on an analytical gas chromatograph in a carbon disulphide solvent. Lactic and pyruvic acid peaks were identified by esterified standards of these two compounds.

Tests were also made on root tips after 2- and 4-h incubation in N_2 (passed through alkaline pyrogallol) for CO_2 evolution as well as ethyl alcohol and lactic acid accumulation. Two seedlings from each treatment (flooded and drained pots) were removed after 90 days, their roots surface sterilized with mercuric chloride (0·02 per cent) substituted for formaldehyde, and roots kept in mercuric chloride for 2 min, then rinsed as before; the distal 1·0 cm of each root tip was then severed. Approximately 100 root tips were cut from each seedling and separated into two equal groups. Each group of 50 roots was placed in a 15-ml reaction flask containing 1·0 ml of sterilized distilled water and 0·2 ml of 20 per cent pyrogallic acid in the centre well. Flasks were attached to a Lardy Warburg respirometer and equilibrated with N_2 for 30 min. Two flasks that contained only 1·0 ml of sterilized water and 0·2 ml of 20 per cent pyrogallic acid served as controls.

CO_2 evolution was measured hourly at a constant volume, with an agitation rate of 110 strokes per min at 25 °C. After 2 and 4 h of N_2 incubation, two flasks from each treatment and one blank were removed, roots were macerated in an ice bath, and the root extract combined with the 1·0 ml of water from the reaction flasks. These extracts plus the blank were centrifuged at 1000 *g* for 5 min, then analysed for lactic acid and ethyl alcohol as before. The experiment was replicated three times.

The absence of any increase in the rate of evolution of CO_2 or in ethanol production with time was considered as evidence that microbial contamination was not significant. In the lactic acid determinations, surface sterilization of roots and use of sterilized equipment thereafter was assumed to hold microbial activity to a negligible level.

Results and discussion

Although the swamp tupelo seedlings were grown in hydroponic solution before being placed in the closed liquid-culture system, those in N_2 initiated new roots within 3 days, but no new roots developed in the aerated treatment. The rate of development of new roots in N_2 was comparable to root extension in the aerated

media. The new roots originated primarily from the tap root and the original secondary root system soon deteriorated. New roots were usually larger in diameter, less branched, and more succulent than the original roots. Similar differences in root systems were observed between flooded and drained pots. Therefore, the extracts from the aerated series and drained pots were from roots that differed morphologically from those in the N_2 series and flooded pots.

TABLE 1. *Lactic acid concentration* in the root tips of swamp tupelo seedlings grown in various root environments*

Moles $\times 10^{-8}$/mg ODW roots

Liquid culture		Sand culture†	
Aerated	Nitrogen	Drained	Flooded
Roots extracted in hot water			
After 9 days‡		After 31 days	
$3{\cdot}7 \pm 1{\cdot}1$	$2{\cdot}9 \pm 0{\cdot}7$	$3{\cdot}3 \pm 0{\cdot}8$	$3{\cdot}3 \pm 0{\cdot}6$
After 22 days§			
$1{\cdot}6 \pm 0{\cdot}1$	$2{\cdot}5 \pm 0{\cdot}4$		
Roots incubated in N_2 and extracted in an ice bath			
		After 2 hours	
		$0{\cdot}6 \pm 0{\cdot}4$	$0{\cdot}5 \pm 0{\cdot}3$
		After 4 hours	
		$0{\cdot}3 \pm 0{\cdot}1$	$0{\cdot}9 \pm 0{\cdot}8$

* Determined by the Barker–Summerson method.
† Average of six seedlings per treatment.
‡ Average of two seedlings per treatment.
§ Average of four seedlings per treatment.

The hot-water extracts from swamp tupelo roots from all treatments contained lactic acid (Table 1) but ethanol was not detected by this method. However, Crawford (1966) was unable to detect ethanol in the roots of *Senecio* spp. unless they were incubated in N_2 and extracted in an ice bath. Likewise, in swamp tupelo, the roots incubated in N_2 and extracted with cold water in an ice bath contained both ethanol and lactic acid (Table 1 and Fig. 1). Incubated roots from flooded pots yielded four to five times as much ethanol as those from drained pots. CO_2 evolution was also greater in flasks containing roots from flooded pots than drained pots. There was little if any difference in lactic acid accumulation between aerated and non-aerated or drained and flooded pots, although after 22 days non-aerated roots had more lactic acid than aerated roots (Table 1). Yields for lactic acid per mg oven-dry weight of root were less in the ice-bath extracts than in the hot-water extracts. Even considering the higher lactic acid yields in the hot-water extracts, the highest ethanol concentrations were about seven times greater than the highest lactic acid concentrations. This ratio is considerably less than the ratio of 17:1 ethanol/lactic acid found in potato tubers (Barron, Link, Klein, and Michel, 1950).

Thomas, Ransom, and Richardson (1956) reported that the ratio of ethanol to CO_2 of several species under anaerobic conditions varied from 0 to 1 but was usually less than 0·7, and Crawford (1966) reported an ethanol/CO_2 ratio of about 0·7 for *Senecio jacobaea* roots. In swamp tupelo the ratio was nearly one (Fig. 1).

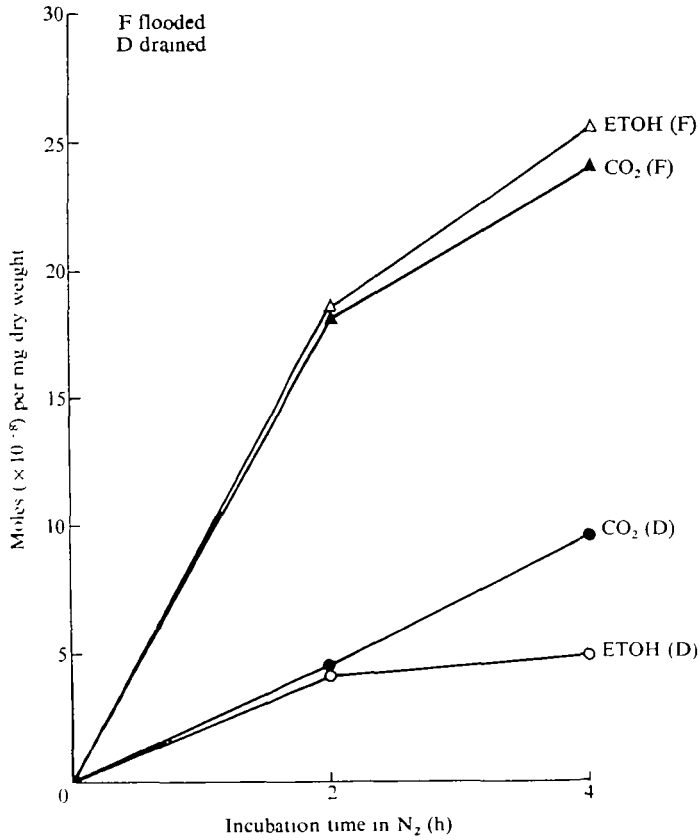


FIG. 1. Ethanol concentration and CO_2 evolution from swamp tupelo root tips from drained and flooded pots after incubation in nitrogen. Each point is the average of six seedlings.

The small differences in ratios between flooded and drained roots are probably due to experimental error since the individual values varied about one. The ethanol pathway appears to be the primary source of CO_2 in swamp tupelo roots under anaerobic conditions.

Chromatographs from the aerated and N_2 series showed peaks with the same retention time as the standards of ethyl esters of lactic acid. Apparently excess lactic acid is leached into the growing medium. Hiatt (1967) reported leakage of organic acid from barley roots under anaerobic conditions. He attributed initial leakage to PEP carboxykinase, but after 1 h leakage was attributed to increased membrane permeability.

Height growth of swamp tupelo seedlings did not vary significantly between 21 per cent O_2 and 100 per cent N_2 in liquid culture or between flooded and drained pots in sand culture (Table 2). However, in N_2 incubation the roots from flooded

TABLE 2. *Effects of different root environments on growth in height and root development of swamp tupelo seedlings*

Growth period days	Number seedlings per treatment	Type gas in liquid culture				
		O_2*	N_2	CO_2†		
		21%	100%	2%	10%	31%
		Height growth (cm)				
9	4	3·3	2·0	..	..	..
9‡	8	2·7	3·4	..	..	..
15	8	..	..	8·8	12·9	4·7
22	8	2·5	2·1	..	..	..
		Number new roots per seedling				
15	8	..	..	15	19	2
		Sand culture height growth (cm)				
		Well-drained pots			Flooded pots	
31	4	7·6			7·0	

* Atmospheric air.

† Each CO_2 gas treatment contained the stated CO_2 percentage plus 1 per cent O_2 and the remainder N_2.

‡ The liquid culture in this experiment contained ½ strength revised nutrient medium (Murashige, T., and Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Pl.* **15**, 473–97). The medium in all other experiments was distilled water, except tap water in the sand culture.

pots accumulated higher concentrations of ethanol than roots from drained pots. This relationship differs slightly from what Crawford (1966, 1967) reported. He found that flood-tolerant species maintained a constant rate of ethanol yield and alcohol dehydrogenase activity under high and low water table conditions, whereas intolerant species increased ethanol yield and alcohol dehydrogenase activity under high water table conditions. He suggested that ethanol accumulated to toxic levels in intolerant species but not in flood-tolerant species. If this is so, then the plants with their roots in anaerobic condition produce an equal amount of shoot growth with only about one-tenth the energy yield in the roots as under aerobic conditions. Therefore, either the energy requirements of roots are far less under flooded conditions than under drained conditions, or under flooded conditions anaerobic respiration is supplemented by aerobic respiration allowed by O_2 transport down the stem. Crawford did not verify O_2 transport from stem to root in the species he used.

OXYGEN TRANSPORT STUDIES

Methods

The oxidation-reduction dye, indigo carmine, was used to test for oxidation around the roots of swamp tupelo seedlings, with their roots in a reduced anaerobic solution (150 ml water, 1 ml of dye, and 1 ml of H_2S-saturated water) and with tops in either atmospheric air or prepurified N_2 in a closed chamber.

Seedlings from drained and from flooded pots were sealed in culture bottles, as previously described, with the dye in a reduced condition (Armstrong, 1967). Four seedlings (two each from drained and flooded pots) were placed inside a clear Plexiglas chamber (27 cm square and 55 cm tall) and four seedlings (two from each treatment) were left outside the chamber. Atmospheric air and prepurified N_2 gas were alternately passed through the chamber as follows: Air → N_2 → Air → N_2 → Air. The roots were inspected at 20-min intervals for the occurrence of a blue halo around the root tip, an indication of oxidation. The experiment was replicated twice in laboratory light at 23 °C.

In another series, six seedlings from flooded pots were sealed in the culture bottles with the dye in a reduced state and stems (except leaves) of four seedlings coated with a 9:1 mixture of paraffin and lanolin. All seedlings were placed in the Plexiglas chamber, subjected to the same gas sequence as above, and roots were inspected for the occurrence of a blue halo.

An additional experiment was performed where 16 six-month-old seedlings of swamp tupelo were grown in the liquid-culture system (full-strength Hoagland's solution). The roots of eight seedlings were aerated, and eight were gassed with the prepurified N_2. The roots of all seedlings were prestained with a 0·1 per cent aqueous solution of crystal violet, and the stems (except leaves) of four seedlings from each treatment were coated to a thickness of approximately 1·0 mm with a paraffin–lanolin mixture (9:1). Growth in height was measured periodically for 22 days, and root growth was observed for the first week. The experiment was conducted in a growth chamber with about 32 000 lx of fluorescent and incandescent light, a 14-h day, and a day–night temperature of 30 and 20 °C respectively.

Some lateral roots from drained and from flooded pots were fixed in FAA, dehydrated in *tert*butyl alcohol, impregnated with Tissuemat, and sectioned at 7 μm thickness on a rotary microtome. The serial sections were stained with aqueous safranin and fast green, and differences in intercellular spaces, suberization, and organization of endodermis studied.

Results and discussion

A blue halo formed around the root tips of previously flooded seedlings with the tops in air and disappeared with the tops in N_2 (Tables 3 and 4). In contrast, no blue halo formed around the root tips of seedlings from drained pots even after 5 h with top in air, but some halo was observed around the central root just below the root collar (1 to 2 cm at most).

TABLE 3. *Oxidation state around the root tips of swamp tupelo seedlings with their roots in a reduced solution of indigo carmine redox dye with the tops in air and nitrogen*

Figures represent number of seedlings

Previous treatment	Tops in Air		Tops in N_2	
	Colour* around root tips			
	Clear	Blue	Clear	Blue
Drained	8	0	4	0
Flooded	0	8	6	0
Flooded—with stems except leaves coated	3	1†	4	0

* The reduced solution is clear and turns blue upon oxidation.

† There was a very faint blue halo around two roots on one seedling. Apparently there was an air leak in the stem coating.

TABLE 4. *Time interval in minutes for change in oxidation state around the root tips in a reduced solution of indigo carmine redox dye of previously flooded swamp tupelo when their tops were alternately gassed with air and nitrogen*

Observations were made at 20-min intervals

Seedling no.	Cycle I		Cycle II*	
	Reduction (N_2)	Oxidation (Air)	Reduction (N_2)	Oxidation (Air)
1	40	80	40	40
2	40	60	40	20
3	60	40	40	20
4	80	40	40	20
5	80	60	40	40
6	80	60	40	20

* After Cycle I (about 2 h) the solution became more sensitive, hence Cycle II probably reflects the time interval more closely than Cycle I.

The paraffin–lanolin coat on the stems of flooded seedlings effectively prevented the oxidation around the root tips of previously flooded seedlings (Table 3); it therefore appears likely that O_2 enters the stems of tupelo through the lenticels rather than the leaves.

The fact that the oxidation around tupelo roots could be turned on and off rapidly by exposing the tops to air or N_2 and that coating stem lenticels with the tops in air prevented oxidation is evidence that the process was O_2 dependent. Armstrong (1967), using an O_2 electrode and the above redox dye, reported that O_2 diffusion plus enzymatic activity dependent upon internal O_2 supply accounted for oxidation around the roots of *Menyanthes* and *Molinia*.

Penfound (1934) found very little intercellular space in the xylem of *Nyssa aquatica* L. and Hook (1968) found that the intercellular spaces in flooded lenticels on swamp tupelo were continuous from the exposed lenticel surface into the cortex and phloem. The pathway for O_2 transport in tupelo appears therefore, to be limited to the cortex and phloem.

Microscopic observations of the roots showed very little suberization (absence of safranin staining) in the epidermal layer of the terminal 2 cm-section in flooded roots, but the entire epidermal layer of roots from drained pots was heavily suberized to within 0·5 cm of the tip. Also, the endodermis was well organized and had distinct Casparian strips within the terminal 2 cm of roots from drained pots, but the endodermis was less organized and Casparian strips were not evident in the same portion of flooded roots. Surprisingly, there were no detectable differences in the intercellular spaces of roots from flooded and drained pots; both contained abundant intercellular spaces within the cortex. It was not determined whether O_2 was transported into roots from drained pots (but did not diffuse through the suberized epidermal layer) or whether O_2 transport into the root was impeded in some means. Regardless of which condition exists, it appears that oxidation of the

rhizosphere is a necessary condition for swamp tupelo to thrive under prolonged flooding.

The apparent differences in the endodermis between roots from flooded and from drained soil may have more implications for nutrient and moisture uptake than for gas exchange. The absence of Casparian strips in the root tips may reduce resistance to moisture uptake as well as energy requirements for active transport of nutrients.

Coating the stems with the paraffin–lanolin mixture did not affect height (non-significant in analysis of variance) or root growth (estimated by eye) any more in N_2 than in an aerated medium. Assuming that the coating effectively blocked O_2 transport to roots as indicated in Table 3, we conclude that energy yield from anaerobic respiration in the roots was sufficient to give comparable top and root growth in the N_2 series as compared with the aerated series.

CARBON DIOXIDE TOLERANCE STUDIES

Methods

Swamp tupelo and sweetgum (*Liquidambar styraciflua* L.) seedlings were grown with their roots in three different levels of CO_2 with 1 per cent O_2 in the closed liquid-culture system. The seedlings were about 2 months old and had been grown hydroponically for 6 days before CO_2 treatments were started. Four seedlings of each species were grown in each CO_2 level (2, 10, and 31 per cent CO_2). The photoperiod was 16 h light, 8 h dark, with a constant day–night temperature of 30 °C. A small bottle of desiccant was attached to the outlet vial on each culture bottle, and transpiration rate was measured by the loss in weight method. Because there were more seedlings (24) than could be handled in the respirometer at one time, the treatments were started at 2-day intervals each at a particular CO_2 level and harvested accordingly after 15-day treatment. Growth in height of seedlings and leaf characteristics were recorded periodically.

At harvest the seedlings were removed from the culture bottles, roots surface sterilized, and the terminal 1 cm of the root severed. Approximately 20 root tips of uniform size from each seedling were placed in 15-ml reaction flasks and rate of O_2 uptake was measured by the direct method in a differential Gilson respirometer (GRP-20) in air. Each flask contained 1·0 ml of distilled water in the outer well and 0·2 ml of 20 per cent KOH in the centre well. Shaking rate was 110 strokes per minute at 30 °C. Uptake was observed at $\frac{1}{2}$-h intervals for 10 h.

Results and discussion

Swamp tupelo roots tolerated much higher concentrations of CO_2 than sweetgum roots without adverse effects. Sweetgum seedlings survived 15 days in 2 per cent CO_2 with only slight chlorosis, although growth in height was imperceptible. However, sweetgum seedlings died within 10 days in 31 per cent CO_2 and within 15 days in 10 per cent CO_2. In contrast, growth of swamp tupelo was not hindered by either 2 or 10 per cent CO_2, but 31 per cent CO_2 retarded root development, growth in height, rate of O_2 uptake by roots, and transpiration rate (Tables 2, 5, and 6).

Rapid growth by swamp tupelo and slow growth by sweetgum with only 1 per cent oxygen in the root environment suggests that differences in CO_2 tolerance of these two species were closely tied to the O_2 requirement of their roots. When species such as sweetgum (intolerant to low O_2 concentrations) are subjected to low O_2 concentrations, it is likely that rate of glycolysis may not be metabolically controlled. Crawford (1966 and 1967) and Crawford and McManmon (1968) have

TABLE 5. *Rate of O_2 uptake of swamp tupelo roots upon return to atmospheric conditions*

Roots were grown under 2, 10, and 31 per cent CO_2 with 1 per cent O_2 for 15 days then respiration rate was measured for 10 h at 2·5-h intervals

Per cent CO_2	Source of roots	Measurement interval (h)			
		0·0–2·5	2·5–5·0	5·0–7·5	7·5–10·0
2	New	4·4	4·8	4·5	3·5
10	New	4·3	4·6	4·0	3·3
10	Original	4·0	3·6	2·9	2·9
31	Original	2·0	1·5	1·6	1·3

Comparisons:
(1) 2 and 10 per cent CO_2, new roots: CO_2 concentration effects N.S. at 0·05 level but significant drop in rate after 7·5 h.
(2) 10 per cent CO_2, new and original roots: source of root effects N.S. at 0·05 level but significant drop in rate after 5·0 h.
(3) 10 and 31 per cent CO_2, original roots: CO_2 concentration effects significant at 0·05 level and a significant drop in rate after 2·5 h.

TABLE 6. *Transpiration rate of sweetgum and swamp tupelo seedlings under three concentrations of CO_2 in the root environment*

Loss of water mg/mg oven dry weight*

Per cent CO_2	Species			
	Sweetgum		Swamp Tupelo	
	Average	Range	Average	Range
2	101	77–131	120	78–138
10	110	94–124	129	118–136
31	83	55–100	95†	88–103

* Treatment averages are the result of four observations.
† Significantly different from the 10 per cent CO_2 value at the 0·15 level.

shown that, within several genera, tolerance to prolonged flooding is related to the ability of individual species to maintain metabolic control of ethanol production. Within genera tested they found that intolerant species accumulated excessive quantities of ethanol under low O_2 concentrations, but tolerant species maintained a near-constant rate of accumulation, regardless of O_2 concentration. However, other flood-tolerant species may increase anaerobic respiration with a decrease in O_2 concentration. Taylor (1942) has shown that respiration in rice shifts toward increased ethanol production below 7 per cent partial pressure of O_2. Similarly, swamp tupelo increases ethanol production in flooded roots, and relative concentrations of lactic acid may be higher after prolonged absence of O_2.

The effect of 31 per cent CO_2 on reducing the respiration rate of roots was irreversible. Norris, Wiegand, and Johanson (1959) found that 10 per cent CO_2 or

higher reduced the respiration rate of onion roots and that the effect was irreversible. In swamp tupelo the decline in rate of O_2 uptake with time; i.e. from 0–2·5 to 7·5–10·0 h, indicates that under high CO_2 concentrations the carbohydrates of roots may be lower than at lower CO_2 concentrations.

Reduction in number of new roots under 31 per cent CO_2 indicates that this concentration of CO_2 inhibited the initiation of new roots. Mer and Causton (1963) reported that 5 per cent CO_2 stimulated cell division in etiolated mesocotyl of oats in darkness. However, Sorokin (1964) has shown that the presumed beneficial effects of CO_2 on cell division are probably due to bicarbonate and that CO_2 is detrimental to cell division. In tupelo there appears to be a small stimulation of growth in height and root initiation at 10 per cent CO_2, but 31 per cent CO_2 inhibited both processes.

After 15-day treatment the original roots of tupelo seedling were coated with a white flakey substance in 10 per cent and 31 per cent CO_2 but new roots were free from these coatings. The coating may have been related to oxidation around new roots and the absence of oxidation around the original roots. The flakey substance was probably carbonates and bicarbonates of iron, manganese, and magnesium. These compounds are known to form in the soil–water solution in the presence of CO_2 [Robinson (1930), International Rice Research Institute (1964), and Ponnamperuma, Martinez, and Loy (1966)].

Although growth in height, root respiration rate, and transpiration rate were retarded at 31 per cent CO_2, the detrimental effects were not as pronounced as those observed in the stagnant-water treatments by Hook (1968). Therefore, it appears that high concentrations of CO_2 in stagnant flooded soil may have additional or compounded effects on tupelo growth that were not observed in the liquid-culture experiment.

CONCLUSIONS

Under flooding swamp tupelo seedlings develop new roots that accelerate anaerobic respiration rate in the absence of O_2, oxidize their rhizosphere, and tolerate high concentrations of CO_2. The combination of these adaptations of the new roots appears to be sufficient to account for flood tolerance in swamp tupelo. The absence of any one of these root adaptations would appear to reduce the flood tolerance of a species.

LITERATURE CITED

APPLEQUIST, M. B., 1960. Soil-site studies of southern hardwoods. In *Eighth Annual Forestry Symposium. Louisiana State Univ.*, 1960, 49–63.

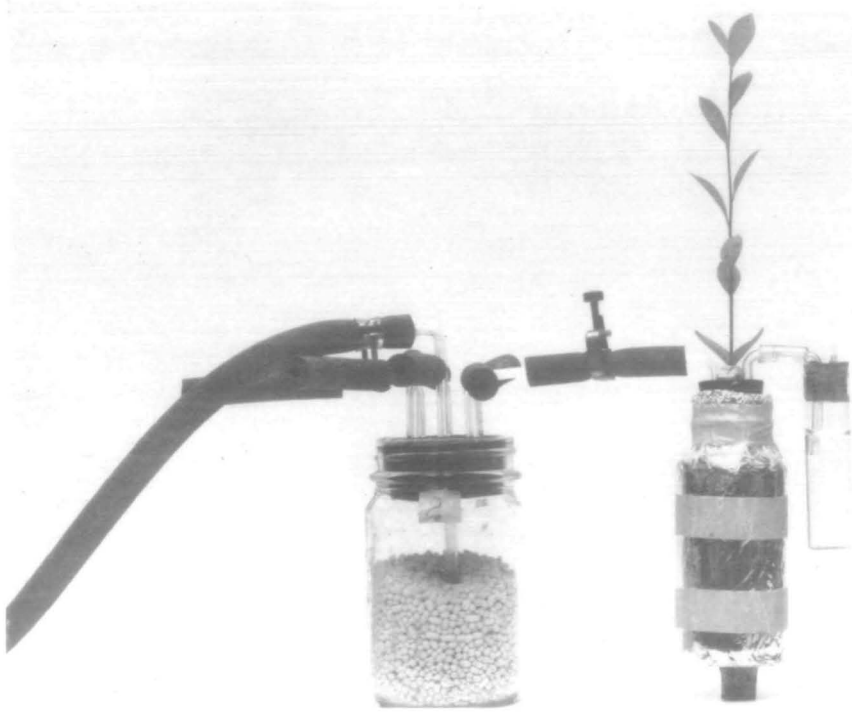
ARMSTRONG, W., 1964. Oxygen diffusion from the roots of some British bog plants. *Nature, Lond.* **204**, 801–2.

—— 1967. The oxidizing activity of roots in waterlogged soils. *Physiologia Pl.* **20**, 920–6.

—— 1968. Oxygen diffusion from roots of woody species. Ibid. **21**, 539–43.

BARBER, D. A., EBERT, M., and EVANS, N. T. S., 1962. The movement of ^{15}O through barley and rice plants. *J. exp. Bot.* **13**, 397–403.

BARKER, S. B., and SUMMERSON, W. H., 1941. The colorimetric determination of lactic acid in biological material. *J. biol. Chem.* **138**, 535.



Liquid culture apparatus with single culture bottle attached.

HOOK, BROWN, AND KORMANIK—PLATE 1

BARRON, E. S. G., LINK, G. K. K., KLEIN, R. N., and MICHEL, B. E., 1950. The metabolism of potato slices. *Archs. Biochem.* **28,** 377–98.

BRINK, N. G., BONNICHSEN, R., and THEORELL, H., 1954. A modified method for the enzymatic microdetermination of ethanol. *Acta pharmac. tox.* **10,** 223.

CRAWFORD, R. M. M., 1966. The control of anaerobic respiration as a determining factor in the distribution of the genus *Senecio*. *J. Ecol.* **54,** 403–13.

—— 1967. Alcohol dehydrogenase activity in relation to flooding tolerance in roots. *J. exp. Bot.* **18,** 458–64.

—— and MCMANMON, M., 1968. Inductive responses of alcohol and malic dehydrogenases in relation to flooding tolerance in roots. Ibid. **19,** 435–41.

DICKSON, RICHARD E., HOSNER, JOHN F., and HOSLEY, NEIL W., 1965. The effects of four water regimes upon the growth of four bottomland tree species. *Forest Sci.* **11,** 299–305.

GREENWOOD, D. J., 1967. Studies on the transport of oxygen through the stems and roots of vegetable seedlings. *New Phytol.* **66,** 337–47.

HIATT, A. J., 1967. Effects of anaerobiosis and respiratory inhibitors on loss of organic acids, amino acids, and K^+ from barley roots. *Pl. Physiol. Lancaster,* **42,** Suppl. vii.

HOOK, DONAL D., 1968. *Growth and development of swamp tupelo* (Nyssa sylvatica *var.* biflora *(Walt.) Sarg.) under different root environments.* Ph.D. Dissertation, University of Georgia. Microfilm No. 69–9493.

—— and BROWN, CLAUD L., 1967. Lactic acid production in the roots of *Nyssa sylvatica* var. *biflora* (Walt.) Sarg. Abs. *Proc. Ass. South. agric. Works, Inc.*, New Orleans, 280–1.

HUIKARI, O., 1954. Experiments on the effect of anaerobic media upon birch, pine, and spruce seedlings. *Commun. Inst. Forest. Fenn.* **42,** 1–13.

INTERNATIONAL RICE RESEARCH INSTITUTE, 1964. Annual Report. 335 p. Manila Hotel, Manila.

JENSEN, CREIGHTON R., LETEY, J., and STOLZY, L. H., 1964. Labeled oxygen: Transport through growing corn roots. *Science. N.Y.* **144,** 550–2.

LAING, H. E., 1940. Respiration of the rhizomes of *Nuphar advenum* and other water plants. *Am. J. Bot.* **27,** 574–81.

LEYTON, LEONARD, and ROUSSEAU, L. F., 1958. Root growth of tree seedlings in relation to aeration. In *Physiology of forest trees.* Ed. Kenneth V. Thimann, Ronald Press Co. N.Y. 467–75.

MER, C. L., and CAUSTON, D. R., 1963. Carbon dioxide: A factor influencing cell division. *Nature, Lond.* **199,** 360–3.

NORRIS, W. E., JR., WIEGAND, JOHN D., and JOHANSON, LAMAR, 1959. Effect of carbon dioxide on respiration of excised onion root tips in high oxygen atmospheres. *Soil Sci.* **88,** 144–9.

PENFOUND, W. T., 1934. Comparative structure of the wood and the 'knees', swollen bases, and normal trunks of the tupelo gum (*Nyssa aquatica* L.). *Am. J. Bot.* **21,** 623–31.

PONNAMPERUMA, F. N., MARTINEZ, ESTRELLA, and LOY, TERESITA, 1966. Influence of redox potential and partial pressure of carbon dioxide on pH values and the suspension effects of flooded soils. *Soil Sci.* **101,** 421–31.

ROBINSON, W. O., 1930. Some chemical phases of submerged soil conditions. Ibid. **30,** 197–217.

SOROKIN, CONSTANTINE, 1964. Carbon dioxide and cell division. *Nature, Lond.* **20,** 1252–4.

TAYLOR, D. L., 1942. Influence of oxygen tension on respiration, fermentation, and growth in wheat and rice. *Am. J. Bot.* **29,** 721–38.

TELLER, J. D., 1958. Colorimetric enzymatic determination of ethanol. *Abstract of Papers 134th Meeting Am. chem. Soc.* 64C.

THOMAS, M. T., RANSOM, S. L., and RICHARDSON, J. A., 1956. *Plant physiology* (4th edn.). J. & A. Churchill, London.

VAN RAALTE, M. H., 1940. On the oxygen supply of rice roots. *Annls. Jard. bot. Buitenz.* **50,** 99–113.