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POPULATION DIFFERENTIATION ALONG A FLOOD FREQUENCY GRADIENT: PHYSIOLOGICAL ADAPTATIONS TO FLOODING IN *NYSSA SYLVATICA*[1]

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Abstract. Throughout the southeastern United States the hardwood *Nyssa sylvatica* (sensu lato) is distributed along a soil moisture gradient from upland sites, which are never flooded, to floodplains, which are periodically flooded and drained, to permanently flooded swamps. Population differentiation with respect to flood tolerance and related physiological attributes was investigated using 1-year-old seedlings grown in a greenhouse from seed collected along this gradient.

Upland plants were very intolerant of flooded soils. Their root systems deteriorated, root respiration rates dropped and, after a year under such conditions, survival was poor and those that did remain were greatly stunted and had accumulated large concentrations of many nutrient elements.

In contrast swamp plants were quite tolerant of flooded soils. Upon flooding, parts of the original root system were lost but new roots were initiated which had an increased capacity for alcoholic fermentation. Many of these new roots were more succulent, larger in diameter, and less branched than drained roots. Such succulent roots however were only a temporary response to short-term flooding; plants flooded for a year did not have such roots, rather the root system superficially resembled drained roots. Concomitant with this return to drained-like roots was an increase in internal oxygen transport to the roots and a drop in alcoholic fermentation.

Floodplain plants under drained conditions allocated less biomass to roots than to shoots and had high respiration rates, traits similar to upland plants. Under flooded conditions they initiated new roots with medium respiration rates, allocated less biomass to roots than to shoots, significantly increased oxygen transport to the roots and had high survival, traits similar to swamp plants. Thus, the floodplain population produced a distinctly flood-tolerant phenotype; but not nearly as tolerant of flooded conditions as the swamp phenotype. Floodplain plants differed from swamp plants in transporting less oxygen to the roots under drained conditions, initiating fewer succulent-type roots and not accelerating alcoholic fermentation upon flooding and after a year under flooded conditions having less total biomass, less oxygen transport to the roots and a greater accumulation of Fe and Mn in the roots. The floodplain plants apparently have been selected to be similar to upland plants under drained conditions and swamp plants under flooding and one consequence of this is that their tolerance of flooded conditions is intermediate. It is argued that one of the more important trade-offs in adapting to flooded conditions is that high internal oxygen transport carries with it a "cost" in terms of excessive water loss under water stress conditions.

Key words: adaptive strategies; alcoholic fermentation; anaerobic; ecotypes; environmental heterogeneity; Georgia; oxygen diffusion; respiration; roots; South Carolina; swamps.

INTRODUCTION

Variation within species has long been recognized by naturalists, but its ecological and evolutionary role was poorly understood until Turesson's (1922, 1925) work. He showed that natural selection could produce, within the same species, genetically differentiated populations specifically adapted to particular environments. Turesson's ecotype concept emphasizes the adaptive role of intraspecific variability and today, largely through the work of Clausen et al. (1940, 1948), there is some foundation for predicting the types of changes expected from 1 environment to the next. A good example is *Nyssa sylvatica* Marsh. (tupelo or blackgum), a broadleaf deciduous tree occurring in a wide variety of habitats in the southeastern United States. In the mountains it establishes best on well-drained "subxeric" sites (Whittaker 1956), whereas in the coastal plain it is found in swamps (Fig. 1), many of which remain flooded throughout the year (Penfound 1952, Applequist 1956). Due to different selection pressures, one would predict that if seeds from these 2 populations were sown in a common environment the seedlings would exhibit genetic differentiation for a variety of characters, in particular ones associated with tolerance to flooding.

When environmental conditions are relatively homogeneous, i.e., "always" drained or flooded, a prediction such as this may be intuitive. However, there are populations of *Nyssa sylvatica* in the piedmont that occur in floodplains which are periodically flooded in the spring and drained in the summer. Given our current understanding of the evolutionary responses to heterogeneous environments (coupled with a confused literature on flood-tolerance in plants) a clear prediction about the pattern of population differentia-

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FIG. 1. A stand of *Nyssa sylvatica* (var. *biflora*) in a permanently flooded swamp on the coastal plain of Georgia.

tion in a floodplain is difficult. Since this habitat alternates between drained and flooded conditions, the evolutionarily stable situation may involve a mixture of upland- and swamp-like plants. In this event we might predict the proportion of swamp types by the frequency of flooding (see Levins 1968), or the proportion of upland types by how severely they are affected by flooding (see Templeton and Rothman 1974). On the other hand, since this habitat is in some sense intermediate, we might expect a population of genotypes which produce an intermediate-like phenotype. If so, then in what ways would they be intermediate, and mechanistically why?

With these questions in mind, this investigation was designed to examine the population differentiation of *Nyssa sylvatica* along a flood frequency gradient characteristic of upland, floodplain and swamp habitats. Using seedlings grown under controlled conditions 3 questions were addressed: 1) How do the 3 populations differ with respect to tolerance of flooded conditions? 2) What physiological mechanisms can account for differing tolerances? and 3) What is the adaptive significance of this pattern of differentiation? To better understand the significance of question 2 it should be useful to briefly review what is known concerning adaptation to flooded soils.

Review of physiological adaptations to flooding

Upon flooding, several parameters of the soil environment change radically (Armstrong 1975). Due to the very low solubility and low rate of diffusion of oxygen in water, the primary effect of flooding is to reduce the aeration of the soil, and if microorganisms are very active it may become nearly anaerobic. Additionally, microorganisms will make use of other electron acceptors for respiratory oxidations and consequently numerous mineral elements become reduced. Manganese and Fe are 2 such elements and are particularly critical because the change in valence on conversion to the manganous (Mn^{2+}) and ferrous (Fe^{2+}) ions results in forms which are more soluble in the soil

solution and therefore accumulate in large concentrations. Thus, rooted plants face 2 major problems in flooded soils. One is the severe limitation on normal aerobic root respiration and another is the potential accumulation of toxic quantities of certain elements.

Explanations of flood tolerance have centered on 3 areas. One has focused on the toxicity of the reduced soil environment and has argued that flood tolerance stems in part from the ability of certain species to avoid excessive accumulation of elements such as Fe and Mn. The other 2 have been concerned with the low oxygen conditions of flooded soils. One holds that plants compensate for hypoxic conditions through metabolic changes in the roots, and another maintains that plants overcome such conditions by internally transporting oxygen down to the roots.

Of these explanations, that of metabolic changes in roots is the most complex and controversial. Two metabolic theories have been proposed, both involving the terminal stages of glycolysis (Fig. 2). One theory is based on the observation that upon flooding, several flood tolerant plants accelerate the terminal stages of the alcoholic fermentation pathway. This is well documented in lowland rice, a plant cultivated in flooded soils. Specifically, increased rates of anaerobic respiration and ethanol production and increased pyruvate decarboxylase and alcohol dehydrogenase activities have been found in the roots upon the introduction of anaerobic or hypoxic (low oxygen) conditions (Taylor 1942, John and Greenway 1976, Wignarajah et al. 1976). Similar responses have been observed for other flood tolerant species (Grineva 1963) including swamp populations of *Nyssa sylvatica* (Hook et al. 1971). Presumably, this acceleration of alcoholic fermentation is adaptive because without it pyruvate and NADH would accumulate and this would shut down glucose catabolism. Thus, alcoholic fermentation maintains glycolysis as a compensatory energy source in the absence of aerobic respiration (Hochachka and Somero 1973:44–46).

A second theory argues that those plants which accelerate alcoholic fermentation upon flooding are the ones least tolerant of flooding (Crawford 1966, 1967, Crawford and McManmon 1968). By accelerating alcoholic fermentation, these plants accumulate large quantities of ethanol which is potentially toxic to the tissues (Karlsen 1925, Chirkova 1976, Fulton and Erickson 1964). Plants tolerant of flooded conditions avoid increased ethanol production apparently by shunting carbon off from PEP and storing it as malate (Fig. 2) which is not toxic (Crawford and Tyler 1969, McManmon and Crawford 1971). This second theory is based on the following observations: 1) the relative change, from drained to flooded conditions, in alcoholic fermentation rates, is greater for nontolerant plants than for tolerant ones, and 2) under flooded conditions the roots of flood tolerant species have increased concentrations of malic acid whereas nontolerant species do not. This pathway apparently does not involve PEP carboxykinase (McManmon and Crawford 1971); consequently no net energy is generated (Mazelis and Vennesland 1957), and it is adaptive strictly as a short-term means of avoiding ethanol poisoning.

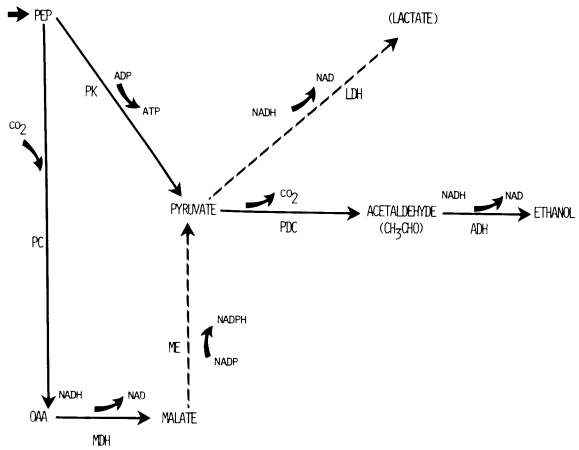

FIG. 2. Terminal stages of glycolysis and potential pathways under low oxygen conditions; PEP = phosphoenolpyruvate, PK = pyruvate kinase, LDH = lactate dehydrogenase, PDC = pyruvate decarboxylase, ADH = alcohol dehydrogenase, PC = PEP carboxylase, MDH = malic dehydrogenase, ME = "Malic Enzyme" (dashed lines indicate pathways which are of doubtful importance in many plants).

Unfortunately, the data in the literature can be interpreted to support either 1 of these theories (in addition to the above, see Hageman and Flesher 1960, Schwartz 1969, Bolton and Erickson 1970, Marshall et al. 1973, Linhart and Baker 1973, Chirkova et al. 1974, Francis et al. 1974, Wignarajah and Greenway 1976, Brown et al. 1976, Baskin and Baskin 1976).

Physiological studies in the present investigation focused on the following: 1) an examination of the effect of long-term (1 yr) flooding on the accumulation of mineral elements in roots, 2) metabolic studies of aerobic respiration rates, anaerobic alcoholic fermentation rates, and changes in concentrations of malic acid in roots under drained conditions and over a time course of flooding; at 1 week, 1 mo, and 1 yr, and 3) changes in rates of internal oxygen transport to the roots under short-term and long-term flooding. Since upland plants suffered very high mortality rates and were nearly dead after extended periods of flooding, the oxygen transport experiments were restricted to the floodplain and swamp plants.

MATERIALS AND METHODS

Populations.—Seeds were collected from the 3 population types along an elevational gradient as follows.

Upland plants occurred on drained sites in the mountains of north Georgia and the piedmont of central Georgia. Floodplain plants occurred on alluvial sites in the piedmont, and swamp plants were taken from more-or-less permanently waterlogged sites on the coastal plain of south Georgia and South Carolina (see Keeley 1977 for precise locations). Each of the 3 habitats was represented by at least 2 geographically separated populations, and each population was represented by seeds from 2 to 15 trees. For convenience, all seedlings from upland sites will be referred to collectively as the "upland population," and, likewise, seedlings of the several collections of floodplain seeds and swamp seeds will be referred to as the "floodplain population" and the "swamp population," respectively.

This delineation of 3 *Nyssa sylvatica* habitat types is an oversimplification. There are trees in the mountains growing along streams and ones on xeric sandhills in the coastal plain, as well as all degrees of intermediate situations (e.g., Hook and Stubbs 1967). No attempt was made to deal with these situations.

Taxonomically, populations from well-drained sites have been treated as var. *sylvatica* and those from flooded habitats as var. *biflora* (Walter) Sargent (Eyde 1966). This is based on differences in numbers of flowers per peduncle, fruit size and leaf shape. No attempt was made in this study to utilize these taxonomic groupings since many of the trees, particularly in the piedmont, combined flower, fruit and leaf characteristics in a variety of ways.

Greenhouse.—The fruits (drupes) were depulped and stored wet at 5°C for 6 months. The duration of cold treatment required for germination was <2 months for the coastal populations, but increased sharply with elevation. Six months' cold treatment produced good germination with all populations.

Seeds were germinated in 15-cm clay pots and later thinned to 2 seedlings/pot. These pots were situated on a greenhouse bench in a randomized design. All seed was sown in May 1975 and divided into 2 groups. One was for analysis of survival and growth under continuous drained and continuous flooded conditions and was harvested after 16 mo. For those pots to be flooded, a tank ≈35 cm tall was constructed on top of the bench and the pots placed inside. Because *Nyssa sylvatica* seeds will not germinate underwater (Debell and Naylor 1972), flooding treatment was begun with the water level below the soil surface, thereby producing a fully saturated soil. After 4 months the water level was raised to ≈5 cm above the soil surface. The other group was utilized for physiological studies between 12 and 16 mo of age. In addition to continuous drained and continuous flooded conditions a series was also grown under drained conditions for ≈1 yr and then flooded for 1 week or 1 mo. Therefore all plants were ≈1 yr old at the time of study.

Laboratory

Nutrients.—Root samples from each population grown under drained conditions and 1 yr of flooding were analyzed for a spectrum of nutrients. Approximately 1 g oven-dry weight of the small (<1 mm diameter) secondary roots were ashed, dissolved in 20% nitric acid and analyzed for 18 macro- and micro-nutrients with a plasma emission spectrophotometer.

Metabolism.—Roots were prepared for each experiment by washing repeatedly in tap water followed by surface sterilization in 0.02% mercuric chloride for 2 min and 4 rinses in distilled water. In all experiments only the terminal 2–3 cm of a root were used.

Aerobic respiration was measured with a Warburg respirometer (Umbreit et al. 1964). Approximately 100 root sections were floated in 3 ml of 0.05 M citrate–0.1 M phosphate buffer (pH 5.4) with 0.25% glucose. Vessels were shaken at 110 strokes/min and maintained at 25 ± 0.05°C. Respiration rates were linear for up to 4 h indicating there was no substantial contamination of microorganisms and substrate was not limiting. After each experiment root sections were blotted and weighed fresh, then oven-dried and weighed again.

Alcoholic fermentation was assayed 2 ways: 1) an *in vivo* assay of ethanol production by root sections under anaerobic conditions and 2) an *in vitro* assay of alcohol dehydrogenase (ADH; EC 1.1.1.1.) activity from root sections directly out of the soil.

Ethanol production was measured in 250-ml flasks with ≈1 g of root sections in 30 ml of the citrate-phosphate buffer. Flasks were flushed with nitrogen (filtered through pyrogallol) for 20 min, then closed off and shaken at 110 strokes/min and 25 ± 1°C. Preliminary experiments indicated no detectable amount of ethanol is produced by the roots without anaerobic incubation (see also Hook 1968). After 4 h incubation, root sections were blotted and weighed fresh then ground with sand in a mortar with 10 ml buffer. This homogenate was filtered through 4 layers of cheesecloth and centrifuged for 20 min at 10 000 rpm. The supernatant was added to the incubation buffer and enzymatically assayed for ethanol (Bergmeyer 1974: 1499–1502). The deproteinization step was found to be unnecessary by running internal controls (addition of known quantities of ethanol to root extracts) so the nondeproteinization method was used. Fresh/dry weight ratios were calculated for a large number of samples and used to convert ethanol production to the same dry weight basis as aerobic respiration. Lactate, which is an endproduct of anaerobic respiration in some plants, was not assayed since it is a relatively minor product in *Nyssa sylvatica* where the ratio of $ETOH/CO_2$ is nearly 1.0 (Hook et al. 1971).

To measure ADH activity, root sections were ground with sand in a mortar with 0.1 M Tris buffer

TABLE 1. Survival and total biomass (g oven dry weight, ODW) for 16-mo-old *Nyssa sylvatica* seedlings from the upland, floodplain and swamp populations grown under drained and flooded conditions

	Upland					Floodplain					Swamp					
	Survival		Biomass			Survival		Biomass			Survival		Biomass			F_s for
	%	N	$\bar{x}$	SD	N	%	N	$\bar{x}$	SD	N	%	N	$\bar{x}$	SD	N	Biomass
Drained	73	30	10.84	(4.62)	22	100	16	13.32	(5.37)	16	65	40	13.66	(5.45)	26	$F_s = 1.86^{NS}$
Flooded	27	30	4.11	(3.22)	8	94	16	7.12	(3.60)	15	98	40	11.70	(4.09)	39	$F_s = 16.75^{***}$
			$F_s = 13.99^{***}$					$F_s = 14.02^{***}$					$F_s = 2.74^{NS}$			

NS $P > .05$.
*** $P < .001$.

(pH 8.0) in an ice bath. This homogenate was filtered through 4 layers of cheesecloth and centrifuged at 2°C and 12 000 rpm for 20 min. The clear supernatant was assayed for specific activity, using acetaldehyde as substrate, at 25°C (Racker 1957:501). The final concentrations in the cuvette were: ≈100 mg protein, 95 mM Tris, 66 mM NADH and 33 mM acetaldehyde. This provided saturating concentrations for the coenzyme and substrate. Protein was determined with the Lowry Method as modified by Bergmeyer (1974:172–174).

Malic acid concentrations were determined as follows. Root sections were ground in distilled water, filtered through 4 layers of cheesecloth, centrifuged at 2°C and 12 000 rpm for 20 min and then filtered once again through cheesecloth to remove lipids. The supernatant was deproteinized and assayed for malic acid with the procedure described by Bergmeyer (1974:1585–1589). Results were expressed on a protein basis using the Lowry Method.

Oxygen transport.—The apparatus used for measuring oxygen transport rates consisted of 2 chambers, 1 for the shoots and 1 for the roots. The intact root systems were surface sterilized and placed in the bottom chamber filled with 1.0 l of tap water. An experiment was initiated by flushing the bottom chamber with nitrogen until anaerobic, sealing it off and flushing the top chamber with 100% oxygen.

Rates of oxygen transport were measured by the rate of oxygen diffusion into solution. This was determined polarographically using a Clark-type oxygen electrode with a polarizing voltage of 0.8 V, and the output was followed on a continuous recorder. All experiments were done at room temperature (23 ± 0.5°C) under constant stirring with a magnetic stirring bar to avoid local depletions of oxygen around the electrode. As pointed out by Armstrong (1971) this measure of oxygen transport rate can be confused by differences in root respiration rates. In general, respiration rates were broadly similar across treatments, and within treatments, the floodplain and swamp plants were very similar, thus for comparative purposes the respiration factor should cancel.

In order to account for differences in seedling size, oxygen transport rates were expressed on the basis of the ODW of all the secondary roots. Determinations for each treatment were replicated and rates of diffusion calculated from the mean of the 2 slopes.

Statistical.—In all cases sample sizes were determined by the availability of seeds from each population, limitations on space and ultimately by survival. Data were analyzed with one-way analysis of variance using the fixed effects model, testing main effects against error. In a few cases the data failed to meet the assumption of homogeneity of variances (F_{max} test); these cases were reanalyzed with the nonparametric Kruskal-Wallis test. Since this applied to only a few cases, and the conclusions remained unchanged, these tests were not included. All pairwise comparisons with a prior hypothesis were made with a 1-tailed t-test.

RESULTS

Flood tolerance experiments.—Under drained conditions all 3 populations had reasonably good survival, ranging from 100% for the floodplain plants to 65% for the swamp plants, and there was no significant difference in total biomass (Table 1). Under flooded conditions, upland plants had over ⅔ mortality and those few that survived produced ⅔ less biomass. The swamp and floodplain plants suffered little or no mortality though the floodplain plants showed a highly significant reduction in biomass which was intermediate between the upland and swamp populations.

Under flooded conditions the upland plants had reductions in both aboveground and belowground biomass whereas the floodplain and swamp plants showed no significant reduction in aboveground biomass, but both showed a highly significant reduction in belowground biomass (Table 2). There were highly significant differences across populations in root/shoot ratios under both conditions. The upland and floodplain populations both allocated more biomass to roots than to shoots under drained conditions. Under flooded conditions all 3 populations showed reductions in

TABLE 2. Biomass (g ODW) partitioned into aboveground (stems and leaves) and belowground (roots) for 16-mo-old seedlings from the three populations under drained and flooded conditions

		Upland			Floodplain			Swamp			
		$\bar{x}$	SD	N	$\bar{x}$	SD	N	$\bar{x}$	SD	N	
Aboveground	Drained	3.79	(1.75)	20	5.91	(2.65)	16	6.82	(3.32)	26	$F_s = 7.08^{**}$
	Flooded	1.91	(1.24)	8	4.19	(2.18)	15	6.78	(2.37)	39	$F_s = 19.56^{***}$
		$F_s = 7.61^{*}$			$F_s = 3.87^{NS}$			$F_s = 0.00^{NS}$			
Belowground	Drained	7.04	(3.23)	20	7.41	(3.48)	16	6.84	(2.51)	26	$F_s = 0.18^{NS}$
	Flooded	2.20	(2.17)	8	2.93	(1.55)	15	4.93	(1.98)	39	$F_s = 10.39^{***}$
		$F_s = 15.16^{***}$			$F_s = 20.94^{***}$			$F_s = 11.75^{***}$			
Root/Shoot	Drained	2.05	(1.01)	22	1.39	(.89)	16	1.07	(.34)	26	$F_s = 9.48^{***}$
	Flooded	1.08	(.50)	8	0.71	(.19)	15	0.74	(.20)	39	$F_s = 6.91^{**}$
		$F_s = 6.56^{*}$			$F_s = 8.32^{**}$			$F_s = 24.98^{***}$			

^{NS} $P > .05$.
* $P < .05$.
** $P < .01$.
*** $P < .001$.

root/shoot ratios with the floodplain and swamp populations allocating much less to roots than to shoots.

Physiology experiments

Root morphology.—Integral to the physiology experiments are the major changes observed in root systems. Within a few days after flooding, the root systems of 1-yr-old upland plants began to discolor and they became progressively more necrotic with continued flooding. Little healthy root tissue remained on plants which had survived a full year of flooding. The 1-yr-old floodplain and swamp plants responded similarly in that portions of the original "drained" root system deteriorated soon after flooding, though deterioration was most extensive for the upland plants and least for the swamp plants.

These 1-yr-old floodplain and swamp populations, however, differed qualitatively from the upland plants in the initiation of new roots soon after flooding. In general, new roots were produced more prolifically on the swamp plants than on the floodplain plants. Most of these new roots were morphologically quite distinct from "drained" roots in that they were initiated directly from the tap root, were very white and brittle, and were much larger in diameter, less branched and more succulent than roots initiated under drained conditions (Fig. 3).

These were short-term changes in the floodplain and swamp root systems. Plants which had been flooded continuously for 1 yr did not possess any of these large succulent roots; rather the entire root system, superficially at least, resembled drained roots. This difference in root systems is illustrated by changes in succulence at different stages of flooding (Table 3). Further observations indicated that these succulent roots were not initiated by plants grown from seed under waterlogged conditions. Also, 1-yr-old plants which initiated such roots upon being flooded, eventually lost them if kept under flooded conditions for a year.

Thus, for any given plant the "root system" consisted of a heterogeneous collection of roots varying with respect to succulence and health in addition to the normal variation in age, size, shape, suberization,

TABLE 3. Ratios of fresh weight/dry weight, for root sections from seedlings from the 3 populations, grown under drained conditions and across a time course of flooding

	Upland			Floodplain			Swamp			
	$\bar{x}$	SD	N	$\bar{x}$	SD	N	$\bar{x}$	SD	N	
Drained	5.6	(0.5)	8	7.1	(0.6)	8	6.6	(0.8)	10	$F_s = 11.12^{**}$
Flooded one week	5.8	(0.7)	8	7.6	(0.8)	8	8.3	(0.3)	8	$F_s = 32.53^{***}$
Flooded one month	4.8	(0.4)	4	9.9	(1.2)	8	10.2	(1.2)	8	$F_s = 36.56^{***}$
Flooded one year	5.3	(1.4)	8	7.6	(0.8)	8	7.2	(0.5)	8	$F_s = 13.02^{***}$
	$F_s = 1.36^{NS}$			$F_s = 15.57^{***}$			$F_s = 36.31^{***}$			

^{NS} $P > .05$.
** $P < .01$.
*** $P < .001$.

FIG. 3. A succulent root produced within the 1st month of flooding by 1-yr-old floodplain and swamp plants. It is morphologically and physiologically quite distinct from roots produced under drained conditions (in the background). Also, note the proliferation and hypertrophy of lenticels below the water line.

TABLE 4. Nutrient concentrations (μg/g ODW) in secondary roots of 16-mo-old seedlings from the 3 populations under drained and flooded conditions. Stars between drained and flooded values signify F_s probability for 1-way analysis of variance (blank indicates $P > .05$)

	Upland			Floodplain			Swamp		
	Drained		Flooded	Drained		Flooded	Drained		Flooded
P	2370		3180	5620		6130	3850		4420
K	12 800		19 200	18 600	*	24 800	16 800		19 200
Ca	7780		6820	7840		6000	5720	*	4540
Mg	6100	***	2540	9210		10 460	6610		7210
Fe	1425	***	34 080	615		2890	497		389
Mn	482	***	4195	300	**	2385	190	***	965
B	29	**	231	27		45	45		53
Cu	25		33	143		98	121		137
Zn	96		97	175		160	93		118
Na	410	**	1380	1450		2000	1130		1190
Al	4540		6170	3150		3340	2250	**	1350
Si	1270		1480	1070		1260	840	***	550
Pb	17	**	95	21	**	37	15		13
Pt	11	**	112	18	*	45	14	**	21
Co	1.2	***	54.4	1.3		3.0	0.3		0.8
Cr	7.0	**	32.7	4.7	*	29.2	9.5	*	19.9
Ni	3.5	***	14.5	2.9	*	4.9	5.1		5.5

* $P < .05$.
** $P < .01$.
*** $P < .001$.

etc. With regard to the physiology experiments it was decided that the most ecologically meaningful measure would be one which sampled the healthiest appearing root tips from the entire root system. Therefore, roots of the various types were sampled in proportion to their frequency, so for example the greater the production of succulent roots on swamp plants after 1 mo of flooding meant a greater sampling of such roots than for floodplain plants. One consequence of this sampling regime is that the "health" of the roots was not always the same from one plant to another, viz. upland plants under drained conditions vs. flooded conditions. It is presumed that the aerobic respiration rates are a reasonably objective measure of the "health" of the root system. Due to the very significant differences in succulence (Table 3) all measures were expressed on either a dry weight or a protein basis.

Nutrients.—Upon flooding, nutrient concentrations in the roots generally increased (Table 4). There was a trend of greater increases, as well as increases in more nutrients, from the swamp to the floodplain to the upland populations. Under flooding the swamp plants had significant increases in only 3 nutrients, the floodplain plants had increases in 6 nutrients and the upland plants had increased concentrations of 10 nutrients. A similar pattern is seen with respect to Fe and Mn. Under flooded conditions the upland population had an order of magnitude greater concentration of Fe than the floodplain population, which in turn, had an order of magnitude greater concentration than the swamp population. The floodplain plants had half as much Mn as the upland plants and the swamp plants half as much as the floodplain plants.

Metabolism.—Under drained conditions the upland plants had a significantly greater root respiration rate than the swamp plants, but after 1 week of flooding there was no difference among populations, due to a drop in the upland plants (Table 5). With continued

TABLE 5. Aerobic respiration rates (μl O_2/hr/g ODW) in excised roots from 1-year-old seedlings from the 3 populations, grown under drained conditions and across a time course of flooding

	Upland			Floodplain			Swamp			
	$\bar{x}$	SD	N	$\bar{x}$	SD	N	$\bar{x}$	SD	N	
Drained	1167	(261)	4	1028	(104)	4	766	(186)	6	$F_s = 5.61$*
Flooded 1 week	957	(190)	4	807	(120)	4	800	(85)	4	$F_s = 1.62^{NS}$
Flooded 1 month	574	(75)	2	1302	(187)	4	1140	(195)	4	$F_s = 11.23$**
Flooded 1 year	383	(114)	4	771	(68)	4	686	(151)	4	$F_s = 12.39$**
	$F_s = 13.42$***			$F_s = 14.70$***			$F_s = 6.13$**			

NS $P > .05$.
* $P < .05$.
** $P < .01$.
*** $P < .001$.

flooding aerobic respiration rates for the upland population continued to drop, so that after 1 yr of flooding root respiration was ≈70% < under drained conditions. Swamp plants increased their respiration rate slightly after 1 mo of flooding but by the end of 1 yr under flooded conditions they had respiration rates comparable to drained plants. Floodplain plants were similar to the upland plants under drained conditions in that both were significantly > the swamp population ($P < .05$). However, the floodplain population responded like the swamp plants under all conditions of flooding.

Respiratory quotients (ratios of CO_2 given off to O_2 taken up) which are a measure of the type of substrate being oxidized, are shown in Fig. 4. Oxidation of carbohydrates gives an RQ = 1.0, whereas lipids and proteins will be ≈0.7–0.8. The floodplain and swamp populations did not show any significant changes in RQ across treatments, and apparently respiratory substrates were largely carbohydrates. The upland population showed a significant drop in RQ after 1 mo of flooding ($P < .05$), indicating perhaps a switch to lipid metabolism, whereas after a year of flooding the RQ was significantly >1.0 ($P < .05$).

Ethanol production rates (Table 6) showed that under drained conditions the upland population had the lowest rate of ethanol production, but within the 1st day after flooding, all populations had accelerated ethanol production, and there was no significant difference among populations at this time. The upland plants sustained this accelerated rate of ethanol production through the first week of flooding, however continued flooding resulted in a drop. After 1 yr of flooding these upland plants showed ≈80% reduction in ethanol production. The floodplain and swamp populations continued to have increased levels of ethanol production beyond the 1st week, reaching very high levels within the 1st month of flooding. However, these high levels were not sustained and after a year of flooding ethanol production rates were below those of drained plants.

Another measure of the capacity for alcoholic fermentation is given by ADH activity shown in Table 7. Under drained conditions and after the 1st week of flooding there was no significant difference among populations. After 1 mo of flooding the upland populations showed reduced ADH activity while the swamp population showed increased activities and the floodplain population remained approximately the same as drained conditions. After a year of flooding all 3 populations had ADH activities significantly below drained conditions ($P < .05$).

Malic acid concentration in the roots (Table 8) showed no significant difference between drained and flooded conditions for the upland population, whereas the floodplain population had a significant increase under flooding and the swamp plants had a highly significant 5-fold increase within the 1st month of flooding and this same level was maintained through a full year of flooding. There was a significant difference between populations after 1 mo and 1 yr of flooding. At both points, the floodplain population was distinct-

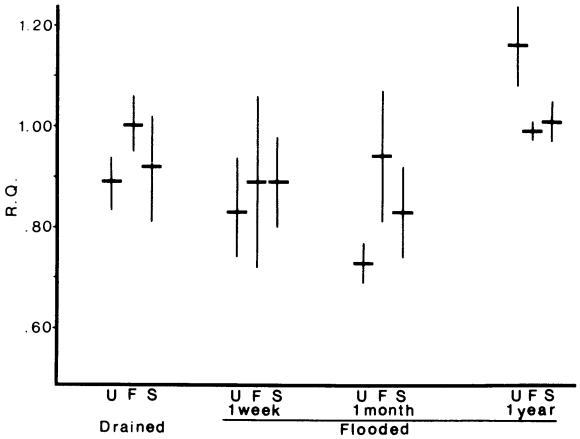

FIG. 4. Respiratory quotients (CO_2/O_2) of excised roots from the upland (U), floodplain (F), and swamp (S) plants under drained and flooded conditions (vertical lines represent 1 SD around the mean).

TABLE 6. Ethanol production rates (μM ETOH/hr/g ODW), under anaerobic incubation, in excised roots from 1-year-old seedlings from the 3 populations, grown under drained conditions and across a time course of flooding

	Upland			Floodplain			Swamp			
	$\bar{x}$	SD	N	$\bar{x}$	SD	N	$\bar{x}$	SD	N	
Drained	16.3	(5.1)	4	28.0	(3.8)	2	22.7	(4.2)	4	$F_s = 4.84^{*}$
Flooded 1 day	30.3	(6.9)	12	34.9	(8.1)	2	33.5	(8.2)	7	$F_s = 0.59^{NS}$
Flooded 1 week	29.5	(0.4)	2	...	...	...	42.3	(3.9)	2	$F_s = 21.30^{*}$
Flooded 1 month	19.9	(3.2)	4	67.8	(12.8)	2	57.7	(17.1)	2	$F_s = 19.50^{**}$
Flooded 1 year	3.5	(3.5)	6	17.7	(7.5)	2	13.6	(2.2)	4	$F_s = 14.21^{**}$
	$F_s = 25.89^{***}$			$F_s = 12.59^{*}$			$F_s = 14.50^{***}$			

NS $P > .05$.
* $P < .05$.
** $P < .01$.
*** $P < .001$.

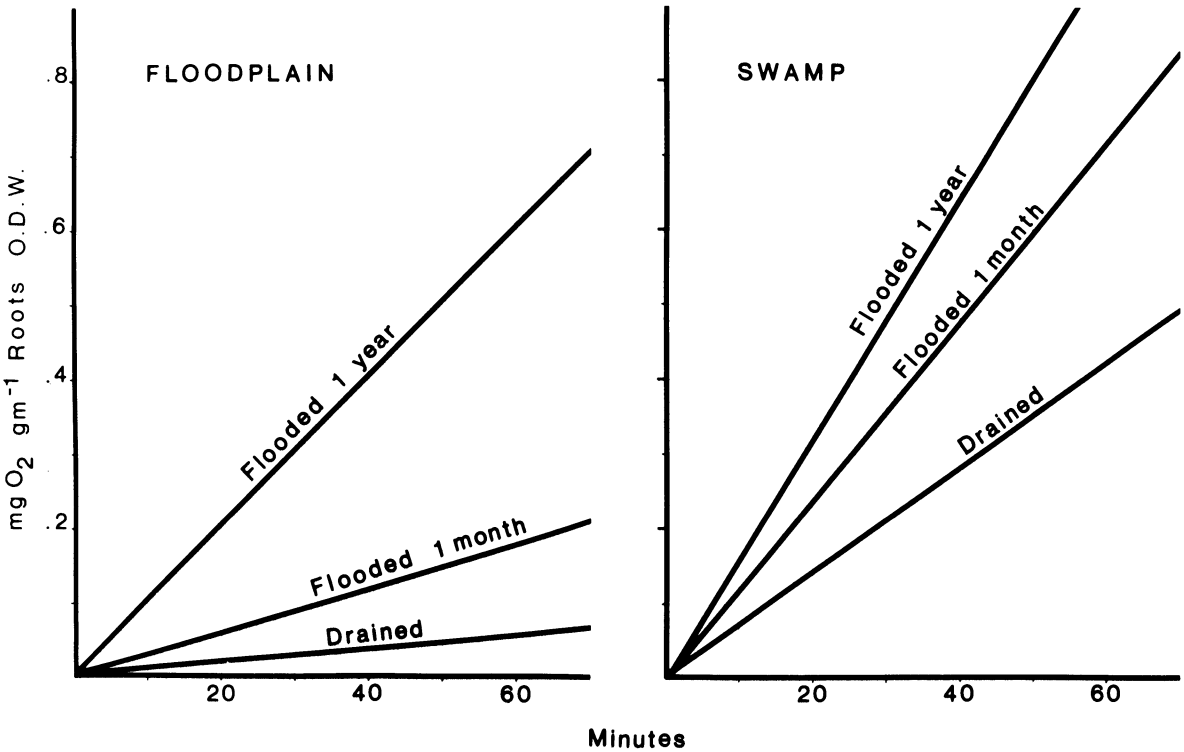

FIG. 5. Rates of oxygen transport, weighted by the ODW of roots for the floodplain and swamp plants, under drained and flooded conditions. Lines were drawn from the mean slope of 2 replicate experiments. See text for statistical treatment of slopes.

ly intermediate between the upland and swamp populations.

Oxygen transport.—Oxygen transport rates are shown in Fig. 5. Under drained conditions, the swamp plants had a significantly greater rate of oxygen transport to their roots than the floodplain plants ($P < .05$). After a year of flooding, the floodplain plants had significantly increased their rate of oxygen transport over drained conditions ($P < .01$). The differences in treatments were not statistically significant for the swamp population, though the trend was the same for both populations: increasing oxygen transport from drained conditions, to 1 mo of flooding, to 1 yr of flooding.

DISCUSSION

Flood tolerance

Along the flood frequency gradient we can delineate 3 distinct population responses to flooded conditions.

Those plants from the upland end of the gradient are very intolerant of flooded soils. Their root system

TABLE 7. Alcohol dehydrogenase activities (nM/min/mg protein) in root extracts from 1-year-old seedlings from the 3 populations grown under drained conditions and across a time course of flooding

	Upland			Floodplain			Swamp			
	$\bar{x}$	SD	N	$\bar{x}$	SD	N	$\bar{x}$	SD	N	
Drained	302	(212)	9	297	(156)	9	317	(142)	9	$F_s = 0.03^{NS}$
Flooded 1 Week	367	(386)	12	168	(110)	12	307	(206)	12	$F_s = 1.84^{NS}$
Flooded 1 month	131	(97)	7	261	(320)	15	496	(213)	15	$F_s = 6.09^{**}$
Flooded 1 year	135	(156)	9	161	(193)	12	62	(40)	16	$F_s = 2.00^{NS}$
	$F_s = 1.98^{NS}$			$F_s = 1.03^{NS}$			$F_s = 18.29^{***}$			

^{NS} $P > .05$.
** $P < .01$.
*** $P < .001$.

deteriorates, no new roots are initiated and root respiration rates drop to very low levels. Under long-term flooding, survival is poor and those that do remain are greatly stunted and moribund. These few remaining plants also have greatly exaggerated accumulations of many nutrient elements. Root RQ shows a significant drop under short-term flooding but is significantly >1.0 after a year of flooded conditions. The accumulation of nutrients under long-term flooding is quite different from what has been observed under short-term flooding (Hosner and Leaf 1962, Dickson et al. 1972). In these studies it was found that within the 1st few months of flooding less flood-tolerant trees showed decreased concentrations of most nutrients. The increases observed in the present study may be a result of a large proportion of dead roots which would nonselectively take up nutrients (Kramer 1951). The changes in RQ suggest that under short-term flooding there is a shift to dependence upon storage products, perhaps as a result of a reduction in translocation as observed for other non-flood-tolerant plants (Kramer 1951, Aung 1974, Nuritdinov and Vartapetian 1976). One explanation for the high RQ after a year of flooding would be the occurrence of alcoholic fermentation under apparently aerobic experimental conditions. This has been shown in other root studies to occur whenever oxygen entry to the cell is hindered (Ramshorn 1961). In this case the very high Fe concentrations would be suspect since Fe compounds will precipitate out and completely occlude the intercellular spaces of roots (Armstrong and Boatman 1967, Green and Etherington 1977).

In contrast to the upland population those plants from the other end of the gradient are very tolerant of flooded soils. Although parts of the original root system deteriorate upon flooding, the swamp plants initiate new, morphologically distinct, roots. These succulent roots have been described previously for swamp populations of *Nyssa sylvatica* (Hook et al. 1971) and roots of similar morphology are initiated upon flooding by a variety of flood tolerant plants (e.g., Weaver and Himmel 1930, Alberda 1953, Valoras and Letey 1966, Bristow 1975). However, what has not been reported previously is that these roots are only temporary and eventually are replaced by roots superficially resembling drained roots. Throughout the duration of flooding, swamp plants maintain a healthy root system with respiration rates comparable to drained roots. After a year of flooding they have the highest survival, the highest total biomass, and increased concentrations of the fewest nutrients coupled with the lowest concentrations of Fe and Mn. This inverse correlation between the accumulation of Fe and Mn and growth in flooded plants has been observed for other species (Jones and Etherington 1970, H. Jones 1971, R. Jones 1972*a*, Bell and Tallis 1974, Ladiges and Kelso 1977), but the causal relationship is unclear. High concentrations of these 2 nutrients are toxic per se, and thus could reduce growth directly (Martin 1968, R. Jones 1972*b*). On the other hand, plants which can transport oxygen internally down to the roots are capable of oxidizing reduced compounds in the rhizosphere, thereby avoiding excessive uptake of them (Teal and Kanwisher 1966, Armstrong 1967, Sheikh 1973); in this instance, better growth may be a direct result of sustained aerobic metabolism.

The floodplain population is distinct in that depending upon conditions, it resembles either the upland plants or the swamp plants or is intermediate. Under drained conditions floodplain plants allocate more biomass to roots than to shoots and have high respiration rates, traits similar to upland plants. Under flooded conditions they initiate and maintain a healthy root system with medium respiration rates, allocate less biomass to roots than to shoots, and have high survival, traits similar to swamp plants. They are distinctly intermediate in total biomass and accumulation of nutrients such as Fe and Mn under flooded conditions. Thus, the floodplain population produces unique genotypes capable of producing a distinctly flood tolerant phenotype but not nearly as tolerant of flooded conditions as the swamp phenotype.

It is reasonable that plants which occur in a periodically flooded environment should be tolerant of flooded soils. Perhaps not so clear is why under flooded conditions the growth of these floodplain plants should be intermediate to that of plants from either end of the gradient. Perhaps by looking at the mechanisms responsible for flood tolerance, we can answer this question.

The role of metabolic mechanisms in flood tolerance

Malate production.—There is little evidence from this study to support the theory that flood tolerance results from a metabolic switch away from ethanol production to malate production. Several points argue this. 1) Within the 1st week of flooding, all 3 populations accelerate ethanol production (Table 6). 2) After 1 mo of flooding, the lowest rate of ethanol production (Table 6) and ADH activity (Table 7) is found in the non-flood-tolerant upland population, in conjunction with the lowest accumulation of malic acid (Table 8). 3) The highly flood tolerant swamp plants, after 1 mo of flooding, have high rates of ethanol production and ADH activity, concomitant with a high concentration of malic acid. 4) After a year of flooding, the swamp population has a high malic acid content coincident with low alcoholic fermentation, but, this time-frame is incompatible with the hypothesis of a metabolic switch from anaerobic ethanol production to anaerobic malate production. Dark fixation of CO_2, involving PEP carboxylase (McManmon and Crawford [1971] report that PEP carboxykinase plays little or no role in the roots of flood-tolerant species) and reduction to malate, results in a zero net energy gain, and this is

TABLE 8. Malic acid concentration (mg malic acid/mg protein) in the roots of 1-year-old seedlings from the 3 populations grown under drained conditions and across a time course of flooding

	Upland			Floodplain			Swamp			
	$\bar{x}$	SD	N	$\bar{x}$	SD	N	$\bar{x}$	SD	N	
Drained	4.15	(2.07)	5	3.45	(1.44)	5	3.86	(1.73)	5	$F_s = 0.20^{NS}$
Flooded 1 week	3.15	(2.16)	5	4.52	(2.74)	22	3.51	(1.76)	15	$F_s = 1.18^{NS}$
Flooded 1 month	2.29	(0.89)	5	6.09	(4.93)	10	14.95	(10.87)	10	$F_s = 5.68^{*}$
Flooded 1 year	5.23	(4.66)	10	8.93	(5.79)	15	14.66	(11.82)	15	$F_s = 4.02^{*}$
	$F_s = 1.00^{NS}$			$F_s = 3.91^{*}$			$F_s = 6.18^{***}$			

^{NS} $P > .05$.
* $P < .05$.
*** $P < .001$.

incompatible with the good growth of these plants during a year of flooding (Table 1). The scheme, implicating malate as an anaerobic end product, is a temporary survival strategy dependent upon a return to aerobic conditions. Yet, in the swamp plants, malate levels remain high under long-term flooding. 5) Additionally, metabolism of malic acid gives an RQ = 1.33 (James 1953:85). Thus, if malate were being stored for metabolism upon return to aerobic conditions, one would predict that the swamp plants, flooded for 1 yr, would have an RQ somewhat > the 1.01 observed (Fig. 4).

It is apparent that malate accumulates independently of alcoholic fermentation and its role as an anaerobic end product of glucose catabolism seems doubtful. An hypothesis worth considering is that malic acid accumulates as a balance for ionic disequilibrium within the cell. This is suggested by several observations. One is the repeated demonstration of increased organic acid (primarily malic acid) production in roots in response to excess cation uptake (Hiatt and Leggett 1974). The altered soil chemistry under flooding could provide a rooting medium that would upset the ionic balance of plants growing in it. For example, upon flooding, soils switch from a nitrate to an ammonium based nitrogen economy, and accumulate high concentrations of CO_2 and the divalent forms of Fe and Mn, as well as undergo a multitude of other changes (Ponnamperuma 1972), most of which can affect the internal pH of the cell (Kirkby and Mengel 1967, Chang and Loomis 1945, Nason and McElroy 1963).

TABLE 9. Effect of exogenously applied ethanol on root respiration rates (μl/h/g ODW) for swamp and floodplain plants under drained and 1 month flooded conditions. Root systems were surface-sterilized and incubated for 24 h in aerated Hoagland's Solution with 3M ETOH (experimental) or without (control), $N = 4^a$

	Q_{O_2}	
	Drained	Flooded 1 month
Control	1131	1183
3M ETOH	436	701
% Control	38.5*	59.2*

[a] Floodplain and swamp populations were not significantly different and were combined.
* t-test on arcsin transformations of percentages gave a $P < .05$, that H_A: μ drained < μ 1 month flooded.

Alcoholic fermentation.—All 3 populations respond similarly to flooding by accelerating ethanol production (Table 6) and having portions of their original root system deteriorate. In light of the known toxicity of ethanol to plant tissues (Karlsen 1925, Grineva 1963, Fulton and Erickson 1964) it is conceivable that accelerated ethanol production causes the deterioration of the original root system. In addition to direct toxic effects, ethanol may also act to increase fungal infection by attracting the zoospores of certain fungal pathogens (Allen and Newhook 1973).

If ethanol production accounts for the deterioration of the original root systems, then it must be explained how the new roots produced by the floodplain and swamp populations are able to accelerate ethanol production and yet still remain healthy. One hypothesis is that these roots transfer the ethanol to their environment, either directly from their roots (Grineva 1963, Bolton and Erickson 1970) or by being transported up the stem and liberated through the lenticels (Chirkova and Gutman 1972) or stomates (Kenefick 1962). Another hypothesis is these roots are able to tolerate higher concentrations of ethanol than are drained roots. It is true that willow roots are more tolerant of ethanol than those of a less flood-tolerant poplar (Chirkova 1976) and rice roots are more tolerant of ethanol than wheat roots (Taylor 1942), but there is little evidence for temporal changes in a species' tolerance. This was tested by examining the effect of externally applied ethanol on aerobic root respiration rates. The results shown in Table 9 indicate that the new roots initiated under flooding are less affected by ethanol than those roots produced under drained conditions. Although this is not definitive evidence of the effect of internally produced ethanol, it does support the hypothesis that these roots are more tolerant of ethanol.

To summarize, under short-term flooding there is an acceleration of ethanol production accompanied by a deterioration of portions of the original root system.

This is common to all populations. With continued flooding the upland plants' root system continues to deteriorate and there is a drop in ethanol production. After a year of flooding most upland plants are dead, and those few remaining have an ethanol production rate ≈80% < drained plants, paralleling a similar drop in aerobic respiration rates, very likely a result of the overall necrosis of the root system.

Upon flooding, the floodplain and swamp plants initiate new roots capable of higher rates of ethanol production and perhaps more tolerant of ethanol. Thus, short-term survival of flooding is related to the acceleration of alcoholic fermentation, providing a compensatory energy source. The ADH activity data (Table 7) support this conclusion for the swamp population but not the floodplain population; viz., at the end of 1 mo of flooding the swamp plants showed accelerated ADH activity whereas the floodplain plants did not. The most likely explanation for this discrepancy would lie in sampling error due to the small number of replicates (2) in the ethanol production experiments. The much larger sample size (15) in the ADH experiments must certainly present a more accurate representation of the floodplain population. Thus, it appears that floodplain and swamp plants respond differently to short-term flooding.

If this difference between populations is due to different selection pressures in the 2 habitats, then a similar pattern of ADH activities might be observed in another tree species occurring in these 2 habitats. To test this prediction, *Nyssa aquatica* L., a species quite distinct from *N. sylvatica,* but frequently sympatric with it in floodplains and swamps was selected (see Keeley 1977 for location of seed sources). The ADH activities for *Nyssa aquatica* and *N. sylvatica* are shown in Fig. 6. After 1 mo of flooding the swamp populations of both species had higher ADH activity than their respective floodplain populations, which failed to accelerate ADH activity at all. This is substantial evidence that the population differences in ADH activity are the result of attributes selected for by the 2 habitats.

Under 1 yr of flooded conditions both the floodplain and swamp populations responded similarly in that ADH activity, as well as ethanol production, were significantly < under drained conditions (this is also true for *Nyssa aquatica,* Fig. 6). Since all of these plants survived long-term flooding, have reasonably good growth rates, and maintain a healthy root system with reasonably good respiration rates, the drop in alcoholic fermentation cannot be attributed to the death of the root system (as is likely the case in the upland plants). There must be some form of control acting to keep fermentation rates low.

The patterns of alcohol fermentation observed for the floodplain and swamp populations raise 2 questions: 1) What accounts for the accelerated ADH activity in the swamp population, but not in the floodplain population in response to short-term flooding, and 2) What sort of control is operating to lower ADH activity in both populations under long-term flooding? The most likely explanations for reductions in ADH activity are: 1) a metabolic switch which shunts carbon off into some product other than ethanol, e.g., malate, or 2) anatomical changes which result in a greater oxygen concentration inside the roots so there is a greater dependence on aerobic respiration. There is very little support for the 1st hypothesis in explaining either the short-term differences between populations or the long-term changes in both populations (see discussion in previous section). On the other hand, developmental changes in anatomy which increase the internal aeration of the stem and root system may in fact account for both of these patterns.

Oxygen transport.—Intuitively it would seem that ADH activity (and alcoholic fermentation in general) should be at a maximum under anaerobic conditions and decrease monotonically with increasing oxygen concentration within the root (Fig. 7, curve a). However, recent experiments indicate the relationship between ADH activity and oxygen concentration may not be so simple. Wignarajah and Greenway (1976) found by flushing the root system of maize with different concentrations of oxygen that, beginning from near anaerobic conditions, ADH activity increased with increasing oxygen concentration reaching maximum activity between 5–10% O_2 (Fig. 7, curve b). Further increases in oxygen resulted in a sharp decline in ADH activity. The reduced activity at near anaerobic conditions was likely due to a reduction in protein synthesis though they did not rule out inhibition or degradation of the enzyme. This relationship between ADH activity and oxygen concentration observed for maize has also been observed for barley; however, in rice ADH activity tended to increase monotonically with decreasing oxygen concentration (Wignarajah et al. 1976). One could argue that the data for maize and barley are more definitive than the data for rice, since rice transports much more oxygen to its roots (Alberda 1953, Armstrong 1967, 1969) than maize or barley (Barber et al. 1962, Jensen et al. 1967, Luxmoore et al. 1970*b*, 1970*c*). Thus it is unlikely that the internal root environment of the rice plants used by Wignarajah et al. (1976) was close to anaerobic, even though the rooting medium was flushed with nitrogen. This explanation would be supported by the observation that maize roots, flushed with nitrogen, showed a further drop in ADH activity when the tops were also flushed with nitrogen (Wignarajah and Greenway 1976).

At present no such experiments have been done for *Nyssa,* but in all likelihood ADH activity is related to internal oxygen concentration in 1 or the other of these 2 patterns. Regardless of which is the case the reduction in ADH activity in both the floodplain and the swamp plants under long-term flooding can be ex-

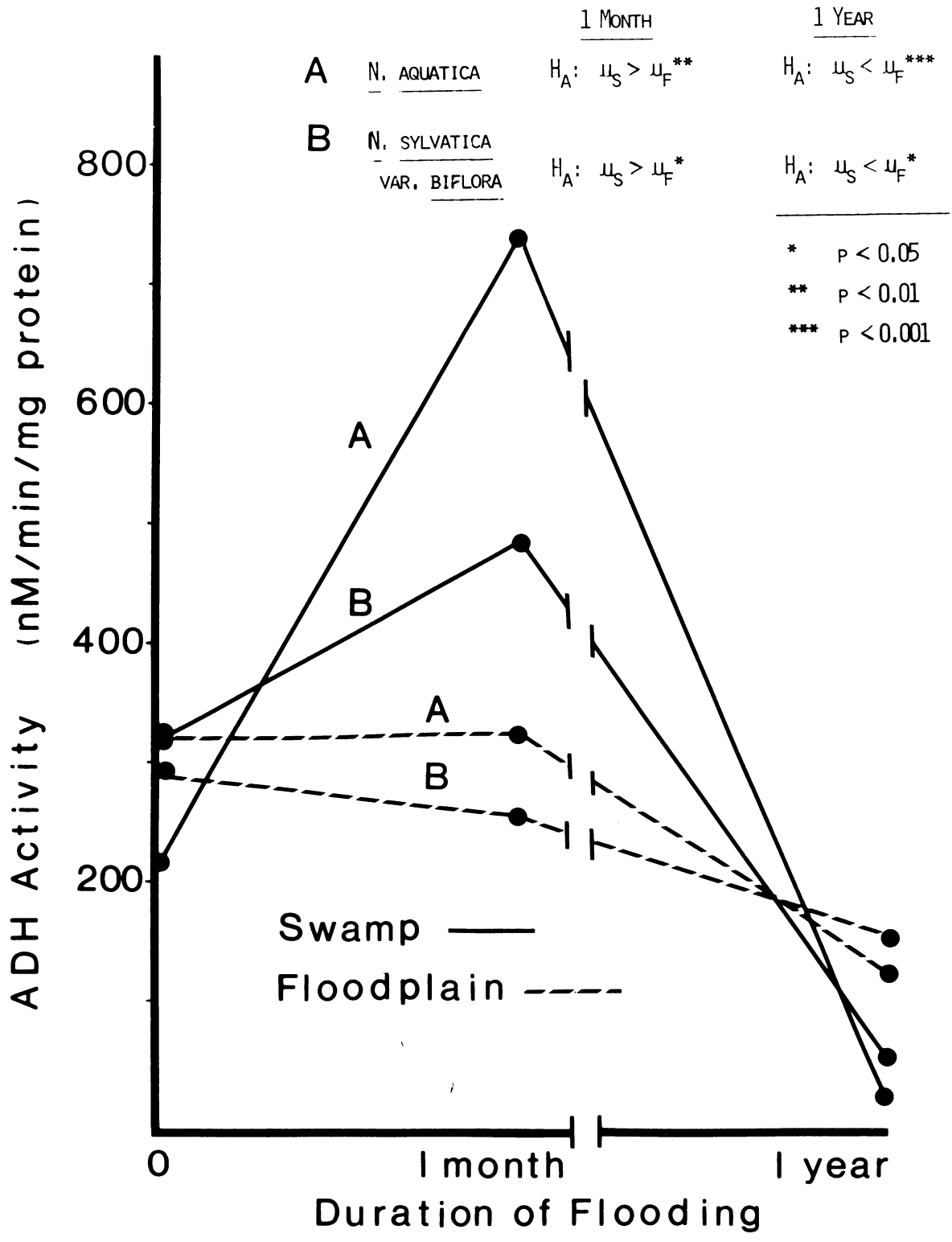

FIG. 6. Alcohol dehydrogenase activities in plants from swamp and floodplain populations of *Nyssa aquatica* and *N. sylvatica* under drained conditions, short-term and long-term flooding (N = 4, 4, 4 – *N.a.*; N = 9, 15, 16 – *N.s.*).

plained as a return to aerobic respiration due to increased oxygen concentration within the root system; Fig. 5 shows both populations increase rate of oxygen delivery to the roots the longer they are flooded. Indeed, developmental changes in anatomy which increase the internal aeration of the stem and root system, and in turn increase the capacity for aerobic respiration, would seem to be a requisite for continued growth under long-term flooding. The time frame for anatomical changes is also in line with the long-term acclimation to lowered alcoholic fermentation. This explanation is appealing too since there are obvious morphological changes in *Nyssa sylvatica* under flooding which would affect the internal aeration of stem and roots. For example, there is a proliferation of lenticels at the base of flooded stems (Fig. 3) as well as a number of anatomical changes which do increase oxygen transport to the roots (Hook et al. 1970*a*, 1971, 1972). This is all the more likely since developmental differences in the volume of intercellular space, dependent upon the oxygen environment around the roots, have been demonstrated for other plants; e.g., barley (Bryant 1934) and rice (Katayama 1961, Armstrong 1971).

The differences in ADH activity between the floodplain and swamp populations in response to short-term flooding are not as easily explained. If we assume a monotonic relationship between ADH activity and oxygen concentration (Fig. 7, curve a) we would have expected the floodplain plants, with the lower rate of oxygen transport after 1 month of flooding, to have had a higher ADH activity. This clearly was not the case. If we assume a convex relationship (Fig. 7, curve b), we could explain the differences this way. After a month of flooding the floodplain plants with their relatively low rate of oxygen transport might be expected to fall somewhere near the left end of the abscissa. The swamp plants would fall along the abscissa somewhere to the right of the floodplain plants. In this case we might expect the swamp plants to have a higher ADH activity which in fact was the case. Thus the failure of the floodplain plants to accelerate ADH activity under short-term flooding might be viewed as a consequence of being very poorly aerated.

An additional point needing to be addressed concerns the fact that ADH activities in the floodplain and swamp plants, after a year of flooding, were lower than drained plants. Based on the preceding arguments it would appear that plants flooded for a year are better aerated internally than are the drained roots either internally or externally. On the surface this seems unreasonable, but it may not be so. Aeration of the soil will vary temporally and spatially, depending upon soil characteristics and drainage patterns (Grable 1966, Greenwood 1969) and these patterns are likely to be accentuated in small clay pots. Undoubtedly there are periods of time after watering in which the soil is poorly aerated. This sort of variation can be observed in

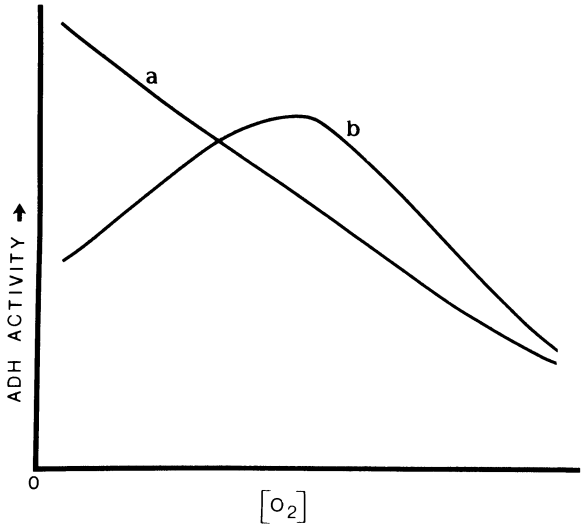

FIG. 7. Two possible models of the relationship between ADH activity and oxygen availability in roots.

a pot; roots from the bottom of "drained" pots have significantly greater ADH activity than ones from the surface ($P < .05$, $N = 4$). Similar variations would be expected in root systems under field conditions in response to localized shifts in soil aeration.

Under long-term flooding, both the floodplain and swamp plants are capable of transporting enough oxygen to sustain their roots by aerobic respiration. In addition, as demonstrated for other plants (Armstrong 1967, 1972), radial oxygen diffusion out of the roots may oxidize the highly reduced rhizosphere and thus prevent the uptake of toxic quantities of ions such as Fe^{2+} and Mn^{2+}. For example, the higher oxygen transport rates (Fig. 5) observed for the swamp plants very likely accounted for the lower concentrations of Fe and Mn (Table 4), relative to the floodplain plants. If oxygen transport and Fe and Mn are causally related (inversely) then very high concentrations of these elements in upland plants suggests, as might be expected, that these plants transport very little oxygen to their roots.

The mechanism of oxygen transport to the roots is undoubtedly gaseous diffusion through aerenchyma or intercellular spaces rather than any sort of active transport (Evans and Ebert 1960, Barber et al. 1962, Teal and Kanwisher 1966, Armstrong 1967, 1969, Luxmoore et al. 1970*a*, Coutts and Armstrong 1976). In *Nyssa sylvatica*, oxygen enters flooded plants through the lenticels at the base of the stem and diffuses down to the roots through intercellular spaces in the phloem or xylem (Hook et al. 1970*a*, 1970*b*, 1971).

Adaptive significance of population differences

The upland plants have little capacity for surviving flooded conditions. This is typical of plants seldom exposed to such conditions in their natural setting.

TABLE 10. Comparison of ADH activities (nM/min/mg protein) between recently emerged seedlings and seedlings older than 1 year of age, across a time course of flooding, for the swamp population of *Nyssa sylvatica*

	1–4 Months			1–1½ Years			
	$\bar{x}$	SD	N	$\bar{x}$	SD	N	
Drained	194	(86)	2	317	(142)	12	$F_s = 1.31^{NS}$
Flooded 1 week	113	(54)	3	307	(207)	12	$F_s = 2.47^{NS}$
Flooded 1 month	154[a]	(116)	4	496[b]	(214)	15	$F_s = 9.24^{**}$
Flooded 4 month	258[a]	(98)	4	621[b]	(164)	4	$F_s = 14.42^{**}$
Flooded 8 month	...	...	...	411[b]	(281)	4	...
	$F_s = 1.49^{NS}$			$F_s = 3.04^{*}$			

[a] Flooded continuously since the cotyledon stage.
[b] Approximately 1 year old at the initiation of flooding.
^{NS} $P > .05$.
* $P < .05$.
** $P < .01$.

At the other end of the flood frequency gradient are the swamp plants, well adapted to flooded soils. These plants are genetically programmed to transport oxygen to their roots in appreciable quantities regardless of the soil conditions of the immediate environment (Fig. 5). If they are suddenly flooded after growing under drained conditions, the aeration of the roots will not be sufficient to allow complete dependence upon aerobic respiration. Thus, there is an acceleration of alcoholic fermentation which acts as a compensatory energy source (Fig. 6). Under continued flooding these plants acclimate in 2 ways. Oxygen transport increases and thus the availability of oxygen for respiration, as well as for oxidation of the rhizosphere, increases. Concomitant with this is a decrease in the oxygen demand as a result of reduced root biomass (Table 2). As the ratio of oxygen availability to oxygen demand increases, the dependence upon alcoholic fermentation decreases.

This describes the sequence of events for a swamp seedling germinating and growing under drained conditions prior to being flooded. It may be that the developmental sequence of events for a swamp seedling germinating under waterlogged conditions would result in a ratio of oxygen availability to oxygen demand high enough to preclude any dependence upon alcoholic fermentation. This is suggested by the observation that seedlings grown from seed in waterlogged soil never initiate the succulent type root which is associated with high alcoholic fermentation. This hypothesis was investigated by testing the following prediction: seedlings flooded for 1 to several months will not accelerate ADH activity if flooded at the cotyledon stage, whereas seedlings grown under drained conditions for a year prior to flooding will. The results shown in Table 10 uphold this prediction. There was no significant change in ADH activity with flooding in recently emerged seedlings whereas 1-yr-old seedlings showed significant changes across the time course of flooding. Thus, 1-yr-old seedlings flooded for one to several months have a highly significant increase in ADH activity over recently emerged seedlings flooded for similar durations of time. However, with continued flooding these older seedlings apparently are capable of increasing oxygen transport to the roots as indicated by the drop in ADH activity after 8 mo of flooding.

These data suggest that the capacity to tolerate and eventually acclimate to flooding, by increasing oxygen transport to the roots, is dependent upon age at the initiation of flooding. This would be supported by the observation that older seedlings are often less flood tolerant than young ones (Crawford 1972). If so, there may be a point in the life cycle of these trees in which developmental changes cannot occur fast enough to survive flooding. There are many tree species which are normally flood tolerant but have populations which succumb when mature individuals are suddenly flooded. Gill (1970) has suggested this is due to ecotypic differences, but the data from this study indicate developmental constraints could account for this phenomenon.

With respect to the swamp populations of *Nyssa* it is conceivable that, because these plants may spend nearly their entire life cycle in waterlogged soils, alcoholic fermentation may be selectively irrelevant to them. There seems to be little justification for the belief that long-term survival in swamps is dependent upon the maintenance of alcoholic fermentation (Hook et al. 1971, 1972, Hook and Brown 1973). Rather, the hypoxic or anaerobic conditions are avoided through internal oxygen transport sufficient to maintain aerobic respiration as well as oxidation of the rhizosphere. Under long-term flooding, one should expect a strong correlation between flood tolerance, high internal oxygen transport and low alcoholic fermentation. As already seen, this is true for the swamp ecotype in comparison to the floodplain ecotype of *Nyssa sylvatica*. It is likely true for interspecific comparisons as well; e.g., swamp populations of *Nyssa sylvatica* and *N. aquatica*. The latter species had significantly lower ADH activity than *N. sylvatica* ($P < .05$) after 1 yr of flooding (Fig. 6) which suggests *N. aquatica* has a

greater capacity for internal oxygen transport which would account for its greater flood tolerance (Applequist 1956, Harms 1973).

Floodplain plants are naturally subjected to both drained and flooded soils. Under drained conditions the upland and floodplain plants allocate more biomass to roots than to aboveground parts whereas the swamp plants do not (Table 2). Additionally, under drained conditions both the upland and floodplain plants have much higher respiration rates than swamp plants (Table 5) and the floodplain (and very likely the upland) plants are transporting little oxygen to their roots (Fig. 4, and discussion in previous section). Upon flooding less biomass is allocated to roots for both the floodplain and swamp plants unlike the upland plants. Across the time course of flooding the floodplain plants parallel the swamp plants in respiration rates, initiation of new roots and anatomical changes leading to increased oxygen transport.

A reasonable question to ask about the floodplain strategy is: If flooding is such a "severe" aspect of the environment, why hasn't there been selection for a "maximin" strategy (e.g., Templeton and Rothman 1974), viz. why don't the floodplain plants transport oxygen to their roots under all conditions so that when flooding occurs they are initially better aerated. Their failure to do so puts them at a competitive disadvantage under flooded conditions (Table 1). The answer to this must be that adaptations for survival under flooding carry with them a "cost" for survival under drained conditions. What are the costs? They may be related to the 2 generally opposing demands of aeration and water balance. Hook and Brown (1972) have argued that one of the functionally more important anatomical features of swamp trees is the relatively pervious cambium which affords internal aeration of the stem. They suggested "it is likely that the absence of large intercellular spaces in the cambium of mesic or xeric species is advantageous, inasmuch as excessive water loss by this pathway could result in desiccation of the cambium and newly formed xylem derivatives during periods of water stress." This hypothesis is supported by the report that the majority of transpirational water loss occurs through the stem lenticels in a flood-tolerant willow in striking contrast to the very low lenticulate water loss for a non-flood-tolerant poplar (Chirkova and Gutman 1972).

I hypothesize that plants in well-drained habitats, where water is limiting and air is not, have been selected for reduced internal aeration as a means of competitive superiority under occasional water stress. For similar reasons there has been selection for greater allocation of biomass to roots and higher root respiration rates. As a consequence, these plants are at a severe disadvantage under flooding when air is limiting. Plants under continuous hydric conditions, where air is limiting and water is not, have been selected for increased aeration of the stem and roots. Parallel with this is a reduction in the demand for oxygen by reductions in root biomass and respiration rates. Consequently, swamp plants should be at a competitive disadvantage under water stress conditions.

Although it is not possible at this point to evaluate the effect of internal aeration on transpiration, there is evidence that adaptation to flooding carries with it a cost under drained conditions. Not only has it been observed that many swamp trees are particularly sensitive to water stress conditions (Bergman 1920, Dickson et al. 1965, Dickson and Broyer 1972) but, there are studies which demonstrate an inverse correlation between flood tolerance and drought tolerance. For example, Bannister (1964*b*) has shown that the flood tolerant *Erica tetralix* L. succumbs to much smaller water deficits than flood-sensitive shrubs. In addition to death under water stress, some flood-tolerant species exhibit drought sensitivity in terms of greatly reduced photosynthetic gain under small water deficits (Regehr et al. 1975). Thus, even though a flood-tolerant species was to survive drought conditions, it may be competitively excluded from such sites (Bannister 1964*a*). Of particular interest to this study is the fact that increasing flood tolerance, correlated with decreasing drought tolerance, can be observed within the same species dependent upon growing conditions (Bell and Tallis 1974). Specifically, Tomar and Ghildyal (1975) have shown resistance to water transport in nonflooded rice plants is twice as high as in flooded ones, and that nonaerenchymous roots of nonflooded plants have ≈ 17 times more resistance than aerenchymous roots of flooded plants.

In conclusion, it would seem that evolution in floodplains has selected against strategies totally committed to 1 condition or the other. Thus, selection may have preserved genotypes capable of acclimating to either drained or flooded conditions, with the result that the phenotypes are "optimally" adapted to neither; perhaps they reflect the optimum compromise.

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