Alcoholic Fermentation in Swamp and Upland Populations of Nyssa sylvatica: Temporal Changes in Adaptive Strategy

Jon E. Keeley, Eldon H. Franz

American Naturalist, Volume 113, Issue 4 (Apr., 1979), 587-592.

Your use of the JSTOR database indicates your acceptance of JSTOR's Terms and Conditions of Use. A copy of JSTOR's Terms and Conditions of Use is available at http://www.jstor.org/about/terms.html, by contacting JSTOR at jstor-info@umich.edu, or by calling JSTOR at (888)388-3574, (734)998-9101 or (FAX) (734)998-9113. No part of a JSTOR transmission may be copied, downloaded, stored, further transmitted, transferred, distributed, altered, or otherwise used, in any form or by any means, except: (1) one stored electronic and one paper copy of any article solely for your personal, non-commercial use, or (2) with prior written permission of JSTOR and the publisher of the article or other text.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

American Naturalist is published by University of Chicago Press. Please contact the publisher for further permissions regarding the use of this work. Publisher contact information may be obtained at http://www.jstor.org/journals/ucpress.html.

American Naturalist
©1979 University of Chicago Press

JSTOR and the JSTOR logo are trademarks of JSTOR, and are Registered in the U.S. Patent and Trademark Office. For more information on JSTOR contact jstor-info@umich.edu.

©2001 JSTOR

http://www.jstor.org/
Tue Apr 17 13:39:01 2001

ALCOHOLIC FERMENTATION IN SWAMP AND UPLAND POPULATIONS OF *NYSSA SYLVATICA*: TEMPORAL CHANGES IN ADAPTIVE STRATEGY

JON E. KEELEY* AND ELDON H. FRANZ†

Department of Botany and Institute of Ecology, University of Georgia, Athens, Georgia 30602

Although much of the biosphere has evolved to function aerobically, many organisms can tolerate periods of low O_2 availability. Hochachka and Somero (1973, pp. 32–33) have identified three strategies of adaptation to anaerobic environments. Some organisms compensate for the lack of available O_2 by maintaining high capacities for anaerobic generation of ATP (compensatory strategy). Others have the substrate level phosphorylations of glycolysis linked to other substrate level phosphorylations allowing an increased yield of ATP during anoxia (exploitative strategy). Finally, in certain organisms the physiological demands for energy are met through highly effective O_2 delivery to cells for aerobic metabolism, thus minimizing or avoiding the physiological problem of limited O_2 availability (avoidance strategy).

In plants, hypoxic (low oxygen) conditions are encountered by the root system whenever the soil is flooded. Plant species and ecotypes vary significantly in survival under such conditions and this variation is one dimension of niche differentiation among plant populations (Franz and Bazzaz 1977). There is, however, no generally accepted theory of the adaptive strategies of tolerant taxa.

Crawford and his coworkers (Crawford and McManmon 1968; Crawford and Tyler 1969; McManmon and Crawford 1971) have developed a metabolic theory of flood tolerance which is a form of exploitative strategy. It is based on the observation that ethanol production by the roots of intolerant taxa increases in response to 1 mo of flooding, whereas tolerant taxa produce the same amount of ethanol, or less, than unflooded controls. They propose that flood sensitivity is due to an accumulation of toxic concentrations of ethanol in the roots of intolerant taxa. Crawford and the others hypothesize that tolerant plants survive flooding by some homeostatic mechanism that limits the production of ethanol by shunting carbon into nontoxic organic acids, such as malic acid. In addition to avoiding an accumulation of toxic ethanol, the malate may be linked to other substrate level phosphorylations or further oxidized with an alternative electron acceptor (Garcia-Novo and Crawford 1973).

Crawford's theory is consistent with the data from several studies (Marshal et al. 1973; Chirkova et al. 1974; Francis et al. 1974), but not all. Hook et al. (1971) found

* Present address: Department of Biology, Occidental College, Los Angeles, California 90041.

† Present address: Environmental Research Center, Washington State University, Pullman, Washington 99163.

Am. Nat. 1979. Vol. 113, pp. 587–592.
© 1979 by The University of Chicago. 0003-0147/79/1304-0008$00.75

that after 1 mo of flooding, ethanol production increased over fivefold in the roots of the flood tolerant *Nyssa sylvatica* Marsh. var. *biflora* (Walt.) Sarg. These authors concluded, in contrast to Crawford, that for tree species indigenous to swamps, the maintenance of accelerated rates of alcoholic fermentation during flooding is one of the primary adaptations for survival in flooded habitats. A similar five-fold increase in ethanol production after 6 days of anaerobiosis has been observed for the flood tolerant rice (John and Greenway 1976). These studies indicate that in two widely different species adaptation to flooding may be dependent upon an enhancement of alcoholic fermentation, apparently as a compensatory energy source under hypoxic conditions.

We report here a comparison of the metabolic responses to flooding of the flood tolerant *Nyssa sylvatica* var. *biflora* with *N. sylvatica* var. *sylvatica*, a closely related taxon seldom found in flooded habitats (Radford et al. 1968) and not tolerant of experimental flooding (Keeley 1977). Additionally, since trees of southeastern lowland swamps often are continuously flooded for years at a time, we followed ethanol production over a flooding time course from 24 h to 1 yr. The inclusion of 1-yr-flooded plants is particularly significant, since no study we know of has followed metabolic responses to flooding over a time course of more than a few weeks, and as will be shown these plants exhibit a definite long-term acclimation response to flooding.

METHODS

All experiments were with 1-yr-old seedlings grown from seed in a greenhouse. Seeds were collected from across a broad geographical range in Georgia; var. *sylvatica* on drained sites from the piedmont to the mountains and var. *biflora* on wet sites from the piedmont to the coast. Seeds were germinated in 15 cm clay pots filled with sandy loam soil. Flooding was with tap water to a level of 3–5 cm above the top of the pot. Alcoholic fermentation was assayed as follows. The terminal 1–2 cm of roots were incubated in 0.05*M* citrate–0.1*M* phosphate buffer (pH 5.4) with 0.25% glucose under nitrogen for 4 hr. After incubation the root sections were blotted and weighed fresh, then ground in a mortar, filtered through cheesecloth, and centrifuged for 20 min at 10,000 rpm. This supernatant was added to the original buffer and assayed for ethanol (Bergmeyer 1974*b*, pp. 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.

RESULTS AND DISCUSSION

Immediately upon flooding the ethanol producing capability of both upland and lowland varieties increased significantly ($P < .01$) compared to that of drained plants (table 1), and there was no significant difference between varieties through the first week of flooding. However, after 1 mo of flooding the ethanol production by *Nyssa sylvatica* Marsh. var. *biflora* increased slightly, whereas it decreased in var. *sylvatica*. Thus, after 1 mo of experimental flooding the lowland flood tolerant *biflora* was capable of producing a significantly greater amount of ethanol than its upland

TABLE 1

ETHANOL PRODUCTION DURING NITROGEN INCUBATION BY ROOTS OF *Nyssa sylvatica* VARIETIES GROWN UNDER DRAINED AND FLOODED CONDITIONS
(μM ETOH/4 h/g fresh weight)

	sylvatica			*biflora*			
	$\bar{X}$	SD	N	$\bar{X}$	SD	N	t test†
Drained	11.60	3.64	4	14.41	2.39	6	NS
Flooded 24 h	21.65	4.94	12	20.11	4.58	9	NS
Flooded 1 wk	20.37	.27	2	20.40	1.88	2	NS
Flooded 1 mo	16.71	2.67	4	25.02	5.62	4	*
Flooded 1 yr	2.61	2.64	6	8.10	2.20	6	**

† Varieties were compared statistically with the two-tailed t test for H_0: $\mu_{sylvatica} = \mu_{biflora}$; NS = $P > .05$.
* $P < .05$.
** $P < .01$.

relative. The most striking change was the very significant ($P < .001$) drop in ethanol production from 1 mo to 1 yr for both varieties. After this period of flooding, ethanol production by var. *sylvatica* was much less ($P < .01$) than by var. *biflora*.

These results do not support Crawford's theory for explaining tolerance and intolerance of flooding. Both varieties of *N. sylvatica* responded immediately to flooding with an increase in the rate of ethanol production. After 1 mo of flooding, contrary to what Crawford's theory would predict, the flood tolerant *biflora* had a greater rate of ethanol production than the intolerant *sylvatica*.

Hook's conclusion that accelerated anaerobic respiration is responsible for survival in perennially flooded swamps is also not supported. The fact that the growth of *biflora* is not decreased by long-term flooding (Keeley 1977) while ethanol production drops to levels lower than drained plants indicates relatively little reliance on anaerobic respiration.

Observations on morphological and anatomical changes in the roots provide clues which may explain the significance of these metabolic changes. Within a week after flooding the secondary root system of both varieties began to deteriorate. However, var. *biflora* initiated new roots which were morphologically quite distinct from drained roots. These new roots, which originated along the length of the tap root, were several milimeters in diameter, very brittle, and quite succulent (see also Hook et al. 1971); roots of similar morphology are initiated upon flooding by a variety of plants (e.g., Weaver and Himmel 1930; Bryant 1934; Alberda 1953; Bristow 1975). After 1 mo of flooding these new roots were capable of accelerated ethanol production (table 1). Variety *sylvatica* did not initiate these new roots, and as the original root system continued to deteriorate the ethanol production declined. After a year of flooding *sylvatica*'s root system was highly necrotic; this probably accounts for the low ethanol production at this point.

Variety *biflora* underwent a second change in its root system between 1 mo and 1 yr of flooding. The larger diameter "fleshy" roots produced soon after flooding were replaced by smaller finely divided roots. Morphologically the roots present after 1 yr of flooding were very similar to roots of drained seedlings. In addition they were

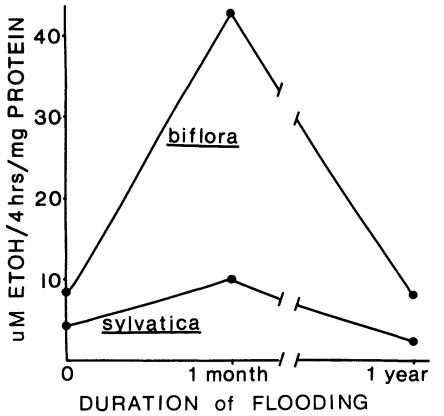

FIG. 1.—Ethanol production/mg of protein for the roots of *Nyssa sylvatica* varieties versus duration of experimental flooding. Ethanol production was converted to a protein basis from the average of four protein to fresh weight determinations per treatment, using the Lowry method as modified by Bergmeyer (1974*a*, pp. 172–174).

quite healthy; thus the drop in ethanol production after 1 yr of flooding does not result simply from death of the root system.

Since the roots produced under the different treatments are morphologically distinct, ethanol production was converted from a fresh weight to a protein basis (fig. 1). We found that the new roots initiated by var. *biflora* within the first month of flooding have less protein per unit fresh weight ($P < .05$) than drained roots and are capable of producing five times more ethanol per unit protein. After 1 yr of flooding these roots have been replaced by others with a higher protein content ($P < .05$) but a much lower ethanol producing capability (fig. 1). This second set of new roots, present after 1 yr of flooding, have nearly identical rates of ethanol production per unit of protein as drained roots (8.46 μM/4 h/mg vs. 8.07 μM/4 h/mg, respectively).

Thus, in *N. sylvatica* var. *biflora* adaptation to flooding is a result of sequential changes in metabolism and anatomy. The initial response to flooding is to produce roots which accelerate anaerobic respiration. This compensatory strategy is adaptive under short-term flooding episodes. This is in line with Hochachka and Somero's (1973, p. 46) argument that the compensatory strategy of accelerating anaerobic respiration is successful for circumventing short-term anaerobic conditions but is a "poor one for the exploitation of O_2-free environments on a sustained basis." As the duration of the flooding period increases, the roots acclimate metabolically to the new conditions. This acclimation response may represent a switch to an exploitative type of strategy as outlined by Crawford; however, the very long period of time required for this switch makes a metabolic change seem unlikely. More probable would be anatomical changes which result in larger air spaces in both stems and roots, thus producing internal aeration and a return to aerobic respiration. It has been demonstrated for a number of species that cortical breakdown leading to a more efficient O_2 delivery to the roots can be induced by flooding (Armstrong 1971*a*, 1971*b*).

TABLE 2

RATES OF INTERNAL OXYGEN TRANSPORT, WEIGHTED BY OVEN DRY WEIGHT OF ALL SECONDARY ROOTS (mg O_2/h/g ODW), FOR 1-YR-OLD SEEDLINGS OF *Nyssa sylvatica* var. *biflora*, $N = 4$

	Drained	Flooded 1 mo	Flooded 1 yr
$\bar{X}$	2.32**	4.52	7.97**
SD	2.14	3.91	2.58

NOTE.—The experimental procedure was to flush the root media with nitrogen and then measure the rate of O_2 diffusion from the roots with the tops sealed off and exposed to an atmosphere of 100% O_2.
** $P < .01$ that for H_A, $\mu_{1\,yr} > \mu_{drained}$ with the one-tailed t test.

If this hypothesis were true we would predict an increasing rate of internal oxygen transport from the stems to the roots with increasing duration of flooding. This prediction was tested experimentally with the polargraphic technique described in Keeley (1977). The results shown in table 2 uphold the prediction. Variety *biflora* shows a highly significant increase in rate of oxygen transport after a year of flooding, providing good evidence that the drop in alcoholic fermentation under long-term flooding is due to a switch to aerobic respiration.

The responses observed for the flood-tolerant var. *biflora* are not unique. Short-term acceleration of alcoholic fermentation is characteristic of other flood-tolerant species (e.g., John and Greenway 1976). Experiments carried out over a long enough time course demonstrate that at least two other species indigenous to flooded habitats respond, similarly to var. *biflora*, with a short-term acceleration of alcoholic fermentation coupled with a long-term drop under continued flooding (Keeley, unpublished data). Thus, while survival of short-term flooding episodes may be dependent upon accelerated alcoholic fermentation, the adaptive strategy for surviving in permanently flooded habitats is neither a compensatory nor an exploitative one, but an avoidance strategy. The appropriate adaptive strategy therefore is dependent upon the structure of the environment.

SUMMARY

Both the upland and swamp varieties of *Nyssa sylvatica* respond initially to flooding with an acceleration of ethanol production. Under continued flooding the roots of the upland variety *sylvatica* have decreased rates of ethanol production, very likely a result of the progressively worsening necrosis of the root system. Few of these plants survive a full year of flooding. The swamp variety *biflora* survives and grows well under flooded conditions due to sequential metabolic and anatomical changes in the roots. Within a month after flooding, var. *biflora* initiates new roots with greatly accelerated rates of ethanol production, perhaps providing for a short-term compensatory energy source. Long-term acclimation to flooding involves a replacement of these roots and increased oxygen transport to the roots. The hypoxic conditions of the soil environment are avoided, and these rates of ethanol production are similar to those of drained plants.

ACKNOWLEDGMENTS

We thank B. L. Haines, S. C. Keeley, D. B. Walker, R. M. M. Crawford, and A. M. Torres for helpful suggestions and criticisms. Support was provided by the Department of Botany, University of Georgia, Athens.

LITERATURE CITED

Alberda, T. 1953. Growth and root development of lowland rice and its relation to oxygen supply. Plant Soil 5:1–28.

Armstrong, W. 1971*a*. Oxygen diffusion from the roots of rice grown under non-waterlogged conditions. Physiol. Plant. 24:242–247.

———. 1971*b*. Radial oxygen losses from intact rice roots as affected by distance from the apex, respiration and waterlogging. Physiol. Plant. 25:192–197.

Bergemeyer, H. W., ed. 1974*a*. Methods of enzymatic analysis. Vol. 1. Academic Press, New York.

———. 1974*b*. Methods of enzymatic analysis. Vol. 3. Academic Press, New York.

Bristow, J. M. 1975. The structure and function of roots in aquatic vascular plants. Pages 221–236 *in* J. G. Torrey and D. T. Clarkson, eds. The development and function of roots. Academic Press, New York.

Bryant, A. E. 1934. Comparison of anatomical and histological differences between roots of barley grown in aerated and in non-aerated culture solutions. Plant Physiol. (Bethesda) 9:389–390.

Chirkova, T. V., I. V. Khazova, and T. P. Astafurova. 1974. Metabolic regulation of plant adaptation to conditions of temporary anaerobiosis. Sov. Plant Physiol. (Eng. Transl. Fiziol. Rast.) 21:82–86.

Crawford, R. M. M., and M. McManmon. 1968. Inductive responses of alcohol and malic dehydrogenases in relation to flooding tolerance in roots. J. Exp. Bot. 19:435–441.

Crawford, R. M. M., and P. D. Tyler. 1969. Organic acid metabolism in relation to flooding tolerance in roots. J. Ecol. 57:234–243.

Francis, C. M., A. C. Devitt, and P. Steele. 1974. Influence of flooding on the alcohol dehydrogenase activity of roots of *Trifolium subterraneum* L. Aust. J. Plant Physiol. 1:9–13.

Franz, E. H., and F. A. Bazzaz. 1977. Simulation of vegetation response to modified hydrologic regimes: a probabilistic model based on niche differentiation in a floodplain forest. Ecology 58:176–183.

Garcia-Nova, F., and R. M. M. Crawford. 1973. Soil aeration, nitrate reduction and flooding tolerance in higher plants. New Phytol. 72:1031–1039.

Hochachka, P. W., and G. N. Somero. 1973. Strategies of biochemical adaptation. Saunders, Philadelphia.

Hook, D. D., C. L. Brown, and P. P. Kormanik. 1971. Inductive flood tolerance in swamp tupelo (*Nyssa sylvatica* var. *biflora* (Walt.) Sarg.). J. Exp. Bot. 22:78–89.

John, C. D., and H. Greenway. 1976. Alcoholic fermentation and activity of some enzymes in rice roots under anaerobiosis. Aust. J. Plant Physiol. 3:325–336.

Keeley, J. E. 1977. The role of environmental heterogeneity in population differentiation along a flood frequency gradient. Ph.D. diss., University of Georgia.

McManmon, M., and R. M. M. Crawford. 1971. A metabolic theory of flooding tolerance: the significance of enzyme distribution and behavior. New Phytol. 70:299–306.

Marshall, D. R., P. Broue, and A. J. Pryor. 1973. Adaptive significance of alcohol dehydrogenase isozymes in maize. Nat. New Biol. 244:16–17.

Radford, A. E., H. E. Ahles, and C. R. Bell. 1968. Manual of the vascular flora of the Carolinas. University of North Carolina Press, Chapel Hill.

Weaver, J. E., and W. J. Himmel. 1930. Relation of increased water content and decreased aeration to root development in hydrophytes. Plant Physiol. (Bethesda) 5:69–92.