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# CONTRASTING EFFECTS OF ANOXIA ON RHIZOME LIPIDS IN *IRIS* SPECIES

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**Key Word Index**—*Iris pseudacorus*; *Iris germanica*; Iridaceae; rhizome lipid composition; fatty acid; neutral lipid; polar lipid; membrane lipid composition; anoxia adaptation.

**Abstract**—A 14-day period of anoxia resulted in losses of polar lipids, particularly their saturated fatty acid components, from the anoxia-tolerant species *Iris pseudacorus*. By complete contrast, the anoxia-intolerant, closely related species *I. germanica*, although possessing a highly similar lipid profile, exhibited no changes in lipid composition in response to anoxia. The consequences of the lipid alterations in *I. pseudacorus* for membrane function and their possible role in adaptation to anoxia are discussed.

## INTRODUCTION

*Iris pseudacorus* L. (yellow flag) occupies habitats such as lakeside muds which are characterized by poor oxygen availability. During the winter months following shoot dieback, oxygen transport to the rhizome is interrupted, and this might be expected to lead to extended periods of either hypoxic or anoxic stress within this organ. By contrast the cultivated *Iris germanica* L., probably of Mediterranean origin, is typically a plant of well-drained soils. In this study *I. germanica* var. Quechei was used as an anoxia-intolerant control.

It has been demonstrated[1, 2] that oxygen is essential for maintaining plant membrane integrity, although the biochemical basis for this is not yet understood. In animals the site of anoxic damage to mitochondria has been shown to be the membrane phospholipids[3]. Molecular oxygen is required for the biosynthesis of unsaturated fatty acids which are known to be essential to membrane structure and function[4]. The possibility therefore exists that in some anoxia-intolerant plant species a factor contributing to cell death may be the inability to synthesize new, or conserve existing, unsaturated fatty acids. This present study was undertaken with the object of comparing what changes (if any) occurred in an anoxia-tolerant species (*I. pseudacorus*)[5] and the closely related anoxia-intolerant species *I. germanica*[6] when subject to anoxic stress.

## RESULTS

During 14 days anoxia, neither species exhibited any growth. In *I. pseudacorus* L. rhizomes, total lipid significantly declined (Table 1). This decrease reflects significant reductions in polar lipids, the neutral fraction remaining unaltered (Table 2). All polar lipid fatty acids decreased significantly. The greatest losses were of the saturated fatty acids, chiefly palmitic and stearic (Figs 1 and 2). In contrast to *I. pseudacorus*, the total lipids of *I. germanica* were not significantly decreased (Table 1). Similarly there were no significant losses of either the polar or neutral frac-

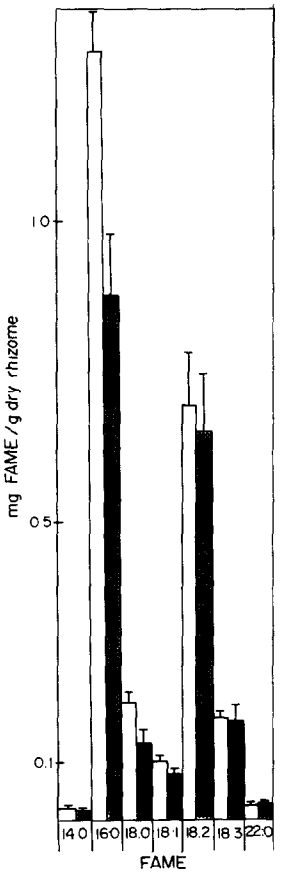


Fig. 1. Neutral lipid fatty acid methyl esters (mg/g dry wt rhizomes) of *I. pseudacorus* incubated under aerobic and anaerobic (shaded) conditions at 20 ± 2° for 14 days. Mean of five and seven replicates for aerobic and anaerobic treatments respectively. Results of Student's *t* test between aerobic and anaerobic treatments; 14:0, non-significant, 16:0, $P < 0.02$; 18:0, non-significant; 18:1, non-significant; 18:2, non-significant; 18:3, non-significant; 22:0, non-significant; error bars = s.e.m.

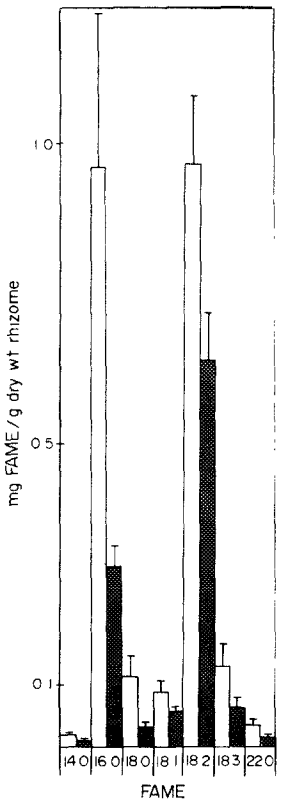


Fig. 2. Polar lipid fatty acid methyl esters (mg/g dry wt rhizomes) of *I. pseudacorus* incubated under aerobic and anaerobic (shaded) conditions at 20 ± 2° for 14 days. Mean of five and seven replicates for aerobic and anaerobic treatments respectively. Results of Student's *t* test between aerobic and anaerobic treatments; 14:0, $P < 0.05$; 16:0, $P < 0.01$; 18:0, $P < 0.05$; 18:1, $P < 0.05$; 18:2, $P < 0.05$; 18:3, $P < 0.05$; 22:0, $P < 0.05$; error bars = s.e.m.

tions (Table 3) or of the individual fatty acids from the above classes (Figs. 3 and 4).

## DISCUSSION

Anoxic conditions would undoubtedly inhibit *de novo* synthesis of unsaturated fatty acids by the mechanisms known to operate in higher plants[7, 8], although the biosynthesis of saturated acids could continue. If turnover of the fatty acyl side chains of membrane lipids is occurring, then in the absence of utilization of unsaturated fatty acids from non-membrane lipid, a preferential depletion of unsaturated fatty acids might be expected in response to anoxia.

It is surprising therefore, to observe in *I. pseudacorus* that under anoxia it is the saturated acids which decrease most markedly. One possible explanation for this result might be the activity under anoxia of an acyl hydrolase specific for the 1 position on polar lipids. It has been shown[9, 10] that this position is occupied preferentially in certain phospholipids and glycolipids by a saturated acid. This activity would thus result in the release of free saturated fatty acids, which might then, in the absence of further degradation, appear in the neutral lipid fraction. However, Fig. 1 shows that there is no compensatory rise in the saturates in the neutral lipids under anoxia. How the saturated acids released under anoxia from the polar fraction might be catabolized in the absence of a mechanism for the regeneration of $NAD^+$ is unclear.

The significant alteration observed in the overall ratio of saturated to unsaturated fatty acids (Table 4), which might be expected to lead to an increase in membrane fluidity in the absence of other compensatory mechanisms, may have important consequences for membrane function. Such mechanisms are unlikely to include the insertion of sterols, as sterol biosynthesis would probably also be inter-

Table 1. Total lipid content (mg/g dry wt rhizome) of *I. pseudacorus* and *I. germanica* rhizomes incubated under aerobic and anaerobic conditions at 20 ± 2° for 14 days

| | Aerobic control | | | | Anaerobic treatment | | | | Significance |
|---|---|---|---|---|---|---|---|---|---|
| Species | $\bar{x}$ | s.d. | s.e. | *n* | $\bar{x}$ | s.d. | s.e. | *n* | (*t* test) |
| *I. pseudacorus* | 11.2 | 1.4 | 0.6 | 5 | 9.4 | 1.7 | 0.6 | 7 | $P < 0.05$ |
| *I. germanica* | 67.5 | 7.6 | 3.4 | 5 | 71.2 | 3.1 | 1.4 | 5 | Non-significant |

Table 2. Polar and neutral lipid content (mg/g dry wt rhizome) of *I. pseudacorus* rhizomes incubated under aerobic and anaerobic conditions at 20 ± 2° for 14 days

| | Aerobic control | | | | Anaerobic treatment | | | | Significance |
|---|---|---|---|---|---|---|---|---|---|
| Lipid class | $\bar{x}$ | s.d. | s.e. | *n* | $\bar{x}$ | s.d. | s.e. | *n* | (*t* test) |
| Polar | 4.43 | 0.96 | 0.43 | 5 | 2.55 | 1.04 | 0.39 | 7 | $P < 0.01$ |
| Neutral | 6.75 | 0.87 | 0.39 | 5 | 6.49 | 1.19 | 0.39 | 7 | Non-significant |

Table 3. Polar and neutral lipid content (mg/g dry wt rhizome) of *I. germanica* rhizomes incubated under aerobic and anaerobic conditions at 20 ± 2° for 14 days

| | Aerobic control | | | | Anaerobic treatment | | | | Significance |
|---|---|---|---|---|---|---|---|---|---|
| Lipid class | $\bar{x}$ | s.d. | s.e. | *n* | $\bar{x}$ | s.d. | s.e. | *n* | (*t* test) |
| Polar | 34.52 | 5.11 | 2.28 | 5 | 30.74 | 4.31 | 1.93 | 5 | Non-significant |
| Neutral | 27.08 | 12.05 | 5.39 | 5 | 25.18 | 12.43 | 5.56 | 5 | Non-significant |

Table 4. Saturated/unsaturated fatty acid methyl esters (by wt) from polar lipids of *I. pseudacorus* and *I. germanica* rhizomes incubated under aerobic and anaerobic conditions at 20 ± 2° for 14 days

| | Aerobic control | | | | Anaerobic treatment | | | | Significance |
|---|---|---|---|---|---|---|---|---|---|
| Species | $\bar{x}$ | s.d. | s.e. | *n* | $\bar{x}$ | s.d. | s.e. | *n* | (*t* test) |
| *I. pseudacorus* | 0.91 | 0.28 | 0.14 | 5 | 0.46 | 0.1 | 0.04 | 7 | $P < 0.01$ |
| *I. germanica* | 0.76 | 0.28 | 0.2 | 5 | 0.77 | 0.22 | 0.10 | 5 | Non-significant |

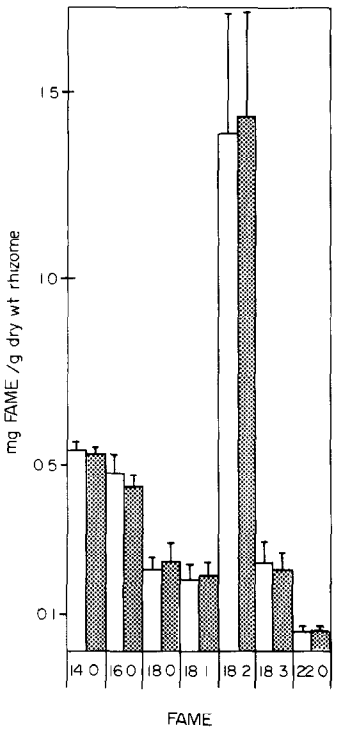


Fig. 3. Neutral lipid fatty acid methyl esters (mg/g dry wt rhizomes) of *I. germanica* incubated under aerobic and anaerobic (shaded) conditions at 20 ± 2° for 14 days. Mean of five replicates (both treatments). Results of Student's *t* test between aerobic and anaerobic treatments; all non-significant. Error bars = s.e.m.

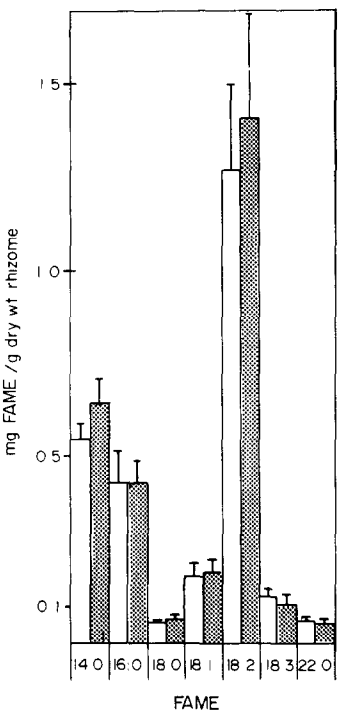


Fig. 4. Polar lipid fatty acid methyl esters (mg/g dry wt rhizomes) of *I. germanica* incubated under aerobic and anaerobic (shaded) conditions at 20 ± 2° for 14 days. Mean of five replicates (both treatments). Results of Student's *t* test between aerobic and anaerobic treatments; all non-significant. Error bars = s.e.m.

rupted under anoxia[11]. The preferential loss of polar lipids is also probably indicative of changes in membrane composition and is consistent with the loss of phospholipids observed in rice and wheat under anoxic conditions[12].

The situation in *I. germanica* completely contrasts with the changes seen in *I. pseudacorus*. Although the fatty acids of the two species are very similar in the aerobic controls, the lack of significant changes in either lipid classes or fatty acid composition raises the question of whether the observed alterations in *I. pseudacorus* lipids represent adaptation to anoxia. Vartapetian *et al.*[13] found that the fatty acid composition of anaerobically and aerobically germinated

rice coleoptiles was similar; however, only the saturated acids incorporated label from [$^{14}$C]acetate, whereas all fatty acids were labelled in air. It must be remembered that although rice is flood tolerant, its viability under anoxia is severely limited[5]. In contrast, others[14] have shown that, as in this work, anaerobiosis results in an increased ratio of unsaturated to saturated acids in both rice and wheat. This change is interpreted as an adaptive mechanism in the former. If the lipid changes in *I. pseudacorus* rhizomes represent an adaptation to anaerobic conditions it will be of interest to determine: (1) if this situation in anoxia-tolerant species is a generalized membrane phenomenon or whether specific subcellular membranes are preferentially involved; (2) whether such a process represents a primary physiologically significant response to anoxia or is a relatively unimportant consequence of some other adaptive process.

## EXPERIMENTAL

*Iris pseudacorus* L. was collected locally and planted out for 2 months in sand in a glasshouse at *ca* 20° with supplementary light under 16 hr days. *Iris germanica* var. Quechei L. was supplied by the University Botanic Garden and planted out in a similar manner. Before treatment rhizomes were trimmed to 9 cm in length, washed and roots and leaves removed. They were then placed on moist filter paper in darkened plastic containers. Anaerobic treatments were carried out in the Anaerobic Workbench (Forma Scientific Ohio, U.S.A.) at 20° ± 2° under a gas regime of 85% $N_2$; 10% $H_2$; 5% $CO_2$. The atmosphere was constantly circulated over a Pd catalyst to remove any traces of $O_2$. The efficacy of the anaerobic system was monitored using methylene blue indicator. The aerobic controls were placed in an incubator at 20° in an air atmosphere.

*Lipid extraction.* After trimming to 8.5 cm to remove any possible bacterial contamination from cut surfaces, rhizomes were grated into liquid nitrogen to minimize lipid degradation due to endogenous lipase activity and freeze-dried for 24 hr. Samples were then milled and immediately before extraction with hexane–*iso*-PrOH[15] the samples were re-hydrated with distilled $H_2O$ (3 ml/g tissue dry wt).

Lipids were fractionated by chromatography on acid-washed Florisil[16]. Pigments and neutral lipids were eluted with $CHCl_3$. Polar lipids were eluted with MeOH. All solvents were freshly re-distilled and BHT (butylated hydroxytoluene) 50 mg/l. was added as anti-oxidant.

*Analysis of fatty acids.* Fatty acid methyl esters were prepared according to Christie[17] but with 1.5% MeOH–$H_2SO_4$ as transesterification reagent, in the presence of methyl heptadecanoate int. standard. Fatty acid methyl esters were analysed by GLC isothermally at 176° and 255° using a (2 m × 4 mm i.d.) glass column packed with SP-2330 (Supelco, Inc. Bellefonte, PA, U.S.A.) with a $N_2$ flow rate of 45 ml/min. Compounds were identified by comparison with standards.

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