

WHOLE PLANT ADAPTATIONS TO FLUCTUATING WATER TABLES

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Abstract: Why some plants are damaged by flooding and others are not, is not a question that can be answered by citing any one particular mechanism or sequence of events. Flood-tolerant plants like obligate aquatic species can survive inundation but differ in that they are also adapted to withstand the consequences of becoming unflooded. Flooding implies a transitory state so that when water tables drop, adapted species have to be able to survive being deprived of the physical support of flood-water as well as re-exposure to a normal air supply. A review of flooding tolerance mechanisms reveals that tolerant species combine a range of adaptations which, depending on the life strategy of the species, can play different roles in enabling intact plants to adjust to both rising and falling water levels. Flooding is also a seasonal stress with many temperate plant communities being subjected to high water tables in winter. The mechanisms that confer tolerance of winter flooding also differ from those that allow plants to grow when flooded during the growing season. This review argues therefore, that reductionist investigations, which examine isolated organs or individual processes, may not be the most suitable method to apply to understanding the complexity of reactions that are needed to survive flooding. Instead, a holistic approach is advocated which examines the reactions of whole plants to changing water levels at different seasons of the year.

INTRODUCTION

Limitations of investigations based on isolated plant parts

The search for fundamental causes to any unsolved problem always requires precise and well-defined experimentation. Consequently, in flooding research, most studies concentrate on particular processes, such as aeration or anaerobic respiration in defined organs such as roots or rhizomes in the hope of finding common properties that define tolerant species (ARMSTRONG et al. 1994). Despite decades of such research into flooding tolerance in higher plants (CRAWFORD et al. 1994, JACKSON et al. 1990, KOZŁOWSKI 1984) there is still no evidence of any common lethal target in flood-intolerant plants, and equally, no common universal solution to prolonged flooding in tolerant species. The diversity of responses to flooding would suggest that, depending on the life strategy of the species, many different mechanisms may be involved in adapting plants to survive periods of inundation. It is equally probable that no one mechanism on its own is adequate for ensuring survival, given that to survive, the flood-tolerant plant has to be able to withstand first rising water tables and, ultimately, face the dangers inherent in re-exposure to a normal oxygen supply.

The ability of plants to withstand flooding and the return to the non-flooded condition will depend on a suite of attributes that may act independently of each other as water tables rise

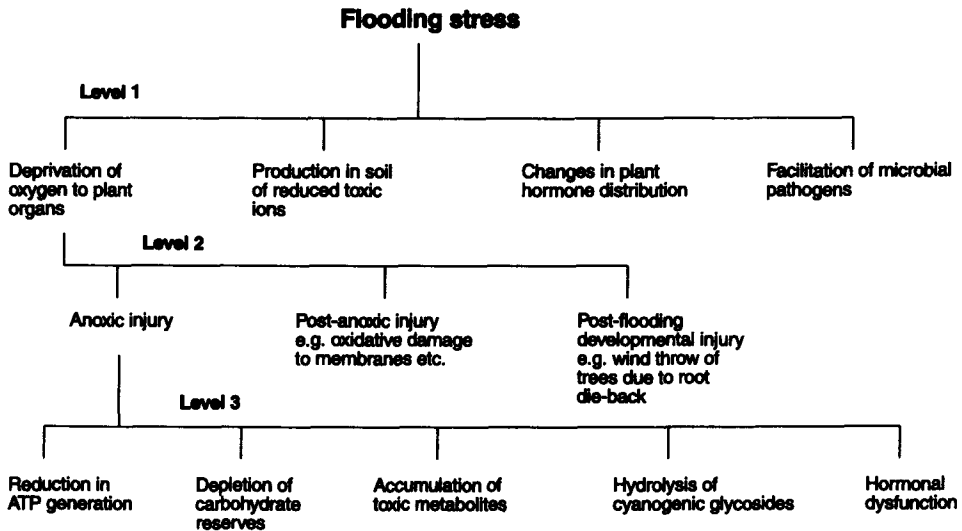


Fig. 1. A diagrammatic attempt at segregating the many changes brought about by flooding. Level 1 lists 4 ways in which flooding stress can impinge on plant growth and survival. In level 2 just one of these possible impacts, namely deprivation of oxygen, is subdivided into 3 possible components. In level 3 just one of the level 2 impacts is further subdivided into 6 possible consequences of anoxic stress.

and fall. The application of standard scientific method, using the normal reductionist approach, in searching for specific lethal targets or processes in relation to flooding may be therefore misplaced and investigations examining whole plants in relation to changing water table levels might yield more useful information.

Defining flooding stress

An unfortunate aspect of some stress physiology studies in plants comes from a frequent lack of specificity in relation to the stress under investigation. Environmental stresses, as they act on plants, are usually described in broad terms, such as cold, heat, drought, or flooding. Each of these terms can give rise to a number of more specific stresses which, if examined further, reveal a variety of potentially injurious effects, either on specific organs or on the development of the plant as a whole. Flooding injury provides a striking example of how one general term can cover a variety of particular stresses such as oxygen deprivation, exposure to reducing compounds in the soil, interference in hormone production and transport, and facilitation of microbial attack. Fig. 1. is an attempt to classify flooding stress in terms of its impact on plant survival. At level 1 four possible causes of injury are listed as possible immediate consequences of oxygen deprivation. By taking just one of these areas, e.g. oxygen deprivation of plant tissues, it is possible to define three sub-headings (level 2) where a temporary lack of oxygen can lead to either immediate or subsequent injury. Repetition of this analysis by a further subdivision of anoxic injury (level three) shows a selection of distinct areas of plant metabolism which can suffer and produce terminal injury from flooding. To cite two extreme examples: death can be rapid as when cyanogenic glycosides are hydrolysed

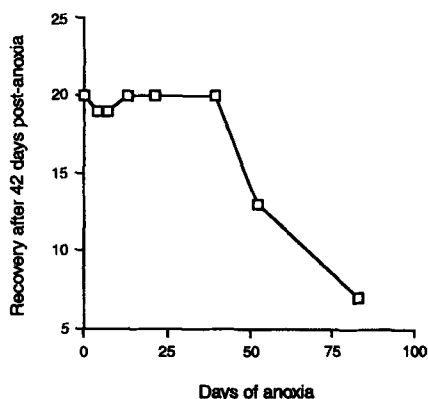
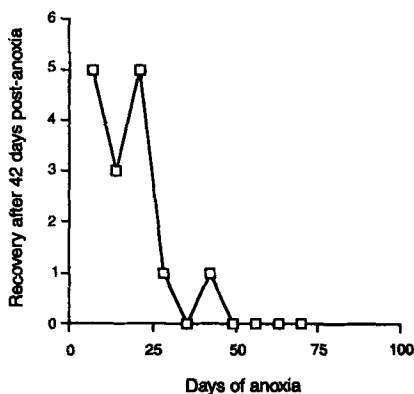
(a) *Deschampsia beringensis*(b) *Festuca vivipara*

Fig. 2. Comparison of length of viability under anoxia of grass seedlings of (a) an arctic population of *Deschampsia beringensis* HULT. from Alaska and (b) a sub-arctic population of *Festuca vivipara* L. from Iceland.

intact plant to withstand flooding. When the plant as a whole is not immediately killed by flooding, then new adventitious roots with increased facilitation for oxygen movement can develop, which allows the plant to come into equilibrium with a change of regime to higher water tables (JACKSON & DREW 1984). However, when studied as detached organs, roots do not exhibit any great degree of diversity in relation to their tolerance of oxygen deprivation. When the relative anoxia-tolerance of roots of different species is compared, it is generally found that oxygen deprivation is eventually fatal to most active roots, irrespective of the flooding tolerance of the species under investigation (CRAWFORD 1982). Even in wetland species, there are few cases of prolonged survival over months of absorptive roots entirely

under anoxia when fruit trees such as apricots are flooded (ROWE & BEARSELL 1973) or can be delayed when it is determined by the length of time needed to bring about carbohydrate depletion (Fig. 2). Post-flooding symptoms, such as root die-back, can cause a delayed death, particularly in trees. Such an example is found in the phenomenon of wind-throw in forest trees (the greatest risk to timber production in oceanic climates such as Scotland) where the die-back of roots, as a result of winter-flooding, reduces the ability of the trees at a later date to withstand strong winds.

Selection of target organs and organelles

Logical examination of the nature of any stress can be used to make a priori assumptions as to what organs or tissues are likely to be affected. It is therefore not surprising that very many comparative studies of flooding injury in plants are carried out on roots. Roots are the first plant organs to be submerged by rising water levels and exhibit a wide range of responses in terms of morphological development depending on the ability of the

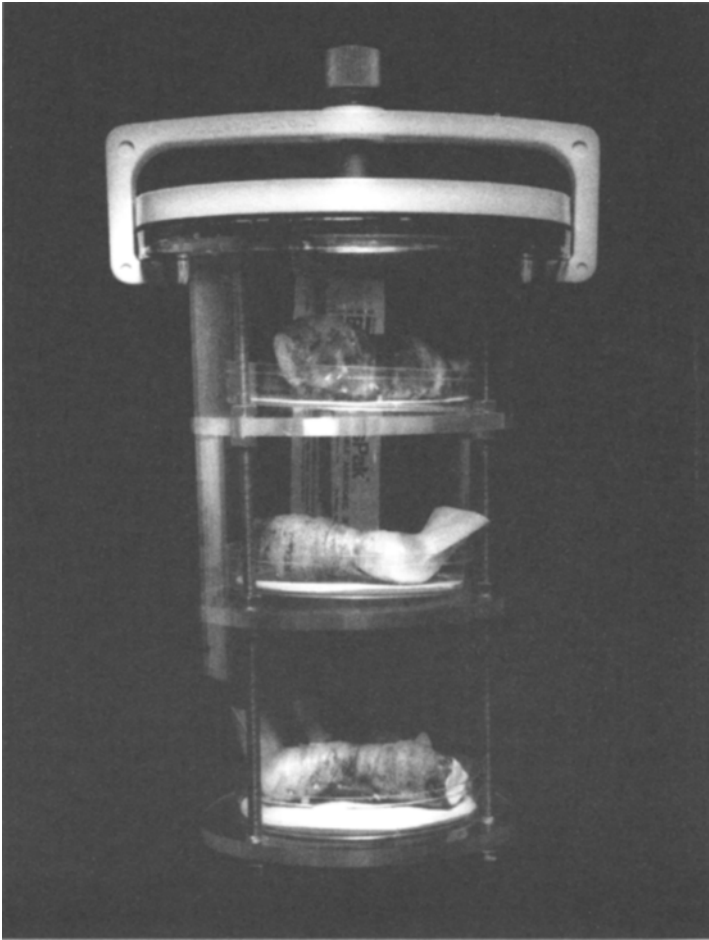


Fig. 3. Rhizomes of *Iris pseudacorus* L. undergoing prolonged anaerobic incubation inside an anaerobe jar containing a gas mixture of hydrogen, nitrogen and carbon dioxide in the presence of a catalyst which ensures the reduction by hydrogen of any oxygen that may enter the system. Under such conditions *Iris pseudacorus* rhizomes can be kept alive for more than 6 weeks. Anaerobic life can be further extended if the jars are placed in an anaerobic incubator at 10 °C (Reproduced with permission from CRAWFORD 1992).

deprived of oxygen. Examination of mitochondrial structure in the roots of the wetland species *Alisma plantago-aquatica* L., *Lycopus europaeus* L. and *Glyceria fluitans* (L.) R.BR. (VARTAPETIAN & ANDREEVA 1986) demonstrated that 24 hours of anoxia was sufficient to cause irreversible destruction of mitochondrial membranes. However, just because the absorbing roots have died, this does not necessarily imply that all parts of the plant in question are equally sensitive to oxygen deprivation. It is sufficient to achieve tolerance of anoxia allowing the whole plant to recover, if those parts of the plant that serve regeneration survive oxygen depletion (BRAENDLE 1990, BRAENDLE & CRAWFORD 1987, CRAWFORD 1992). Thus, the long-term survival under oxygen deprivation of the essential underground organs of

flood-tolerant plants is not achieved by having tolerant absorptive roots, but by having organs such as rhizomes, tubers or tap roots, that are able to regenerate new roots once the flooding stress has passed (Fig. 3).

The seasonal aspect of the flooding stress is extremely important ecologically although this is sometimes neglected by agriculturally-orientated research where the main concern is the survival of plants in the growing season. High water tables occur in most temperate plant communities with greater frequency in winter, and it is the ability to survive prolonged inundation in the dormant season, when oxygen diffusion from shoot to root is either absent or less active than in summer, that determines the long-term survival of the species in a wetland site. To obtain a comprehensive view of flooding tolerance in plants in general, which is not confined to annual crop species, it is necessary to examine not the rate at which sensitive organs die, but the capacity of the whole plant to regenerate after a period of flooding or anoxia. Such an approach takes into account seasonal differences and uses a longer term outlook in assessing the capacity for recovery. This type of investigation is very different from that used in some crop studies, where for example observing immediate root tip death can be the sole diagnosis of flooding tolerance (ROBERTS et al. 1989). The ability of many species to lose certain organs on inundation (e.g. roots and leaves) and yet survive flooding (BRAENDLE & CRAWFORD 1987) demonstrates the diversity of response to flooding and the multiplicity of strategies which are involved in ensuring the ultimate survival and presence of species in flood-prone habitats.

Sub-cellular targets of flooding injury

Many investigations seek for a common factor in flooding tolerance by examining cell organelles, or even parts of the organelles such as membranes. A series of studies on the maintenance of intact mitochondria in rice roots under anoxia has shown that for this species (*Oryza sativa* L.) a period of anoxia of 48 h does not irreversibly cause the mitochondria to lose their functional capacity for aerobic respiration (VARTAPETIAN et al. 1976). These experiments also showed that it was not any particular property of the mitochondria that conferred this resistance to anoxia. The survival of the anaerobic mitochondria was due to the whole plant, namely the ability of the rice seedling as a whole to transport organic compounds even in the absence of oxygen.

This same argument can also be applied to studies which look in detail at sub-cellular structures such as membranes under anoxia. Lipid degradation is a common post-anoxic occurrence in flood-sensitive species. Conversely, membrane stability under anoxia with minimal free lipid production and an absence of post-anoxic peroxidation damage are striking features of species tolerant of long-term anoxia such as *Acorus calamus* L. (see WEBER & BRÄNDLE 1996). After 70 days of anoxia, lipids from *Acorus calamus* rhizomes show only minimal alteration in saturation level with the principal change being a shift from 18:3 to 18:2 fatty acids. Furthermore, the absence of any substantial free fatty acid generation in these post-anoxic tissues indicates that these rhizomes have endured prolonged periods of anoxia with minimal membrane degradation. However, there is no evidence as yet to show that this resistance to anoxic and post-anoxic injury is due to the lipid constitution of membranes in anoxia-tolerant species. No functionally related differences have been reported so far in the composition of lipids between tolerant or intolerant species. As with the observations on

mitochondrial viability after a period of anoxia, it is more probable that the stability of the membranes in the tolerant species is a function of the general ability of this species to maintain all its tissues in a healthy state even after a prolonged period of anoxia.

ADAPTATION TO STRESS BY ACCLIMATISATION OF THE INTACT PLANT

In vitro examination of the responses of isolated plant parts to flooding or anoxia gives only partial answers to how some plants can adapt to flooding and others not. This is to be expected as the viability of different plant parts is a mutually dependent process and adaptation to stress normally takes place only in the intact plant. Acclimatisation to any stress takes time and the shortened life of detached organs therefore will tend to preclude any capacity for acclimatisation. Hardening of plants to the risk of a future stress is a well known horticultural practice and can be achieved when intact plants are exposed to a partial stress which induces adaptation or hardening to the ultimate stress. This phenomenon is well known in drought (DAVIES et al. 1990) and cold hardening (KÖRNER 1988). In relation to flooding, a period of hypoxia given to intact plants allows roots to survive a subsequent period of anoxia to which they otherwise would have succumbed (SAGLIO et al. 1988, ANDREWS et al. 1993).

Fluctuating water tables: the double stress from flooding and unflooding

Flooding implies a transitory change from low to high water tables which will eventually be expected to revert to the normal level. A flood-tolerant plant is therefore one in which the plant can adapt first to being flooded and subsequently re-adapt to the unflooded condition. The transitory nature of the high water level distinguishes flood-tolerant species from aquatic plants. True aquatic species, although surviving total immersion in water, will not survive when the water table drops. Aquatic species cannot therefore be used as examples of flood-tolerant plants, as they are not subject to the cycle of rising and falling water tables. Even though aquatic species are tolerant of permanent inundation, they cannot survive being un-flooded so cannot be classed as plants that can survive a period of temporary inundation (Fig. 4).

Tolerance of fluctuating water tables demands a high degree of phenotypic plasticity, both morphologically and physiologically. If this were not the case, then plants which might have adapted to being flooded would be in danger of becoming unviable when flood-waters recede and they are exposed once again to normal concentrations of oxygen and the normal risks of desiccation. Adaptation to any stress, either by morphological or physiological means, usually results in making the plant less fit to the unstressed condition. Every adaptation has its cost in terms of general fitness. As species adapt to a more restricted environment they accumulate properties which disadvantage them in the unstressed habitat. Drought adaptations such as small leaves, or hairiness make the drought-tolerant plant less able to compete if the drought stress fails to occur. Similarly, adaptations to ultra-short growing seasons in late cold sites in the high Arctic requires a degree of habitat specialisation that can exclude such ecotypes from warmer sites even a few metres away (CRAWFORD & ABBOTT 1994). Morphological adaptations to flooding can also prove disadvantageous when water tables drop or if the habitat dries out and exposes the plants to drought. Thick, poorly branched aerenchymatous roots, developed in response to flooding, have a small surface: volume ratio, which may limit the water and nutrient absorptive powers of roots when the plant returns to normal conditions (KONČALOVÁ

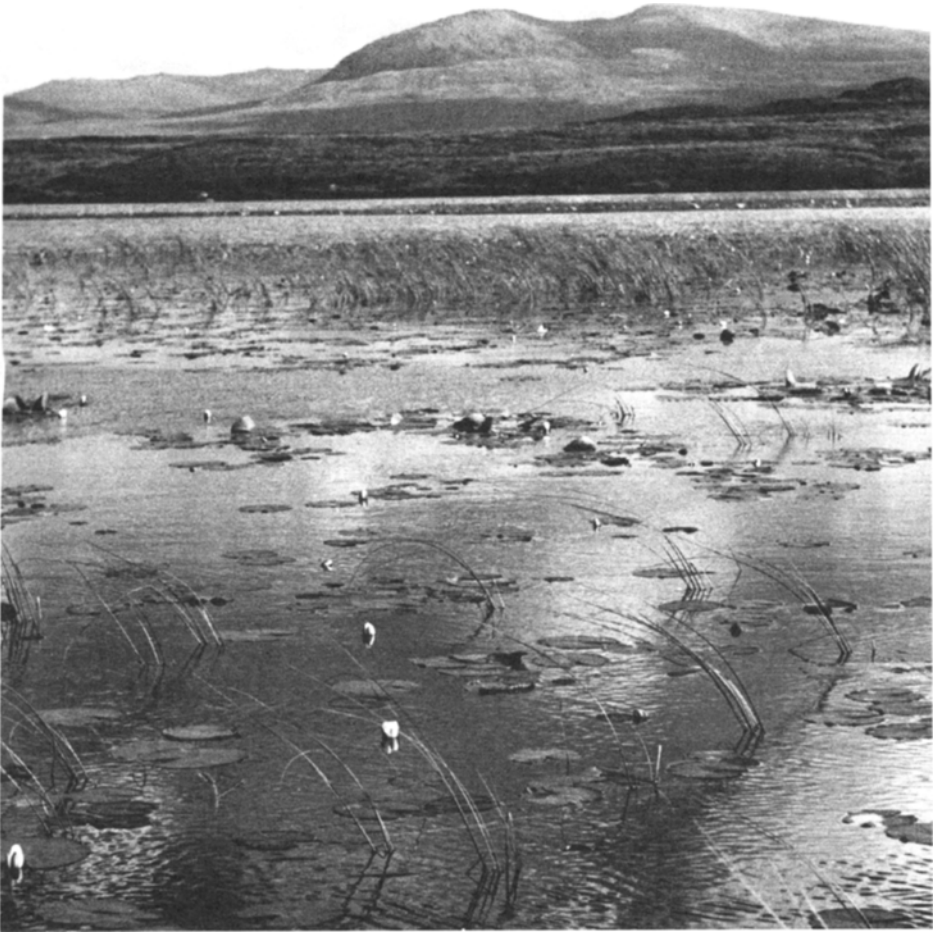


Fig. 4. The white water-lily (*Nymphaea alba* L.) growing in a loch on the Moor of Rannoch, Scotland. This photograph of a true aquatic species is shown to emphasize the argument that aquatic species are not flood-tolerant as they lack the amphibious properties of flood-tolerant species which can be flooded and then unflooded and still survive.

1990). Species that develop a mat of adventitious roots at the soil surface when flooded are in danger of suffering from desiccation injury when the water table drops unless they have preserved their deeper anchoring roots (GILL 1970). Plants which have responded to flooding by extensive shoot extension may physically collapse when the water table drops.

Flood-tolerant plants also have to adjust to both a reduction in the availability of oxygen followed by a restoration of the normal supply once water tables drop. In some cases fluctuations in water table levels can impose a period of hypoxia (limited oxygen supply) or even anoxia (total absence of oxygen) which is then followed by re-exposure to normal aerobic conditions. Some tissues that have been deprived of oxygen show irreversible damage when

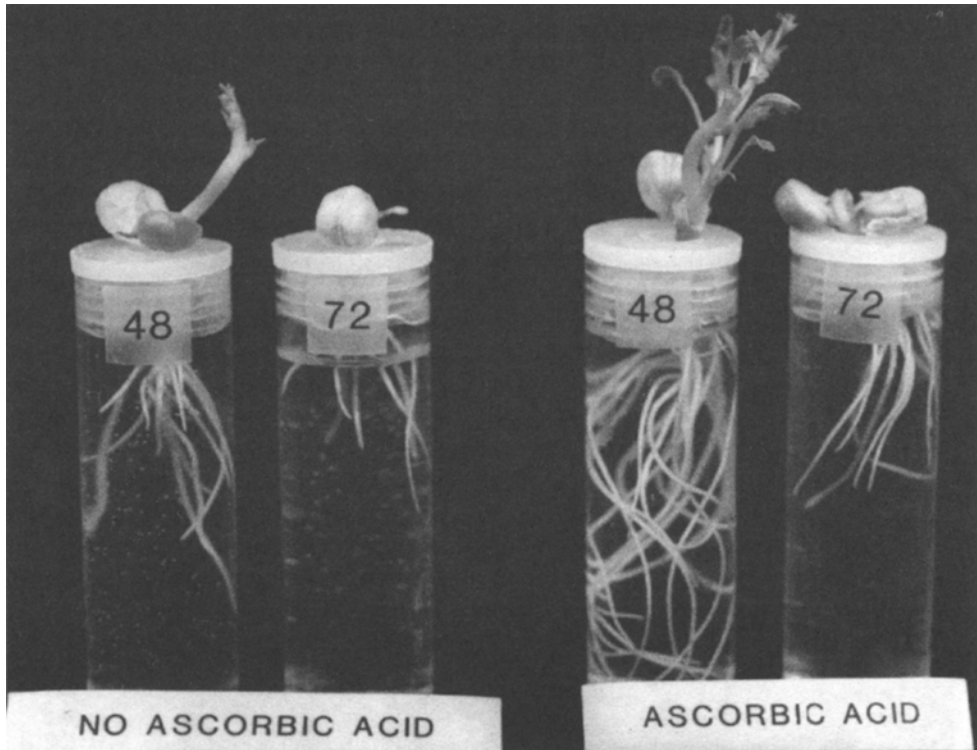


Fig. 5. Ascorbic acid acting as an antidote to anoxic injury. The chickpea seedlings on the right were transferred to a 10 mM solution of ascorbic acid (pH 5.6) before emerging from anaerobic treatments of 48 and 72 h, respectively. The antioxidant properties of ascorbic acid appear to have minimized post-anoxic injury and aided recovery particularly in the less damaging condition of the anaerobic incubation limited to 48 h (reproduced with permission from CRAWFORD 1992).

returned to air. The post-anoxic injury involves rapid peroxidative damage which is brought about by active radical generation and the formation of toxic oxidative products such as acetaldehyde (ALBRECHT & WIEDENROTH 1994, CRAWFORD 1992, PFISTER-SIEBER & BRAENDLE 1994, STUDER & BRAENDLE 1988, VAN TOAI & BOLLES 1991).

Species which are able to survive sudden re-exposure to air after a period of oxygen deprivation use a variety of protection mechanisms to minimise the risks of post-anoxic injury. Many rhizomatous species from wetland habitats are naturally well-defended against post-anoxic injury by a variety of defence mechanisms which can be grouped under two headings (1) enzymatic and (2) anti-oxidant. Enzymatic protection against post-anoxic injury has been studied in the over-wintering tubers of the anoxia tolerant species *Iris pseudacorus* L. and compared with the intolerant *I. germanica* L. The tolerant species differs from the intolerant in being able to increase superoxide dismutase (SOD) activity during a month-long period of anaerobic incubation and thus enters the post-anoxic phase well-equipped to counteract the potential hazards of superoxide generation (MONK et al. 1987). There appears to be much variation in wetland species as to which type of anti-oxidant activity is present

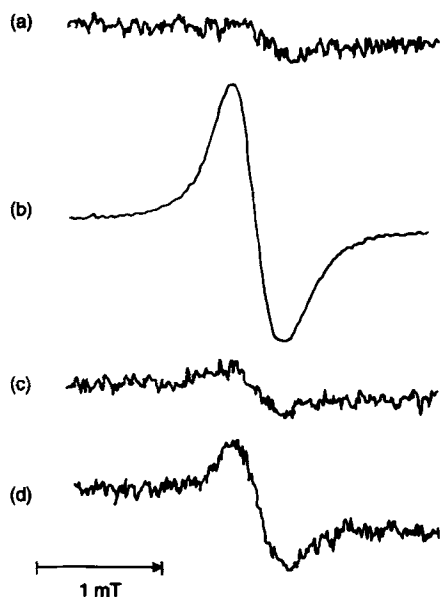


Fig. 6. Electron paramagnetic resonance (EPR) spectra from (a) aerobic and (b) anaerobic rhizomes of *Iris germanica*. *I. germanica* is tolerant of anoxia and on restoration of oxygen after a period of anoxia generates a signal (b) which indicates the generation of active radicals. Treatment of the anaerobic rhizomes with anti-oxidants (c) 5 mM ascorbic acid and (d) 5mM reduced glutathione, 10 minutes before-exposure to air shows a significant reduction in the amplitude of the active radical signal, particularly in the case of ascorbic acid which is more easily absorbed (for references see text). No signal was observed in the anoxia tolerant *Iris pseudacorus* – not shown. (mT = milli Tesla).

oxidative cell damage (ALSCHER 1989). The enzymes dehydroascorbate reductase (DHAR; EC 1.8.5.1) and monodehydroascorbate reductase (MR; EC 1.6.5.4) are involved in the cellular defence against phytotoxic oxidation by maintenance of the reduced active forms of glutathione and ascorbate, respectively; MR catalyses the reduction of monodehydroascorbate (MDA) to ascorbate and DHAR plays an important role in the H_2O_2 detoxification system (FOYER & HALLIWELL 1976). In soybean (*Glycine max* (L.) HERR.) short-term anoxia results in the production of superoxide radicals, while longer treatments induce high levels of superoxide dismutase activity and thus provide a greater protection when the plants return to air (VAN TOAI & BOLLES 1991). By contrast, the defence system against post-anoxic injury in rice appears weaker as it is activated only when the plants return to air (USHIMARU et al. 1992).

Thus, in both anoxic and post-anoxic stress it is the well-being of the intact plant that confers the ability to survive fluctuating water tables by controlling overall defence

in their perennating organs (WOLLENWEBER-RATZER & CRAWFORD 1994). Many species appear to rely on anti-oxidants such as ascorbic acid, α -tocopherol and glutathione. These defences are also dependent on enzymatic activity as ascorbic acid and glutathione are only active as anti-oxidants in the reduced state. Ascorbic acid is a common biological antioxidant and its role in counteracting post-anoxic injury in plants has been demonstrated for *Cicer arietinum* L. (CRAWFORD & WOLLENWEBER-RATZER 1992, Fig. 5). Glutathione and ascorbate occur in millimolar concentrations in the plant cell, where in the reduced form they are active as antioxidants. Perfusion of anoxic *Iris germanica* rhizomes with 5 mM ascorbic acid (Fig. 6) just before re-exposure to air can reduce the amplitude of the signal generated by active radical production (CRAWFORD et al. 1994). There is strong evidence that the cellular glutathione and ascorbate levels increase as a response to phytotoxic oxidative processes and that high levels of antioxidants confer resistance to



Fig. 7. Harvesting birch sap in early spring from a mature tree of *Betula pubescens* EHRH. This source of a sugar-rich sap has long been known and was extensively used by the Norse in medieval times for the making of birch wine. In well-grown trees with good reserves a litre or more of sap can be tapped in one hour.

mechanisms against both internally generated damaging substances such as hydrogen peroxide or superoxide radicals and the harmful consequences of taking up soluble reduced iron, which is an ever-present danger in flooded soils and can lead to increased active radical generation in leaves (HENDRY & BROCKLEBANK 1985).

Seasonal responses to flooding or anoxia

In perennial species the most frequent exposure to changes in water table levels arise as a result of seasonal changes. Here again, it is the properties of the whole plant that contribute to the seasonal adjustment to different water table levels. This is evident both morphologically and physiologically. Over-wintering stands of *Phragmites australis* (CAV.) TRIN. ex STEUD. usually have the old season's stalk either intact or broken off usually above the high water

Table 1. A comparison of the ethanol and malic acid contents of sap from birch trees (*Betula pubescens* EHRH.) growing in wet and dry soils. The samples were taken in early April before bud break and each figure is the mean of three samples (CRAWFORD 1972).

Soil type	Water status of soil	Ethanol μMols^{-1} litre of sap	Malic acid μMols^{-1} litre of sap
Peat	Wet	13.5 \pm 0.75	3850 \pm 340
Peat	Dry	9.9 \pm 2.09	1870 \pm 330
Sand	Wet	31.9 \pm 9.4	3170 \pm 130
Sand	Dry	13.4 \pm 4.5	2490 \pm 360

level. This broken stalk can assist ventilation of the underground parts of the plant by the "ventura effect" in which wind blowing across the cut end of the stalk creates a pressure gradient which assists the ventilation of the below-ground parts of the plants (ARMSTRONG et al. 1992).

Seasonal changes also take place in plants both in their metabolic activities and in the reserves that are available to sustain them through periods of stress. The differential expression of glycolytic enzyme activity and availability of reserves in rhizomes of *Acorus calamus* L. is discussed in detail by WEBER & BRÄNDLE (1996). A number of tree species demonstrate very clearly the interdependence of different parts of the whole plant in surviving seasonal stress. The common birch (*Betula pubescens* EHRH.) is a particular case where laboratory experiments on parts of the plant would fail to illustrate how the integrated adaptation of the whole tree and the annual redistribution of carbohydrate reserves between shoots and roots aids the seasonal cycle of over-wintering in wet flood-prone soils. In autumn, birch, in common with many other tree species, translocates considerable starch reserves to the over-wintering roots. In spring, the hydrolysis of this starch takes place at such a rate as to drive a sugar-rich sap upwards in the xylem sap. This phenomenon has long been known and was extensively used by the Norse in medieval times for the making of birch wine (Fig. 7). In well grown trees with good carbohydrate reserves a litre or more of sap can be tapped in one hour. Not only is rising spring sap rich in soluble sugars, it also contains ethanol and malic acid in considerable amounts, particularly in wet habitats (Tab. 1). Depending on the species varying amounts of other hydrogen rich compounds also occur such as citrulline, ornithine, arginine, etc., which all serve as transporters of hydrogen from the potentially hypoxic root to the well-aerated aerial portions of the tree (CRAWFORD 1992). This transport of hydrogen upwards serves the same purpose with regard to the ratio of reduced to oxidised metabolites in the roots as the diffusion of gaseous oxygen downwards. Trees which can grow in soil where water tables remain high in early spring are still capable of having a root metabolism that is active enough to provide the energy for a rapid flow of rising sap. In deep rooted species this spring root activity appears to take place in a hypoxic or even anaerobic soil environment with little access to air by internal diffusion. The maintenance of anaerobic respiration is expensive in terms of carbohydrate reserves. However, the autumnal provisioning of tree roots with extensive starch deposits appears to be related to this need for maintaining anaerobic activity in spring, particularly in habitats with poorly aerated soils (Fig. 8).

Table 2. List of species tested in Spitsbergen for anoxia tolerance. Intact plants were kept under total anoxia in anaerobe jars for 7 days at 5 °C in the dark. Plants were judged to be anoxia-tolerant if after 48 h re-exposure to air they showed no wilting or discolouration of their shoots. Nomenclature follows RØNNING (1978).

Anoxia tolerant (Including leaves)	Anoxia intolerant
<i>Saxifraga caespitosa</i>	<i>Equisetum arvense</i>
<i>S. oppositifolia</i>	<i>Saxifraga hieracifolia</i>
<i>S. foliosa</i>	<i>S. cernua</i>
<i>Ranunculus sulphureus</i>	<i>Ranunculus pygmaeus</i>
<i>Cardamine nymani</i>	<i>Oxyria digyna</i>
<i>Huperzia selago</i>	<i>Pedicularis hirsuta</i>
<i>Eriophorum scheuchzeri</i>	<i>Cochlearia groenlandica</i>
<i>Juncus biglumis</i>	<i>Polygonum viviparum</i>
<i>Carex misandra</i>	<i>Poa alpina</i> (mature plant – plantlets only tolerant)
<i>Luzula arctica</i>	<i>Draba oxycarpa</i>
<i>L. arcuata</i> subsp. <i>confusa</i>	
<i>Dryas octopetala</i>	
<i>Puccinellia vahliana</i>	
<i>Alopecurus borealis</i>	
<i>Poa alpina</i> (plantlets)	
<i>Deschampsia alpina</i>	
<i>Salix polaris</i> (leaves not tolerant)	

Whole plant tolerance of anoxia

Very few studies have considered the effects of anoxia on the whole plant. Leaves are usually the last organs to be inundated by rising water levels and the first to show adverse effects from flooding (Fig. 9). Leaf-wilting has long been observed as one of the first indications of oxygen deprivation on roots. In most species leaves are highly intolerant of anoxia and are usually the first organs to suffer irreversible damage as a result of oxygen deprivation.

However, such is the adaptability of plants to almost every type of extreme condition, that there are terrestrial plants that have foliage that can withstand prolonged periods of oxygen deprivation. Studies of whole plant tolerance of anoxia in the high Arctic (CRAWFORD et al. 1994) have found populations of some common high arctic vascular plant species in Spitsbergen that have a surprisingly high anoxia tolerance (Tab. 2). Particularly striking has been the capacity of these high latitude populations to maintain turgid, green leaves throughout the period of anoxia and into the post-anoxic recovery phase. Prolonged anoxia tolerance had never been reported previously for green leaves, which normally lose turgor and wither rapidly when deprived of oxygen. Tests on more southern populations from Norway, Iceland, and Scotland of species found to be tolerant of anoxia in Spitsbergen, failed to detect an equivalent ability to survive oxygen deprivation. The adaptive significance of having leaves that are tolerant of anoxia probably comes from the frequency with which lowland coastal populations of some arctic plant species are encased in ice for long periods, sometimes from November through to June. Ice-encasement prevents movement of oxygen to the leaves and consequently

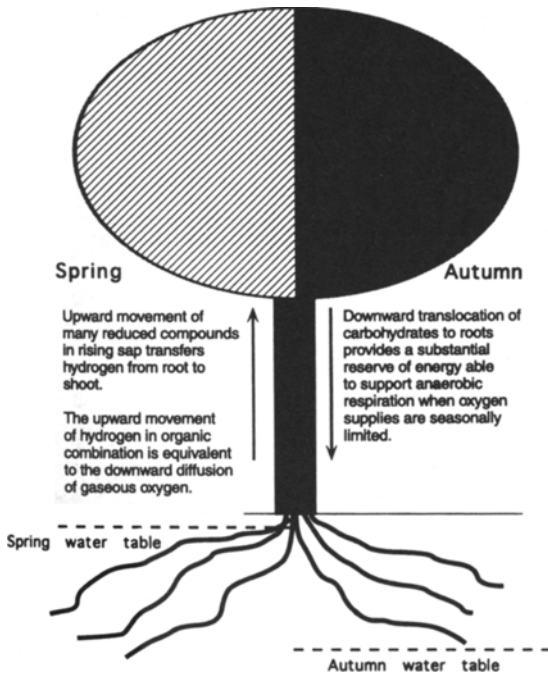


Fig. 8. Diagrammatic representation of the seasonal cycle of the movement of carbohydrate as found in many trees and discussed in the text in relation to the ability of birch trees to produce a strong upward flow of sap even when soils are fully saturated or flooded and aeration to the roots is impeded. In autumn, starch reserves are translocated downwards to the over-wintering roots. In spring, the hydrolysis of this starch is available to meet the energy demands of hypoxic roots showing evidence of strong anaerobic activity. The upward moving sap contains many reduced compounds. This upward movement of organically bound hydrogen is equivalent metabolically to the downward movement of gaseous oxygen which would only reach the lower roots with difficulty at this time of the year.

which after only 48 h of anoxia already shows pronounced injury to the terminal portions of its leaves (Fig. 11).

Seed resistance to soaking injury: a property of the intact seed?

Soil waterlogging can result in poor field emergence in a number of crops among which the pulses (peas and beans) are particularly sensitive. Poor germination under these conditions is usually described as soaking injury. This term covers a number of physical and metabolic stresses potentially damaging to germination. The presence of excess water impedes oxygen diffusion and in some species induces excessive anaerobic activity which can lead to high concentrations of ethanol followed by cell damage and attack by soil pathogens (CRAWFORD

deprives the entire plant of access to oxygen for periods that can be as long as 9-10 months in certain years (Fig. 10).

The above observation of anoxia tolerance in green leaves has prompted further studies to determine if this phenomenon can be found in any species outside the Arctic (CRAWFORD, unpubl.). Examination of the tolerance of a number of wetland plants such as *Iris pseudacorus*, *Schoenoplectus lacustris* (L.) PALLA and *Carex rostrata* STOKES ex WITH. reveals that in most species leaves have a very limited tolerance of anoxia. However, there is one interesting exception. *Acorus calamus* has already been referred to as a species with remarkable tolerance of anoxia. The intact plant when placed in an anaerobic incubator can maintain green turgid leaves after 65 days of anoxia and shows no peroxidative damage on return to air. This contrasts with *Iris pseudacorus* (a species with rhizomes that are highly tolerant of prolonged anoxia)



Fig. 9. The initial effect of flooding on seedlings of *Picea abies* (L.) KARST. (left, flooded: right, unflooded) caused a stimulation of shoot growth. This was eventually followed by needle death and eventually the death of the entire plant.



Fig. 10. An arctic shore community of *Carex misandra* R.BR. Plant communities in this type of habitat are frequently ice-encased for many months resulting in a complete interruption of access to gaseous oxygen. This species is one of several high Arctic species in which the entire plant (shoot and root) is tolerant of prolonged anoxia.

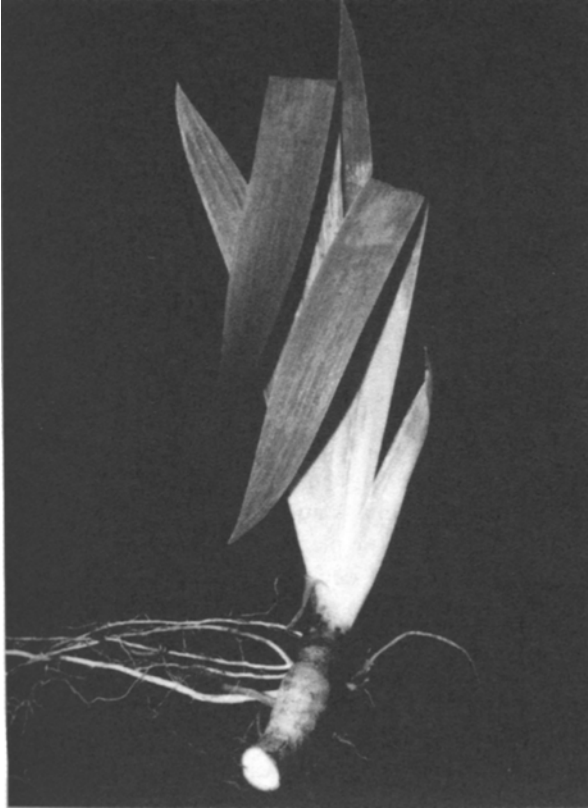


Fig. 11. Anaerobic injury as sustained by the tips of *Iris pseudacorus* leaves after 48 h anoxia. *Iris pseudacorus* has rhizomes that are highly tolerant of anoxia but in common with most flood-tolerant temperate species tested so far the leaves are rapidly injured. Only *Acorus calamus* among temperate flood-tolerant species has been found to maintain healthy turgid green leaves after 65 days of anoxia. See also Tab. 2 for a list of arctic species which have anoxia-tolerant foliage.

1977). In seeds with permeable seed coats, soaking can lead to a rapid uptake of water with excessively rapid tissue hydration leading to a physical disruption of seed tissues (POWELL & MATTHEWS 1978).

BARTON (1950) first observed that dissolved carbon dioxide could reduce the sensitivity of the seeds to soaking. Recent studies have confirmed this suggestion and shown that carbon dioxide saturation of the soak-water reduced injury in *Phaseolus vulgaris* L. seeds (PRETORIUS & SMALL 1991, 1992, 1993, SMALL et al. 1991). A number of theories have been examined as to why this phenomena occurs (SMALL et al. 1991). Changes in pH due to the formation of carbonic acid (H_2CO_3), have been discounted, as lowering the pH without the addition of CO_2 did not improve resistance to soaking. Also, the beneficial effects of CO_2 did not appear to be due to ethylene antagonism, as the further addition of ethylene to the system did not increase injury. Further studies (PRETORIUS & SMALL 1991) showed that protein content of French bean seeds suffering from

soaking injury was markedly reduced, due to a reduction in embryonic synthesis. By looking at the progress of radioactively labelled carbon along many metabolic pathways in both treatments of seeds (PRETORIUS & SMALL 1992) it was suggested that it is the lack of oxidative pentose phosphate (OPP) pathway activity which appears to be a major factor in the onset of soaking injury. This theory, based on the depression of the OPP pathway is similar to explanations that have been put forward for the method by which secondary dormancy operates in seeds. In *Phaseolus vulgaris* seeds soaking injury can be reduced by saturating the soak-water with carbon dioxide. Explanations for the cause of this effect have hitherto been primarily metabolic involving ethylene production, depression of the oxidative pentose phosphate pathway and sugar leaching. A re-examination of the role of carbon dioxide in preventing soaking injury in seeds of *Phaseolus vulgaris* and *Pisum sativum* L. suggests that physical

changes rather than metabolic processes may be responsible for the reduction of soaking injury by carbon dioxide saturation of the soak-water. When seeds of *Phaseolus vulgaris* are soaked in CO₂-saturated water, carbon dioxide accumulates inside the testa causing a high proportion of the seeds to rise to the surface. No gas accumulation took place in seeds soaked in aerated water. Seeds perforated with two pin-holes through the testa accumulated no gas and lost the protection that carbon dioxide saturation provided against over-rapid rehydration (Fig. 12, CRAWFORD & GODBER, unpubl.). Thus the ability of these seeds to resist soaking injury when placed in carbon dioxide saturated water, is not related to any particular metabolic process brought about by the presence of carbon dioxide, but to the intact nature of the whole seed which is able to prevent excessively rapid hydration due to the liberation and trapping of carbon dioxide from the CO₂ saturated water.

CONCLUSIONS

Surviving flooding is a multi-faceted challenge for wetland plants. To overcome the stresses of rising and falling water tables demands a high degree of integration in the responses of roots and shoots both morphologically and physiologically. A number of processes discussed above which are vital to viability, operate only in the whole plant and cannot be investigated

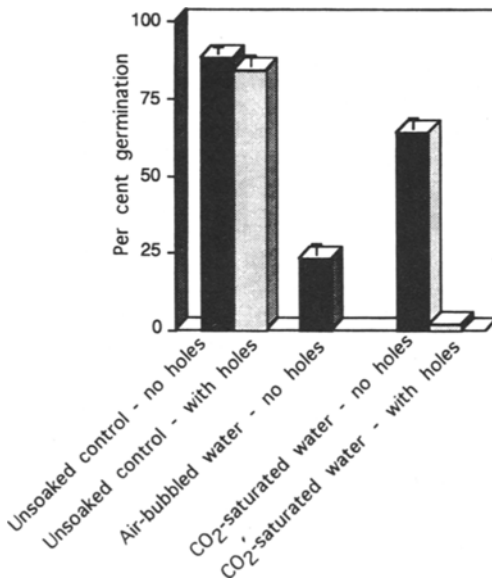


Fig. 12. Effect on subsequent viability of soaking seeds of *Phaseolus vulgaris* that are either intact or have been perforated with two pin holes in water that is (a) aerated and (b) saturated with carbon dioxide. Note that the making of the pin holes reverses the protective effect of carbon dioxide in the soak-water (CRAWFORD & GODBER, unpubl.).

using detached organs or isolated organelles and exposing them to restricted aeration. Adaptive mechanisms, which include the synthesis of enzymes or the growth of new organs, will also only operate in intact plants. Similarly, the translocation of carbohydrate to the roots of over-wintering trees is a prerequisite for anoxia tolerance and cannot be observed in detached organs. Likewise, the ability to transport an oxygen debt from root to shoot can be investigated only in whole plants. Adaptations that are related in any way to translocation, transpiration or environmental sensing through hormonal effects will also only be manifested when the plant is intact. To achieve long-term flooding tolerance, as in over-wintering rhizomes and tubers, it is the intact plant that is able to sustain vital regenerative tissues during the period of anoxia and sense when it is appropriate to renew shoot growth. Emergence from an anaerobic to an aerobic environment also requires metabolic readjustment after weeks or months of oxygen starvation. An understanding of these many adjustments

both in form and function is not readily obtained from reductionist experimentation, it requires as well a holistic appreciation of plant adaptations to changing environments.

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