*Acta Bot. Neerl.* 43(4), December 1994, p. 307–358

REVIEW

# Mechanisms of flood tolerance in plants

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*Key-words:* flooding, submergence, environmental stress, plant hormones, adaptation, review.

## INTRODUCTION

It has been estimated that wetlands occupy approximately 6% of the earth's land surfaces (Maltby 1991). They comprise fresh, brackish and salt-water marshes, inland and coastal swamps, flood plains, and lowland and upland mires (fen, bogs), as well as agricultural wetlands such as rice paddy. On a smaller scale there are constructed wetlands valued for their role in the purification of domestic, agricultural and industrial

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*Abbreviations*: ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboyxlic acid; AVG, aminoethoxyvinylglycine; CK, cytokinin; ETH, ethylene (ethene); GA, gibberellin; IAA, indole acetic acid.

effluents (Cooper & Findlater 1990). In addition to these examples, occasional soil flooding and even a degree of submergence can be a common though unwelcome feature of non-wetland agriculture.

Wetlands are usually characterized by permanent or long-term soil flooding, but often the vegetation may be wholly or partly submerged; in the lower reaches of coastal marshes this may be a daily or twice daily occurrence. Indeed, since wetlands are found world-wide and at most altitudes, flooding regimes can vary enormously; not only do the seasonal timing, duration and depth of flooding differ, but so too do light and temperature regimes and sediment type—factors which strongly interact with flooding to influence vegetation. The large numbers and wide variety of vascular plants indigenous to wetlands reflect this variety in habitat features; species range from wholly submerged aquatics of pools, rivers, coastal and lake margins through to the large tree species of the flood plain forests of the Americas and Africa. In view of this variety in both plant and habitat, a whole spectrum of flood-related stresses might be anticipated; similarly one might expect to find a number of flood-tolerance strategies.

Research into wetlands and plant adaptation to flooding has increased considerably in the past two decades. This has been driven partly by the explosion in post-graduate education in the developed countries, by increased funding for ecophysiological and agricultural research, by the realization that such areas are among the most productive, by the growing awareness of the many threats to wilderness areas and the need for a greater understanding of the vegetation to underpin wetland management schemes, by the agro-economics of rice production, by a desire to reduce flood damage in non-wetland crops, and latterly by the advances in molecular biology and genetical engineering which promise the controlled introduction of flood tolerance into currently intolerant agronomic species. This high level of activity is reflected in the large numbers of recent articles and books on wetlands and aspects of flood tolerance of which the following are examples (Kozlowski 1984; Crawford 1987, 1992; Hook *et al.* 1988; Bowes 1989; Blom 1990; Drew 1990; Armstrong *et al.* 1991c; Jackson *et al.* 1991; Jensen & Madsen 1991; Voesenek *et al.* 1992; Blom *et al.* 1994; Voesenek & Van der Veen 1994).

Grime (1989) argues that the objectives of ecological and evolutionary research are best served by using the term 'stress' to describe external constraints limiting resource acquisition, growth or the reproduction of organisms. This is the definition of stress adopted here. However, in the area of flood tolerance, the consequences of oxygen stress may exclude species by their effects during periods when there is no growth or resource acquisition in the normally accepted sense (Crawford 1992).

For plants, the primary constraint imposed by flooding is impeded gas-exchange, since diffusive resistance to most gases is approx. 10,000 × greater in water than in air. Furthermore, in the case of oxygen there is also a 30-fold drop in concentration between the gaseous and the dissolved states. In flooded soils this increased resistance to gas-transport coupled with microbial demand usually ensures that aerobic conditions can extend from <1 mm to no more than a few centimetres from the soil surface (Armstrong *et al.* 1991c), and in freshly flooded soils this situation may arise in a matter of hours (Turner & Patrick 1968), the effect being hastened at high temperatures. In tidal marshes, soil aeration can vary both daily, seasonally and topographically (Armstrong *et al.* 1985) and the zonations of some plants are clearly related to aspects of flood tolerance.

Roots are particularly sensitive to oxygen deficiency and with the exception of the seminal root of *Trapa natans* (Menegus *et al.* 1992), there are no records of sustained growth by anoxic roots, although survival by anaerobic metabolism can be enhanced by sugar supplements and by hypoxic pretreatments (Vartapetian *et al.* 1978; Webb & Armstrong 1983; Saglio *et al.* 1988; Johnson *et al.* 1989). Anoxia also probably inhibits the germination of most seeds although there are examples of a beneficial influence in some cases (Keeley 1988; Côme *et al.* 1991). Oxygen stress in roots is accompanied by a decline in energy levels (Saglio *et al.* 1980; Davies *et al.* 1987), by a more rapid depletion of carbohydrate reserves (Saglio *et al.* 1980), by cytoplasmic pH changes (Davies *et al.* 1987; Roberts *et al.* 1984) and by ultrastructural changes (Davies *et al.* 1987; Vartapetian 1991; as a consequence of these and other factors, cell maintenance may be disrupted, nutrient acquisition impaired (Drew 1988; Atwell & Steer 1990) and hormonal changes induced (see section on hormones). Further to this, flooded soils are characteristically deficient in certain nutrients, and owing to the anaerobic microbial activity they may develop phytotoxic concentrations of others (Armstrong 1982; Koch *et al.* 1990; Snowdon & Wheeler 1993). Nutrient deficiencies can be exacerbated by alternating flood and dry periods (Ponnamperuma 1984). Additionally the anaerobic decomposition of organic matter can release organic phytotoxins such as the volatile organic acids (Wang *et al.* 1967; Kovacs *et al.* 1989). Excellent reviews dealing with the chemistry of flooded soils are those of Ponnamperuma (1972, 1984), Gambrell & Patrick (1978), and Gambrell *et al.* (1991).

When flooding extends to a total or partial submergence of aerial shoots and foliage the consequences for the vegetation are even more far reaching than for soil flooding alone. In the first instance the effects of the soil flooding will be intensified by the lengthening of diffusion paths for gas exchange with the atmosphere. In addition, however (and even more important for most species), is the restriction placed upon $CO_2$ supply for photosynthesis (Bowes 1989; Madsen 1993); allied to this is the even greater impedance to respiratory oxygen supply and to the escape of photosynthetically produced oxygen. Again, because of its low solubility, a build-up of ethylene is likely in the submerged system. An important consequence of the impeded oxygen transport is that the oxygen relations of the leafy shoot (and in many cases, the roots and rhizosphere) may undergo substantial diurnal oscillations (see later). Submerging waters also reduce photosynthetic activity by reducing the incident radiation: light is attenuated exponentially with depth, the ratio of red/far red light increases and at solar angles of 41·4° direct solar radiation is completely reflected by a smooth water surface. Turbidity will also attenuate the radiation particularly in the UV A & B bands, while a proportionally greater depletion of far-red to red light is observed (Holmes & Klein 1987).

In view of the enormous variety of flooding regimes that can occur it would be most surprising if tolerance to flooding was based on a single adaptive feature or strategy. Indeed, it is becoming increasingly evident that tolerance, even within a single species, derives from an amalgam of features both structural and metabolic, and often intimately linked to the growth cycle and reproductive strategies (Haldemann & Brändle 1986; Blom *et al.* 1990; Voesenek & Blom 1992; Voesenek *et al.* 1992). For some species, however, of equal importance may be a mechanism to help survive what has become known as post-anoxic injury—a phenomenon of an immediate post-flood period when previously anaerobic tissues become aerobic once more—the result can be a build-up of potentially damaging super-oxide radicals (Hendry & Brocklebank 1985).

Broadly speaking, flooding resistance in plants is achieved by one or more features which improve gas exchange, as well as various metabolic features which help maintain a sufficiency of energy production to sustain cell integrity and avoid irreparable damage under oxygen stress; the latter in some species extends to a limited degree of anaerobic growth to help establish (seedlings) or re-establish (rhizomes) contact with the atmosphere. In addition to this, response to flooding is often under hormonal control. Consequently, this review is divided into three sections: (i) tolerance involving the promotion of gas-transport—essentially an anoxia-avoiding strategy; (ii) tolerance involving metabolic adaptation—essentially an accommodation strategy, and (iii) tolerance involving hormonal action.

## TOLERANCE INVOLVING GAS TRANSPORT

For the long-term survival of plants in flooded soil, the roots must obtain oxygen, and several successful strategies can be identified by which this takes place. They include: (i) the exploitation of the surface aerobic layers—surface rooting; (ii) enhanced development of internal gas-space to facilitate gas-phase diffusion in the roots, diffusion and convection in the shoots (& rhizomes), and oxygenation of the rhizosphere; and (iii) the rapid extension of various parts of the shoot system (sometimes anaerobically) to establish (or re-establish) gas-phase connection between the plant and atmosphere in response to submergence, or at the start of the growing season, or at germination. The gas exchange of normally-submerged species may also be improved by a lack of leaf cuticularisation, while in normally emergent species the gas exchange of submerged foliage may be improved by surface gas-films which adhere to the leaf. In the event of partial submergence these provide a direct connexion with the atmosphere.

### *Surface rooting*

The depth to which oxygen will be detectable in waterlogged soil is a function of the microbial and chemical oxygen demand and the diffusive resistance (Armstrong 1979). The extent to which roots might utilize this oxygen is predictable by mathematical modelling (Armstrong 1979; Armstrong *et al.* 1991a; De Willegan & Van Noordwijk 1989). It will depend upon the degree and distribution of oxygen demand within the roots, the resistance to radial diffusion across the roots, and on the narrowness of the root: the narrower the root, the greater will be the surface area to volume ratio and the more likely will it be that radial oxygen diffusion from the soil will accommodate the root's respiratory demand. Mathematical modelling also shows that, by acting as a buffer, an oxygen presence in the surface layers can reduce the radial oxygen loss from those roots which receive a supply by internal gas-phase diffusion from the shoot. In this way more of the oxygen which enters the root from or via the shoot becomes available to help extend the rooting depth, and hence the exploitable soil volume by extending the length of root which can be aerated. The latter might be more enhanced by high root numbers per unit volume since the likely effect of some radial oxygen loss from many roots will be to reduce the total loss from any individual root; high root densities can also result in a general raising of the redox state of the surface horizons by the overlapping of adjacent rhizospheres (Justin & Armstrong 1987). A certain degree of flood tolerance can thus be achieved by the development of fine roots in the surface aerobic layers of a flooded soil, and although the exploitable soil volume might be somewhat restricted and shoot growth somewhat retarded, the uptake of phototoxins can be minimized (Laan *et al.* 1989b). Surface rooting is a recognizable strategy among a significant proportion of wetland species (Justin & Armstrong 1987), and for non-wetland plants it is possibly the only significant long-term strategy of flood tolerance other than stock or seed dormancy. The reasons for the surface rooting habit have not been thoroughly researched. It may be that in some cases those roots which penetrate below the aerobic zone simply die. On the other hand, root orientation is frequently altered by flooding: horizontal rooting is common, as is upward growth (Armstrong & Boatman 1967) and it seems reasonable to suppose that this might be under hormonal control (Reid & Bradford 1984, and below).

### *Gas-space development and diffusion*

By far the most effective way for roots and rhizomes to obtain oxygen when in a flooded soil is by gas-phase transport from (viz. photosynthetic $O_2$), or via (viz. atmospheric oxygen) the shoot system. In most plants, whether they be wetland or non-wetland types, a gas-space continuum can be found, primarily within the cortical tissues. This usually stretches from the stomata (or lenticels) to within a few microns of the root—root cap junction (Armstrong 1979). It has been demonstrated that even where this gas-space system occupies only a small fraction of the total root cross-sectional area, and is composed of non-aerenchymatous intercellular spaces only (e.g. in pea 1·5–4%), sufficient oxygen will diffuse through it (Armstrong *et al.* 1982, 1983) to support root respiration and effect significant root growth through sterile but anaerobic media (Healy & Armstrong 1972; Webb & Armstrong 1983). In waterlogged soils, however, there are additional sinks for oxygen: the microbial and chemical oxygen demands, and these act effectively as competitors with the root for this internal oxygen supply. Oxygen is thus lost from root to rhizosphere and it must be strongly emphasized that, contrary to what is often supposed, the root does not just release oxygen which is surplus to its own requirements. For example, in terms of diffusive resistance, the non-porous meristematic and mature stelar tissues of the root may be further from the internal path than are the microbes of the rhizoplane and rhizosphere. The result may be preferential oxygen release to the rhizosphere, and the length of root which can be sufficiently aerated is reduced (Armstrong & Beckett 1987). Lateral roots can release many times more oxygen than they consume (Armstrong, Armstrong & Beckett 1990) but this severely limits their growth.

Low root porosities which are a predominant feature of non-wetland plants are probably never sufficient to support root penetration of more than a short distance below the surface aerobic horizons of a flooded soils. This conclusion is supported both by the results of mathematical modelling (Armstrong 1979; Armstrong & Beckett 1987) and by the relationship between root porosity and rooting depths of plants in flooded soil (Justin & Armstrong, 1987 and Fig. 1). In pea, oxygen leakage from root to soil can reduce the rooting depth from $\geqslant 100$ mm in an anaerobic sterile medium to $\leqslant 30$ mm in a freshly waterlogged loam; even freshly de-oxygenated but stagnant dilute agar medium (0·05% w/v) is sufficient to demonstrate this external sink (Armstrong *et al.* 1983). Again in pea, roots which have grown to lengths in excess of 100 mm in a drained loam will die back to lengths of $\leqslant 40$ mm if the soil becomes flooded.

The deeper rooting found in the majority of wetland species can be attributed to the much higher root porosities developed in these plants. Principally this is the result of aerenchyma formation (Justin & Armstrong 1987). However, the gas-space provision in many wetland species may be higher than non-wetland plants even prior to aerenchyma development due to a different cell configuration—cubic as opposed to hexagonal cell packing (Justin & Armstrong 1987). The maximum porosity achievable with cubic

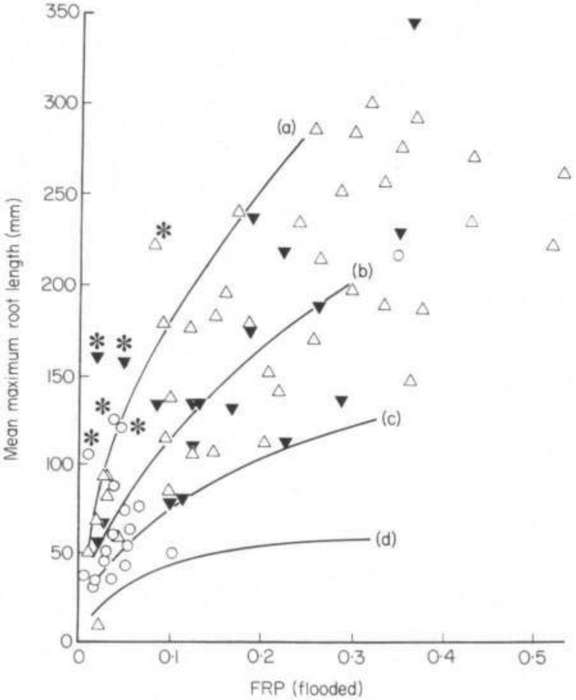


**Fig. 1.** For a range of non-wetland (○), intermediate (▼) and wetland (△) species, shows the relationship between fractional root porosity (FRP) and mean maximum root length achieved in flooded soil. Each point represents a different species. Species marked with an asterisk displayed horizontal roots. For comparison, curves (a), (b), (c) and (d) are derived from electrical modelling of root aeration (Armstrong 1979) with input data as follows: (a) root respiration, $M_R=30$ mg m$^{-3}$ s$^{-1}$, no radial leakage from root to soil (applicable to all root radii); (b) $M_R=30$ mg m$^{-3}$ s$^{-1}$, root radius, $r=0{\cdot}5$ mm, soil oxygen demand, $M_S=53$ mg m$^{-3}$ s$^{-1}$; (c) $M_R=120$ mg m$^{-3}$ s$^{-1}$, $r=0{\cdot}5$ mm, $M_S=53$ mg m$^{-1}$; (d) as for (c) but $r=0{\cdot}1$ mm. After Justin & Armstrong 1987.

packing is 21·4%, with hexagonal packing it is 9·3%, and hence it is quite conceivable that meristem architecture might play a hitherto unrecognized but important role in flood tolerance (see also Baluška *et al.* 1993).

Aerenchyma can be found in roots, rhizomes, stems and leaves, but it is most common in roots where, in some grasses, it has been shown to be under hormonal control (see later). In some wetland species it may be confined to the roots; however, one species is known, *Hydrocotyle vulgaris*, in which the rhizome is highly aerenchymatous and the roots strictly non-aerenchymatous (and extremely short). In some species aerenchyma is produced in response to flooding; in others, e.g. rice, it is an almost constant feature of the root system, although it may become more extensive under flooding (Justin & Armstrong 1991a). Aerenchyma may be lysigeneous—involving cell collapse and some degree of cell separation (common, particularly among the Gramineae and Cyperaceae), or can be shizogeneous (good examples in the Polygonacae): here cells separate but do not collapse, and consequently they continue to form an oxygen sink. Possibly as a result of this extra oxygen sink, plants which form schizogeneous aerenchyma, whilst including very successful wetland species (Laan *et al.* 1989a), are often not so deeply rooting as lysigeneous types (Justin & Armstrong 1987).

Aerenchyma essentially serves two purposes, both of which facilitate gas transport: (i) it greatly reduces diffusive resistance to longitudinal (axial) transport of gases from shoot to root; and (ii) it causes a reduction in oxygen demand per unit volume in the organ in which it occurs. Of the two, however, it is the reduced resistance to transport which accomplishes most in terms of enhanced aeration, a conclusion arrived at by modelling studies in which the anatomical and respiratory demand characteristics of non-wetland and wetland root systems were intermixed (Armstrong 1979; pp. 292–297). In roots, although there may be a convective flow component (Beckett *et al.* 1988; Koncalova *et al.* 1988), aerenchyma primarily enhances the diffusion of oxygen and carbon dioxide, and increases the potential for aerating the rhizosphere; it has also been shown to provide a conduit for methane loss from the sediments (Dacey & Klug 1979; Chanton *et al.* 1993; Sorrell & Boon 1994), and a route for the transport of nitrogen as well as oxygen to roots to support nitrogen fixation by diazotrophic bacteria such as *Azospirillum* (Ueckert *et al.* 1990). In leaves, aerial shoots and rhizomes, aerenchyma greatly enhances oxygen transport towards the root—a flow which may be both diffusive or convective (see below).

The crucial role of aerenchyma in improving root aeration under flooding is readily demonstrated by mathematical modelling (Armstrong 1979). Also, (i) rooting depths of a whole range of species have been found to increase with root porosity in a manner predictable on the basis of improved aeration (Fig. 1, and Rogers & West 1993), (ii) the development of aerenchyma has been shown to lead to higher energy charge and ATP levels in maize roots (Drew *et al.* 1985) and (iii) for similar root lengths consistently higher apical oxygen concentrations (monitored non-destructively) have been found in aerenchymatous as opposed to non-aerenchymatous roots (Armstrong 1979; Thomson *et al.* 1990). Examples of the high apical oxygen levels and sustained growth of rice roots in anaerobic media as a result of aerenchyma development are to be found in Webb & Armstrong (1983) and Armstrong & Webb (1985).

Some of the complexity which underlies the internal aeration of root systems in flooded soils has been demonstrated by the development of mathematical models which attempt to accommodate the multicylindrical nature of the system (Armstrong & Beckett 1987): structurally the root consists of various tissue cylinders, e.g. stelar, cortical and hypodermal/epidermal. External to the root is the additional rhizosphere cylinder. Axial gas-phase diffusion occurs primarily through the cortex, and gas exchange with the other tissues and rhizosphere is necessarily by radial liquid-phase diffusion from and to the cortex. Respiratory oxygen demand and diffusivity can vary radially between cylinders as well as axially along cylinders. Consequently, the oxygen concentration at any locus will depend upon the degree and distribution of oxygen demand and diffusive resistance throughout the whole root and rhizosphere. The proximity of other roots, and the occurrence of secondary (lateral roots), increases the complexity as does their proximity to one another: lateral root development is bound to increase the axial resistance to diffusion through the cortex of the primary root, and the oxygen demand of the laterals and their rhizospheres will compete for oxygen with the primary root. No attempt has yet been made to assess these proximity effects, but the multicylindrical modelling has highlighted the likelihood of sub-apical anoxic stelar cores, a consequence of basipetal reductions in stelar and endodermal oxygen permeability; this has since been supported by the results of enzyme (Thomson & Greenway 1991) and oxygen micro-probe (Armstrong *et al.* 1994) studies. Whether this can be classed as a flood tolerance feature must be open to debate, but reduced oxygen uptake by the stele will make oxygen more available at the root apex and hence will lead to greater soil penetration. However, these findings suggest the need for caution in interpreting ADH activity in roots as being indicative of a deleterious oxygen stress (Meldelssohn & McKee 1987; Burdick & Mendelssohn 1990).

Whilst cortical aerenchyma development, by its enhancement of aeration, is of obvious value in the flooding resistance of non-woody plants, its usefulness as a flood-tolerant feature in woody species may be relatively short-lived because of secondary growth. In woody roots, secondary cortex normally arises from a secondary meristem developed sub-apically in the pericycle of the stele. As a consequence of this the primary cortex of the more apical parts (the major route for gas-flow to the apex) may soon be isolated from the shoot. It has not been established whether there is ever radial pore space continuity between the pericycle (or secondary cortex) and primary cortex in woody roots, although there is some evidence that radial oxygen movement (perhaps in the liquid phase) from an aerenchymatous pericycle to primary cortex may be sufficient to support the growth of the primary apex of lodgepole pine in flooded soils (Coutts & Phillipson 1978a,b). For many plants secondary growth may be the single most detrimental characteristic in terms of flood tolerance, but where the secondary cortex becomes aerenchymatous (e.g. as in the tap root of some of the genus *Rumex*) gas transport may be sufficient to support the development of aerenchymatous non-woody lateral roots and flood tolerance is maintained (Justin & Armstrong 1987; Laan *et al.* 1990).

A common response to the onset of flooding by both wetland herbs and flood tolerant trees is the proliferation of new crops of aerenchymatous roots, particularly adventitious. These serve at least in the short term, to help supplement the water and nutrient requirements of the shoot system (Kozlowski 1984; Hook 1984). In trees, the response is invariably associated with intense localized phellogen activity just below and above the water table, giving hypertrophied strongly hydrophobic lenticels. If above the water table, these lenticels should serve to increase gas-flow to the roots; below the water table the roots emerge from these points. As water tables fall, exposing more of the lenticels, their hydrophobic nature ensures immediate gas-phase connexion through the bark. In some plants, soil flooding appears to suppress or very much restrict secondary growth in the roots, and the aerenchyma of the primary cortex remains functional (Justin & Armstrong 1987). In the stem of the herbaceous vine *Mikania scandens*, however, an unusual proliferation of aerenchymatous cortical tissue and epidermis plus stomata parallels the normal secondary development of the vascular tissues under flooded conditions (Moon *et al.* 1993).

The role of internal gas transport in the flood tolerance of the large flood-plain tree species remains to be established. It may be that many of these plants rely principally on a much reduced metabolic rate, and substantial carbohydrate reserves to survive flood periods (Schlüter & Furch 1992; Crawford 1992), but in the majority of cases the successful strategy is probably a combination of morphological, anatomical and metabolic adaptations (Joly 1991). It has recently been shown that young plants of the amazonian palm *Astrocaryum jauri*, which can survive submergence for up to 300 days, do so by a combination of anaerobic metabolism and continuing internal aeration sustained by some underwater photosynthesis (Schlüter *et al.* 1993). Regarding oxygen transport, it is possible that dead gas-filled elements of the xylem could form a conduit for oxygen transport in many species, and it is interesting that Hook & Brown (1972) were able to show that in flood-tolerant North American species the cambium of the stele was permeated by radial intercellular gas spaces, whereas in flood-intolerant species it was not. Radial intercellular spaces in the phellogen of adler have recently been identified as the pores responsible for supporting a thermal transpiration pressurisation which might be involved in some convective flow (Buchel & Grosse 1990). It has been suggested that wetland tree species might be aided by convective flows of gases from shoot to root; the implications of such gas flows are considered in the next section.

### *Root–shoot, root–rhizome and root–root junctions*

Cortex to cortex connections at root–shoot, root–rhizome and root–root junctions have rarely been studied in any detail, and it is not certain how commonplace gas-phase connections might be. A lack of gas-phase connection might seriously impede root aeration. Just how serious this might be would depend upon the path length, its sectional area, the oxygen diffusivity of cell wall materials and oxygen demand within the junction and beyond. It might be sufficient to exclude plants from flooded soils. It has occasionally been suggested that even certain species of wetland plant either lack gas-phase continuity or have critically high diffusive resistances across root–rhizome and root–shoot junctions (*Puccinellia festuciformis*, Stelzer & Lächli 1980; *Carex rostrata*, Fagerstedt 1992). However, *Puccinellia* was later shown to have quite adequate gas-phase continuity in the form of loose cellular packing in the root–shoot junction deep within the stem cortex (Justin & Armstrong 1983). Also, it was found that oxygen transport from shoot to root was quite adequate to support the normal oxygen demands of the roots in anaerobic media. The root–shoot junction in the sedge *Eriophorum angustifolium* is very similar to that of *Puccinellia* (Gaynard & Armstrong 1987), that of the root–rhizome junction in *Phragmites* consists of a stellate parenchyma having a porosity of as much as 50% (Armstrong & Armstrong 1990), while gas-space connections also occur in the adventitious root–lateral root junctions of *Phragmites* (Armstrong 1992). Porous root–shoot junctions occur also in maize and *Menyanthes trifoliata* (S. Lythe & W. Armstrong, unpublished data). Clearly, more information is required on this subject. We presently believe that some gas-space connection across junctions is probably the norm but that high stem-cortex resistance might be a more critical factor in some species.

### *Convective gas flows*

Since the length of any plant organ which can be supported by internal diffusive aeration depends upon the degree and distribution of the oxygen demand and upon the degree and distribution of diffusive resistance, it follows that, to penetrate more than 300 mm into flooded soil the roots must be very porous, have very low oxygen demand, and release only limited amounts of oxygen to the rhizosphere (Armstrong 1979). In some wetland species, however, the roots arise from rhizomes which themselves may be more than 0·5 m below the surface of the sediment, while the above-ground parts may also be partially submerged to varying degrees. This poses the question 'how in these circumstances can root, rhizome and rhizosphere aeration be maintained?' The answer seems to lie with pressurized (convective) gas-flows. Our current awareness of the existence of such flows began with reports of pressure flow in the water lily Nuphar (Dacey 1980, 1981), and since then flows in other water lilies (Schroder *et al.* 1986; Mevi-Schutz & Grosse 1988; Grosse *et al.* 1991) and other plants (Raskin & Kende 1983a, 1985; Armstrong & Armstrong 1990; Armstrong *et al.* 1992; Brix *et al.* 1992) have been recorded. The subject has been reviewed by Armstrong *et al.* (1991a), and two categories of convection have been recognized: non-throughflow and throughflow, the latter seeming to be by far the more important in terms of flood tolerance.

Non-throughflow convections occur when the whole (or part) of the $CO_2$ evolved in respiration remains dissolved rather than reciprocating the oxygen inflow by diffusing back to the atmosphere via the plant's gas-space system. The dissolved $CO_2$ might be lost directly to the transpiration stream or be leaked to the submerging waters; in some instances it could be fixed (e.g. to malate). The pressure deficit which this will create 'sucks' air into the plant from the atmosphere. However, the velocity varies along the aeration path, being highest at the point where the gases enter the plant, and zero at the limit of the aerated path. The flow might even be reversed if there is a significant amount of photosynthetic oxygen production (Raskin & Kende 1985), or if there is anaerobic $CO_2$ production beyond the aeration limit (W. Armstrong, P.M. Beckett & S.H.F.W. Justin, unpublished data). A certain degree of non-throughflow convection may occur wherever there is partial flooding, but its importance as an aeration and flood tolerance mechanism may be only marginal: claims that it might be the major mechanism for aeration in deep-water rice (Raskin & Kende 1983a, 1985) were recently refuted (Beckett *et al.* 1988). Analyses revealed a dependency of convection upon diffusion, and showed that, for all practical purposes, it should be possible to ignore convection completely in calculating aerobic path lengths within the submerged organ. Non-throughflow convection in mangroves has been known for some years (Scholander *et al.* 1955), and a substantial pressure deficit (e.g. 5–6 kPa, Curran *et al.* 1986) is created by carbon dioxide solubilisation during periods of tidal submergence. The non-throughflow convection in mangroves may occur in two stages—a rapid inflow of gases on re-exposure during the ebb-tide, followed by a much slower inflow dictated by any on-going deficiency in the ratio of $O_2$:$CO_2$ exchange with the plant's internal atmosphere. How important the first of these stages might be is questionable since the pressure deficit is redressed by an inflow of air rather than oxygen, and the sudden inrush of air will only directly reach the proximal parts of the aeration path. Curran *et al.* 1986 have concluded that it is of little importance, and suggest that the oxygen-storage facility of the aerenchyma is critical to the maintenance of aerobic respiration during tidal submergence. The second of these convection stages, being akin to that in deep-water rice, is probably also of little importance (Curran *et al.* 1986; Beckett *et al.* 1988). At present it is difficult also to envisage how convections in tree roots induced by thermal transpiration (Schroder 1989; Grosse *et al.* 1992) could be anything other than non-throughflow in the submerged parts, and the pressures recorded are so small that they should have an almost negligible effect in terms of increasing diffusion to the roots. W. Armstrong, S. Moir & S. Docherty (unpublished data) found that applying gas pressures artificially to the cut ends of willow and alder stems marginally increased radial oxygen loss from the roots in proportion to the increased density of oxygen in the pressurized gas; marked increases in oxygen release only occurred when the pressures were sufficient (>2000 Pa) to induce bubbles of air to be extruded through the submerged lenticels, i.e. under throughflow conditions.

Throughflow convections, so called because gases entering a plant at a point above the water-table are convected into the submerged parts before 'exhausting' (venting) back to the atmosphere at some other point, can be generated in at least three ways: (i) by humidity-induced diffusion, HID (often thermally enhanced) (Dacey 1981; Armstrong & Armstrong 1990; Brix *et al.* 1992); (ii) by thermal transpiration (often termed thermo-osmosis) (Dacey 1981; Schroder *et al.* 1986); and (iii) by Venturi-induced

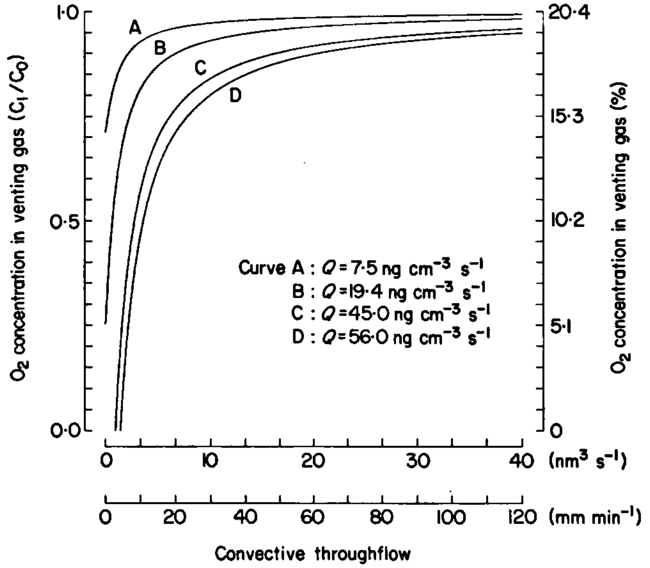


**Fig. 2.** Mathematical modelling of throughflow convection in *Phragmites*. For different levels of oxygen demand, shows how increasing raes of convection can be expected to influence the [$O_2$] concentration in the gases vented from a 500 mm length of rhizome of 60% porosity: curve A, oxygen demand of 7·5 ng $cm^{-3}$ $s^{-1}$ equivalent to rhizome respiration only; curve B, as A, but with extra respiratory demand equivalent to that of 10 adventitious roots plus attendant laterals (600 per root); curve C, as B, but with additional demand due to oxygen consumption in the rhizosphere (soil oxygen demand 50 ng $cm^{-1}$ $s^{-1}$); curve D, as C, but with demand in the rhizosphere raised to 500 ng $cm^{-3}$ $s^{-1}$. After Armstrong, Armstrong & Beckett 1992.

suction (Armstrong *et al.* 1992). With throughflow convection there is little diminution in the flow velocity along the aeration path, and since flow rates can be very substantial indeed (15 $cm^3$ $min^{-1}$ for water-lily petioles and for single *Phragmites* shoots; internal velocity *c.* 800 mm $min^{-1}$), there can be little doubt that they are of considerable importance in flood tolerance. Often the oxygen concentrations developed within the rhizome system will be made much higher (i.e. close to atmospheric) by convection than by diffusion alone; consequently, diffusion of oxygen from rhizome to root and rhizosphere can be substantially enhanced (Armstrong & Armstrong 1990; Armstrong *et al.* 1992 and see Fig. 2). Furthermore, $CO_2$ and gases such as $CH_4$ emanating from the sediments will be removed more readily (Dacey & Klug 1979; Sorrell & Boon 1994).

Both humidity-induced convection (HIC) and thermal transpiration require the presence of a micro-porous partition having a greater resistance to Poiseuille flow than to diffusion. In HIC the constant humidification on the inner side of the partition (in *Phragmites* the living leaf-sheath stomatal surface), creates a sub-stomatal atmosphere in which the concentrations of $O_2$ and $N_2$ are less than in the external atmosphere. Since the depth of the stomatal pore is relatively short ($\leqslant 10\ \mu m$), and since internal evaporation will continue to buffer the water vapour component of the internal atmosphere, a rapid diffusive inflow of $O_2$ and $N_2$ is promoted, and the sub-stomatal atmosphere will tend to pressurize as the $O_2$ and $N_2$ concentrations within the plant tend to equalize with those outside. Consequently, the pressuring sub-stomatal gases will begin to convect back to the atmosphere along the path of least resistance; in the case of *Phragmites* this is generally through the rhizome, and venting is via the broken ends of dead culms; in water lilies the gases pass to the rhizome before venting along another route in either the pressure-generating leaf (Mevi-Schutz & Grosse 1988) or a different leaf. In *Phragmites*, because of the high resistance to any Poiseuille backflow through the stomata of the inflow shoot, static pressures up to at least 1 kPa may develop within the plant if convection to the rhizome is deliberately blocked. In 14 species of emergent macrophytes tested by Brix *et al.* (1992) eight produced static pressures in the range 200–1300 Pa and air flows of 0·2 to >10 $cm^3$ $min^{-1}$ per shoot.

It was thought originally that pore diameters ⩽0·1 μm were required to enable HIC, but recent work (Armstrong 1992, pp. 139–199; Armstrong & Armstrong 1994) has shown that this is not so: provided that the evaporative surface (e.g. the sub-epidermal cell layers) is very close to the basis of the pores, significant flows should be attainable even with pore diameters up to 3 μm. It should be noted that even very small pressure differentials are sufficient to drive considerable gas-flows in plants provided that throughflow resistances are not too great. In the case of *Phragmites*, even with relatively low dynamic pressure differentials (⩽50 Pa) it has been estimated that by HIC up to 0·3 kg of $O_2$ $day^{-1}$ might be convected into the rhizome system per $m^2$ of reed bed area, whereas by diffusion perhaps only 1/100 to 1/30 of this amount could be transported. However, it must be acknowledged that much of the convected gas might go unused and be vented back to the atmosphere.

Thermal transpiration (thermo-osmosis) is a term used to describe the diffusion of dry gases through microporous partitions under the influence of a concentration gradient induced by a temperature differential across the membrane. The gases move against the temperature gradient and, as they accumulate on the warmer side, against the pressure gradient that is created. As with HIC, however, the pressures which can be generated usually fall short of those which seem theoretically possible, and this is probably due to a certain 'leakiness' of the membrane because of greater than ideal pore sizes. In water lilies, the warming of leaves by sunlight or by the waters on which they float can create the potential for a thermal transpiration component, and it may be that thermal transpiration is frequently of considerable significance in floating-leaved species (Grosse *et al.* 1991; Groose & Bauch 1991).

Whether convections are generated by humidity-induced diffusion or by thermal transpiration, the flows tend to show a diel variation. Daytime conditions tend to enhance flow both by the energy input, which creates temperature differentials and enhances internal humidification, and through stomatal opening. For the most part, however, HID is probably of greatest significance in emergent macrophytes since it will function even if the temperatures within the plant are lower than ambient.

Venturi-convection has been found so far in only one plant—*Phragmites australis* (Armstrong *et al.* 1992), although there is no reason to suppose that it might not occur in other species. It is driven by the suction pressures developed at the exposed broken ends of wind-blown tall dead persistent flowering culms. Air is drawn into the rhizomes via stubble close to ground level where the wind-currents are less. Venturi-convection will operate at any time of day, night or season provided that there is sufficient wind, and since the suction developed is a function of $wind\text{-}speed^2$, gusting assumes some importance.

Convections will be of greatest benefit to plants with creeping rhizomes and partially submerged shoots and should help to support deeper growth in anaerobic sediments. There is every reason to believe that they play a major role in the flood tolerance of some plants, but the full benefits of convection, and the extent to which they occur in the plant kingdom remain to be discovered. The mechanisms and structures responsible for convective flows should be borne in mind when seeking to generate greater degrees of flood tolerance in plants.

### *Shoot and foliage submergence*

If the aerial shoots and foliage of plants are submerged to any degree the consequences can be far-reaching in terms of aeration, assimilation and hormone balances: even the normally aquatic species (the submersed aquatic macrophytes or SAM) can be limited in their productivity, due to the high aqueous resistance to $CO_2$ and $O_2$ diffusion in submerging waters (Bowes 1987; Sand-Jensen 1987; Keeley 1987; Jensen & Madsen 1991; Madsen 1993).

In recent years, much research has been focused on the survival strategies of SAM species, and a whole variety of adaptive features has been identified. These include: (i) a general absence of cuticularization which facilitates the easy transference of $O_2$, $CO_2$, and even $HCO_3^-$ across leaf surfaces; (ii) very high porosites; (iii) high photosynthetic:non photosynthetic cell ratios; (iv) high surface:volume ratios; (v) various degrees of C4-acid metabolism; and (vi) fixation of $CO_2$ derived from the sediments by internal diffusion via the roots.

In general, much less information is available concerning the effects of submergence on the normally emergent macrophyte, wetland or non-wetland plant. However, it can be deduced that diffusive aeration to below-ground parts will be most acutely affected by partial submergence if the aerial parts immersed are of low porosity and non-photosynthetic; woody species, therefore, might be particularly at risk. Few studies have addressed this point, but it has been shown that immersion of only 30 mm of stem above the root–shoot junction was sufficient to cause complete cessation of oxygen release from the adventitious roots of *Salix fragilis* cuttings (Armstrong 1968). There is still no clear understanding of the survival mechanisms operating in tree species in flood plains such as the Amazon basin, although it can be anticipated that anaerobic metabolism and dormancy are likely to be of considerable significance (see above, and Joly 1991).

Where plants are foliated to ground level, as in the grasses and sedges, cuticularization of leaves and confinement of gas-transfer sites to the stomata can severely restrict gas transfer in submerged conditions and additional factors assume importance. For example, in many plants the foliar gas-space is supplemented by gas-films which cling to the submerged leaf surfaces (Raskin & Kende 1983a, 1985). In the case of partial submergence, these connect with the atmosphere and will thus enhance diffusive gas exchange (and some convective flow, see above) from (e.g. $O_2$ at night; $CO_2$ during the day) the atmosphere. Equally important, however, they may facilitate $O_2$ and $CO_2$ transfer into the plant from the submerging waters since gas, rather than fluid, impinges on the stomata and fills the leaf grooves containing the stomata. Transfer rates will depend upon (i) the concentration of the gases in the submerging waters, and (ii) the degree of agitation of the waters: the greater the degree of agitation the thinner will be the boundary layers and the more rapid the exchange. At night, agitation will enhance oxygen transfer from water to plant, and during the day will enhance $CO_2$ transfer from the water increasing both assimilation and photosynthetic oxygen production. It has been demonstrated that, at laboratory temperatures, there can be sufficient oxygen transfer across leaf surfaces in the dark to maintain root aeration and extension growth provided streaming rates in the water are significant (Fig. 3). If the waters are not

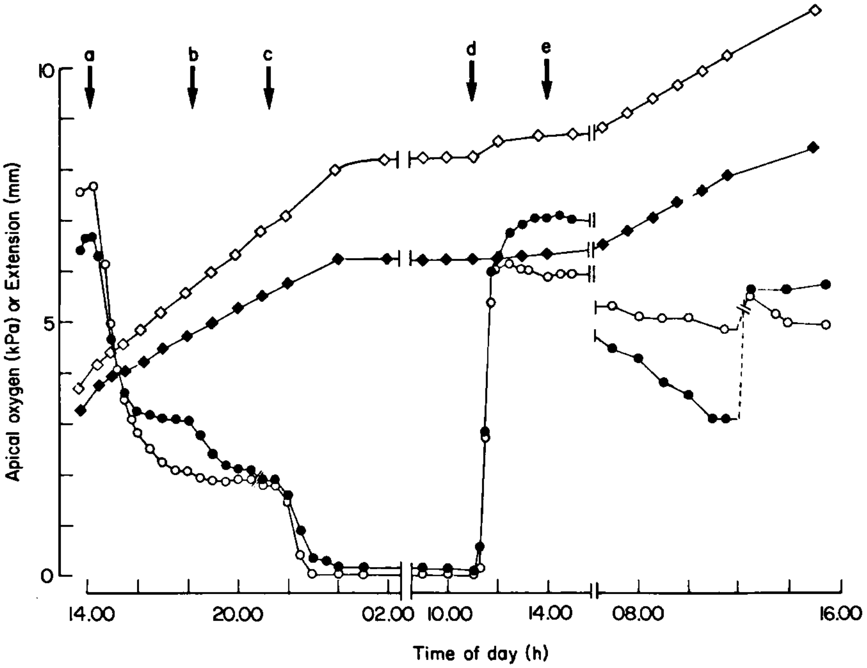


**Fig. 3.** The effects on root oxygen (lower graphs; measured as in Fig. 4) and root extension (upper graphs) in two mature rice plants subjected a 23°C to the following treatments: (a) shoot system submerged in air-saturated water streaming at 29·5 mm $s^{-1}$; (b) streaming rate 5 mm $s^{-1}$; (c) streaming halted; (d) water drained to expose shoot; (e) shoots illuminated. Roots at all times in stagnant anaerobic medium. S.H.F.W. Justin, W. Armstrong & P.M. Beckett, unpublished data. Note that gas exchange between leaves and water under the two streaming water regimes maintains oxygen concentrations in the roots at a level sufficient to support extension.

agitated, however, the likelihood is that root growth will be rapidly halted because of an insufficient oxygen supply. Again, if temperatures are high (32°C) there may a diel requirement for photosynthetic oxygen to support root growth (Fig. 4), even though the waters may be agitated and air-saturated (Waters *et al.* 1989). If gas films are minimal and waters stagnant, dissolved $CO_2$ levels may have to attain many times more than the air-saturation value before there is appreciable oxygen generation by photosynthesis (Gaynard & Armstrong 1987; Sand-Jensen 1987). However, although photosynthesis can raise oxygen levels in submerged leaves sufficiently to maintain a significant oxygen concentration in the roots (Fig. 4), it would be wrong to assume that this in any way indicates a sufficiency of assimilation rate for sustained plant survival: carbohydrate starvation leading to reduced respiratory demand can also lead to elevated oxygen concentrations in roots. High $CO_2$ concentrations in submerging waters should help sustain assimilation rates, but in stagnant waters, because of the impedance to oxygen loss, high leaf oxygen concentrations may result with deleterious consequences because of increased photorespiration. On the other hand in emergent macrophytes, $CO_2$ derived from the sediment and transported into the aerial parts via the aerenchyma may stimulate photosynthesis both by its improved availability and by reducing photorespiration (Constable *et al.* 1992; Brix 1990) More information on these points, on gas exchange and submergence tolerance in non-SAM species, and on the possible contribution of C4 acids is required. Useful papers on the water quality in deep water

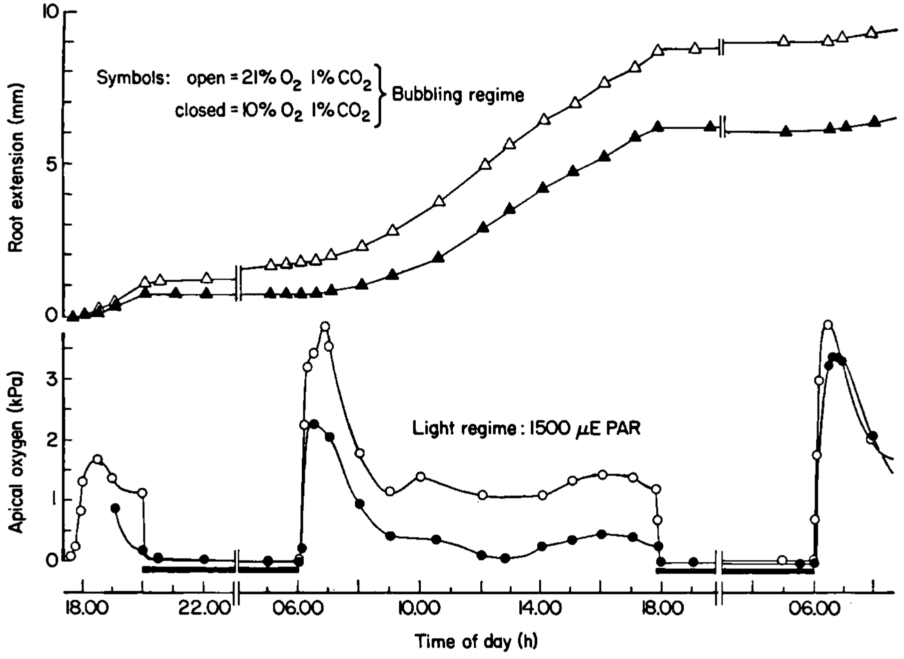


Fig. 4. Photosynthesis-dependent diurnal variation in the oxygen regime in the root apices of rice plants submerged at 32°C. Roots in stangant anaerobic medium, water around the shoots in water bubbed with gas mixtures as shown. Upper two graphs show root extension, the lower two show root surface oxygen partial pressures 2–7 mm from the root tip and dependent upon radial leakage from the roots' cortical gas space to a sheathing polarographic oxygen electrode. Armstrong & Setter, unpublished data, but see also Waters *et al.* 1989.

rice fields and the oxygen, $CO_2$ and carbohydrate relations of the deep water rice plant are those of Setter *et al.* (1987a,b and 1988).

Leaf, shoot and root aerenchyma may serve for a time as an oxygen reservoir to buffer plants against any sudden impedance to gas exchange consequent upon submergence. Nevertheless, the reservoir function of aerenchyma is probably of value only at relatively low temperatures and in situations such as coastal salt-marshes where submergence is only of a few hours duration each day. Curran *et al.* (1986) concluded that at 15°C, the aerenchyma within the roots of *Avicennia marina* contains sufficient oxygen to maintain aerobic conditions over the period during which the root system is isolated from the air by tidal submergence of the shoot. However, the fall in oxygen concentrations within the roots was accompanied by a reduction in respiration. This may have been a consequence of the development of stelar anoxia (Armstrong & Beckett 1987). If, as a result, aerobiosis in the cortex was prolonged, this might enhance flood tolerance. At summer temperatures of 25°C, Curran *et al.* calculated that roots could become anoxic during tidal submergence, but predicted that there might be a gradual adjustment to warmer conditions by the formation of new pneumatophores and more aerenchyma; they also found some evidence of dark fixation of $CO_2$ to malate. Hovenden & Allaway (1994) have recently reported new pathways of gas conductance ('subrisules') in the pneumatophores of *Avicennia*.

An excellent study into the effects of tidal submergence on aeration was that of Gleason & Zeiman (1981). They grew the salt-marsh grasses *Spartina alterniflora* and

*S. patens* in a tidal model, and among other things concluded that photosynthesis contributed significantly to oxygen supply during flooding: under equivalent flooding, oxygen in the shoots of both species declined to considerably lower concentrations during darkness than during illumination. Also, despite the aerenchyma reservoir factor, *S. alterniflora* shoot base lacunae became virtually anoxic during midsummer high tides in darkness. This finding suggested that oxygen deprivation during such periods may act as a physiological barrier to seaward extension of the range of *S. alterniflora* by inhibiting the growth of below-ground organs. Oxygen concentrations in *S. patens* shoot bases declined to considerably lower values than in *S. alterniflora* under equivalent light, flooding and temperature conditions, a finding which suggested that the apparent inability of *S. patens* to colonize the regularly flooded low marsh habitat of *S. alterniflora* may be related to the deleterious effects of submergence on aeration.

The effects of photosynthesis, and the relatively limited capacity of the aerenchyma oxygen reservoir to maintain aerobiosis in non-SAM species, have also been noted by Gaynard (1979), Gaynard & Armstrong (1987), Smith *et al.* (1984) and Waters *et al.* (1989). In the first of these studies on the sedge *Eriophorum angustifolium* it was noted that if 9/10 of the foliage was submerged, root aeration was considerably better even in low light than in darkness. It was concluded that aerial contact by the leaf tips which maintained a $CO_2$ transfer to support photosynthesis was responsible. Similar effects have been noted recently in studies on the growth and survival of *Rumex* species in a Dutch river ecosystem (Laan & Blom 1990).

Finally, reference should be made to the hormonally induced rapid extension growth of shoots or petioles which occurs in some species (see later). The growth, which helps re-establish aerial contact, is clearly a major flood tolerance mechanism.

### *Radial oxygen loss and the rhizosphere*

In flooded soils, provided that there is a gas-space continuum between root and shoot, leakage to the rhizosphere from aerobic parts of roots is inevitable unless there is a critical combination of relatively low oxygen permeability and relatively high respiratory oxygen demand in the radial path through the non-porous outer layers of the root. Thus, even roots of relatively low porosity are likely to effect some oxygenation of the rhizosphere. However, such leakages may severely curtail the length to which the roots may be aerated internally (Armstrong 1979; Armstrong & Beckett 1987) and rooting depths and exploitable soil volume might be very restricted.

As well as encouraging an aerobic microflora, radial oxygen loss to the rhizosphere can effect a variety of oxidative reactions beneficial to plants: examples include the conversions of $\mathrm{MnII} \rightarrow \mathrm{MnIV}$, $\mathrm{FeII} \rightarrow \mathrm{FeIII}$, and $H_2S \rightarrow S$. In this respect, rhizosphere oxygenation is probably of equal importance for flood tolerance as aerenchyma development or anaerobic metabolism.

The precipitation of hydrated iron oxides is the most frequently observed example of rhizosphere oxidation by roots (Armstrong & Boatman 1967; Green & Etherington 1977; Laan *et al.* 1989; St-Cryr & Crowder 1988; Armstrong *et al.* 1992; Snowden & Wheeler 1993; St-Cyr, Fortin & Campbell 1993). This helps to protect against the absorption of toxic amounts of the ferrous ion, but may also help to reduce the intake of arsenic and heavy metals such as zinc and cadmium which can become adsorbed or complexed to the rhizosphere iron deposits (Laxen 1983; Otte *et al.* 1989, 1991).

Hydrogen sulphide, a potent phytotoxin (Koch *et al.* 1990), may be oxidized directly, or be scavenged as the relatively insoluble ferrous sulphide (Engler & Patrick 1975; Winter & Kickuth 1989).

Radial oxygen loss will also support aerobic nitrifying bacteria (Hansen & Andersen 1981; Hoffmann 1990; K. Buis 1994, personal communication) or aerobic nitrogen-fixing bacteria (Uckert *et al.* 1990) in the rhizosphere. The potential for nitrification in the rhizosphere is a major consideration underlying the current use of wetlands for the purification of domestic and agricultural effluents. Here, the ammonia from urea may be nitrified in the aerobic conditions of the rhizosphere, subsequently being either absorbed by the plants, or denitrified and evolved as nitrogen gas after diffusing to anaerobic sites beyond the rhizosphere. The extent to which constructed wetlands can effect this process is still the subject of investigation and debate. However, there is no doubt that it occurs. Buis *et al.* (1994) have developed an elegant combination of experiment and mathematical model to assess the dimensions of, and nitrifying activity in, the oxidized rhizosphere.

Much still remains to be learned of rhizosphere oxygenation and its effects. In submerged or partially submerged species the oxygenation and oxidation will follow a diel cycle (Smith *et al.* 1984; Waters *et al.* 1989) and it can be envisaged that this will result in a 'pulsing' of the rhizosphere, but how this influences vigour and survival needs to be determined. In Western Australia, the survival of a subterranean clover species in periodically flooded soils has been attributed to radial oxygen loss from the roots of Kikuyu grass. This would seem to amount to type of flood tolerance symbiosis, the Kikuyu grass either supplying the clover roots with oxygen, or buffering against oxygen losses from the clover (Setter & Belford 1990).

The sites of radial oxygen loss from roots are relatively easy to identify by the use of indicator dyes, e.g. methylene blue (Armstrong & Armstrong 1988; Armstrong *et al.* 1992). Because of their high surface to volume ratio, and their high numbers, lateral roots can be the major source of sediment oxidation by roots. Although the young growing parts of adventitious roots are also a major site for oxygen release, critical combinations of relatively low oxygen permeability and relatively high respiratory oxygen demand in the epidermal/hypodermal layers, can reduce oxygen leakage in the sub-apical parts of such as rice and *Phragmites* to almost nil, despite the occurrence of high concentrations in the cortex. In such roots the bulk of the oxygen released in basal regions may be largely confined to the laterals. For *Phragmites*, it has been calculated that oxygen loss from a single lateral can amount to 1/30 of the total released from the matured parent adventitious root. The amount released by the complement of basal laterals on an adventitious root could amount to 40 × that from the adventitious root itself (Armstrong *et al.* 1990).

Regarding the quantities of oxygen released, these must vary enormously from species to species, plant to plant and root to root, and will probably undergo enormous seasonal variation in conjunction with root maturation. For example, the epidermal/hypodermal tissue cylinders often become very much less permeable to oxygen in sub-apical regions due to suberization and/or lignification. This has for a long time been regarded as an adaptation to flooding (Yamasaki 1952), and it not only helps conserve oxygen but it may also be important for reducing phytotoxin entry (Armstrong 1979). In maize roots, PAL and peroxidase activity induced by ABA results in lignification, reduced protein, solute and oxygen leakage and increased flood tolerance (Van Toai 1994). In *Phragmites australis*, extremes of lignification extending even to the cortical cells, together with callus development, have been interpreted as defence responses to soil-borne phytotoxins (Armstrong & Armstrong 1994).

Attempts to measure oxygen release from roots have relied either on (i) the use of root-sleeving polarographic electrodes which, by creating their own quantifiable sink activity, can provide very accurate localized measurements on the roots of intact plants (Armstrong 1979), or (ii) the measurement of dissolved oxygen depletion from solution cultures. To obtain meaningful values for oxygen release from whole root systems presents considerable difficulties. Results obtained by measuring changes in the dissolved oxygen concentration of culture solutions supporting whole root systems (Bedford *et al.* 1991; Gries *et al.* 1990; Howes & Teal 1994) must be viewed with caution, since (i) a constant diffusion gradient may not be maintained around the root, (ii) the oxygen sink activity is likely to be weak, and (iii) because of convective thermal circulation of culture solution, the oxygen released from one (more aerobic) part of a root system may be re-absorbed by other more remote and less aerobic parts. Mathematical modelling provides a useful means of estimating the potential for oxygen release, however, and recent studies on *Phragmites australis* have suggested that from 5 to 35 g $m^2 d^{-1}$ could be released into flooded soils. These results accord (1) with measurements in which a strong sink was maintained (a) on individual roots in streaming water experiments (Armstrong *et al.* 1990), and (b) on whole root systems maintained in titanium citrate redox buffer (Sorrell & Armstrong 1994); and (2) with deductions from effluent transformation in the field (Gray *et al.* 1990). They are very much higher than those found by Bedford *et al.* (1991), Gries *et al.* (1990), or Howes & Teal (1994).

## TOLERANCE INVOLVING METABOLIC ADAPTATION

It is likely that all higher plants can survive a certain period without oxygen, and phylogenetically there is no doubt that fermentation processes evolved before aerobic respiration. Indeed, a majority of plants probably have to cope at least once in their life with the adverse effects of flooding or oxygen deprivation. For example, the oxygen supply of imbibed seeds is frequently restricted (Martin *et al.* 1991), and metabolic adaptation mechanisms with respect to energy production are very easily induced in seeds and many other tissues with fermentation processes often becoming active. However, although ethanolic and lactic acid fermentation do occur, in most cases they will not lead to long-term flooding tolerance (Laan 1990). Germinating seeds, even rice, have to gain access to molecular oxygen, otherwise growth will be stopped. Most of the other tissues die because of the lack of intermediates, energy-dependent processes, and perhaps because of the effects of glycolytic end-products acting as phytotoxins. A hindered energy metabolism is quite often shown by very low adenylate energy charge values (Pradet & Raymond 1983).

Survival under anoxia can range from half an hour up to several months. Very sensitive organs are cotton roots and the cyanogenic glucoside-containing roots of peach trees; the most tolerant, probably overwintering wetland plant rhizomes, which sometimes have to endure severe hypoxia or anoxia in water-saturated muds (Brändle & Crawford 1987). However, the metabolic-facilitated survival of flooding is dependent on more than one of the following:

(i) the control of energy metabolism under oxygen deprivation;
(ii) the availability of extensive energy resources;

(iii) the provision of essential gene products and synthesis of macromolecules; and
(iv) protection against post anoxic injury.

*Control of energy metabolism under oxygen deprivation*

The ATP required in anaerobic tissues is obviously generated in glycolytic processes, mainly ethanolic and lactic acid fermentation. Indispensable intermediates are provided in many organisms by a partially functioning TCA cycle (Kennedy *et al.* 1992). But these processes are not always very efficient, e.g. with regard to ATP provision. In non-tolerant underground organs, such as potato tubers, adenylate energy charge (AEC), i.e. (ATP+0·5 ADP)/(ATP+ADP+AMP) is very low under anoxia (AEC<0·3), indicating ATP shortage for ATP-consuming processes (Sieber & Brändle 1991). Sugar supply or a hypoxic pre-treatment of maize roots (*Zea mays* L.) is able to ameliorate energy provision to some extent, but not for prolonged periods (Saglio *et al.* 1988). Glucose supplementation was found to extend the survival *in vivo* of anoxic rice roots form 4 to 44 hours but not for longer (Webb & Armstrong 1983). It seems that carbohydrate transport into oxygen-deprived roots is strongly inhibited or blocked (Perata & Alpi 1993). On the other hand, many wetland plant rhizomes, such as the sweet flag (*Acorus calamus* L.), the reed (*Phragmites australis*) and the bulrush (*Schoenoplectus lacustris* L.), restore high adenylate energy charge values within a few hours (AEC $\simeq$ 0·8, Brändle 1990). An exception is *Iris pseudacorus*, showing low AEC values under prolonged anoxia (Hanhijärvi & Fagerstedt 1994). In most cases, an equilibrium prevails between ATP-producing and -consuming processes, although probably at a lower level than under air. Besides glycolytic ATP, some additional ATP is made available in wetland plant rhizomes by the high activity of starch phosphorylase rather than amylases (Steinmann & Brändle 1984). This has been observed in *Acorus calamus*, *Schoenoplectus lacustris* and in the cattail (*Typha latifolia* L.), as well as in the rice coleoptile (*Oryza sativa* L.). In each case, however, only shoot extension is possible by cell elongation. Otherwise the plant organs are living in a kind of 'anaerobic retreat'. The energy provided is mainly used for maintenance. Sustained growth is dependent upon the particular organ becoming eventually aerated either by achieving contact with atmosphere, or by photosynthesis, in order to get sufficient ATP for growth processes. Normally, root growth and the development of most wetland plant rhizomes is strongly inhibited below about 1% oxygen, and root growth ceases rapidly under anoxia (Webb & Armstrong 1983). In wetland plant rhizomes, however, maximum respiration capacity, and therefore ATP production, is attained at quite low internal oxygen concentrations, e.g. in *Acorus calamus* at 3·4 kPa $O_2$ and *Phragmites australis* at 2·7 kPa $O_2$ (Studer & Brändle 1984). Similarly, the critical oxygen pressures for root growth and respiration can be extremely low, e.g. for rice root respiration $\leqslant$2 kPa (Armstrong & Gaynard 1976) and root growth $\leqslant$0·8 kPa (Armstrong & Webb 1985). To sum up, it can be said that moderately hypoxic conditions probably do not greatly affect wetland plant development.

The glycolytic flux seems to be controlled by a pH-stat, starting with lactic acid fermentation. Subsequently, the slightly lowered pH induces ethanolic fermentation (Davies 1986). ADH (alcohol dehydrogenase) enzymes work best below pH 7. As far as we know, lactic acid fermentation is subsequently strongly inhibited in tolerant tissues, but much less in many non-tolerant. This difference has recently been shown in rice and wheat seedlings (Menegus *et al.* 1991) as well as in *Acorus calamus* rhizomes and potato tubers (Sieber & Brändle 1991). In potato tubers, lactic fermentation still continues, the pH drops quickly far below neutrality ($\Delta$pH=0·8), and the tissue dies within 48–72

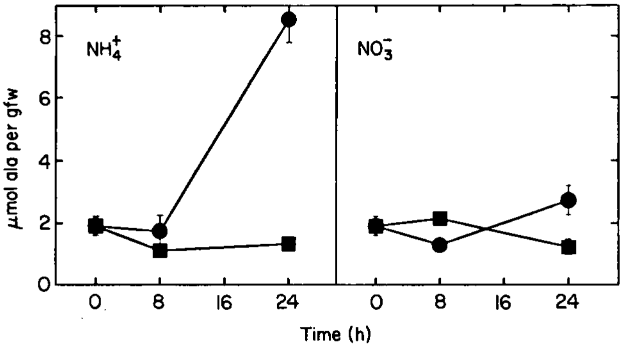


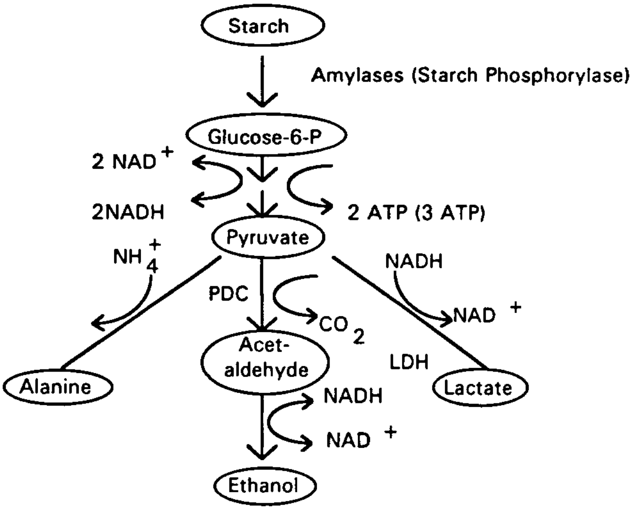
Fig. 5. Time-dependent changes in alanine of concentrations in rhizome tissues of *Acorus calamus* fed with 3·3 mM ammonia or 4 mM nitrate, respectively under aerobic (■) and anaerobic (●) conditions. Mean values and SD of four experiments.

hours. Cytoplasmic acidosis has been shown by NMR technique in several tissues and ADH-mutants of *Arabidopsis thaliana*. It is considered as one of the main causes of cell death under anoxia (Roberts *et al.* 1984). The pH values of adapted species do not drop to the same extent. The pH drop within the cytosol is counteracted by alkalinization processes, e.g. the formation of γ-aminobutyric acid and the accumulation of amides and probably arginine (Crawford *et al.* 1994; Weber & Brändle 1994). Cytoplasmic acidosis induces ATPases and causes the breakdown of the ATP-dependent pH gradient between cytosol and vacuole within a few minutes in non-tolerant tissues. Subsequently, the tonoplast loses is semipermeability and the cells die.

It is not known yet if the fermentative enzymes involved have other properties in wetland species than in dryland species. However, ethanol is the most common glycolytic end-product besides alanine and some lactic acid in wetland plants. Alanine is often formed when abundant $NH4^+$ is available outside and within tissues probably by direct aminotransfer to pyruvate (Fig. 5). Ammonia is immediately detoxified and, at a later date, the amino group can be used to form many other N-rich compounds such as glutamine, asparagine and arginine. The main fermentation pathways are shown in Fig. 6.

Sometimes malate can also be found in reasonable amounts. This diversification of glycolytic end products has been considered as a powerful strategy to lower the toxicity of some intermediate and end products, and to export the oxygen debt out of oxygen-starved tissues into the well aerated tissues. Malate is a non-toxic compound and it may play an important role in flooded trees, but probably to a lesser extent in herbaceous wetland plants. The question as to whether ethanol is toxic *per se* has been a matter of debate for a long time (Crawford 1992). However, several papers have appeared describing tissues which are sensitive to ethanol concentrations below 20 mM (Perata *et al.* 1987, 1988). Recent results have also pointed out that acetaldehyde, the oxidation product of ethanol, could be responsible for ethanol-induced damage (Perata & Alpi 1991) especially in the backwards reactions from ethanol to acetaldehyde in re-aerated tissues (Pfister-Sieber & Brändle 1994). From this point of view we have to consider ethanol as a potential cell toxin. However, in wetland plant rhizomes and also in many dryland species ethanol may never attain toxic concentrations since rhizomes and other thin and porous tissues release ethanol very easily into the surroundings.

To the best of our knowledge ethanolic fermentation is the most efficient pathway to produce considerable amounts of ATP under anoxia in plants. Flood-sensitive dryland



PDC = pyruvate decarboxylase
ADH = alcohol dhydrogenase
LDH = lactate dehydrogenase

**Fig. 6.** Important fermentation pathways in plants. Ethanol is the most abundant glycolytic end product.

species increase their ADH activities considerably, e.g. peas (*Pisum sativum* L.) up to 30 times (Crawford 1978). This increase is accompanied by a remarkable 'Pasteur effect' and extensive reserve consumption. Wetland plants rhizomes often exhibit a 'Pasteur effect' only at the beginning of a flooding period and therefore economize reserves and produce less toxic compounds. Wetland plant rhizomes have large reserves and show a more moderate increase in fermentation (Brändle 1991). The sugar starvation in anoxic root tips, for example, may be because of the effects of oxygen depletion on the unloading of sugar from the phloem as well as of the effects along the phloem transport route (Saglio 1985; Perata & Alpi 1993). Therefore, anoxic roots are quickly depleted of energy and die because of energy starvation (Davies *et al.* 1987); even the adventitious root tips of intact rice plants can be killed by only 4 h of anoxia (Webb & Armstrong 1983). On the other hand, there is good evidence that hypoxically treated maize roots may continue to grow, albeit at a reduced rate, while containing a core of anaerobiosis within the tip region (Gibbs *et al.* 1994). It is thought that metabolic co-operation might exist between cells receiving an adequate oxygen supply and cells in the anoxic zone.

### *Availability of extensive energy reserves*

Bearing in mind the problem with energy metabolism and transport under anoxia, it turns out that only few plant organs are potential candidates for surviving prolonged flooding. Thickened roots, stems and seeds fulfil only one criterion. They contain enough reserves, but roots and also totally flooded stems are for metabolic reasons not flood-tolerant, and they would not be able to regenerate to intact plants. Also, there are relatively few seeds, such as rice, *Echinochloa* and *Erythrina caffra*, that are able to extend their coleoptiles or leaves under anoxia. Without any molecular oxygen they never develop roots and die within a few days. All seeds and achenes have a very limited anoxia tolerance. Nevertheless, starchy seeds are able to germinate at very low oxygen concentration down to 0·01%: they are fairly hypoxia-tolerant. Fatty seeds need about 2% $O_2$. In both cases, however, growth is strongly retarded (Alani *et al.* 1985). Seeds of wetland plants often develop under floating conditions and settle down only when fully germinated (*Lemna* syndrome). The presence of high amounts of reserves is an essential prerequisite, but never the only cause of flooding resistance. It is not surprising that all perennating amphibious plants belong to the rhizome geophytes and usually spread out vegetatively. Wetland plant rhizomes with their adapted energy metabolism, and in their roles as storage and bud-bearing organs, are predestined for prolonged flood survival (Brändle 1991). Overwintering rhizomes contain carbohydrates up to 50% of their dry weight, mostly starch. An exception is *Phragmites australis.* This plant stores more than 50% of the reserves in the form of sucrose, and much less starch. The carbohydrate content during the course of the year shows typical variation. The content is high at the end of the vegetation period, decreases slightly during the cold season and sharply when new shoots, rhizomes and roots develop in late spring and early summer. In healthy rhizomes, the carbohydrates are sufficient to survive the winter season and for the heterotrophic growth in spring (Haldemann & Brändle 1986). At this stage rhizomes are sensitive to defoliation. Later on in the growing season high amounts of sucrose are transported from the green shoot into the rhizome. Protein storage is much lower in wetland plant rhizomes, the contents varying between 4 and 12 mg $g^{-1}$ fresh weight. The proteins undergo the same variation during the course of the year, in winter being 2–3 times higher than in summer. Protein and carbohydrate degradation is correlated with the development processes in the intact plant. For example, additional PDC mRNA occurs in flooded *Acorus calamus* rhizomes at natural sites in early spring and disappears immediately when the shoots emerge. PDC is usually the limiting step in fermentation (Bucher, 1994). The same is true for the enzymes involved, such as amylases, phosphorylases and fermentative enzymes (Steinmann & Brändle 1984). However, rhizomes of *Acorus calamus*, *Phragmites australis*, *Schoenoplectus lacustris* and *Typha latifolia* accumulate amino acids before protein breakdown starts at the end of the overwintering period (Haldemann & Brändle 1988). They fix ammonia from the surrounding anoxic mud to form alanine in the roots (see Fig. 5) and to store mainly arginine in *Acorus calamus*, and asparagine in other species (Fig. 7). These amino acids range within 5–40 μmol $g^{-1}$ fw, and in the case of arginine could counteract efficiently cytoplasmic acidosis within the rhizome. We assume that these amino acids are the main nitrogen source in spring.

The same might happen with sulphide. Anoxic rhizomes of *Acorus calamus* and *Phragmites australis* easily pick up sulphide and convert it to thiols (Fig. 8). In *Phragmites australis* the thiols consist mostly of glutathione and cysteine (Fürtig, personal communication). Both mechanisms allow the storage of N and S and the detoxification of ammonia and sulphide.

Lipids are not stored in wetland plants species in considerable amounts. One of the reasons could be the high oxygen demand during degradation in comparison to carbohydrates.

### *Provision of essential gene products and synthesis of macromolecules*

Cell maintenance and eventual growth require the synthesis and/or the stability of essential macromolecules, such as RNAs, proteins and membrane lipids. Studies at the mRNA level show surprising stability of the aerobic RNA, which remains untranslated

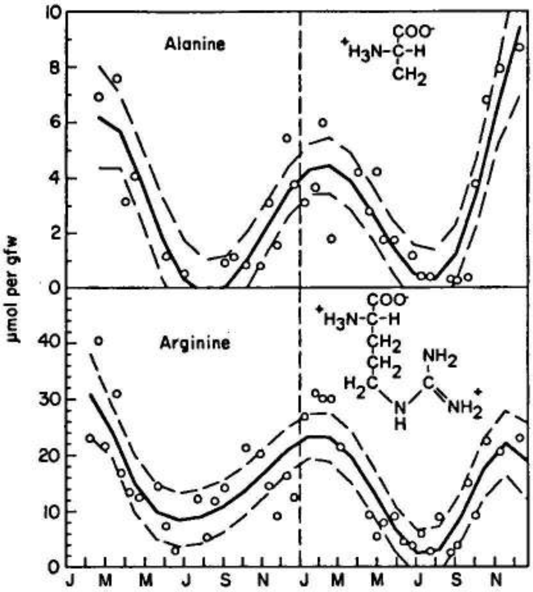


**Fig. 7.** Seasonal-dependent changes of alanine and arginine concentrations in root and rhizome of *Acorus calamus*. Arginine, with four N-atoms is an efficient N-storage compound. after Weber & Brändle 1994.

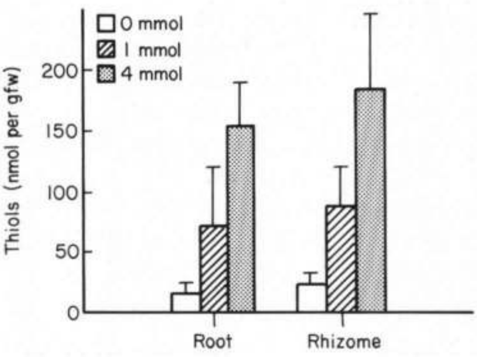


**Fig. 8.** Contents of acid-soluble thiols in roots and rhizomes of *Acorus calamus* fed with 1 and 4 mmol sulphide for 24 h ($n=5$, ± SD).

under anoxia. According to the degree of hypoxia or anoxia new sets of RNA are produced. Under anoxia about 20 stress proteins appear, the significance of which is partially understood (Sachs & Ho 1986).

The best characterized anaerobic protein is the adh1 molecule. It seems to be necessary that at least one functioning AHD isozyme is present in an anaerobic tissue (Ho & Sachs 1989). Otherwise even short oxygen starvation cannot be survived. This is not only due to the lack of energy, but also to the stabilization of intracellular pH by ethanolic fermentation. Most of the investigations in these fields have been done with crop seedlings, e.g. barley, maize, peas and rice, but *Echinochloa* and some wetland plant rhizomes have been investigated also. A common feature of all species is an increase ADH which differs markedly between the species. Wetland species usually show a lower increase on a percentage basis (Crawford 1978). Maize seedlings pre-adapted to hypoxia show under anoxia high AHD levels and high ethanol production (Saglio *et al.* 1988). Survival of the very sensitive root tips extends from 8–9 hours to about 22–96 h (Saglio *et al.* 1988; Johnson *et al.* 1989), but still far too short a period to be considered flood-tolerant.

Among the anaerobic stress proteins some others have been characterized. These mainly consist of glycolysis-related enzymes, alanine aminotransferase, sucrose synthase, and superoxide dismutases (SOD) which probably functions to provide immediate protection after re-aeration (Monk *et al.* 1987a; Ricard *et al.* 1991). After re-oxygenation, these newly synthesized proteins disappear quickly, and so do the anaerobic mRNAs. However, in intolerant species, the anaerobic treatment results in a drastic change in the pattern of proteins as shown on 2D gels (Ho & Sachs 1989): normal protein synthesis is almost completely suppressed. Marsh plant rhizomes behave somewhat differently with regard to their anoxic protein synthesis, but protein synthesis is also strongly inhibited, e.g. in *Acorus calamus* 56%, in *Phragmites australis* 59%, in *Schoenoplectus lacustris* 58% and in *Typha latifolia* 61%. Inhibition is measured as the fixation of a radioactive amino acid cocktail following a 20-h period of anoxic pre-treatment before feeding (M. Bucher, personal communication). For *Acorus calamus* rhizomes, the fingerprint on 2D gels of the labelled soluble proteins synthesized under air and anoxia, shows a large number of identical spots, indicating that this perennating organ is able to synthesize most of the essential proteins. Under anaerobic conditions about 60–70 spots are detectable (Fig. 9).

The differences between air and anoxia also show that metabolic adaptation to anaerobiosis demands a set of new proteins, mainly for glycolytic enzymes. Obviously *Acorus calamus* can maintain protein synthesis under anoxia, albeit at a lower level, but this behaviour could be one of the reasons that enables marsh plant geophytes to survive flooded periods with minimum costs.

With regard to the oxygen sensing and signal transduction system there is very little information available at the molecular level. Recently it was shown that anaerobic transcriptions are triggered not by the metabolic consequences of oxygen limitation, but directly through an oxygen sensor (Appelby *et al.* 1988; Bucher *et al.* 1994). In this connection haemoglobin may fulfil the following role:

$$\text{oxyhaemoglogin} \underset{\text{normoxia}}{\overset{\text{anoxia}}{\rightleftarrows}} \text{haemoglobin} \underset{\substack{\text{transcription}\\\text{factors}}}{\overset{\text{modifies}}{\longrightarrow}} \text{induction of anaerobic polypeptides.}$$

The synthesis of new lipids is very costly, and under oxygen deprivaton desaturation processes are hindered, or without any oxygen, even blocked. This is true for dryland species as well as for wetland species. The strategy with regard to lipids of wetland plant rhizomes can be the preservation of the lipid molecules as long as possible (Henzi & Brändle 1993). In fact, the content and the composition of polar lipids is much more stable in *Acorus calamus* and *Schoenoplectus lacustris* than in *Iris germanica* rhizomes (Table 1).

Some of the individual lipids of wetland species change their internal composition slightly. Usually linolenic acid (C18:3) decreases and is replaced by lower desaturated fatty acids. In *Acorus calamus* the composition recovers within 2 days of re-aeration. Both wetland species show hardly any decay in comparison to *Iris germanica.* We conclude that in adapted species lipids have a high stability, and the small changes

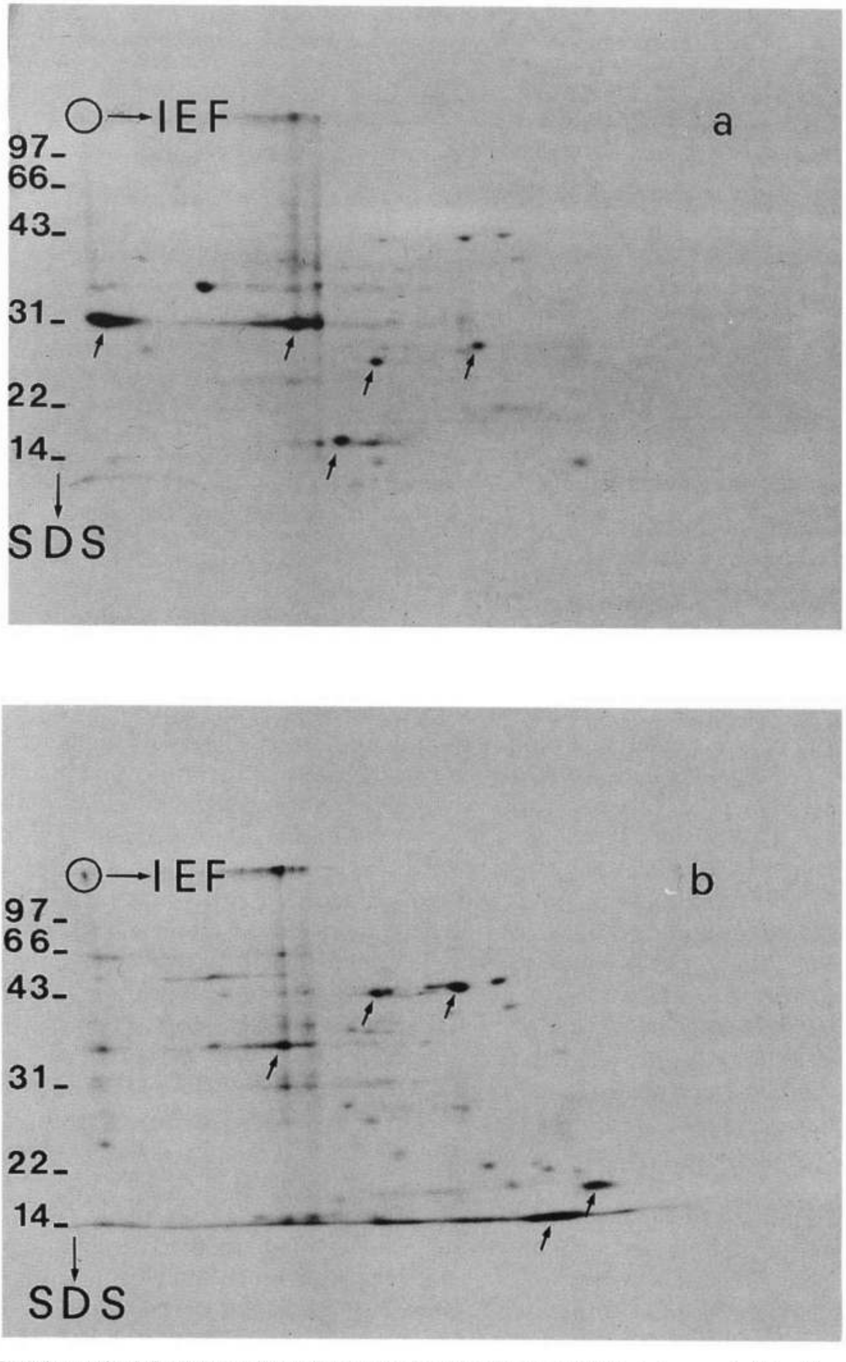


**Fig. 9.** Protein synthesis in rhizome discs of *Acorus calamus*: (a) under air; (b) under anoxia. Two dimensional separation of soluble proteins by IEF, pH 3–10, SDS gel electorphoresis, MW i $10^3$ Dalton, after feeding with a radioactive amino-acid cocktail. Arrow indicate differences (M. Bucher, unpublished).

**Table 1.** Content of polar lipids and free fatty acids under air and under anoxia in rhizomes of *Acorus calamus* (70 days anoxia treatment), *Schoenoplectus lacustris* (35 days) and *Iris germanica* (14 days). After Henzi & Brändle 1993

| | Polar lipids (µm $g^{-1}$ fw) | | Fatty acids (µg $g^{-1}$ fw) | |
|---|---|---|---|---|
| | Control | Anoxia | Control | Anoxia |
| *A. calamus* | 695 ± 44 | 651 ± 38 | <10 | <20 |
| *S. lacustris* | 724 ± 35 | 661 ± 22 | <10 | <20 |
| *I. germanica* | 891 ± 37 | 425 ± 29 | <10 | 138 ± 17 |

within the lipids are not lethal. Therefore, organelles are stable too, and thus can function for longer anoxia periods. Under strictly anoxic conditions we observed at least 25% regeneration in *Acorus calamus* after 10–12 weeks, in *Schoenoplectus lacustris* after 5–6 weeks (20°C). *Iris germanica* rhizomes are dead within about 14 days of anaerobic treatment. Non-rhizomatous tissues die usually within much shorter periods.

### *Protection against post-anoxic injury*

Re-exposure to oxygen after an anoxic treatment or natural flooding can cause severe injuries to tissues and organs (Gutteridge & Halliwell 1990; Crawford 1992). Typical behaviour of plant organs showing post-anoxic injury is as follows. Under anoxia, and just after the anoxic treatment the tissues usually look quite healthy; subsequently, after re-exposure to air they become soft, start to disintegrate and die very quickly. The main reason of this breakdown is probably the generation of superoxide radicals, iron induced-hydroxyl radicals or other reactive oxygen species (Hendry & Brocklebank 1985). Radicals produce mutations and dysfunction of enzymes and oxidative damage to lipids and membranes. Damaged cells leak their solutes and die quickly (Scandalios 1993). In tolerant species, however, peroxidation products of lipid breakdown such as malondialdehyde, ethylene and ethane occur to a much smaller extent than in intolerant species as shown for wheat and potato (Albrecht & Wiedenroth 1994; Pfister-Sieber & Brändle 1994). Possibly, tolerant tissues are better protected against radicals either by the accumulation of antioxidants, e.g. ascorbic acid, glutathione or eventually reduced phenolics, or by the induction of high SOD or active oxygen-species-removing enzymes such as catalase and peroxidase (Larson 1988; Monk *et al.* 1987a,b). Marsh plant rhizomes are rich in phenolic compounds, but the significance of these compounds has not been examined experimentally, so far. In the presence of sulphide, which occurs commonly in the natural habitat, *Acorus calamus* produces considerable amounts of glutathione. Besides the detoxification effect, the glutathione could be interpreted as an antioxidant provision in case of post-anoxic or post-hypoxic periods. Flooded rice seedlings, however, show a weak defence system. They produce high amounts of glutathione only after re-aeration, and the enzymatic defence system, even later on (Ushimaru *et al.* 1992). The localization and the effects of antioxidants remain an unresolved question (Gutteridge & Halliwell 1990). Most animal tissues, but also plant tissues, contain some antioxidants without being protected from short-term (1–3 h) anoxia. In soybean (*Glycine max.* L.), for example, short-term anoxia favours subsequently the production of superoxide radicals. Longer anoxic treatment leads to higher SOD activities and therefore to a more efficient protection. Furthermore, transgenic tobacco plants with additional and over-expressed SOD genes are markedly better protected against oxidative stress (Hérouart *et al.* 1993, Sen Gupta *et al.* 1993). However, exogenously applied ascorbic acid is, from the beginning of treatment, a much more effective protectant than endogenous antioxidants (VanToai & Bolles 1991). This raises the question: 'Is the high efficiency of external ascorbate because superoxide radicals are localized in the outer cell membrane or even in the cell wall?'

Marsh plant rhizomes, e.g. *Acorus calamus* and *Schoenoplectus lacustris* show very slow lipid peroxidation and stable membranes during recovery after 50–70 days of anoxic treatment. In contrast, rhizomes of the less tolerant *Iris germanica* show high peroxidation already after 7–10 days anoxia, and the membranes are completely disintegrated within 2–3 weeks (Henzi & Brändle 1993). Potato shoot survive less than 1 day, and tubers about 2–3 days, showing considerable amounts of post-anoxically formed malondialdehyde, ethane and ethylene (Pfister-Sieber & Brändle 1994). There must be a high protection of membranes in tolerant species, probably caused by a very slow 'turn over' under energy restriction.

Alternatively, the return of oxygen to oxygen-deprived plant organs can release a surplus of acetaldehyde and ethylene from anaerobically accumulated ethanol and ACC. Acetaldehyde is a strong cell toxin, and ethylene could promote early senescence. However, wetland plant rhizomes easily release ethanol, and when re-aerated they also release ethylene through the aerenchyma, thus avoiding high tissue concentrations of potentially harmful compounds. This is not the case in *Iris germanica* rhizomes and potato tubers. They accumulate ethanol (Monk *et al.* 1984; Pfister-Sieber & Brändle 1994). The latter produces an outburst of considerable amounts of acetaldehyde from ethanol. The reaction is probably catalysed by catalase.

$$H_2O_2 + CH_3CH_2OH \xrightarrow{\text{catalase}} 2H_2O + CH_3CHO$$

The perennating parts of wetland plants (rhizomes and stolons) obviously combine a variety of strategies to counteract the hazards of their frequently experienced natural post-anoxic or post-hypoxic conditions. Most of the strategies are present in dryland species too, but it seems to be a question of the extent. However, our knowledge is still very scarce and should be improved in the near future especially with regard to the oxygen sensing systems and the effects of oxidative stress.

## HORMONES AND RESISTANCE TO FLOODING

The persistence of meristems and the plasticity of cell development allows for an open type of growth, and creates options for morphological re-adjustment throughout much of the life of a plant. This developmental plasticity, together with the inextricable connection between form and function in plants, links hormone action with the ability to survive stress, such as flooding or submergence. Changes in morphology induced by environmental perturbation and mediated by hormones and associated factors can be considered as adaptive whenever the re-modelling (e.g. adventitious rooting or aerenchyma formation) confers increased fitness in the face of these perturbations. Evidence linking hormone action and such morphological adaptations to flooding or submergence is now reviewed.

### *Importance of ethylene*

Of the five major hormones (IAA, ETH, GA, CK and ABA), ETH is the hormone most closely associated with developmental responses to inundation. Many species sense they are submerged by reacting swiftly and positively to ETH in ways that enhance survival (for example, by extending shoots more quickly towards the water surface). This is not co-incidental. Submergence in water inevitably increases internal concentrations of ETH within plants. This is because of slower diffusion of the gas in water than in air (diffusion coefficients $1{\cdot}7 \times 10^{-5}\,\mathrm{cm}^2\,\mathrm{s}^{-1}$ and $0{\cdot}16\,\mathrm{cm}^2\,\mathrm{s}^{-1}$ at 20°C, respectively) restricts radial losses. Furthermore, ETH biosynthesis is sensitive to oxygen supply. For example, when inundation under stagnant conditions completely extinguishes oxygen supply, ETH production ceases (Fig. 10) because the last step in biosynthesis, which oxidizes ACC to ETH, is prevented, causing ACC to accumulate (Bradford & Yang 1980a). Furthermore, an unexpected *promotion* of ETH production by partial oxygen shortage has been found in roots of maize and barley, and in the stems of deep water rice (Jackson 1985). It is also possible that ETH produced in flooded soil by micro-organisms enriches flooded plants with the gas (Smith & Russell 1969).

### *Root extension*

Root extension is strongly inhibited by oxygen shortage in all of the species which have been examined, with the exception of *Trapa natans* (Menegus *et al.* 1992). However, submergence can affect root growth even when oxygen is in plentiful supply (Larqué-Saavedra *et al.* 1975), probably because of ETH accumulating within the roots because of the water covering. Different species produce ETH at different rates. It has been calculated that under 5 mm water, roots of a vigorous producer, such as *Sinapis alba* (white mustard), would accumulate sufficient gas to slow extension growth, while a weak producer, such as *Oryza sativa* (rice), would accumulate smaller, growth-promoting amounts (Konings & Jackson 1979). One reason for the relatively slow rates of ETH production by rice roots is a marked insensitivity to IAA (Jackson & Pearce 1991), a hormone that promotes biosynthesis of the gas.

### *Aerenchyma formation*

Internal oxygen transport through interconnected gas-filled intercellular spaces (aerenchyma) allows many species to grow and survive in flooded soils (see previous section). In flood-tolerant species, such as *Senecio* (Smirnoff & Crawford 1983), *Rumex* (Laan *et al.* 1989a,b) and dryland cereals, such as maize (*Zea mays*), wheat (*Triticum aestivum*) (Trought & Drew 1980), barley (*Hordeum vulgare*) (Larsen *et al.* 1986) and others (Justin & Armstrong 1987), poor aeration increases aerenchyma formation. This intercellular space can be created by lysigenous collapse of particular files of cells, as seen in roots and leaf-bases of maize (Jackson 1989). In some wetland species (e.g. *Rumex maritimus*, Laan *et al.* 1989), aerenchyma is formed schizogenously by cell separations that result from changes in the pattern of cell division in the apical meristem or variation in the extensibility between cells as they leave the meristem.

Lysigenous aerenchyma formation in maize is promoted in cortical regions of roots that are partially deficient in oxygen (e.g. 3 kPa oxygen, external equilibrium partial pressure). The effect is mediated by ETH (Drew *et al.* 1979) that is produced in greater amounts under these conditions of partial oxygen shortage (Jackson 1982) as is ACC (Atwell *et al.* 1988). The extra ETH is not a consequence of slower spermine and

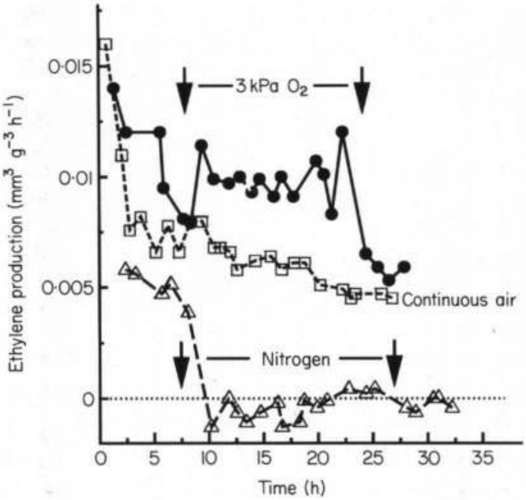


**Fig. 10.** Production of ethylene by single primary roots of 2-day-old seedlings of *Zea mays* grown either in continuous air (20·8 kPa oxygen) or treated for 16 h with oxygen-free nitrogen gas or with 3 kPa oxygen. Arrows show the start and finishing times of the two treatments. Taken from Brailsford *et al.* 1993.

spermidine accumulation releasing more *S*-adenosylmethionine for ACC and ETH production. However, a build-up of putrescine the diamine precursor of these polyamines in partially oxygen-deficient roots favours ETH biosynthesis (Jackson & Hall 1993). Increased ACC and ETH production may also result from increased ACC synthesis in the stelar core of the root that may be anaerobic because of encircling resistances to inward diffusion of oxygen, and as a consequence of oxygen consumption by more peripheral cells (Armstrong & Beckett 1987; Armstrong *et al.* 1994). Work with other species and tissues suggests that at least one ACC synthase gene (Zarembinski & Theologis 1993) could be induced by anoxia in the stele, giving rise to increased ACC synthase activity (Wang & Arteca 1992) and thereby increased amounts of ACC that could move radially into better aerated cells for conversion to ETH (Jackson 1994). The promoting effect of small oxygen partial pressures (3 kPa) on ETH production has recently been confirmed by monitoring ETH output from individual roots of intact seedlings with a highly sensitive photoacoustic laser detector (Fig. 10 and Brailsford *et al.* 1993).

Aerenchyma development can be delayed for several days by growing seedling roots of maize in atmospheres of norbornadiene, a volatile inhibitor of ETH action. Subsequent exposure to ETH has shown that cells up to at least 5 days old can respond to gas by degenerating to create aerenchyma (Brailsford & Jackson, unpublished). A disorientation of the regular arrangement of microtubules heralds this cell collapse (Baluška *et al.* 1993), and electron microscope studies have revealed signs of cell wall degradation (Campbell & Drew 1983). This loss of wall structure has been linked with increases in the level of soluble cellulase β-(1→4)-glucanase) in the roots (Grinieva & Bragina 1993). Cell walls of cereals comprise approximately 30% β-(1→3),β(1→4)-glucan (Fry 1988), and cellulase may degrade this polymer in regions of 10 or more contiguous (1→4)-linked glucose residues. Other biochemical and molecular biological events involved in aerenchyma development remain largely unknown. cDNA cloning and differential hybridization may help identify which genes are activated by ETH to bring about cell collapse. In animal physiology, such programmed cell death is known as apoptosis and is regulated by gene products that either promote or suppress it; intercellular signalling may also be involved (White 1993).

The extent to which ETH promotes the development of aerenchyma in roots of other species has not been tested extensively. Ethylene is active in *Pinus serotina* (Topa & McLeod 1988) and has some involvement in willow (Attwood 1993) but plays little part in regulating aerenchyma of rice (cultivar RB3, Jackson *et al.* 1985), where the sequence of cellular dissembly differs from that of maize (Webb & Jackson 1986). However, roots of the rice cultivar Norin 36 respond positively to ETH, and the extent of aerenchyma under poorly aerated conditions can be decreased somewhat with the ETH antagonist, silver nitrate (Justin & Armstrong 1991a).

The importance of hormones, other than ETH, in aerenchyma development remains uncertain. Konings & de Wolf (1984) found that a synthetic auxin naphthylacetic acid (NAA), ABA and a synthetic cytokinin (kinetin) can inhibit aerenchyma formation, while $GA_3$ promotes it. However, concentrations required for activity are high and sometimes distort root morphology (see Justin & Armstrong 1991b). Furthermore, results have not been consistent. For example, NAA promotes rather than inhibits aerenchyma when effects of the auxin on total root length are taken into account (Justin & Armstrong 1991b). The significance of the inhibition of aerenchyma by ABA reported by Konings & de Wolf (1984) is uncertain since levels of this hormone in roots subjected to severe oxygen shortage do not change much (Wadman-Van Schravendijk & Van Andel 1985; Jackson *et al.* 1988), although the effects of a more subtle decrease in oxygen supply have not been checked. The notable longevity of cells clustering around lateral root primordia is one location where hormones other than ETH could be interfering in ETH action, possibly by inhibiting DNA hydrolysis (Baluska 1990).

Schizogenous aerenchyma formation, a characteristic of many wetland plants, and formed by cell separation rather than by lysigeny (Laan *et al.* 1989), remains neglected experimentally.

### *Promotion of shoot extension by submergence*

*Total submergence of shoots.* Many amphibious and aquatic plants extend their internodes or petioles more quickly when completely submerged in water, thereby shortening the duration of submergence and decreasing the likelihood of asphyxiation by oxygen and carbon dioxide shortage (Laan & Blom 1990), which is especially acute during the night (Setter *et al.* 1988). A survey of several species of *Rumex* has linked their different abilities to survive submergence with, amongst other characteristics, the potential for increased elongation rates under water (Blom *et al.* 1990). Ethylene, a powerful inhibitor of elongation in most plants, is largely responsible for *promoting* fast extension underwater. In the aquatic dicot, *Callitriche platycarpa*, submergence promotes stem extension within 30 min, in association with the accumulation of growth-stimulating amounts of internal ETH (approximately $1\ \mu l\ l^{-1}$) (Musgrave *et al.* 1972). The effects of both submergence and ETH are equally rapid and reversible, involve cell elongation rather than division. These effects can be inhibited by the ETH action inhibitor silver nitrate (Jackson 1982), and require the presence of GAs. Inhibitors of GA synthesis abolish the response to submergence, or ETH, but this can be rescued by addition of exogenous $GA_3$ (Musgrave *et al.* 1972). Much subsequent work has confirmed that a similar mechanism operates in many species, most notably on under-water petiole growth (Ridge 1985). In submerged *Rumex* and *Rannunculus*

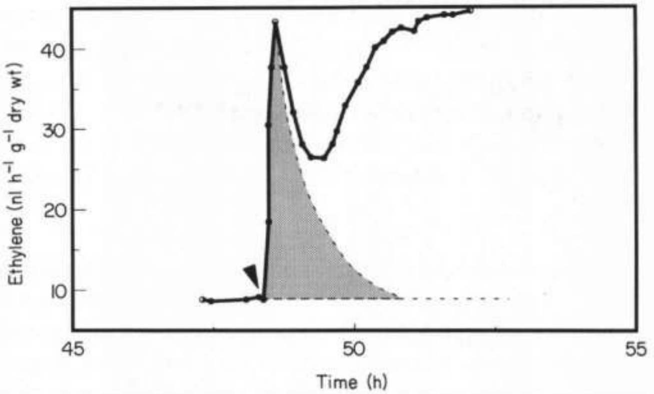


**Fig. 11.** A time of ethylene emanation rates from whole, rooted plants of *Rumex palustris* totally submerged for 24 h and then de-submerged to the soil surface (arrow). The kinetics of the initial sharp rise and fall in ethylene release immediately following de-submergence (shaded segment) show an almost instant release of the gas resulting from removal of the water barrier to outward diffusion. Unshaded areas under the peak represent new ethylene production that is additional to gas released within the first minute. Taken from Voesenek *et al.* 1993.

*sceleratus*, increased amounts of ACC in the leaf blade contribute to raising internal ETH levels (Voesenek *et al.* 1990b). In *R. sceleratus*, ETH promotion of petiole extension (Musgrave & Walters 1973) requires IAA rather than GA. The auxin originates in the leaf lamina; excision preventing the elongation response to ETH unless the detached lamina is substituted by exogenous IAA (Horton 1987). Kang *et al.* (1992) suggest that ETH sensitizes growing cells to endogenous auxin. In *Nymphoides peltata*, the fringed water lily, ETH promotes petiole extension primarily by increasing cell wall extensibility through acidification of the walls (Malone & Ridge 1983), and by stimulating cell division, especially in younger leaves. The action of cell turgor in driving extension by the more plastic cell walls is augmented by longitudinal buoyant tension created by submergence of the low density, aerenchymatous shoots (Musgrave & Walters 1974; Ridge 1987). In *Regnellidium diphyllum*, a tropical water fern, ETH-promoted growth has been linked to increased cell wall extensibility, but in this case the effect is not mediated by wall acidification (Ridge & Osborne 1989).

Until recently, most research in this area has been with excised shoots; few experiments linking ETH and underwater growth having been made with whole plants. However, it is now clear, at least in whole *Rumex palustris* and *R. crispus* plants, positive growth responses to exogenous ETH can substantially reproduce the submergence effect (Voesenek *et al.* 1990a). Appropriately, petiole growth of the closely related species *R. acetosa*, rarely found in flood-prone areas, is retarded rather than promoted by submergence or ETH. Sensitive photoacoustic laser detection of ETH has shown that, as expected, shoots of intact plants that are de-submerged emit a burst of ETH (Van der Sman *et al.* 1991) as the impediment to gaseous diffusion is removed. The kinetics of the release are compatible with the idea that submergence entraps ETH which then dissipates quickly when the plant re-emerges into the aerial environment (Fig. 11). This work has also confirmed that submergence increases ETH production by the plant, and in amounts that exceed substantially those seen in plants growing either in well-drained conditions or with the soil waterlogged but the shoot wholly in air (Voesenek *et al.* 1993). The explanation may involve transport of ACC from the waterlogged root system; the subsequent conversion into ETH being supported by an increase in ACC oxidase activity (Voesenek *et al.* 1990b) that is probably enhanced further by $CO_2$ (Smith & John 1993). Carbon dioxide also can promote elongation in dark-grown *Potamogeton distinctus* and *Sagittaria pymea*, species that also elongate rapidly when submerged or treated with ETH (Suge & Kusanagi 1975). However, in these species, the effect of $CO_2$ appears to be mediated through enhancing ETH action rather than biosynthesis.

*Partial submergence of shoots.* The most intensively studied elongation response to partial submergence is that by internodes of deep-water rice (*Oryza sativa*). The subject has been reviewed in detail (Kende 1987; Jackson & Pearce 1991) and only the more recent papers will be cited here. Twenty-one days after germination, stem elongation by deep water rice commences and is stimulated markedly if the shoot is partially submerged, with a lag period of approximately 200 min. Elongation is more vigorous during the photoperiods (Stunzi & Kende 1989). The resulting greater stem length permits depth accommodation of several metres of water with little or no loss of crop yield (Bekhasut *et al.* 1990). This growth promotion takes place in association with accumulation of up to 1·4 $\mu l\,l^{-1}$ ETH in the central stem lacuna (Stunzi & Kende 1989) caused by water entrapment and by faster ETH production that is induced by small partial pressures of oxygen (4–15 kPa) within submerged plants, especially at night. The extra ETH production is probably a consequence of enhanced ACC synthase activity in the intercalary meristem, or close by (Cohen & Kende 1987). As already mentioned, when shoots are submerged, their internal carbon dioxide concentrations rise (Stunzi & Kende 1989); this enhances ETH action. In common with *C. platycarpa*, underwater elongation by the stems of deep-water rice is underpinned by GAs, which can increase in concentration during submergence (Azuma *et al.* 1990), while levels of inhibiting ABA decrease (Hoffman-Benning & Kende 1992). If the production of GAs is blocked with tetcyclasis, neither submergence, ETH not carbon dioxide can promote growth. Ethylene sensitizes extending cells of the inter-calary meristem to GA, resulting in faster growing cells. This, in turn, stimulates more frequent cell division. The outcome is more cells each with a longer final length (Sauter & Kende 1992a). Gibberellin action and, axiomatically, that of ETH, is enhanced by a lowering of lignin production, and by increases in β-(1→3), β(1→4)-glucans associated with cell wall loosening (Sauter & Kende 1992b). The outer epidermal cells limit the amount of elongation overall, since it is only here that cell wall synthesis takes place during submergence (Kutschera & Kende 1989), and where GA/ETH re-orientate microtubules and cellulose microfibrils into the latitudinal direction that favours cell elongation (Sauter *et al.* 1993). Ethylene action increases the elasticity of the wall matrix which, together with some osmotic adjustment, favours faster and more extensive cell growth (Kutschera & Kende 1988). Osmolites, carbon skeletons and respiratory substrates needed to sustain high growth rates are supplied by an accelerated breakdown of starch in more mature parts of the stem, activated by ETH in association with the appearance of an additional iso-zyme of α-amylase. Further sustenance is derived from photosynthesis, which is stimulated on a unit area basis in those parts of the leaves remaining above water. Approximately 18% of total photosynthate is transported to the growing submerged internodes. *Total* submergence halts stem extension partly because photosynthesis is inhibited by a lack of carbon dioxide (Setter *et al.* 1989) but principally because leaf sheaths surrounding the stem act as a barrier to oxygen entry from the water, giving rise to the anaerobic conditions in which ETH production and action are prevented (Pearce *et al.* 1992).

*Submergence of germinating rice seeds and seedlings.* Total submergence of seeds such as wheat (*Triticum aestivum*), millet (*Eleucine coracanana*) and sorghum (*Sorghum caudatum*), in still water, completely prevents germination, even when the equilibrium partial pressure of oxygen in the bulk solution remains above zero (e.g. 11 kPa; Pearce & Jackson 1991). In contrast, rice germinates with equal rapidity in well aerated and hypoxic states, with the root emerging before the coleoptile. Furthermore, coleoptile elongation is promoted by submergence as a result of the actions of ETH and carbon dioxide entrapped by the water, and by small, but not extinguished, oxygen partial pressures (Ku *et al.* 1970; Raskin & Kende 1983b; Ishizawa & Esashi 1984a; Pearce & Jackson 1991). Ishizawa & Eshashi (1984a) showed that coleoptiles of submerged rice seedlings are indeed enriched with ETH and that a fourth factor (probably buoyant tension) enhances the growth response to the three gases. Buoyant forces may give the coleoptile elongation some sense of direction since the lack of starch-filled amyloplasts in submerged coleoptiles interferes with the normal gravitropic response that depends on amyloplast sedimentation (Kutschera *et al.* 1990).

The elongation-promoting effects of low oxygen and carbon dioxide are not mediated by increased ETH biosynthesis (Raskin & Kende 1983a,b; Pearce *et al.* 1992). However, the promoting effect of carbon dioxide is, nevertheless, dependent on the action of ETH since activity is abolished by applying norbornadiene (Ishizawa *et al.* 1988) that competes with ETH for at least two high affinity binding sites in rice seedlings (Sanders *et al.* 1990). The growth-promoting action of submergence and ETH in rice coleoptiles is associated with enhanced cell osmolarity (Kutschera *et al.* 1990) and increases in wall extensibility (Ishizawa & Eshashi 1984b), and with greater sucrose transport from the endosperm and scutellum to the coleoptile, where unloading is thought to be stimulated (Ishizawa & Eshashi 1988). Evidence that auxin is required for ETH-promoted extension is contradictory (see Jackson & Pearce 1991) although it is clear that exogenous auxin can promote coleoptile growth, both in its own right and through promoting ETH production (Ishizawa & Eshashi 1983).

Promotion of elongation by submergence in shoots is not restricted to coleoptiles of dark-grown seedlings. Extension by the true leaves of rice growing in the light can also be enhanced. The response occurs both in young seedlings at the three-leaf stage and in older plants at the tillering stage (Jackson *et al.* 1987). Premature senescence of older non-growing leaves by submergence may also be a response to entrapped ETH. Varieties of rice known to tolerate complete submergence for the longest time, exhibit the weakest growth response to ETH or submergence in terms of extension growth or senescence. This may indicate the metabolic cost of accelerated growth under water. Thus, when water is too deep for the elongated shoot to reach the surface, survival is compromised by the costs associated with the futile faster growth, and with leaf senescence (Jackson *et al.* 1987).

Elongation of the rice coleoptile is also accelerated and prolonged by the total absence of oxygen (Ohwaki 1967), a situation where ETH production is halted and where applications of ETH or norbornadiene have no effect on extension (Pearce *et al.* 1992), even though putative ETH receptor levels remain as normal (Sanders *et al.* 1990). Clearly, ETH plays no part in this anoxia-stimulated extension process. Auxin is also inactive in anoxic rice coleoptiles (Pegoraro *et al.* 1988). However, growth under

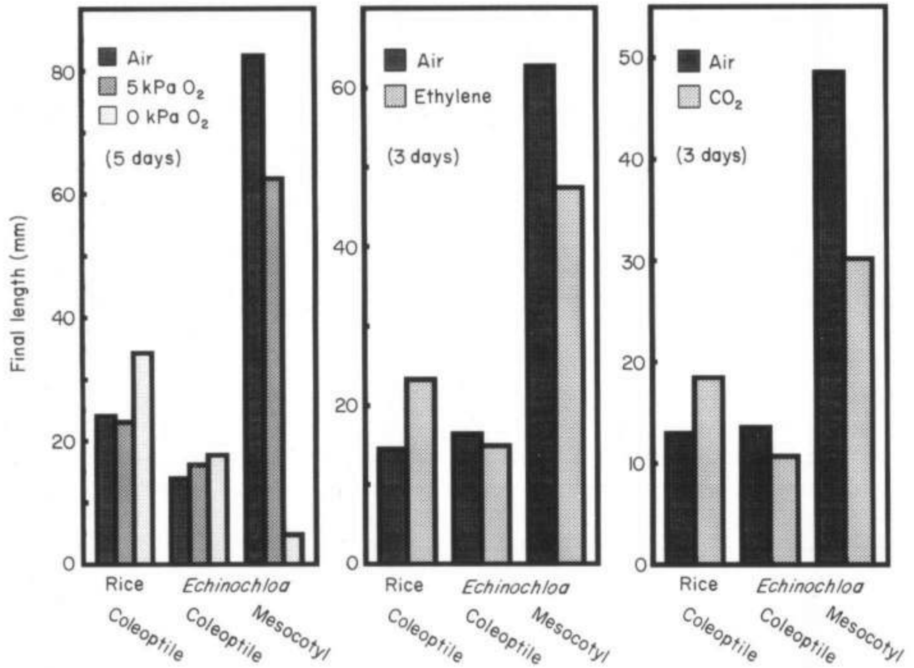


**Fig. 12.** Comparison of lengths of coleoptiles or mesocotyls of seedlings of rice or *Echinochloa oryzoides* exposed for 3 or 5 days to flowing gas mixtures containing 0 or 5 kPa oxygen in nitrogen, 0·1 Pa ethylene or 10 kPa carbon dioxide. Taken from Pearce & Jackson 1991.

anoxia is not entirely free of hormonal regulation. Reggiani *et al.* (1989) showed that putrescine accumulates in anoxic rice coleoptiles and promotes their extension. Horton (1991) has identified a strong inhibiting effect of ABA and also some activity by the auxin-transport inhibitor naphthylphthalamic acid (Horton 1993). Clearly, further studies of IAA and ABA accumulation and action in anoxic coleoptiles are needed (Mapelli & Bertani 1993).

Inhibition of ETH production by anoxia results in the accumulation of ACC (Pearce *et al.* 1992) as a consequence of no oxidation to ETH and enhanced transcription of an ACC synthase gene (Zarembinsky & Theologis 1993). The extra ACC is available for later oxidation to ETH when the coleoptile gains access to some oxygen. This ETH will help maintain fast extension rates (Pearce *et al.* 1992) and may also inhibit post-anoxic oxidative damage by stimulating ascorbate peroxidase activity (Melhorn 1990).

*Echinochloa oryzoides*, a form of barnyard grass, is a rice weed and can complete its life cycle in the same flooded conditions (Kennedy *et al.* 1980). However, comparative studies have revealed some surprising differences between rice and *E. oryzoides*. For example, barnyard grass produces a coleoptile of similar and considerable length (50–60 mm) in air, or under oxygen deficiency, or when enriched with ETH and carbon dioxide. However, the mesocotyl is inhibited by these conditions. Thus, overall, poor aeration lengthens the seedling shoot of rice and shortens but does not abolish shoot extension in barnyard grass (Fig. 12) The coleoptile of barnyard grass is a unique example of an aerobically elongating organ that is unable to respond to ETH.

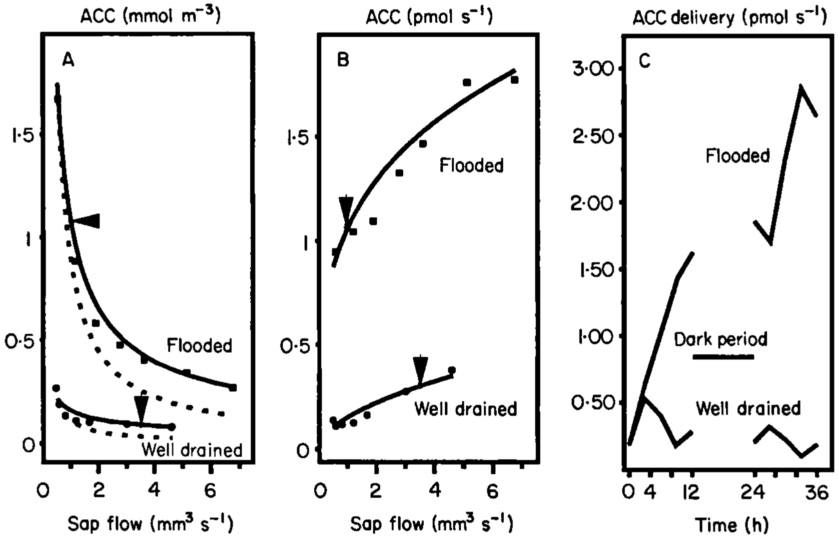


**Fig. 13.** Concentration and delivery rates of ACC in xylem sap flowing from excised roots systems of flooded and well drained tomato plants. (A) Effect of increasing flow of sap through detached root systems grown in well drained or flooded soil for 12 h on ACC concentration. Arrows show concentrations at sap flows comparable with transpiration of intact plants. (B) Effect of increasing flow sap on the delivery rate of ACC from welldrained roots, or roots flooded for 12 h. Arrows show delivery rates at sap flows comparable with transpiration of intact plants. (C) Time course of ACC delivery in sap induced to flow at whole plant transpiration rates. Sap flows were induced by applying increasing pneumatic pressures to the roots, up to a maximum of 0·4 MPa. Sap was taken for ACC analysis within a few minutes of removing the shoot system (Else *et al.* 1995).

### *Morphological adaptations in shoots of flooded plants*

Changes in the flow of hormones between roots and shoots can mediate in shoot responses to root stresses. ‘Positive’, ‘negative’, ‘accumulative’ and ‘debit’ messages can, theoretically, be involved (Jackson 1993). The transpiration stream is the most likely pathway for positive and negative messages, and hormones are good candidates as messenger solutes. One common difficulty, when evaluating claims for specific changes in solute content of xylem sap, is that published measurements are frequently in the form of concentrations rather than amounts moving out of the roots in a given time, i.e. delivery rate. However, concentration is a function of flow rate (Fig. 13a) and is thus unstable even when the amounts delivered from the roots are unchanged. Thus, unless the sap analysed flows at rates close to those of whole plant transpiration (arrows on Fig. 13a), the concentration will not be the same as that in the sap of intact plants because of dilution effects.

A related problem is that flooded plants often transpire more slowly than their well drained counterparts. Thus, even where differences in sap concentration between well drained and flooded plants are accurately estimated, they may simply arise as dilution effects rather than any real differences in output of hormones from the roots. Calculating rates of delivery, by multiplying concentration by sap flow, avoid most of these problems, particularly if sap is first induced to flow at whole plant transpiration rates (arrows in Fig. 13b) by applying an appropriate pneumatic pressure to the roots (Jackson 1993; Else *et al.* 1993, 1995).

*Epinastic curvature of leaves.* Epinasty in tomato plants (*Lycopersicon esculentum*) is the result of enhanced cell expansion on the adaxial surface of leaf petioles causing them to reflex downwards. Extra ETH, formed in the leaves (Jackson & Campbell 1975) by the enzymic oxidation of ACC transported in increased amounts from oxygen-deficient roots in the transpiration stream, promotes this phenomenon during the first 1–3 days of soil flooding (Bradford & Yang 1980a). Increased delivery of ACC from roots to shoots has recently been confirmed and estimates refined using physico-chemical analyses of ACC in sap induced to flow at rates of whole-plant transpiration (Fig. 13c). When ACC delivery is depressed by applying AVG to oxygen-deficient root systems, or if the oxidation of ACC to ETH in the shoots is blocked with cobalt chloride, shoot ETH production and epinasty are decreased in flooded plants (Bradford *et al.* 1982). Shoot tissues of species other than tomato also accumulate more ETH following soil flooding, e.g. *Vicia faba* (El-Beltagy & Hall 1974), *Phaseolus vulgaris* (Wadman-Van Schravendijk & Van Andel 1986), and *Rumex palustris* (Van der Sman *et al.* 1991).

The re-orientation of normally horizontal shoot growth by the diageotropic tomato mutant (Jackson 1979; Bradford & Yang 1980b) and by the stolons of the strawberry clover (*Trifolium fragiferum*) (Bendixen & Petersen 1962), are probably also responses to increased ETH resulting from greater flux of ACC from the anaerobic roots. Until they die, such roots are a rich source of ACC because a lack of oxygen prevents the oxidative conversion of ACC to ETH, and increases the level of gene transcription and activity of ACC synthase (Wang & Arteca 1992; Zarembinski & Theologis 1993).

ACC oxidase in the shoots is generally thought to be present in excess and, thus, not to limit ETH production rates. However, leaves of flooded tomato plants contain higher levels of ACC oxidase than those of well drained plants in association with faster ETH production and epinastic curvature. Furthermore, when accumulation of translatable mRNA for an ACC oxidase is inhibited by insertion of an antisense construct, the leaves of flooded plants give an attenuated epinasty response and synthesise less ETH than wild type counterparts (English *et al.* 1993) even though ACC delivery rates from roots remain unchanged by the antisense transformation. Thus, the last step in ETH biosynthesis appears to place a limit on production. This indicates that promotion of ACC oxidase activity in the leaves (English *et al.* 1993) is a part of the mechanism explaining flooding-enhanced ETH production.

*Stem hypertrophy and adventitious rooting.* In woody species, stem hypertrophy and adventitious rooting are often associated with hypertrophic swelling of lenticels (Fig. 14). The new roots are important for plant survival since removing them (Table 2) can depress shoot growth. Similarly, blocking the lenticels with lanolin interferes with internal aeration (Armstrong, 1968). The causes of the hypertrophy of the stem-base, and of lenticels, is probably ETH (Kawase 1981), which in sunflower plants, increases in concentration in this region (Kawase 1974; Wample & Reid 1979). The effect has been linked with increases in β-(1→4)- glucanase (cellulase) activity, that presumably weakens walls, thus favouring cell expansion (Kawase 1979). Hypertrophy is thought to prolong survival in flooded soils by increasing the porosity at the point of insertion of adventitious roots, thus enhancing aeration.

The formation of new adventitious roots is a much more complex process than hypertrophy and its regulation remains poorly understood. Once more, ETH has been implicated. Applying ACC, or the ETH-releasing compound ethephon, promotes rooting from sunflower hypocotyls (Liu *et al.* 1990). As with hypertrophy, merely

**Fig. 14.** Effects of 40 days' flooding on (above) the production of replacement adventitious roots at the base of stems of *Malus domestica* rootstock MM106, and (below) the development of hypertrophic lenticels. These were made clearer by removing the roots prior to photography (A.Q.M. Al-Husainy & M.B. Jackson, unpublished).

**Table 2.** Effect of daily removal of visible adventitious roots on shoots of *Helianthus annuus* plants grown in well drained or waterlogged soil for 20 days

| | Non-waterlogged | Waterlogged | Waterlogged with adventitious roots removed |
|---|---|---|---|
| Leaves | | | |
| Number | 27·8 ± 1·3 | 27·5 ± 1·3 | 27·4 ± 0·9* |
| Area ($cm^2$) | 1208 ± 52 | 952 ± 63 | 770 ± 71* |
| Fresh wt (g) | 41·0 ± 2·3 | 31·2 ± 2·4 | 22·8 ± 2·8* |
| Stem | | | |
| Height (cm) | 44·4 ± 5·3 | 37·0 ± 4·0 | 37·3 ± 3·3 |
| Hypocotyl diameter (mm) | 11·2 ± 0·5 | 17·1 ± 0·7 | 15·0 ± 0·8* |

Plants were 2 weeks old at the start.
Means of 8 replicates ± standard errors.
*Signficantly different from 'waterlogged' ($P$=0·05).
(A.Q.M. Al-Husainy & M.B. Jackson, unpublished).

trapping of ETH by a water covering, even when aerated, can stimulate root formation, although some oxygen shortage can enhance the effect in *Echinochloa phyllopogon* (Everard *et al.* 1991). Where flooding damages the original roots, the shoot is likely to be enriched with substances no longer exported to the roots because the phloem transport system is largely arrested. Auxin, well-known for promoting root initiation in stems, accumulates in this way (Phillips 1964; Wample & Reid 1979). A third, and largely untested idea is that root formation benefits from less CK and GA moving into the shoot from flooded root systems (see below). In support of this, applying these hormones to flooded plants suppresses root formation in flooded intact tomato plants (Selman & Sandanam 1972), although detailed tests with a range of hormones on 6-day-old de-rooted sunflower seedlings (Fabijan *et al.* 1981) presents a more confusing picture. In view of the significance of adventitious root formation for the survival of flooded plants, it is surprising that a satisfactory explanation for the stimulation by flooding has not been forthcoming.

In plants which already have pre-formed adventitious root primordia, root emergence is stimulated by flooding. In monocots such as maize (Jackson *et al.* 1981) and woody plants such as willow (*Salix fragilis*) (Kawase 1972) ETH favours this outgrowth, although subsequent root extension is inhibited.

*Stomatal closure.* In many species (e.g. *L. esculentum*, *Pisum sativum*, *Phaseolus vulgaris*), flooding of the soil can close somata and this is usually associated with increased concentrations of ABA in leaves. A causal involvement of ABA is indicated by the attenuated stomatal response in mutants with defective ABA biosynthetic pathways (Jackson & Hall 1987), and by tests showing that applying sufficient ABA to raise internal levels to those of flooded plants reduces leaf conductances (Jackson & Hall 1987; Neuman & Smit 1991). Where flooding induces wilting, as happens in *P. vulgaris* (Wadman-van-Schravendijk & van Andel 1985), the extra ABA is probably synthesized in the leaves in response to the water deficit. In several other species, sustained stomatal closure and the associated high ABA levels take place while leaf hydration is little changed or even increased. Thus, some explanation other than loss of leaf hydration is needed to explain the maintenance of stomatal closure and elevated shoot ABA levels. One possibility is that oxygen-starved roots export ABA in increased amounts as a positive message. In support of this, Zhang & Davies (1987) measured increases of 100–700% in ABA in roots of flooded pea plants during the second, third and fourth days of flooding, when foliar ABA was also increasing. The presumed carrier of the root-sourced ABA into the leaves is the transpiration stream and Neuman & Smit (1991) found xylem sap ABA concentration to increase in flooded *Phaseolus vulgaris*. Unfortunately, this result is almost entirely explicable in terms of less dilution arising from slower sap flow rates in flooded plants rather than greater export of ABA from flooded roots (Jackson 1993). A preliminary report that flooding increases ABA delivery from roots to shoots of tomato (Jackson 1993) has proved erroneous because compounds present in flooded plants show similar behaviour to ABA in the immunoassay used. Most other published evidence is against the notion of flooded roots enriching the shoots with ABA. For example, in plants of a *Populus* hybrid, Smit *et al.* (1990) found a decrease in ABA delivery in xylem sap, after 24 h and 48 h of flooding. Similarly, mass spectrometric analyses of ABA delivery in flooded tomato plants have shown decreases in delivery when measurements were made using sap flowing at transpiration rates (Else *et al.* 1994, 1995). This result is compatible with the known requirement for oxygen by the ABA biosynthetic pathway (Zeevaart *et al.* 1991). It is also compatible with reports that ABA does not increase in flooded or anaerobic roots (Wadman-van-Schravendijk & Van Andel 1985; Jackson *et al.* 1988; Shih-Ying & Van Toai 1991), and that grafting ABA-deficient root systems onto wild type shoots decrease neither ABA accumulation in shoots, nor stomatal responses to soil flooding (Jackson 1991).

Since ABA responsible for closing stomata in flooded plants is unlikely to come from the roots it must be of shoot origin. It is probably an accumulation message which builds up in the leaves because phloem export to regions of normally high sink strength are depressed by flooding. However, petiole girdling and related experiments that try to simulate the effect, have met with mixed success (Jackson & Hall 1987; Smit *et al.* 1990; Reece & Riha 1991).

*Stem elongation, leaf expansion and senescence.* It is widely believed that root systems supply the shoots with CKs and GAs needed for growth and leaf longevity. Much of the evidence supporting this view comes from experiments showing that root stresses, such as flooding, decrease the concentrations of these hormones in xylem sap exuding from the cut stump of detopped plants (reviewed in Jackson 1993). Carr & Reid (1969) showed delivery of bioassayed CK activity from roots to decline by 25% during the first 2 days of waterlogging when stem extension by sunflower plants had almost stopped. After 3 days, when lower leaves started to yellow, CK concentration in sap was reduced by 37%, and by 94% after 4 days, when leaf yellowing became more pronounced. However, the idea that the leaf senescence was a result of a shortfall in cytokinin supply from the roots is questioned by the observation that detaching leaves to deprive them of root CKs did not promote their rapid senescence (Barrows & Carr 1969). Neuman *et al.* (1990), using mass spectrometry, confirmed that hypoxic roots of *Poplar* and *P. vulgaris* export only about one-third of the normal amount of the CK zeatin riboside. However, leaf concentrations were not affected by this smaller delivery from the roots. These authors also found that inhibition of stomatal apertures, or of leaf expansion, caused by flooding could not be overcome by supplying zeatin riboside exogenously to make good the shortfall in endogenous hormone. This work suggests that leaf expansion may be limited more by an inhibitor such as ABA or ETH rather than the lack of cytokinin. More work is needed before a convincing case can be made for believing that a decrease in root CKs is damaging for shoot growth of flooded plants. However, there is evidence that photosynthesis may be limited by decreased ribulose bisphosphate activity brought about by a shortage of CKs (Bradford 1983). This possibility deserves further examination.

Evidence that a lack of GAs supplied by roots is physiologically significant for shoot growth of flooded plants is also weak. The level of gibberellin-like activity in bleeding xylem sap of flooded tomato plants is substantially decreased by 1–3 days of waterlogging (Reid & Crozier 1971) but the extent to which this affects stem extension is uncertain. Disquieting aspects of the evidence include the mismatch between changes in the levels of GA activity in the xylem sap and those of the leaves (see also Neuman *et al.* 1990), and results showing that flooded plants are no more responsive to exogenous GA that their well drained counterparts (Reid & Crozier 1971; Selman & Sandanam 1972). Indeed, the opposite is sometimes the case, suggesting that the principal constraint on stem extension is not GA deficiency but rather a reduced capacity to respond to the GA available. Hormones, such as ABA and ETH, may contribute to such an effect.

## CONCLUDING REMARKS

We have reviewed a wide range of metabolic and morphological responses of plants to impeded aeration and the complex physical processes that drive aeration processes. It is important that this large body of information does not obscure the central issue in this review, which is that no higher plant can survive for more than a few hours, days or weeks at most, without gaining access to molecular oxygen or becoming dormant. Four main questions have been dealt with in this connection. Firstly, what are the metabolic 'lesions' that kill plant cells either during anoxia or when they are returned to oxygenated conditions? Secondly, what is the metabolic explanation for the ability of some plant tissues to survive or even grow without oxygen for longer (albeit limited) periods than others? Thirdly, what are the developmental processes that enable inundated plants or plant parts to gain or re-gain access to oxygenated zones quickly enough to prevent their asphyxiation. Fourthly, what are the physical processes, and their interplay with metabolism, that are exploited by plant adaptations to bring about the ventilation of inundated tissues. These four questions relate principally to those parts of the plant which suffer directly from oxygen shortage (e.g. roots or rhizomes in flooded soil and submerged shoots). We have also considered several adaptive responses in parts of the plants not directly exposed to oxygen deprivation. For example, the stomatal closure and leaf epinastic curvature frequently seen in shoots when the roots are subjected to sudden soil waterlogging. Here, the issue has been to identify and quantify the root to shoot communication processes that carry morphogenetically active messages to target tissues some distance from the inundated organs.

Specific progress often stems from an appropriate combination of a sound conceptual framework and new technologies that allow hypotheses to be tested more definitively than hitherto. There can be little doubt that studies of flood tolerance in plants will benefit increasingly from molecular biological methods. These can be expected to (i) reveal genes and gene products that are involved in key responses to poor aeration, (ii) provide probes for locating precisely which cells are responding to stress and when, and (iii) enable roles for particular genes and the enzymes for which they code to be identified by manipulating promoter and structural components of genes and by the use of anti-sense technology. Equally promising is the rapid development of techniques for studying whole plants with a high level of precision and sensitivity but with the minimum disturbance to the plant. These include non-invasive nuclear magnetic resonance for studies of intracellular biochemistry (Fahn, Lane & Higashi 1993), microprobes for measuring inter- and intra-cellular oxygen (Armstrong 1994), photoacoustic laser detection of minute amounts of ethylene (Voesenek *et al.* 1990b), gentle pressurization techniques for obtaining xylem sap samples at chosen sites within the canopy of whole plants (Else *et al.* 1994), and highly sensitive immunoassays for hormones for which only very small tissue samples are needed. Last, but by no means least, is the availability of computing power to build mathematical models that create an interactive quantitative framework to .physiological notions of cause and effect. The past decade has seen enormous strides being made in the understanding of flood tolerance mechanisms, the next decade should see a number of these mechanisms introduced or intensified in non-wetland crop species, through genetic engineering or selection.

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