Chapter 8

Oxygen Transport, Respiration, and Anaerobic Carbohydrate Catabolism in Roots in Flooded Soils

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Summary

Soil flooding is a severe abiotic stress for many plant species (e.g., most crops), whereas well adapted species (e.g., rice (*Oryza sativa*) and other wetland species) usually thrive. Flooded soils are usually anaerobic, so that internal O_2 transport from shoot to roots is crucial for sustaining respiration in submerged organs. Formation of aerenchyma, together with a barrier impermeable to radial O_2 loss in basal zones of roots, act synergistically to enhance O_2 diffusion to the apex of the main axis of roots of many wetland species. In some situations the soil is not anoxic, but provides a restricted O_2 supply to the roots. For roots with aerenchyma, O_2 is usually much more readily available from the intercellular gas-filled pathway, than exogenously from the soil. In both types of situations some cells/tissues may become anoxic, especially the apical regions and the stele since these are at the ends of the longitudinal (gas phase) and radial (predominately liquid phase) diffusion paths, respectively. Anoxia in root tissues becomes even more likely when shoots of plants are submerged by floodwaters. The inhibition of oxidative phosphorylation due to anoxia causes a severe energy crisis. Tolerance of anoxia requires a carbohydrate supply to fuel anaerobic catabolism, and apportionment of the scarce available energy to processes essential to survival, whereas several energy-consuming processes typical for aerobic cells

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may be reduced. In addition to O_2-deficiency, roots in waterlogged soils must also tolerate high CO_2 partial pressures (e.g., up to 43 kPa); however, information on this topic is scant.

I. Introduction

Flooded soils are usually anaerobic (Ponnamperuma, 1984); plants then depend on internal transport of O_2 into the roots, from the shoots still in contact with the atmosphere (Section III.A). When shoots are completely submerged, and in submerged aquatic plants, O_2 derived from photosynthesis in the shoots moves to the roots growing in anaerobic substrates. There are also situations where soils become deficient in O_2 (i.e. hypoxic) rather than anoxic (Section II), so that roots obtain some O_2 from the external medium as well as O_2 from the shoots via diffusion in intercellular gas-filled spaces within the roots. In both types of situations, the apical regions and the stele of roots may receive a sub-optimal O_2 supply, since these are at the ends of the longitudinal (gas phase) and radial (predominately liquid phase) diffusion paths, and both the stele and apex usually have fast rates of respiration (Sections III.A and III.C). The cessation of oxidative phosphorylation during anoxia results in a severe energy deficit, so that anoxia tolerance is presumably required in at least some tissues/organs of plants inhabiting flood-prone environments (Section IV). Unless the roots can access O_2 from the shoots, cells of many species begin to die within a few hours or days (Gibbs and Greenway, 2003).

Soil O_2 deficiency results from the consumption of O_2 by plant roots and soil organisms without appreciable replacement, since the flux of O_2 into soils is ~320,000 times less when flooding occurs, due to the 10^4-fold slower diffusivity and the 32-fold lower solubility of O_2 in water than in air (Armstrong and Drew, 2002). The anaerobiosis occurs in the bulk soil, and not necessarily in the rhizosphere that, in some species, receives substantial O_2 due to radial O_2 loss (ROL) from the interconnected gas-space system in plants (Section III.B). In addition to O_2 deficiency, flooding also changes other factors that may influence the physiology of plants; CO_2 and ethylene (C_2H_4) both accumulate. The P_{CO_2} increased from 1.8–8 kPa in aerobic soil to 18–43 kPa within two weeks of flooding pots containing several rice paddy soils (Ponnamperuma, 1984). In rhizomes of *Phragmites australis* growing in flooded soil, P_{CO_2} was up to 7.4 kPa (Brix, 1988). Furthermore, anaerobic metabolism by soil micro-organisms may produce reduced compounds, such as Mn^{2+}, Fe^{2+}, S^{2-} and carboxylic acids (Ponnamperuma, 1984; McKee and McKevlin, 1993) that are toxic to plants.

The conditions in flooded soils have profound effects on metabolism in roots; the responses of plants being dependent upon species, tissue, and other environmental variables (e.g., temperature). Possible adaptations, acclimations and responses of plant roots to soil flooding are summarized in Fig. 1. There are dramatic differences among species in tolerance to soil flooding. Intolerant species, like pea (*Pisum sativum*), are severely injured within one to four days following the onset of waterlogging (Jackson, 1979); by contrast, wetland species survive, and in many cases grow vigorously, in waterlogged soils (Justin and Armstrong, 1987). Paddy rice (*Oryza sativa*), with water depths maintained between 50 and 100 mm above the soil till seven days before maturity, yielded 8.4 and 13.6 tonnes ha^{-1} in the tropics and subtropics, respectively (Ying et al., 1998). Such yields are impressive, since rooting depths in paddy fields typically do not exceed 300 mm (Section III.A.2). Thus, although soil flooding is a severe abiotic stress for many plant species, well adapted species can thrive in such conditions.

For plants with shoots in air, O_2 will diffuse at least some distance into the roots along intercellular gas-filled spaces and aerenchyma (Section III.A). Aerenchyma refers to large interconnected gas channels that greatly enhance O_2 movement into submerged portions of plants. The P_{O2} within aerenchymatous roots decreases in a curvilinear gradient with distance from the root-shoot junction, since O_2 is consumed along the diffusion pathway (Armstrong, 1979; Armstrong et al., 2000). Furthermore, gradients in $[O_2]$ also occur in the radial direction, due to uptake of O_2 by the cells along the diffusion path (Fig. 2); the steepness of the gradients will depend upon the porosity and O_2-consumption rates in the

Abbreviations: ABA – abscisic acid; AEC – adenylate energy charge = [ATP + 0.5 ADP] / [ATP + ADP + AMP]; COP – critical O_2 pressure; P_{CO_2} – CO_2 partial pressure; pH_{cyt} – cytosolic pH; pH_{vac} – vacuolar pH; P_{O_2} – O_2 partial pressure; Q_{10} – (rate of reaction at temperature T + 10 °C) / (rate of reaction at temperature T); RGR – relative growth rate; ROL – radial O_2 loss

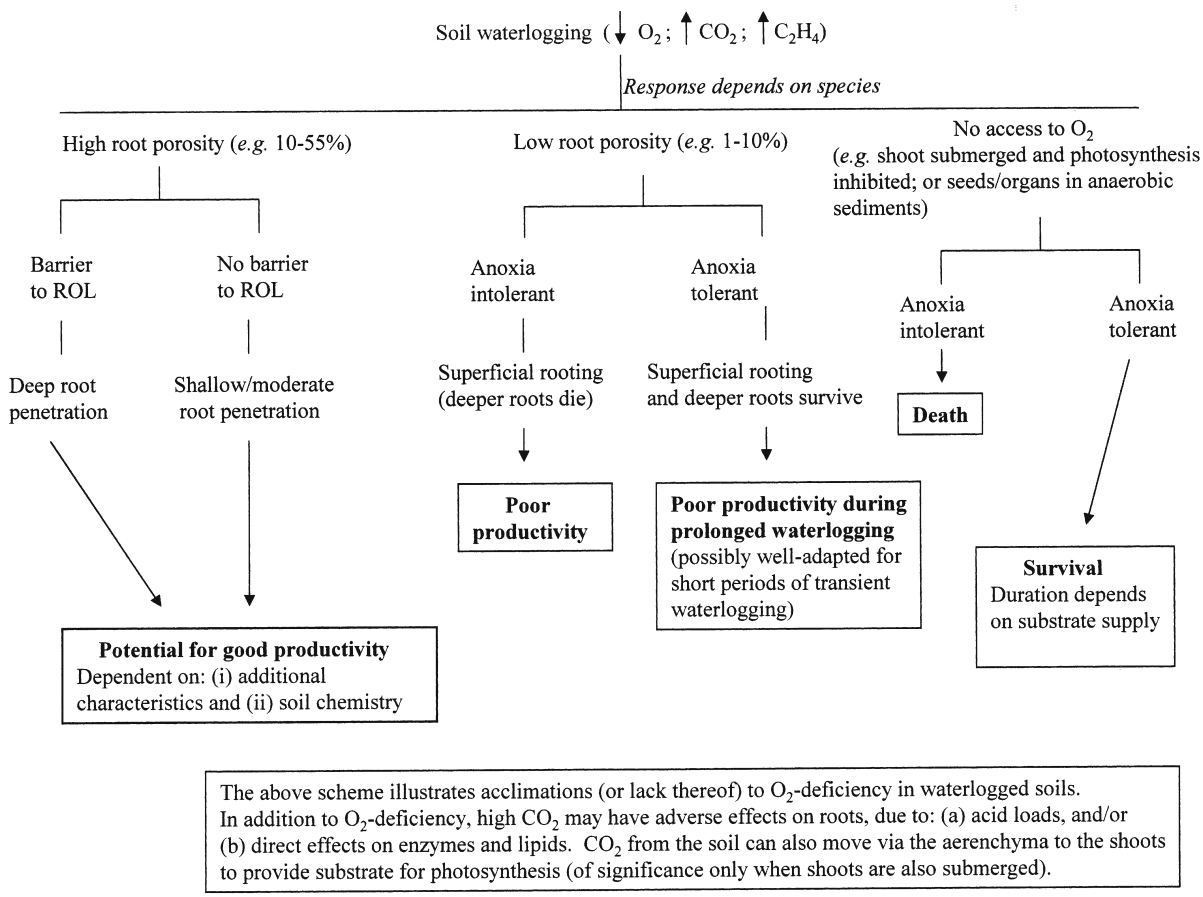

Fig. 1. Scheme showing possible responses, adaptations and acclimations of plants in response to soil flooding.

various tissues. Thus, parts of the root systems and even some tissues within individual roots of plants in flooded soils may suffer anoxia, others will experience sub-optimal [O_2], while yet others may remain fully aerobic, depending on the soil O_2 status, capacity for internal O_2 movement, and root morphology; e.g., surface roots may have access to O_2 while deeper roots suffer anoxia.

Anoxia tolerance might be an important adaptation contributing to the ability of some plants to persist in flood-prone environments (Section IV). Plant species, and organs/tissues, show large differences in tolerance of anoxia (Gibbs and Greenway, 2003). For example, tips of roots of *P. sativum* died within 7 hours, and even with exogenous glucose these tips died after 24 to 36 hours of anoxia (Webb and Armstrong, 1983). On the other hand, rhizomes of the marsh species *Acorus calamus* survived for at least 90 days (Crawford and Braendle, 1996). Glycolysis linked to ethanolic fermentation is the predominant pathway for anaerobic carbohydrate catabolism during anoxia in most plant species (ap Rees et al., 1987), and provides at least some ATP during anoxia (Section IV). Anaerobic carbohydrate catabolism, together with other essential traits (Section IV.A), enable survival during anoxia; the period of survival depending on a supply of carbohydrates, the species, and tissue or organ. Growth during anoxia has only been found in a few cases; shoot extension in anoxia relies on fast rates of anaerobic carbohydrate catabolism fuelled by remobilization of carbohydrate reserves (e.g., coleoptiles of *O. sativa*, Atwell and Greenway, 1987; Perata et al., 1998; stems of *Potamogeton* spp., Summers et al., 2000; Harada and Ishizawa, 2003). Shoot extension enables submerged plants to reach flowing water containing O_2 or adequate light for photosynthesis, or enables the shoot to emerge and establish contact with the atmosphere (Ridge, 1987;

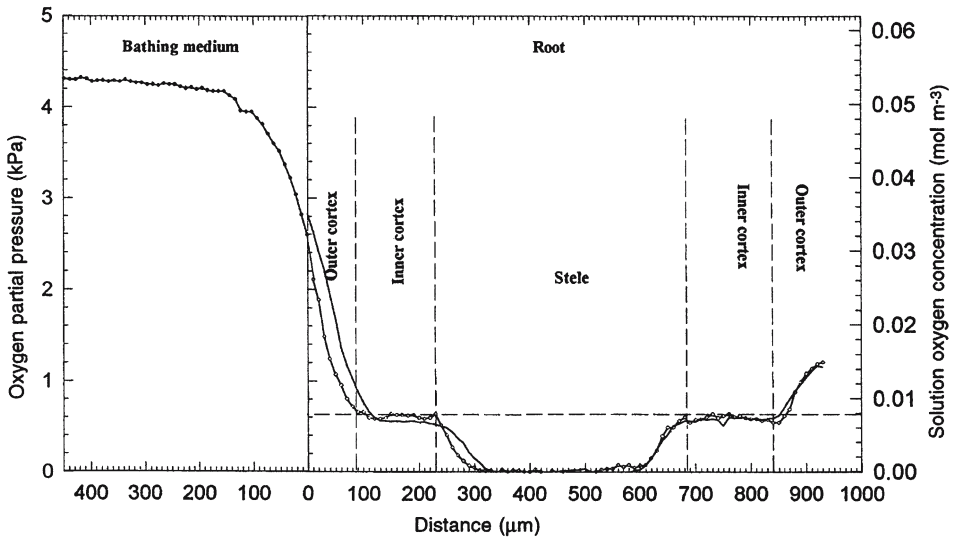

Fig. 2. Radial profile of O_2 partial pressures across an excised primary root of *Zea mays* when in a flowing nutrient solution containing 0.054 mol m^{-3} O_2, at 25°C. Measurements were taken by driving an O_2 microelectrode in steps through the root (75 mm behind the apex, total length 135 mm). The line with symbols is the in-track, and that without symbols is the out-track. Reproduced from Gibbs et al. (1998) in Australian Journal of Plant Physiology with permission of CSIRO Publishing (© CSIRO, 2002).

Voesenek and Blom, 1989). Movement of O_2 from shoot to roots via aerenchyma enables oxidative phosphorylation and development of an aerobic rhizosphere, enhancing root growth and functioning in anaerobic substrates.

This chapter: (i) summarizes knowledge on root aeration, emphasising the importance of both aerenchyma and a barrier to radial O_2 loss (ROL) for internal O_2 movement to the apex of roots in flooded soils; (ii) considers the evidence for, and consequences of, some tissues/cells in roots (e.g., stele and apical regions) being hypoxic/anoxic, while adjacent regions receive adequate O_2 for oxidative phosphorylation, depending on positions relative to the longitudinal (gas phase) and radial (predominately liquid phase) diffusion paths; (iii) evaluates possible limitations of carbohydrate supply to fuel catabolism in roots, as related to anoxia tolerance, and (iv) provides some speculation on the possible effects on respiration in roots of high P_{CO2} typical for flooded soils.

II. Soils with Low, But Not Zero, O_2

In many situations flooded soils become anoxic, and then roots depend completely on internal transport of O_2 from the shoots (Section III.A). However, soils may become hypoxic, rather than anoxic. Soil O_2 deficiency, as opposed to soil anoxia, has been reviewed comprehensively by Drew (1992). This chapter only addresses some key aspects.

Upon flooding, the rate of O_2 depletion from the soil solution and entrapped air pockets will depend on temperature and activity of micro-organisms and plant roots, so depletion will be slow at low temperatures and low content of organic matter (Drew, 1992). For soils in England, waterlogged in different seasons, O_2 almost reached zero at 200 mm depth after 10 to 15 days in January (soil temperature was 4 °C) and after five to six days in May (soil temperature was 11 to 12 °C) (Cannell et al., 1980). In a clay soil during spring in Australia (soil temperature was 11 to 13 °C), O_2 at 125 and 250 mm was depleted within two to four days after flood irrigation (Meyer et al., 1985). The depletion of O_2 over time may allow roots to acclimate prior to the onset of anoxia (Gibbs and Greenway, 2003), and/or be important for survival of roots during the time required to form aerenchyma (Thomson et al., 1990). Other situations of moderate O_2 deficiency in soils were documented for a compacted soil with low air-filled porosity (Blackwell et al., 1985), in a wetland soil with a significant flow of water through the profile (Armstrong and Boatman, 1967), and even in soils under daily trickle irrigation (Meek et al., 1983). Oxygen deficiency would not only develop during transient waterlogging, but

also persist for some time following drainage, particularly in fine-textured soils in which the O_2 flux can remain low for two to four days after drainage (Blackwell, 1983).

In addition to the situations summarized above, Setter and Belford (1990) hypothesized that in some cases O_2 may be available to roots in flooded soils if substantial ROL occurs from roots of neighbors. These authors suggested that O_2 movement through the aerenchyma (being up to 70% of root cross-sections) in winter-dormant kikuyu grass (*Pennisetum cladestinum*) might provide O_2 to roots of subterranean clover (*Trifolium subterraneum*) growing during the wet winter; this species has a relatively low root porosity (e.g., 10 to 14% in roots in hypoxic solution; Gibberd et al., 2001). Unfortunately, the evidence is only anecdotal. Investigation of this possibility is warranted, since this could be a hitherto unrecognized mechanism by which plants with little aerenchyma could grow in flooded soils.

The best method to evaluate the capacity of a soil to supply O_2 to roots is by measurements of O_2 fluxes using bare Pt electrodes, as developed by Blackwell (1983). Using intact plants, the extension rate of oat (*Avena sativa*) roots in flooded soil at 10°C, was decreased by 80% once O_2-flux rates dropped below 0.25 μmol m^{-2} s^{-1}, a value close to the O_2 flux required to achieve maximum respiration rates in excised root tips at 10 °C (Blackwell and Wells, 1983). To compare with units of P_{O2}, and the commonly used terminology 'critical O_2 pressure' (COP), soil O_2 was 15 kPa when the minimum threshold of O_2 flux to sustain root extension was reached (Blackwell and Wells, 1983). The threshold rate of O_2 flux for maximum root extension was determined by measurements taken while O_2 was depleted following soil flooding (Blackwell and Wells, 1983). That is, the system was not in a steady-state, and so the roots might not have fully acclimated to the lower $[O_2]$ (see Drew, 1997; Gibbs and Greenway, 2003). A steady-state was achieved for seminal roots of *Z. mays* in a chamber perfused with nutrient solution at selected $[O_2]$ (Gibbs et al., 1998). At 4 to 5 kPa O_2, O_2 fluxes and root-extension rates were reduced to about half of those at 21 kPa O_2; and the COP for root extension would have been ~10 kPa. These two estimates of COP for extension of roots with exogenously supplied O_2 are one order of magnitude higher than that determined for roots of *O. sativa* with O_2 supplied endogenously via aerenchyma (Armstrong and Webb, 1985). The higher COP for exogenous O_2 is a consequence of the large apparent resistance to O_2 diffusion between a bulk external solution and the interior of roots (Fig. 2). The apparent resistance to radial O_2 diffusion into roots results approximately equally from resistance to diffusion across liquid-phase boundary layers adjacent to roots and 'resistance' across the epidermis/hypodermis; the latter includes a component due to O_2 consumption by these cell layers as well as a physical resistance. Thus, internal O_2 diffusion from the shoots to the root tips, through gas-filled spaces, can be significant for preventing O_2 deficiency, even when some O_2 is present in the external medium.

Importantly, substantial functioning (e.g., nutrient uptake) of seminal roots of *Triticum aestivum* occurred at $[O_2]$ that severely inhibited growth (Kuiper et al., 1994). These seminal roots would have consumed 10 to 35% of the amount of O_2 from the rooting medium when compared to that in aerated controls, as deduced from respiration rates of excised root segments when measured at a range of external $[O_2]$. The roots would also have received some O_2 from the shoots, but this would presumably have been restricted to the upper parts, since seminal roots of *T. aestivum*, at the growth stage tested by Kuiper et al. (1994) did not form aerenchyma when plants were transferred from aerated to O_2-deficient solution (Thomson et al., 1990). Relative growth rates (RGRs) of these O_2-deficient roots were only 33% of those of aerated controls (Kuiper et al., 1994), and much of the dry mass increment in the O_2-deficient roots was probably due to carbohydrate accumulation (Section IV.B.1). Nevertheless, at 11 days after commencement of the low-O_2 treatment the net uptake rates of K^+ and NO_3^- by the seminal roots were still appreciable, being 80% and 47%, respectively (Kuiper et al., 1994). For roots in hypoxic solution, the stele is likely the first tissue to suffer anoxia (Section III.C), a condition that can inhibit xylem loading and therefore solute transport to the shoots (Gibbs et al., 1998).

III. 'Avoidance' of Anoxia: Internal Aeration in Plants

A. Aerenchyma and O_2 Movement

1. Diffusion of O_2 within Roots

When in an anaerobic medium, the capacity for internal O_2 transport will largely determine respiratory activity and therefore energy status in roots,

and, in the longer term, the survival, functioning and growth of roots. Aerenchyma, together with non-aerenchymatous intercellular gas-filled spaces, provides a pathway for movement of gases between shoots and the root tips (Armstrong, 1979; Jackson and Armstrong, 1999; Colmer, 2003a). In roots of many wetland species, aerenchyma is constitutive, and its volume is often further enhanced by soil flooding (Justin and Armstrong, 1987). Formation of aerenchyma following the onset of root-zone O_2 deficiency can take a few days (e.g., adventitious roots of *T. aestivum*, Malik et al., 2003). Roots of some species do not form aerenchyma (e.g., *Brassica napus*, Voesenek et al., 1999), or lose the capacity to form aerenchyma with age/developmental state (e.g., *T. aestivum* seminal roots longer than 100 mm; Thomson et al., 1990). Porosity in roots (i.e. gas-filled intercellular spaces plus aerenchyma) varied from below 1% in some non-wetland species to as much as 53% in a wetland species, for plants grown in flooded potting mix (Justin and Armstrong, 1987). Oxygen moves by diffusion in the aerenchyma in roots (Armstrong, 1979). Convective flows can occur along the shoots and rhizomes of several emergent and floating-leaved wetland species (Section III.A.2), yet even in these species O_2 movement into and along roots is diffusive (Beckett et al., 1988).

The importance of internal O_2 diffusion for maintaining respiratory metabolism in roots when in an anaerobic substrate was demonstrated by measurements of adenylate energy charge (AEC) in root tips of *Z. mays* seedlings transferred into an O_2-free medium after different pre-treatments resulting in roots with, or without, aerenchyma (Drew et al., 1985). Seventy five min after transfer of the intact roots into an O_2-free medium, the AEC in 5 mm root tips was ~0.7 for roots with aerenchyma (porosity 12.7%), compared with ~0.4 in those without aerenchyma (porosity 3.7%). AEC was 0.9 in excised root tips when aerobic (Drew et al., 1985).

Mathematical models predict the dependence of root penetration into anaerobic substrates upon internal O_2 diffusion (Armstrong, 1979; Armstrong and Beckett, 1987). The maximum length (ι) of a root dependent upon internal O_2 diffusion is determined by (Armstrong, 1979):

$$\iota = \sqrt{\left(\left(2D_o\tau\varepsilon\left(C_o - C_1\right)\right)/M\right)} \qquad (1)$$

where D_o = O_2 diffusivity in air, τ = tortuosity factor, ε = the fractional root porosity, $C_o = P_{O_2}$ in air at the root-shoot junction, $C_1 = P_{O2}$ in air spaces just behind the apex at which growth would cease, M = rate of O_2 consumption by the root tissue. Armstrong (1979) cautioned that the assumptions (radially homogeneous tissue, uniform O_2-consumption rates and porosity along the root, absence of ROL) would rarely hold; nevertheless, the equation provides a useful 'benchmark' against which experimental data can be interpreted (Thomson et al., 1992; Gibberd et al., 2001; McDonald et al., 2001).

Equation (1) highlights the importance of fractional root porosity and rates of O_2 consumption on the potential length of the O_2-diffusion path. The importance of high porosity for root growth was shown in a study of 91 species grown in flooded potting mix; species with roots of ≤5% porosity penetrated 30 to 95 mm, whereas those with ≥35% porosity reached 150 to 345 mm (Justin and Armstrong, 1987). Soil temperature is predicted to have a marked effect on the distance O_2 reaches within roots (Armstrong, 1979), since cooler conditions would decrease rates of O_2 consumption along the diffusion path; Q_{10} for O_2 diffusion being 1.1 while for respiration short-term Q_{10} values are 2 to 3 (Chapter 7, Atkin et al.). However, respiration in some species or in some conditions acclimates to changes in temperature (Atkin et al., 2000; Chapter 7, Atkin et al.), so that in these cases the longer-term effect of temperature on the maximum lengths of roots in anaerobic substrates may be less than expected based on the Q_{10} values given above. The possibility that roots might acclimate to hypoxia by decreasing O_2-consumption rates is considered in the next paragraph.

Lambers and Steingrover (1978) hypothesized that roots of waterlogging-tolerant species might decrease rates of O_2 consumption in response to hypoxia, by reduced activity of the alternative oxidase. In leaves of *O. sativa* seedlings submerged in darkness for 24 hours, declines in mRNA levels of nucleus-encoded respiratory genes (coding for alternative oxidase and cytochrome c oxidase) were observed, whereas levels of transcripts of mitochondria-encoded cytochrome c oxidase genes were maintained (Tsuji et al., 2000). For roots of *Hordeum vulgare* seedlings in N_2-flushed solution (with shoots in air), alternative oxidase protein and capacity (assayed for isolated mitochondria) decreased to very low levels, whereas mRNA levels (assayed for whole roots) were relatively constant, indicating possible translational regulation of alternative oxidase protein levels (Szal et al., 2003). Capacity of the cytochrome pathway

was the same for mitochondria isolated from aerated or N_2-flushed roots (Szal et al., 2003). By contrast, anoxia increased alternative oxidase protein level in cultured cells of *Glycine max* (Amor et al., 2000). The adaptive benefit of reduced activity of the alternative oxidase in roots dependent on O_2 supply via aerenchyma (i.e. in 'hypoxic' roots), would be that reduced consumption of O_2 would enhance its diffusion further into the root system before being exhausted. On the other hand, increased expression of the alternative oxidase in anoxia has been suggested to assist in protection against oxidative stress during re-aeration (Amor et al., 2000). In roots of *H. vulgare*, alternative oxidase protein increased again within 24 hours after returning seedlings from N_2-flushed to aerated solutions (Szal et al., 2003). In order to reach more definitive conclusions regarding the adaptive significance of these possible changes in alternative oxidase activity for roots of plants in flooded soils, the responses described above, and in vivo activities of the alternative and cytochrome pathways measured using the ^{18}O-fractionation technique (Chapter 3, Ribas-Carbo et al.), should be evaluated in roots of a wider range of flooding-tolerant and -intolerant species when exposed to a range of $[O_2]$. Rates should be assessed on a tissue fresh mass or on a protein basis, since formation of aerenchyma will reduce rates on a tissue volume basis, while accumulation of non-structural carbohydrates (Section IV.B.1) can be large enough to give a misleading impression when rates are expressed on a dry mass basis.

In addition to higher porosity and lower rates of O_2 consumption, other morphological and anatomical features also enhance the capacity for longitudinal O_2 diffusion in roots. (i) Roots of larger diameter have a lower diffusive resistance per unit length, compared with roots of smaller diameter (Armstrong, 1979; Armstrong et al., 1982). (ii) Lateral roots consume O_2 from the aerenchyma in the parent root, thus lowering P_{O_2} in the aerenchyma, and decreasing diffusion to the apex of the main axis (Armstrong et al., 1983). However, if laterals emerge near the base of roots of high porosity, O_2 consumed by the laterals might have little impact on the P_{O_2} in the root base, and thus on O_2 diffusion to the tip of the main root axis (Armstrong et al., 1990). (iii) Roots with a narrow stele would consume less O_2 per unit length of the diffusion path in the aerenchyma that leads to the tip of the main axis, than roots with a relatively thick stele (Armstrong and Beckett, 1987). Respiration in the stele can be relatively fast; e.g., ~3-fold faster on a volume basis than in the cortex plus outer cell layers in roots of *Z. mays* (Armstrong et al., 1991b), and several wetland species have a relatively narrow stele (McDonald et al., 2002). (iv) A barrier to ROL in the cell layers exterior to the aerenchyma, as shown in the basal zones in roots of numerous wetland species (Armstrong, 1979; Colmer, 2003a), would diminish O_2 losses to the rhizosphere, and thus enhance longitudinal diffusion toward the tip of the main axis (Section III.B). Plant species show much variation in the expression of these morphological and anatomical traits, with a combination of these being expressed in many wetland species, and not, or to a lesser extent, in dryland species (Justin and Armstrong, 1987; McDonald et al., 2002; Colmer, 2003a; Garthwaite et al., 2003).

2. Through-flows of Gases along Rhizomes of Wetland Plants

Convective flows can transport O_2 (and other gases) along the shoots and rhizomes of several emergent and floating-leaved wetland species, but only when pressure gradients are established along the aerenchymatous pathway, and when there is a low-resistance exit to the atmosphere, enabling through-flows (Beckett et al., 1988). For example, in the yellow waterlily (*Nuphar luteum*) growing in 1.5 m of water, flow rates of gases within the aerenchymatous petioles were 40 to 56 ml min^{-1} (Dacey, 1981); these impressive rates of gas flow were aptly described by Dacey (1980) as 'internal winds.' Indeed, through-flows can result in an increase of two orders of magnitude in the effective length of aeration in culms and rhizomes above that possible via diffusion (Armstrong et al., 1991a), enabling some emergent wetland plants such as *Eleocharis sphacelata* to inhabit areas with up to 2 m water depth (Sorrell et al., 1997). Through-flows increase P_{O_2} in rhizomes above those if only diffusion occurs, and therefore enhance O_2 diffusion into roots arising from the rhizomes. For example, in *P. australis* through-flow increased P_{O_2} in the rhizome from ~9 to ~20 kPa, and increased by ~4-fold the ROL from just behind the main apex of an adventitious root arising from the rhizome (Armstrong J et al., 1992).

Rates of through-flow are determined by the pressure gradient and the resistance to flow along the aeration system. The pressure gradient can result from: (i) pressures above atmospheric generated in living shoot tissues (e.g., several wetland species; Brix et al., 1992) due to gradients in H_2O vapor

concentration between the interior and exterior of an enclosed space with the surface of the enclosure containing micro-pores (Dacey, 1981; Armstrong et al., 1996a,b; Grosse, 1996), or (ii) venturi-induced suction caused by wind blowing over the open ends of broken culms, at least in *P. australis* (Armstrong J et al., 1996).

The requirements for pressurization in leaves have been evaluated using physical models. Pressurization occurs when there is humid air in an enclosed space (e.g., a leaf blade or sheath), surrounded by less humid air, and with at least some part of the surface having small enough pores to have significantly more resistance to pressure flow than to diffusion of gas molecules ('Knudsen regime'; Leuning, 1983). Since H_2O vapor occupies space, it dilutes the concentrations of other atmospheric gases in the enclosed space, resulting in concentration gradients of N_2 and O_2, so these gases diffuse in, and increase the total pressure within the enclosed space. Static pressures increase as pore size decreases; nevertheless, pressurization can occur with pores up to 3 μm in diameter, depending on other factors, such as the overall porosity and thickness (i.e. diffusion path length) of the partition between the enclosed space and atmosphere (Leuning, 1983; Armstrong et al., 1996a,b). The static pressure (ΔP_{static}) above atmospheric in a 'closed' system is described by the equation (Armstrong et al., 1996a):

$$\Delta P_{static} = (P_a + P_{wi} - P_{wa}) - P_a \quad (2)$$

where: P_a is pressure of the atmosphere, P_{wi} is H_2O vapor pressure inside the enclosed space, and P_{wa} is H_2O vapor pressure of the atmosphere outside the enclosed space. If the enclosed space has an outlet, then pressurization will result in a flow of gases via the outlet at a rate determined by the pressure gradient and resistance to flow, and $\Delta P_{dynamic}$ will be lower than ΔP_{static}. For a given resistance in the exit pathway, maximum flow rates occur when the diameters of the pores in the surface of the enclosed space are ~0.2 μm (Armstrong et al., 1996a). If pores in the partition are smaller than ~0.2 μm, the static pressure would become even higher than at 0.2 μm, but the flow would be reduced because the resistance to flow through the partition would become a significant part of the resistance of the pathway as a whole. Leaf-to-air temperature gradients (leaf warmer) enhance pressurization, since this increases the gradient in H_2O vapor concentration across the partition, at least in the case of emergent species (Steinberg, 1996; Colmer, 2003a).

An alternative mechanism to positive pressures generated in living shoot tissues is venturi-induced suction caused by wind blowing over tall, broken culms; to date, this mechanism is only documented for *P. australis* (Armstrong J et al., 1996). Wind blowing across an open cylinder causes a localized reduction in air pressure (ΔP_{static}) below atmospheric, as described by Bernoulli's equation (Armstrong J et al., 1992):

$$\Delta P_{static} = -\tfrac{1}{2}\, \rho\, V^2 \quad (3)$$

where: ρ is the density of air and V the wind velocity. ΔP_{static} for broken culms was ~60 % of theoretical values, since the culms are leaky and have jagged rims (Armstrong J et al., 1992). In the intact plant, gas is sucked out of taller, broken culms, and air enters via shorter culms exposed to the lower wind speeds near the water/ground surface (Armstrong J et al., 1992, 1996).

As discussed above, through-flows greatly increase aeration of rhizomes, and therefore O_2 available to diffuse into roots arising from rhizomes. Such flows enable rhizomes and therefore roots, to grow at depths deeper than accessible to roots arising from a root-shoot junction of a non-rhizomatous species, such as *O. sativa*. Maximum lengths of the roots per se in these two cases should be similar (i.e. if porosity, ROL, and O_2-consumption rates were similar), with P_{O2} at the root-rhizome or root-shoot junctions being near atmospheric. As examples, maximum root lengths for *O. sativa* and *P. australis* in a waterlogged potting mix were, respectively, 276 mm and 223 mm (Justin and Armstrong, 1987). However, since rhizomes can grow relatively deep into flooded soils, the potential soil volume explored by rhizomatous species can be larger than for non-rhizomatous species. For example, rhizomes of *Phragmites communis* growing in the field occurred at depths between 250 to 1500 mm below the soil surface (Haslam, 1970). Deep rhizomes, with roots, might give a competitive advantage for nutrient acquisition, at least in flooded soils low in nutrients. Nevertheless, the non-rhizomatous *O. sativa* is very productive in permanently flooded fields, where the plough pan is at 200 mm depth from the soil surface, and maximum lengths of root main axes is 290 mm, presumably due to horizontal growth along the plough pan (Kirk, 2003). Such data show that convective flows and the ability to access soil layers deeper than

those accessible by aerenchymatous roots arising from a shoot base near the soil surface, are not required for high productivity, at least in fertile agricultural soils when these are flooded.

B. Radial O_2 Loss (ROL) from Roots to Flooded Soils

ROL may 'protect' roots against reduced toxins (e.g., Fe^{2+}) often present in flooded soils; in the oxygenated rhizosphere, Fe^{2+} would be oxidized to the insoluble Fe_2O_3 (Begg et al., 1994; Mendelssohn et al., 1995; St-Cyr and Campbell, 1996). Carboxylic acids produced by micro-organisms in anaerobic soils may also be catabolized by aerobic micro-organisms in an oxygenated rhizosphere. However, there is a cost to these putative beneficial effects; ROL along a root axis would diminish the O_2 supply to the apex of the main axis, and therefore reduce rooting depths in anaerobic substrates. For example, Armstrong (1979) estimated that even for roots of *O. sativa* with a barrier to ROL at ≥ 50 mm behind the apex (i.e. with O_2 loss only from the apical 50 mm), ~30% of the O_2 supplied via the aerenchyma might be lost to the rhizosphere.

The flux of O_2 from the root aerenchyma to a soil is determined by: (i) the concentration gradient, (ii) the physical resistance to O_2 diffusion, and (iii) the rate of O_2 consumption in the cells exterior to the aerenchyma. Roots of many, but not all, wetland species contain a 'tight' barrier to ROL in the basal zones (Armstrong, 1964, 1979; Visser et al., 2000; McDonald et al., 2002; Colmer, 2003a), as shown in Fig. 3 for an intact adventitious root of *P. australis* in an O_2-depleted medium (Armstrong et al., 2000). The $[O_2]$ at the root surface was relatively high near the tip, whereas root surface $[O_2]$ was extremely low at 30 mm and further from the apex (Fig. 3). This pattern of $[O_2]$ at the epidermis occurred despite the opposite profile for P_{O_2} in the aerenchyma; as predicted from diffusion in a tube with O_2 consumption along the path (see Armstrong, 1979), internal P_{O_2} was highest near the root base, and declined in a curvilinear gradient toward the root tip (Fig. 3). In the basal zones, the combination of high P_{O_2} in the aerenchyma, but low ROL, indicates the permeability to O_2 movement across the outer cell layers was very low.

Measurements of $[O_2]$ across an intact adventitious root of *P. australis* when in an O_2-depleted medium, showed that the greatest impedance to ROL was in the hypodermis + epidermis (Fig. 4). Oxygen consumption by the hypodermis + epidermis, in addition to a physical barrier against O_2 diffusion, would cause the substantial drop in $[O_2]$ across the outer cell layers of the root (Armstrong et al., 2000).

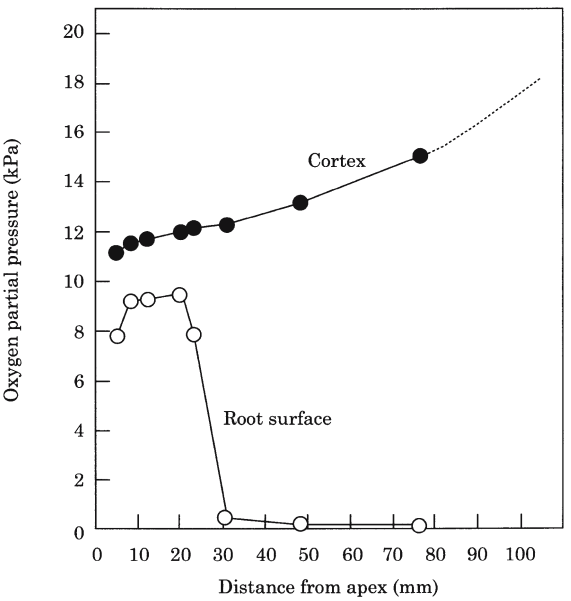

Fig. 3. Longitudinal profile of O_2-partial pressures along the surface and in the cortex of an adventitious root of *Phragmites australis* when in an O_2-depleted solution, at 23 °C. Measurements were taken using an O_2 microelectrode. The shoots were in air, and the intact 100 mm root, as well as the rhizome and other roots, were in an O_2-free solution containing 0.05% (w/v) agar to prevent convection. Reproduced from Armstrong et al. (2000) in the Annals of Botany with permission of the Annals of Botany Company.

A 'tight' barrier to ROL develops constitutively in the basal zones of adventitious roots of many wetland species, but was induced by growth in stagnant deoxygenated medium in several others (Colmer, 2003a), including *O. sativa* (Colmer, 2003b). By diminishing O_2 losses from the basal root zones to the soil, the barrier to ROL enhances longitudinal O_2 diffusion towards the apex of the main axis, and therefore tends to keep higher apical $[O_2]$ than otherwise would be the case; the higher $[O_2]$ will, in turn, increase the capacity for aeration of the soil surrounding the apex. Even in species with a 'tight' barrier to ROL, O_2 losses are substantial near the root tip, and also from laterals (Armstrong J and Armstrong, 1988; Conlin and Crowder, 1989; Armstrong et al., 1990; Sorrell, 1994). The quantitative significance of ROL from laterals for sediment oxygenation by *P. australis* (adventitious root main axes contain a barrier to

ROL in basal zones) was highlighted in mathematical modeling by Armstrong J et al. (1996) that predicted ~97% of O_2 loss from the root system may occur via laterals (based on P_{O_2} of 17 kPa at root-rhizome junction). The restriction of ROL to key sites (laterals and root tips) may be viewed as an 'efficient use' of the limited supply of O_2; this spatial distribution of ROL maximizes root penetration, and functioning of the laterals and tip regions, while protecting them from toxins in anaerobic soils.

Although a barrier to ROL is considered to be adaptive for roots in waterlogged soils, Armstrong (1979) and Koncalova (1990) hypothesized this feature may diminish water and nutrient uptake. Whether a barrier to ROL impedes uptake of water, nutrients, soil-derived gases (e.g., CO_2 and ethylene) and other substances (e.g., 'toxins' such as Fe^{2+}, Mn^{2+} and carboxylic acids often present in flooded soils), would be determined by the anatomical and chemical nature of the barrier, and the possibility of 'passage areas' (Armstrong et al., 2000; Beckett et al., 2001). Unfortunately, information on the nature of the barrier, and whether it impedes movement of substances from soils into roots is scant (Colmer, 2003a). Diffusive permeabilities across epidermal/hypodermal 'sleeves' isolated from roots of the wetland *Carex arenaria*, as compared with 'sleeves' from roots of the non-wetland species onion (*Allium cepa*), were 200-fold lower for water and 500-fold lower for $H_2PO_4^-$ and Ca^{2+} (Robards et al., 1979). In adventitious roots of *O. sativa*, hydraulic conductivity in the radial direction (expressed on a root surface area basis) was 0.2 to 0.5 of that in the primary seminal root of *Z. mays* (Miyamoto et al., 2001). However, experiments with 'sleeves' of the outer cell layers from adventitious roots of *O. sativa* indicated these layers do not limit the rate of water uptake (Ranathunge et al., 2003). Moreover, induction of the barrier to ROL in adventitious roots of *O. sativa* had no effect on the capacity for net NO_3^- uptake from aerobic solution, measured using ion-selective microelectrodes (Rubinigg et al., 2002). To better understand the possible impacts of a barrier to ROL on root functioning, measurements of nutrient and water uptake (in air and anoxia), as well as entry of soil-derived gases and 'toxins', are required for roots of a wider range of wetland species. Uptake rates of these various substances should be evaluated for zones of roots with a barrier to ROL, and for zones (i.e. tip of the main axis and laterals) without a barrier to ROL. A high degree of spatial resolution will be required, since at least in the case of *P. australis*, small O_2-permeable areas can occur in regions that otherwise contain a barrier to ROL (Armstrong et al., 2000).

C. Radial Diffusion of O_2 into the Stele of Roots

In roots dependent on O_2 supplied via the intercellular gas-filled spaces, such as aerenchyma, and in roots dependent on a restricted O_2 supply from the soil, anoxic zones may develop adjacent to zones with sufficient O_2 for oxidative phosphorylation. In both cases, such anoxic zones have been shown for the stele and the root apex.

For submerged tissues, $[O_2]$ gradients will develop between the epidermis and the core of the tissue. Berry and Norris (1949) hypothesized that such gradients could result in anoxia in the core, with more peripheral cells still receiving adequate O_2 for oxidative phosphorylation. Data from excised onion (*A. cepa*) root segments showed that when the $[O_2]$ was below the COP for respiration, the RQ increased, implying a switch of at least some cells to ethanolic fermentation (Berry and Norris, 1949). Severe hypoxia can also occur in the central tissues and/or most distal zones (i.e. just behind the root tip) in roots of *Z. mays* when in an O_2-deficient medium. The evidence for this consists of: (i) measurements of $[O_2]$ within roots using microelectrodes (Armstrong et al., 1994), (ii) higher pyruvate decarboxylase (PDC) activity in the stele versus the cortex, separated from the intact primary root of seedlings in hypoxic or anoxic solutions (Thomson and Greenway, 1991), and (iii) increased [alanine], a product of anaerobic catabolism, in 2 to 3 mm tips sampled from the primary root of seedlings after 24 to 31 hours in O_2-deficient (i.e. 0.06 mol m^{-3} O_2) solution (Gibbs et al., 1995). Moreover, mathematical models indicate that cells in regions of the stele may receive sub-optimal O_2, even in roots reliant on an internal O_2 supply via aerenchyma (Armstrong and Beckett, 1987). Thus, the position of any particular cell within an organ or tissue, relative to the longitudinal (gas phase) and radial (predominately liquid phase) diffusion paths, will be a major determinant of its O_2 supply.

The extent to which the stele becomes O_2 deficient depends upon several factors, such as: (i) the P_{O_2} in the cortical gas spaces (determined by the external $[O_2]$, the O_2 diffusion in the aerenchyma, and the distance from the root-shoot junction), (ii) the resistance to radial O_2 diffusion through the endodermis and stelar

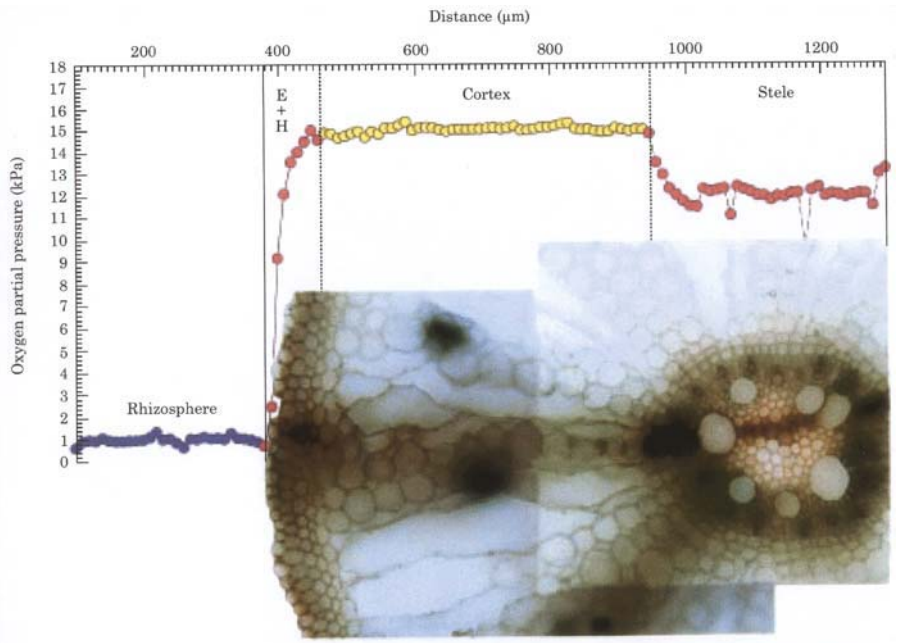

Fig. 4. Radial profile of O_2-partial pressures adjacent to, and inside, an intact adventitious root of *Phragmites australis* when in an O_2-depleted solution, at 23 °C. Measurements were taken using an O_2 microelectrode, by driving the microelectrode in steps toward and through the root. The shoots were in air, and the measurements were taken 100 mm behind the tip of an intact root 160 mm in length. Note the relatively high O_2-partial pressure in the cortex and in the relatively narrow stele, but the steep gradient of O_2-partial pressure within the epidermal/hypodermal cylinder, and very low concentrations in the solution adjacent to the root exterior, indicating that the outer layers have a very high resistance to O_2 loss from the root to the medium. Reproduced from Armstrong et al. (2000) in the Annals of Botany with permission of the Annals of Botany Company.

tissues, (iii) the radius of the stele (i.e. diffusion-path length), and (iv) the rate of O_2 consumption in the stele (Armstrong and Beckett, 1987). In contrast to the relatively high porosity and gas-phase diffusion in the root cortex, the stele usually has negligible porosity, so that radial diffusion is predominately in the liquid phase (Armstrong and Beckett, 1987; Armstrong et al., 1991b). Additionally, the rate of O_2 consumption (per unit volume) in the stele can be about three-fold faster than that in the cortex plus outer cell layers, at least in roots of *Z. mays* (Armstrong et al., 1991b). This combination of liquid-phase diffusion and relatively fast O_2-consumption rates result in steep declines in $[O_2]$ with distance into the stele (Fig. 2).

Roots of many wetland species tend to have a narrow stele; e.g., in *O. sativa* the stele occupies less than 5% of the cross-sectional area of the adventitious roots, as compared with 24 to 36% in *Sorghum bicolor* (McDonald et al., 2002). Therefore, the diffusion path from the aerenchyma to the centre of the stele is relatively short in roots of many wetland species; therefore, P_{O2} in the stele may only decline by a few kPa between the aerenchyma and the centre of the stele (Fig. 4). If O_2 deficiency did occur in the stele, or part thereof, this would have implications for root functioning, especially ion transport to the shoot (Gibbs et al., 1998); therefore, avoidance of this condition might be necessary to maintain vigorous growth in flooded soils.

Submerged plants are subject to temporal changes in O_2 status, as well as the spatial differences in O_2 supply described above. In submerged *O. sativa*, O_2 produced in photosynthesis, diffused in aerenchyma to the roots, resulting in 0.6 to 0.8 mol m^{-3} O_2 in solution at the surface of the elongating zone within a few min after starting the light period, and this increase in $[O_2]$ was followed by resumption of root extension (Waters et al., 1989). By contrast, following the onset of darkness, $[O_2]$ at the root tip declined rapidly to almost zero, and root extension slowed almost immediately and had virtually ceased within ~1 hour; furthermore, ethanol was then produced by the roots (Waters et al., 1989). Similarly, O_2 produced during photosynthesis in submerged aquatic and marine plants resulted in marked diurnal

changes in [O_2] in roots (Sorrell and Dromgoole, 1987; Christensen et al., 1994; Pedersen et al., 1995; Connell et al., 1999).

The occurrence of anaerobic carbohydrate catabolism in roots of intact plants during times of waterlogging and submergence makes it relevant to discuss, in the next section, mechanisms of anoxia tolerance in plants.

IV. Anoxia Tolerance in Plants

Anoxia tolerance has been reviewed comprehensively (Drew, 1997; Gibbs and Greenway, 2003; Greenway and Gibbs, 2003). The central feature of anoxia is an energy crisis; rates of ATP production have been assessed from rates of anaerobic catabolism, and range between 3% to as high as 38% of rates in aerobic tissues (Gibbs and Greenway, 2003). For *Z. mays* root tips, ATP production in anoxia was ~7% of the rate when fully aerobic (sample perfused with solution bubbled with 100 kPa O_2), measured using in vivo saturation-transfer ^{31}P-NMR spectroscopy (Roberts et al., 1985); this result was similar to rates assessed from data on anaerobic carbohydrate catabolism (Gibbs and Greenway, 2003). Anoxia tolerance has been shown for several plant organs, such as coleoptiles, stems, tubers, and rhizomes of wetland species. Although anoxia tolerance may also be of adaptive significance for roots of plants in flood-prone environments, further work is required to provide conclusive evidence.

Anoxia tolerance is crucial for rhizomes and storage organs of marsh species buried in anoxic mud over winter. Rhizomes of the marsh species *Acorus calamus* survived anoxia for more than 90 days (Crawford and Braendle, 1996). In spring, growth of shoots commences, and O_2 becomes available when the shoots start photosynthesis in illuminated water or when the shoots reach the atmosphere (Crawford and Braendle, 1996). In *Potamogeton pectinatus*, elongation of stems emerging from tubers was much faster in anoxia than in air (Summers and Jackson, 1996). Anoxia tolerance is also necessary during establishment of plants from seeds in flooded soils. The coleoptile of *O. sativa*, and of the paddy weed *Echinochloa oryzoides*, elongated during five days of anoxia, imposed three days after imbibition (Pearce and Jackson, 1991).

In the absence of comprehensive data we can only speculate if anoxia tolerance is also adaptive for roots of plants in waterlogged soils, and for plants submerged during flooding. We suggest four situations:

(i) During the initial period of waterlogging, when aerenchyma in roots of many species has not yet developed (e.g., *Z. mays*, Konings, 1982; *T. aestivum*, Thomson et al., 1990).

(ii) For roots exposed to transient waterlogging. In anoxia-intolerant species, roots below the water table decompose (Jackson and Drew, 1984), but whether roots shown to be anoxia-tolerant can survive under the same conditions has not been tested.

(iii) For roots of submerged plants during nighttime, since photosynthesis is the main O_2 source resulting in marked diurnal fluctuations in O_2 supply to the roots (e.g., *O. sativa*; Waters et al., 1989). Ethanol production in roots of submerged *O. sativa* occurred during the night (Waters et al., 1989), implying anoxia tolerance in roots is one component of submergence-tolerance.

(iv) During long-term waterlogging when parts of the stele might be anoxic, even in some species with aerenchymatous roots (Section III.C). Fast rates of anaerobic carbohydrate catabolism in the stele may then contribute to energy required for nutrient transport to the shoot (e.g., in xylem loading), and for transport of sugars to the root cells (Saglio, 1985).

A. Anaerobic Carbohydrate Catabolism: Fast and Slow Modes

There are two modes of carbohydrate catabolism as related to anoxia tolerance in plants. In certain anoxia-tolerant organs/tissues, rates of carbohydrate catabolism during anoxia can be substantially faster than when in air (Mode 1: 'Fast mode' with accelerated glycolysis; i.e. a 'Pasteur Effect'); yet in other anoxia-tolerant organs/tissues catabolism can be much slower than when in air (Mode 2: 'Slow mode,' sometimes called 'metabolic arrest') (Gibbs and Greenway, 2003).

A prominent case for the mode with fast rates of anaerobic catabolism is the stems of the aquatic plant *P. pectinatus*, in which elongation is promoted

by anoxia (Summers and Jackson, 1996). The stems have an estimated rate of glycolysis six times faster in anoxia than in air (based on CO_2-evolution rates; Summers et al., 2000). Mobilization of carbohydrate reserves in the tubers (described for *P. distinctus*; Harada and Ishizawa, 2003), and translocation to the stems, almost certainly provides the required substrates. The fast rates of glycolysis linked to ethanol production, and perhaps some energy production via sucrose synthase, would provide the ATP required for extension growth (Gibbs and Greenway, 2003).

The most extreme example of the mode with slow anaerobic catabolism is germinating lettuce (*Lactuca sativa*) seeds, which can survive anoxia for at least 14 days without any measurable CO_2 evolution, indicating very slow rates of anaerobic catabolism (Pradet and Bomsel, 1978). The 'slow' mode conserves carbohydrate reserves, and thus prolongs the duration of survival; the duration also depending on the initial levels of carbohydrates. The 'slow' mode of anaerobic catabolism may not be of relevance for roots of plants with shoots in air, since levels of non-structural carbohydrates often increase upon exposure of the roots to O_2 deficiency (Section IV.B.1), but may become important during complete submergence when non-structural carbohydrates in plants are usually depleted (Section IV.B.2). The other, less extreme, examples of plant tissues/organs with a tendency toward this slower mode of carbohydrate catabolism are discussed in Gibbs and Greenway (2003); in these cases glycolysis appears to be down-regulated following acclimation to anoxia (e.g., in the coleoptile of *O. sativa*; Colmer et al., 2001).

Anoxia tolerance in tissues with either mode of carbohydrate catabolism will also require other adaptive characteristics (Drew, 1997; Chang et al., 2000; Gibbs and Greenway, 2003); even the 'fast' mode produces considerably less ATP than oxidative phosphorylation, and cells would still suffer an energy shortage. These other adaptive characteristics include:

(i) A reduction in energy required for maintenance which, for anoxia-tolerant tissues, have been assessed to be two- to nine-fold lower in anoxic than in aerated tissues (Greenway and Gibbs, 2003). Such reductions may be achieved by decreases in protein turnover, ion fluxes, and other processes (Greenway and Gibbs, 2003).

(ii) Direction of the restricted supply of energy to crucial processes for survival; e.g., regulation of pH_{cyt} (Greenway and Gibbs, 2003).

Greenway and Gibbs (2003) suggested that without these acclimations, membrane selectivity declines, culminating after some time in cell death due to the loss of integrity of the tonoplast (Zhang et al., 1992), causing release of hydrolytic enzymes from the vacuole.

Both respiration and anaerobic catabolism require adequate substrates; these might become limiting because of reductions in photosynthesis in plants in waterlogged soil and when submerged. The next section examines levels of non-structural carbohydrates in plants in flooded soils.

B. Substrate Availability: Soluble Sugars and Starch

Photosynthesis declines in many species soon after waterlogging occurs. In the case of submergence, photosynthesis is also usually reduced, with the degree of inhibition depending on the characteristics of the flood-waters. In this section we discuss whether these declines in photosynthesis result in a deficiency of carbohydrates for respiration (and ethanolic fermentation), or whether in the case of plants in waterlogged soil the decreased photosynthesis results from feedback due to increased levels of carbohydrates that accumulate due to reduced consumption resulting from decreased growth. In that case, carbohydrates should not be deficient, provided that transport of sugars continues even to the root tips. The available data indicate that carbohydrates are usually ample in plants with shoots in air and roots in O_2-deficient media (Section IV.B.1), while the opposite applies to plants when shoots are submerged also (Section IV.B.2). The inference that carbohydrates may limit survival and growth of submerged plants requires testing by adding exogenously supplied sugars.

1. During Waterlogging

Plants exposed to a low $[O_2]$ in the root medium usually have higher levels of non-structural carbohydrates in both leaves and roots, than plants in aerated conditions (Setter et al., 1987). For plants in nutrient solutions flushed with N_2, non-structural carbohydrates increased in four species from either flooding-prone or well-drained habitats (Albrecht et al., 1997), and in *T. aestivum* (Barrett-Lennard et al., 1988; Albrecht et al., 1993). For plants in water-

logged soils, non-structural carbohydrates increased 5-fold in leaves of *T. aestivum* (Malik et al., 2002), while in roots of *Medicago sativa* sucrose increased from 2–6% to 11–16% of the dry mass (Barta 1988; Castonguay et al., 1993); in leaves starch increased 2.5- to 6-fold reaching over 20% of the dry mass (Castonguay et al., 1993). The accumulation of non-structural carbohydrates occurs, despite reductions in rates of photosynthesis in plants with roots in waterlogged soils or in hypoxic nutrient solution (Castonguay et al., 1993; Albrecht et al., 1997; Malik et al., 2001, 2002). Setter et al. (1987b) argued that for plants with roots in O_2-deficient media, the evidence favored the scenario as shown in Fig. 5; that is, non-structural carbohydrates would accumulate primarily due to reduced utilization because growth is inhibited, as also concluded by others (Barta, 1987; Castonguay et al., 1993; Albrecht et al., 1997). Reductions in photosynthesis in plants in waterlogged soil may then result from feedback regulation by the high concentrations of non-structural carbohydrates in leaves (Fig. 5).

In contrast to the preceding, Albrecht et al. (1997) suggested that in *Senecio aquaticus* (a species that inhabits flooding-prone soils), reduced utilization of carbohydrates in growth and catabolism did not account for high accumulation of fructans, because after 9 days in N_2-flushed solution culture there was only 'slightly altered biomass production.' We disagree with this view. Firstly, plant dry masses were reduced by ~20%; assessed from fresh mass data presented by Albrecht et al. (1997), but since the fresh:dry mass ratios were not given, we used values of 9.5 for shoots (Lambers, 1976) and 9.7 for roots (calculated from Crawford, 1966). Furthermore, growth of *S. aquaticus*, assessed as dry mass minus non-structural carbohydrates, was reduced by 51% (assessed from Albrecht et al., 1997). The large reduction in growth (i.e. dry mass excluding non-structural carbohydrates) of *S. aquaticus* in a N_2-flushed solution is somewhat unusual for a wetland species. The responses described above for *S. aquaticus* differ from an earlier study in which root dry mass was not reduced for plants in N_2-flushed solutions for 23 days (Lambers and Steingrover, 1978). Furthermore, soluble sugars did accumulate (up to two-fold) in roots of *S. aquaticus* during the first few days; however, subsequently soluble sugar concentrations were lower (~60% of values in aerated controls) for the remainder of the experiment (Lambers et al., 1978). The reason for these differences in results between the two studies is not known. We

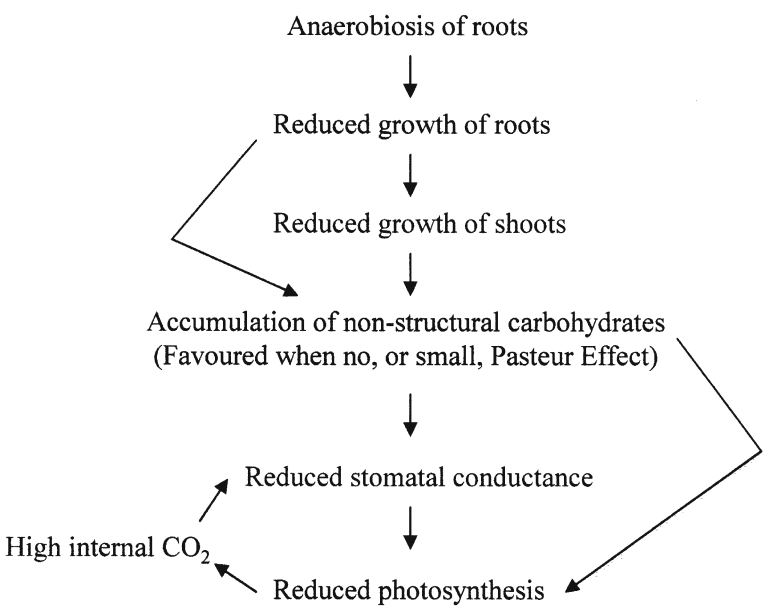

Fig. 5. Hypothesis to account for accumulation of non-structural carbohydrates in shoots and roots of plants with roots in an O_2-deficient medium. Based on Setter et al. (1987). 'Pasteur Effect' is accelerated glycolysis.

conclude that the data on *Senecio* species published by Albrecht et al. (1997) are consistent with the hypothesis that carbohydrate accumulation in plants exposed to root-zone O_2-deficiency is due to reduced growth decreasing utilization of carbohydrates. An additional contribution to carbohydrate accumulation might be reduced catabolism in hypoxic roots, if the activity of the alternative oxidase declines (discussed in Section III.A.1).

Increases in non-structural carbohydrates in plants grown with a low [O_2] in the root-zone are not universal; levels of carbohydrates in plants tend to be variable and depend on environment and time of day. As one example, in *O. sativa* with roots in N_2-flushed solutions, the sum of hexoses and sucrose increased only during summer, and not during winter, presumably due to differences in rates of photosynthesis associated with light intensity and day length (Limpinuntana and Greenway, 1979). Furthermore, 2-mm tips of seminal roots of *T. aestivum* had lower [total sugar] when in N_2-flushed, as compared with aerated solution, even though all other tissues had higher [total sugar] (Barrett-Lennard et al., 1988). The main axes of these seminal roots had lost the capacity to elongate (established when returned to aerated solution), so the failure to accumulate sugars in the apical 2 mm of roots might have resulted from severe injury; anoxic roots of *T. aestivum* suffered substantial losses of solutes, including sugars, to the medium (Greenway et al., 1992).

Carbohydrates accumulated in plants during waterlogging may be important for recovery upon soil drainage (Albrecht et al., 1993, 1997); e.g., in both seminal and adventitious roots of *T. aestivum* the RGRs for ethanol-insoluble dry mass over the first four days after return of plants from N_2-flushed to aerated solutions were 37% faster than those in continuously aerated plants (Barrett-Lennard et al., 1988). Furthermore, after 14 days of waterlogging the total accumulated non-structural carbohydrates in *T. aestivum* were the same or higher than the amount required for adventitious root growth during the first 7 days after drainage (Malik et al., 2002). Whether these reserves are of adaptive value is not sure. During the first few days after drainage, stomatal opening and photosynthesis remained at less than 35 to 50% of values in plants in continuously drained pots (Malik et al., 2001). Photosynthesis during the first few days of recovery may have been down-regulated due to feedback from high tissue carbohydrates; then there would be little benefit from accumulated carbohydrates. Alternatively, the accumulated carbohydrates may be crucial for rapid re-growth of roots if there is an inherently low capacity for photosynthesis during post-waterlogging. Leaf N, P and K concentrations were low in *T. aestivum* after 15 days of waterlogging (Trought and Drew, 1980), and these low levels may decrease photosynthetic capacity, so that the carbohydrates accumulated during waterlogging would be an important source of substrates during the time taken for photosynthetic capacity to recover following drainage.

Summing up, the hypothesis that intact plants with roots in O_2-deficient media are usually not limited by carbohydrate supply needs to be confirmed for wetland species that do not suffer substantial reductions in growth when in anaerobic rooting media. Future work should focus on the carbohydrate status of tips of roots in their most rapid phase of elongation, avoiding samples taken from roots that have reached their maximum lengths as set by the internal O_2 diffusion from the shoots (Section III.A.1). Furthermore, whether exogenous sugars can stimulate growth should also be tested. Such tests are required, because high carbohydrate levels do not rule out a possible response to exogenous sugars, as shown for anoxic *T. aestivum* roots (Waters et al., 1991) and slices of tissue from the tap root of *Beta vulgaris* (Zhang and Greenway, 1994).

2. During Submergence

In contrast to the accumulation of non-structural carbohydrates in plants during waterlogging (Section IV.B.1), carbohydrate depletion typically occurs during complete submergence, at least for *O. sativa* (Setter et al., 1987; 1997). The adverse consequence of depletion of carbohydrates for submergence tolerance in *O. sativa* has been demonstrated by manipulation of [non-structural carbohydrates] in tissues before submergence using a variety of techniques (reviewed by Jackson and Ram, 2003). It was concluded that decreases in [non-structural carbohydrates] during submergence of *O. sativa* were due to continued catabolism (and growth), while photosynthesis was reduced. Photosynthesis being limited by: (i) low irradiance, and (ii) inadequate CO_2 supplies (Jackson and Ram, 2003). It is therefore not surprising that the carbohydrate status would differ between plants completely submerged, and plants with roots in waterlogged soil and shoots in air.

Here we only discuss further one aspect of the

response of tissue [carbohydrate] to complete submergence, which is relevant to catabolism. In *O. sativa*, submergent-tolerant cultivars retained a higher concentration (total dry mass basis) of non-structural carbohydrates at the end of submergence than intolerant cultivars (Mallik et al., 1995; Singh et al., 2001; Ram et al., 2002; Jackson and Ram, 2003). So, the decreased survival in the intolerant cultivars might have been associated with a deficiency of substrate for respiration and/or anaerobic catabolism. The higher non-structural carbohydrates in the tolerant cultivars at the end of submergence has been attributed to a combination of a higher initial [carbohydrate] and slower consumption during submergence (Mallik et al., 1995; Chaturvedi et al., 1996; Singh et al., 2001; Ram et al., 2002). There is persuasive evidence for slower decreases in concentration of non-structural carbohydrates in the tolerant cultivars (Singh et al., 2001); after 15 days submergence, decreases in total non-structural carbohydrates were 1.6 times greater in shoots of submergent-intolerant compared with tolerant cultivars. A similar difference between cultivars after 7 days submergence was reported by Mazaredo and Vergara (1982). There are three possible mechanisms that could cause these differences in the rate of decreases in concentrations of non-structural carbohydrates during submergence:

i) Catabolism might be faster in the intolerant than in the tolerant cultivars. A slowing down of catabolism associated with submergence tolerance would be consistent with down-regulation of ethanol formation in anoxia-tolerant tissues during anoxia (Greenway and Gibbs, 2003).

ii) Faster decreases of [non-structural carbohydrates] in submergent-intolerant cultivars might be due to their faster shoot elongation (Mallik et al., 1995; Chaturvedi et al., 1996; Singh et al., 2001; Ram et al., 2002; Jackson and Ram, 2003). However, it is unknown whether this faster elongation is associated with increases in structural dry mass, such as proteins, cell walls and/or organic solutes for an increased volume; such changes would themselves decrease the non-structural carbohydrate concentration, when expressed on a total dry mass basis.

iii) Photosynthesis during submergence might be faster in the tolerant cultivars; this needs to be evaluated in experiments using a range of exogenous CO_2 and irradiances, but such data are presently not available.

Resolution of these possibilities is worthwhile, especially considering the clear cultivar differences in response to submergence in this important crop.

Another possible factor interfering with respiration in plants in flooded soils are high P_{CO_2}, and this will be briefly considered in the next section.

V. Effects of High Partial Pressures of CO_2 (P_{CO_2}) in Flooded Soils on Respiration

Root environments usually have substantially higher P_{CO_2} than the atmosphere (Cramer, 2002). For example, the P_{CO_2} in the gas-phase 0.3 m below the surface of a well-drained soil ranged between 0.08 kPa in winter to 1.6 kPa in summer (Johnson et al., 1994). Upon flooding, P_{CO_2} in soils (and roots) becomes even higher, since escape of CO_2 evolved from soil organisms and subterranean parts of plants is drastically curtailed because of the slow diffusion of gases in water. As an example of the high P_{CO_2} that can occur in flooded soils, P_{CO_2} reached 18 and 43 kPa in pots containing flooded soils from rice fields with pH 5.7 and 6.3, respectively (Ponnamperuma, 1984). Yet, surprisingly little is known about the responses of roots to these high P_{CO_2}. In this section we have only reviewed the aspects directly related to effects of high (i.e. several kPa) P_{CO_2} on respiration; the most relevant data available are for callus cells derived from shoots, but with P_{CO_2} suddenly raised.

Partial pressures of CO_2 in roots will be determined by rates of CO_2 evolution by the cells, CO_2 exchanges with the soil, and any movement of CO_2 to the shoots, either by longitudinal diffusion in aerenchyma or by mass flow in the xylem. Permeability of membranes to CO_2 is very high (Espie and Colman, 1982), so exchange of CO_2 between roots and soil would be fast, unless the impermeable layer to ROL which occurs in roots of many wetland species (Section III.B), is also impermeable to CO_2. Even then, the root apex would still be exposed to high P_{CO_2}. Reliable data on P_{CO2} within roots in flooded soils are not available. However, in rhizomes of *P. australis* in soil flooded for two months, P_{CO_2} was 1.2 to 3.2 kPa at 0 to 20 cm depth and 6.7 to 7.4 kPa at 50 to 80 cm depth (Brix, 1988). By inference, P_{CO_2} would have been at least as high in the roots arising from these rhizomes.

In the very different context of global warming,

the effects of increased atmospheric P_{CO_2} (i.e. ~70 Pa, as compared with current atmospheric P_{CO_2} of 36 Pa) on respiration, and other processes, have been reviewed (Drake et al., 1997, 1999). Such data do not seem relevant for predicting responses of roots to the much higher P_{CO2} in flooded soils; these can be three orders of magnitude higher than current atmospheric P_{CO2}. Metabolism could be affected by very high (i.e. several kPa) P_{CO_2} due to an acid load (i.e. a tendency to acidify the cytosol) (see Bown, 1985), or direct effects of CO_2 and/or HCO_3^- on enzymes and/or lipids (Mitz, 1979; Lorimer, 1983). The $[HCO_3^-]$ in the various compartments will depend on the P_{CO_2} and pH, as described by the Henderson-Hasselbalch equation (Segel, 1976). To emphasize the differences between cells exposed to elevated P_{CO_2} in the atmosphere (e.g., 70 Pa; Drake et al., 1997) and cells exposed to P_{CO_2} in flooded soils (e.g., ~7 kPa in rhizomes; Brix, 1988), we compare the degree of acid loading under these two situations. Assuming pH_{cyt} of 7.5, $[HCO_3^-]$ in the cytosol of cells exposed to 7 kPa P_{CO_2} could have risen to 63 mol m^{-3}, whereas at P_{CO_2} of 70 Pa (i.e. approximately double present atmospheric P_{CO_2}), the $[HCO_3^-]$ in the cytosol would be only 0.63 mol m^{-3} (calculated using the Henderson-Hasselbalch equation). Thus, even if P_{CO2} in the atmosphere doubled, the acid loading would be negligible, whereas for cells of organs in flooded soils the acid loading is of the same order of magnitude as the buffering capacity of 20 to 100 mol m^{-3} (pH unit)$^{-1}$ in the cytosol of plant cells (Kurkdjian and Guern, 1989).

The inhibitory effect of high (i.e. several kPa) P_{CO_2} on respiration has been studied using callus cells of *Dianthus caryophyllus*. Uncoupled respiration in the presence of SHAM (i.e. an estimate of the maximum capacity of the cytochrome pathway) was inhibited by 50% when 20 mol m^{-3} CO_2/HCO_3^- was added (Palet et al., 1991). Given that the pH in solution increased from 5.7 to 6.0 following addition of the HCO_3^- (see Table 1 in Palet et al., 1991), $[CO_2]$ would have been 12 mol m^{-3}, and P_{CO_2} 35 kPa; calculated using pK = 6.1 for $HCO_3^- \leftrightarrow CO_2$ (Segel, 1976) and conversion of CO_2 to P_{CO_2} at 25°C according to Armstrong (1979). That only the cytochrome pathway remained operational during this experiment was verified by addition of 1 mol m^{-3} cyanide at the end of the experiment which abolished O_2 uptake (Palet et al., 1991). The limiting reactions responsible for the reduced O_2 uptake at high $[CO_2/HCO_3^-]$ are not known. Cytochrome oxidase may have been directly inhibited (Palet et al., 1991), a feasible suggestion since in isolated mitochondria in state 3 the flux control coefficient reached 0.47 to 0.66 (Padovan et al., 1989). Alternatively, glycolysis could have been inhibited. Furthermore, a large inhibition of succinate oxidation at high $[HCO_3^-]$ has been found in isolated mitochondria from *Z. mays* (Cerwick et al., 1995). These hypotheses need to be explored for fibrous roots exposed to a range of P_{CO_2}, and include pre-treatments during which P_{CO_2} is raised gradually to assess possible acclimation to high P_{CO_2}.

VI. Conclusions

Waterlogging and flooding restrict gas exchange between the soil and atmosphere, so O_2 becomes deficient and usually absent altogether, while CO_2 increases. Cessation of oxidative phosphorylation during anoxia causes a severe energy crisis. In wetland species, tubers and rhizomes are anoxia-tolerant, while coleoptiles and stems even grow during anoxia. Anoxia tolerance requires a carbohydrate supply to fuel ethanolic fermentation, and apportionment of the scarce energy to processes essential to survival. In some cases, rates of glycolysis in anoxia are faster than in aerobic tissues (a 'Pasteur Effect'); e.g., in shoots that elongate to escape anoxia ('escape' is achieved if the shoot reaches an O_2 source).

In the majority of cases the cessation of oxidative phosphorylation stops growth and often causes death of cells within a few hours or days. Adaptation to flooded environments is centered on obtaining O_2, and complemented by a capacity to cope with a restricted O_2 supply to part of the roots. Some soils become hypoxic, rather than anoxic, and thus supply measurable, though insufficient O_2, to roots. Even then, an adequate supply of O_2 via aerenchyma is required for plants to thrive. Maximum root lengths are determined by the capacity for internal O_2 diffusion to the apex of the main axis; in turn, influenced by the volume of aerenchyma, respiration rates, and rates of ROL to the soil. In some wetland species, through-flows of gases greatly increase aeration of rhizomes, and the supply of O_2 available to diffuse into roots. This allows a substantial increase in rhizome depths, and therefore of the volume of soil explored by roots arising from these rhizomes.

In roots of some species, including those with aerenchyma, anaerobic carbohydrate catabolism may occur in the stele and in the terminal tip (i.e. tissues at the end of the O_2-diffusion paths), while oxidative phosphorylation continues in the cells with access

to O_2, derived either from the external solution or via the cortical aerenchyma. Thus, anoxia tolerance is presumably a component of tolerance of roots to soil waterlogging and flooding, at least in some species. Currently, nearly all the data on this aspect are for roots of *Z. mays*. Future work needs to establish the degree to which these patterns of O_2 supply, and aerobic versus anaerobic catabolism, occur in tissues of roots of other species. The degree of O_2 deficiency in roots of wetland species might be much less than in aerenchymatous roots of non-wetland species; roots of wetland species typically have more aerenchyma and a much narrower stele, and these features can mitigate development of anoxic zones (e.g., see Fig. 4 for *P. australis*).

An important issue for plants in waterlogged soils, or when submerged, is whether substrates are sufficient for catabolism, be it respiration or ethanol formation. Non-structural carbohydrates usually increase in roots of plants in waterlogged soils with shoots in air, while the opposite applies when plants are submerged. The accumulation of carbohydrates in plants in waterlogged soils can be attributed to reduced consumption due to growth being inhibited. Conversely, in submerged plants photosynthesis is inhibited, so carbohydrates are depleted. The inference that carbohydrate deficiency might limit survival of submerged wetland species requires testing; e.g., by feeding exogenous sugars. In addition, for roots of plants in waterlogged soils it remains feasible that carbohydrate supply to apices might become limiting.

Another important feature of a flooded environments is a high P_{CO_2}, which is therefore bound to become high in roots. However, surprisingly little is known about the response of plants to P_{CO_2} in the range of 5–35 kPa. In this review we have only dealt with the aspects directly related to respiration, and even on this aspect there is little known. Research should focus on long-term effects, rather than use sudden applications of high P_{CO_2}, since the latter do not occur in nature.

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