New Phytol. (1999), **144**, 133–142

Juncus bulbosus as a pioneer species in acidic lignite mining lakes: interactions, mechanism and survival strategies

ABAD CHABBI

Department of Soil Protection and Recultivation, Brandenburg Technical University of Cottbus, Faculty of Environmental Science, P.O. Box 101344, D-03013 Cottbus, Germany (*tel 49 355 781177*; *fax 49 355 781170*; *e-mail* chabbi@TU-Cottbus.de)

Received 24 May 1999; *accepted 15 June 1999*

SUMMARY

Bulbous rush (*Juncus bulbosus*) initiates plant colonization in extremely acid lakes resulting from coal mining operations. Various analytical techniques (methylene blue/agar method, Ti^{3+}-citrate solution) X-ray diffraction (XRD), scanning electron microscopy (SEM), and Energy-dispersive X-ray (EDX) were used to assess the mechanisms and strategies employed by *J. bulbosus* to overcome the extreme conditions. The plant releases oxygen into the rhizosphere in turn increasing the redox potential and inducing iron oxide plaque formation. XRD showed that the iron oxide of the plaque is mainly goethite that has been developed in the presence of CO_2; SEM showed that there is a micro-space between the roots and sand grains which is inhabited by microorganisms. Furthermore, SEM-EDX studies on internal iron distribution demonstrate that iron toxicity is delayed by the physiological and biochemical structure of the plant. It is suggested that *J. bulbosus* uses a variety of mechanisms and strategies (morphological, physiological and biochemical adaptation) which are mainly complementary and which interact with each other to help *J. bulbosus* to manage its growth and survival in an extreme environment.

Key words: mining sediment, iron uptake, endodermis, iron plaque, toxicity, *Juncus bulbosus*.

INTRODUCTION

Bulbous rush (*Juncus bulbosus*) is the pioneer species and dominant macrophyte in the Lusatian lignite mining lakes (Pietsch, 1973). The extreme and hostile site conditions resulting from pyrite oxidation suggest the presence of adaptive mechanisms that enable *J. bulbosus* to persist in sediments and water bodies where low pH (2.5–3) and concentrations of dissolved iron, manganese and aluminium exceed the levels that would kill most species (Table 1). The mining lakes are therefor an extreme environment for plant growth. Until now, most studies of *J. bulbosus* have been conducted in moderately acidic lakes, particularly in Sweden, the Netherlands, and Scotland, that have resulted from acid depositions and which are clearly different from lignite mining lakes. Those studies focused on the establishment and expansion of *J. bulbosus* (Hinneri, 1976; Roelofs, 1983; Van Damm, 1988), on the interaction of plant growth with pH values (Wortelboer, 1990), and on the role of CO_2 in the survival of *J. bulbosus* (Roelofs *et al.*, 1984; Wetzel *et al.*, 1984; Svedäng, 1992). To my knowledge, there have been no studies of how *Juncus* plants survive in mining sediment or what ecophysiological and biochemical traits might aid survival.

It is hypothesized that *J. bulbosus* has developed numerous adaptation mechanisms that interact with each other to help to cope with the extreme conditions of acid lignite-mining lakes. Here, data are presented which comprise the first elaborate study of the morphology, and which relate to the physiology and to the biochemical traits which enable growth of *J. bulbosus* in an extreme environment.

MATERIALS AND METHODS

Sites

The investigations were carried out in Senftenberg See (Lake SFB) and the Koyne-Plessa (Lake 108 and Lake 109) mining district (State of Brandenburg) in eastern Germany (lat 45°46′ N, long 45°48′ E). These lakes are the result of decades of lignite

Table 1. *Chemical conditions and characteristic of* Juncus bulbosus *waters*

Lake n°	EC/µS	DOC	pH	Al^{3+}	Fe^{3+}	Mn^{4+}	Ca^{2+}	Mg^{2+}	K	Na^{+}	Zn^{2+}	Cl^{-}	NH_4^{+}	NO_3^{-}	PO_4^{3-}	SO_4^{2-}
107*	4132	5.50	2.53	6.07	45.14	0.54	21.74	5.13	0.07	0.32	0.01	0.20	0.13	0.17	0.00	67.51
SE ±	0.25	0.22	0.02	0.20	1.58	0.02	0.46	0.12	0.00	0.01	0.00	0.01	0.02	0.00	0.00	1.41
108	1501	0.77	2.86	0.42	1.99	0.05	6.20	1.38	0.10	0.31	0.00	0.40	0.00	0.02	0.00	10.91
SE ±	0.22	0.11	0.03	0.02	0.08	0.00	0.05	0.02	0.01	0.02	0.00	0.01	0.00	0.00	0.00	0.31
109	798	0.94	3.53	0.15	0.21	0.03	7.05	1.81	0.13	0.38	0.00	0.43	0.00	0.01	0.00	11.26
SE ±	0.14	0.14	0.01	0.02	0.02	0.00	0.10	0.03	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.09
SFB	598	0.59	3.32	0.13	0.34	0.02	3.75	1.13	0.16	0.69	0.00	0.69	0.00	0.02	0.00	6.26
SE ±	0.18	0.01	0.01	0.02	0.01	0.00	0.02	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.04

*Unvegetated.
All values except dissolved oxygen concentration (DOC: mg l^{-1}) and electrical conductivity (EC: µS cm^{-1}) are (mM (eq) l^{-1}) (Chabbi *et al.*, 1998).

mining. The water levels fluctuate largely according to levels of ground water. The substrate is mainly Pleistocene-Sand with little Tertiary material (Senftenberg See) or Tertiary material rich in pyrite (Koyne-Plessa district). *Juncus bulbosus*, in both floating and submersed stands, is the dominant macrophyte of the littorals of these lakes.

Redox profiles

Soil redox potentials (E_h) were measured in vegetated and unvegetated lakes using brightened platinum electrodes and a calomel reference electrode. The measured potential (mV) was corrected by adding + 242 mV. Each electrode was checked before use with quinhydrone in pH 4 and 7 buffers (the reading for quinhydrone is 218 or 224 mV, respectively, at 25°C). Four replicate electrodes were used at depths of 2, 4, 6, 8 and 10 cm (which correspond to a good portion of the rooting zone of *Juncus bulbosus* L.) and two replicate electrodes at depths of 10, 12, 14, 16, 18 and 20 cm, where less variability was observed.

Plant material

On 5 August 1997, turgid and structurally intact living roots were collected at the sampling sites from acid lignite-mine sediment rich in iron. Roots were carefully collected with a stainless steel shovel, the root and soil kept intact, placed in plastic bags, transported to the laboratory and stored overnight at 4°C. Root and soil were separated using de-ionized water. Root material with iron plaque (iron oxide around the roots) was used for several analyses: oxygen release, powder X-ray diffraction (XRD) and scanning electron microscopy (SEM).

Measurement of radial oxygen loss from roots

The rate of oxygen release from *J. bulbosus* roots which were still attached to the plant was estimated colormetrically with Ti^{3+}-citrate solution. This technique was described by several authors (Delaune *et al.*, 1990; Sorrell *et al.*, 1993). Ti^{3+}-citrate was prepared by bubbling N gas through the medium (to prevent exposure of the solution to air) according to the method of Zehnder and Wuhrmann (1976). Roots were washed in tap water, rinsed in de-ionized water and gently blotted dry on tissue paper. Root systems were then immersed (one per flask) in 100 ml 25 %-strength Hoagland's solution containing Ti^{3+}-citrate. Six hours after immersion absorbance of the partly oxidized Ti^{3+}-citrate solution was read at 527 nm on a spectrometer. Released oxygen was calculated by the formula:

$$ROL = c(y - z)$$

(where ROL = radial oxygen loss in (µmol g^{-1} d. wt h^{-1}, c = initial volume of Ti^{3+}-citrate added to each

flask, in ml, y = concentration of Ti^{3+}-citrate solution of control (without plant) and z = concentration of Ti^{3+}-citrate after 6 h (with plants)).

Rhizosphere oxidation experiment

Oxygen leakage from *J. bulbosus* roots was examined (four replicates) by the methylene blue/agar method described by Trolldenier (1988). A solution containing 0.2% (w/v) agarose and 1 mM $CaCl_2$ was prepared by heating (to not more than 70°C). The mixture was cooled to 40°C in a cold bath. A stock of the redox indicator methylene blue (10 mg l^{-1}) was added. This solution was reduced using 0.6 g l^{-1} sodium dithionite ($Na_2S_2O_4$) and gently shaken until it was colourless. The solution was transferred to clear acrylic viewing boxes (20 cm × 20 cm × 1 cm) and purged with N gas to prevent oxidation of leucomethylene blue.

When the methylene-agar solution had cooled to 25°C, roots were immersed in the liquid in the viewing boxes. The surface of the agar was immediately covered with a 2.5-cm-deep layer of paraffin oil to avoid direct contamination with atmospheric oxygen. The part of the plant shoot in contact with paraffin oil was protected by a layer of parafilm. The entire experimental unit was transferred to a growth chamber where changes in colour and redox potential of the agar solution were monitored for 6 h.

Oxidation of the methylene-blue, indicated by a blue halo formation, and changes in redox potential were recorded systematically every 10 min for 70 min. Brightened platinum electrodes were inserted alongside selected roots and into the adjacent bulk agar to measure redox potential (E_h), which was calculated by adding the potential of the calomel reference electrode (+ 244 mV) to the mV reading.

Powder X-ray diffraction (XRD) of the rhizosphere of Juncus bulbosus

Roots with iron oxide plaque were quick-frozen and freeze-dried. After 24 h, the iron plaque was separated from the roots and gently ground by hand. X- ray diffraction analyses of the powdered specimens were conducted using Co-$K\alpha$ radiation as described by Bigham *et al.* (1990).

Scanning electron microscopy (SEM)

For SEM, fresh root segments (10 mm from apex) with iron plaque were fixed with 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 4°C over night. After gentle washing with buffer solution, segments were postfixed with 1% (w/v) OsO_4 for 2 h, dehydrated with acetone and embedded in epoxy resin (Spurr, 1969). After polymerization at 70°C for 24 h, specimens were cut into 4-mm sections and mounted on aluminium specimen mounts with epoxy resin. After gentle grinding, the specimens were polished and coated with carbon, then investigated with a SEM (ZEISS DSM 962, Zeiss, Jena, Germany) at 20 kV with a working distance of 25 mm using a backscattering electron (BSE) detector and an energy-dispersive X-ray (EDX) detector (Link ISIS, Oxford Instruments, Oxford, UK).

RESULTS AND DISCUSSION

Root oxygen release pathways and redox status

The release of oxygen from the root to the surrounding sediment has been postulated as the mechanism that prevents the movement of potentially toxic substances (e.g. Fe^{2+}) from the root surface or within the root to the shoots (Armstrong, 1979; Carlson & Forrest, 1982; Chabbi *et al.*, 1998). The rate of radial oxygen loss (ROL) from *J. bulbosus* roots using Ti^{3+}-citrate (Fig. 1) was 1.31 ± 0.07–1.58 ± 0.04 μmol g^{-1} d. wt h^{-1}. Although measurement of ROL with Ti^{3+}-citrate provides a quantitative measure of oxygen release from the whole root system, it provides no information on its distribution. The methylene-blue/agar technique showed which specific roots were releasing oxygen and the distribution of this release along an individual root axis. *J. bulbosus* roots showed oxygen release primarily from the sub-apical region. No oxygen release was exhibited along the remaining root length even after observation for 6 h. The results indicate that oxygen release along the whole root is limited and, consequently, oxygen supply to the root apex is more efficient in terms of oxidizing the substrate. The development of important blue-halo formation around the apex is caused by the leakage of the oxygen which oxidizes the leucomethylene blue in the rhizosphere. The measurement of changes in redox potentials (E_h) in the

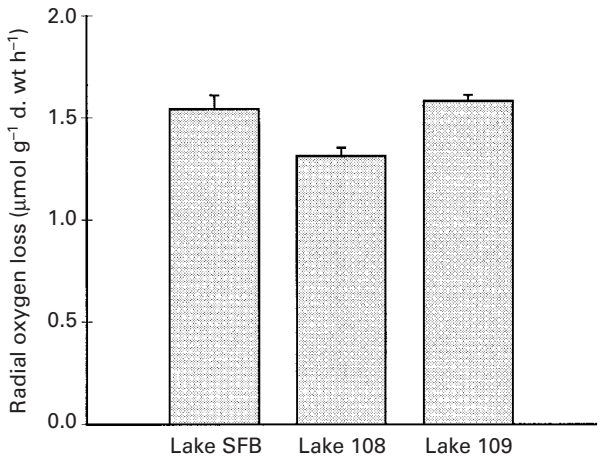

Fig. 1. Radial oxygen loss (ROL) from *Juncus bulbosus* in a 6-h period. Values are the mean ± SE ($n = 3$).

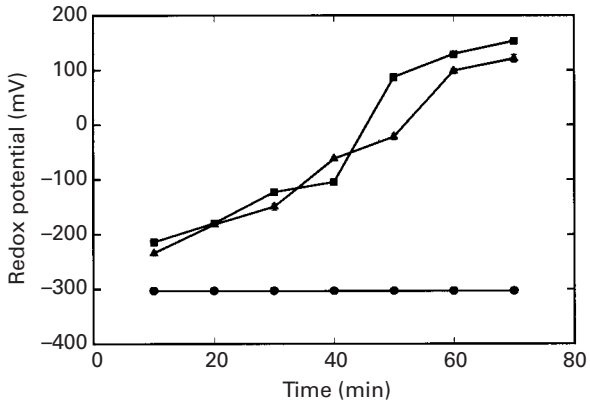

Fig. 2. Time course changes in rhizosphere redox potential (E_h) when root systems of *Juncus bulbosus* were placed in reduced methylene blue-agar solutions. *J. bulbosus* from Lake SFB, closed triangles; *J. bulbosus* from Lake 109, closed squares; control, closed circles. Platinum electrodes were inserted close to the root tip or several centimetres away from a root, outside the halo-formation zone (control). Values are the means ± SE ($n = 3$). Note that the SE bars are smaller than the symbols. Root length, 10–15 cm.

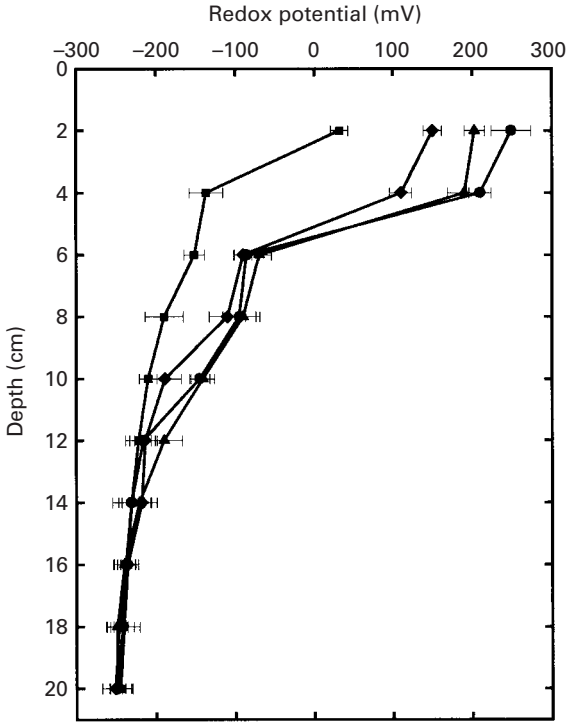

Fig. 3. Profiles of redox potential (E_h) in sediment of different lakes with and without *Juncus bulbosus*. Lake SFB, closed circles; Lake 108, closed diamonds; Lake 109, closed triangles; nonvegetated, closed squares. Profiles displayed from 2–10 cm were determined with four replicate electrodes and those from 10–20 cm with two replicate electrodes. Values are the means ± SE ($n = 2$–4).

methylene-blue agar medium at the root surface over time indicated higher redox potentials in the rhizosphere (*c.* +150 to +190 mV) when the platinum electrodes were inserted close to the root tips, inside the zone of halo formation. This demonstrates that oxygen release from *Juncus* roots was capable of changing the redox status of strongly reduced medium (reduced methylene-blue/agar = −300 mV). Redox potentials continued to increase over time in the rhizosphere of *J. bulbosus* roots (Fig. 2). This finding is in agreement with the Ti^{3+}-citrate measurement and can be interpreted to signify that the *Juncus* plants introduced enough oxygen into the soil to alter its redox state.

The rate of oxygen leakage of 1.58 μmol g^{-1} d. wt h^{-1} is higher than that in Roelefs *et al.* (1984) whose data show relatively low oxygen efflux from *J. bulbosus*, possibly because of the use of a different, less effective methodology. However, it is also possible that in their study, less root area was oxygen-permeable. The difficulty of measuring the rate of root oxygen release by wetland plants is still a matter of debate, since there is not a widespread literature on the topic. Considerable care must be taken in the design of experiments that address rate of oxygen release by wetland plants (Sorrell & Armstrong, 1994). In this study, measurements of rate of oxygen release were made on plants uprooted from lake sediment and transferred to conditions *in situ* using Ti^{3+}-citrate and methylene-blue/agar. Studying plants uprooted from lake sediment and used within 24 h after collection rather than those which have been grown in hydroponic culture in a glasshouse might minimize errors in the estimation of the rate of natural root oxygen release. Ti^{3+}-citrate and methylene-blue/agar induced an oxygen demand and E_h conditions (−300 mV) that might encourage the establishment of natural root oxygen release rates. The combination of both methods might overcome difficulties in quantifying the rate of oxygen release and time course of redox changes in the rhizosphere of *J. bulbosus*.

Rice plants and others macrophytes can release oxygen and raise sediment E_h in the root zone (Boone *et al.*, 1983; Justin & Armstrong, 1987; Armstrong *et al.*, 1992; Wigand *et al.* 1997), but in extreme conditions and at low pH some rice plants, for example, looked unhealthy (Alam, 1981) and *Luronium natans* or *Ranunculus ololeuco* decayed and lost their structure (Maessen *et al.*, 1992). The rate of oxygen release from roots was not measured under realistic field conditions here, but field measurement of E_h showed higher values in the root zone of *J. bulbosus* than in the sediment beneath it or in unvegetated sediment (Fig. 3). This must indicate (i) that the *Juncus* plants have released enough oxygen to increase the E_h into root zone and (ii) that *J. bulbosus* is able to survive and to alter its E_h in the sediment in spite of the environmental conditions of acid mining lakes (Table 1). Under laboratory conditions, Janiesch (1991) observed an increase in oxygen release in *Carex* species with increasing Fe^{2+} concentration, but the physiological reasons for this were not known. Bedford *et al.* (1991) mentioned that several sinks for oxygen could exist in the

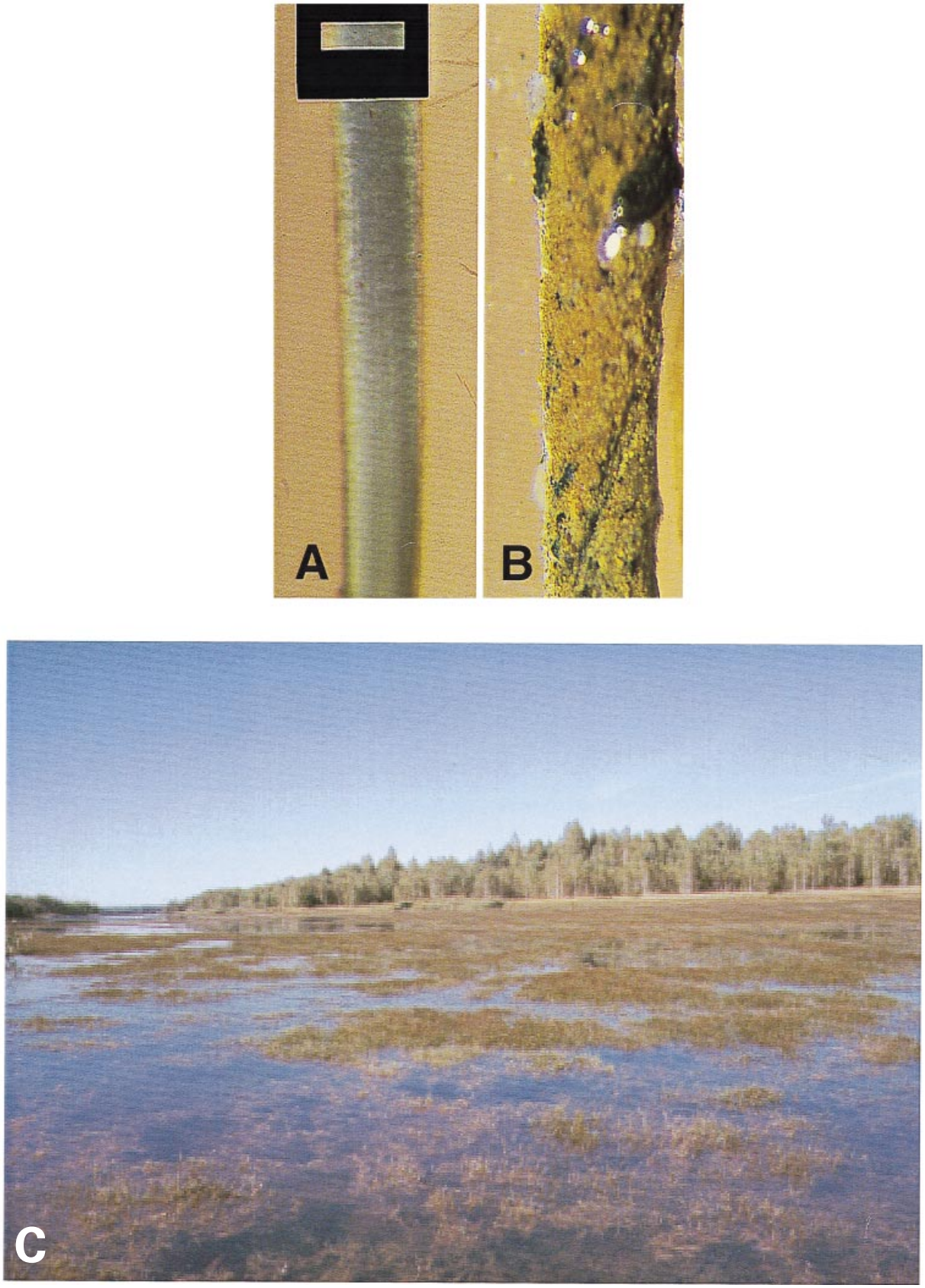

Fig. 4. Roots of *Juncus bulbosus*. (A) Without iron plaque (white root). (B) With iron plaque clearly visible as a reddish brown precipitate. Scale bar, 200 μm. (C) Aerial parts of a dense stand of *J. bulbosus* in Lake SFB.

rhizosphere and that the reduced form of iron reacts quickly with oxygen and seems to be a strong sink for oxygen. Begg *et al*. (1994) documented that in the soil an oxygen sink is enhanced by diffusion of ferrous iron towards the roots and its reaction with oxygen.

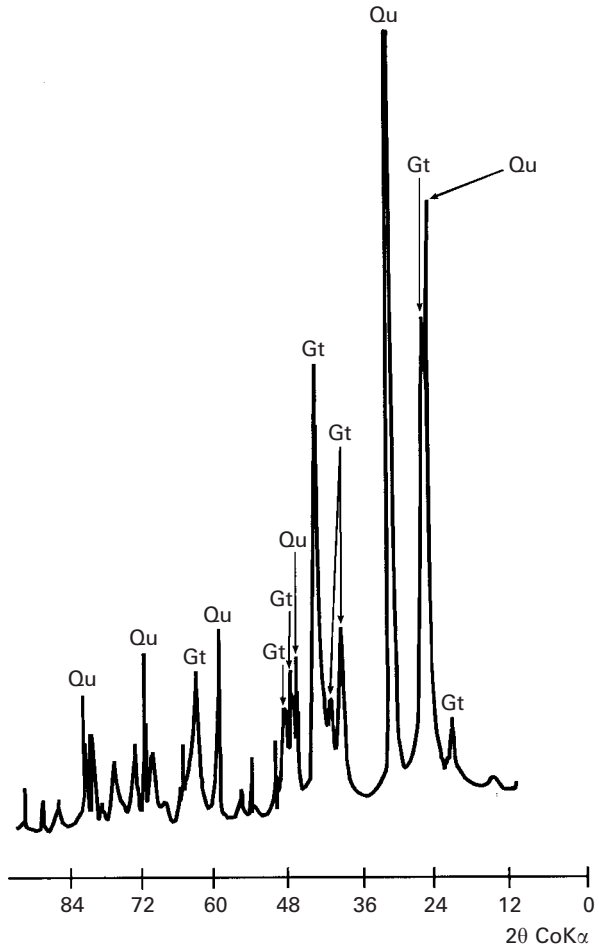

Fig. 5. X-ray diffraction pattern of oxidized root channels from *Juncus bulbosus* growing in acid lignite mine sediment. Gt, goethite; Qu, quartz.

Characterization of iron plaque

Juncus bulbosus was characterized by the presence of an extensive plaque surrounding the roots (Fig. 4). The data suggest that sediment oxygenation and subsequent increase of E_h results in oxidation of Fe^{2+} to Fe^{3+} which leads to the formation of iron oxide plaques. The oxide deposit is shown by XRD to be rich in goethite (Fig. 5) which has an average crystal size (mean coherence length perpendicular to (110)) of only *c.* 10 nm, as calculated from the corrected full width at half height of the (110) reflection using the Scherrer formula (Schwertmann & Fitzpatrick, 1977). This type of plaque, consisting of very small goethite crystals, forms commonly from oxidation of Fe^{2+} in surface environments at ambient temperatures under circumneutral conditions (for a review, see Cornell & Schwertmann, 1996). Chen *et al.* (1980) were the first to observe goethite in rice root deposits. Schwertmann (1959) and Carlson & Schwertmann (1990) demonstrated in laboratory experiments that CO_2 in the oxidation environment favoured goethite (alpha-FeOOH) over lepidocrocite (gamma-FeOOH) formation. By thin-sectioning a root concretion (called a pipe stem), Schwertmann & Fitzpatrick (1977) showed that the inner part close to the root was rich in goethite, whereas lepidocrocite dominated in the outer part. From this it is concluded that the release of CO_2 by the *Juncus* root–microorganism interaction fostered goethite formation.

It is assumed that inorganic C is a limiting factor for primary production in extremely acidic lakes (Goldman *et al.*, 1974). Moreover, much evidence in the literature indicates that rates of C uptake by roots of aquatic plants (Søndergaard & Sand-Jenssen, 1979) can be higher than uptake by their shoots, particularly in acid lakes. The extreme acidity (pH < 3.5) combined with shallowness of acid lakes leads to a weak concentration of CO_2 (*c.* 0.43 mg C l^{-1}, A. Chabbi unpublished material) around the submerged leaves of *J. bulbosus*. Nixdorf *et al.* (1998) reported a very weak concentration of DIC in the study lakes which was below the level of detection (< 0.5 mg C l^{-1}). A subsequent study (Kapfer, 1998) documented that DIC produced by microbial activities (e.g. sulphate reduction, denitrification, respiration) in the sediment is rapidly lost to the atmosphere. There is evidence however, to demonstrate that inorganic C controls the growth dynamics of *J. bulbosus* and that its competitive ability increases with increase in CO_2 in the system (Roelofs *et al.*, 1984; Wetzel *et al.*, 1984; Svedäng, 1992). The question then is how *J. bulbosus* avoids inorganic C limitation in acidic mining lakes.

Examination by SEM of transverse sections of oxidized root channels (10 mm from apex) show that the root is surrounded by quartz (Fig. 5) and Fe oxides (Chabbi *et al.*, 1997). Between the root and the sand grains there is a micro-space (Fig. 6A–C). This unusual space is inhabited by colonies of microorganisms (Fig. 6B–D). The result suggests that the microbial component probably consists of true rhizobacteria on the root surface beneath the iron plaque, as opposed to typical 'iron-associated bacteria', which would be expected to be literally coated with iron. So far as could be ascertained, evidence of this particular association has not been reported before. The presence of these organisms might be associated with the amount of material released from roots (root exudation patterns), which increase with plant stress, as demonstrated by several researchers (Hall *et al.*, 1978; Hall & Moore, 1979; Curl & Trelove, 1986), thereby providing microorganisms available substrates for metabolism. These microorganisms might metabolize the exudates to different extents and thereby cause an increase in the release of CO_2 in the rooting medium. Furthermore, the formation of iron plaques might prevent the loss of inorganic C from the system. It is possible that *Juncus* conserves some of its C by exploiting the accessory CO_2 produced in the micro-space. This would accord with the finding of Wetzel *et al.*, (1984) who concluded that a substantial part (a quarter to

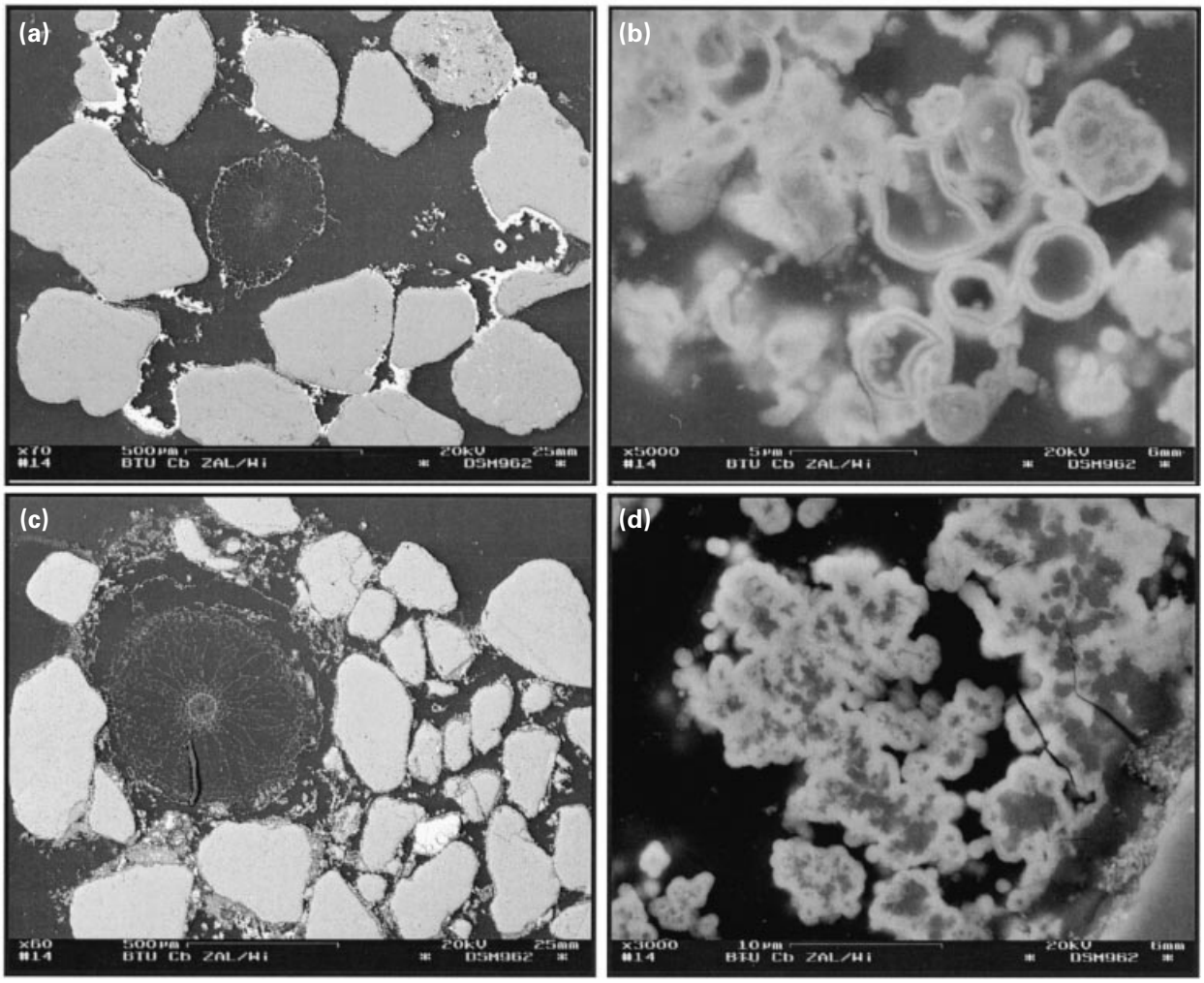

Fig. 6. Scanning electron micrographs of *Juncus bulbosus* root with iron plaque (10 mm from apex). (A) Lake SFB. (C) Lake 108. Visible free space between the surface root (centre) and mineral component. (B) and (D) microbial component between surface root and mineral component/red precipitate in (A) and (C).

a third) of the CO_2 fixed photosynthetically by *J. bulbosus* is derived from root uptake.

It is well known that ferrous iron oxidation and proton release from roots generates acidification (Ahmad & Nye, 1990; Begg *et al*., 1994). These two sources of acidity can produce large changes in pH close to roots and may exacerbate the problem of the plant in already acid sediments. However, net CO_2 assimilation might remove some of the acidity produced in Fe^{2+} oxidation and in the cation–anion intake imbalance from the micro-space as follows: $HCO_3^- + H^+ \rightarrow CO_2 + H_2O$ (Begg *et al*., 1994).

Internal iron distribution

The formation of iron plaques is considered by a number of researchers to be a mechanism of protection against the entry of reduced phytotoxic elements into the root cells (Armstrong 1967; Mendelssohn & Postek, 1982; Snowden & Wheeler, 1995; Chabbi *et al*., 1998). The REM show that rhizodermis and exodermis cells are characterized by high light BSE-contrasts (showing white in Fig. 7A), indicating high iron content (Fig. 7C). Inside the root, the central cylinder (parenchyma) is enclosed by an endodermis, which can be clearly seen as a formation of white spots (Fig. 7A, B). This area shows encrustation (light BSE-contrast) in the outer cell walls of the central cylinder. An elevated iron content in this region and a lower content within the stelar cylinder (Fig. 7C, D) might derive from the role of the endodermis (white spots). These observations were made by energy-dispersive X-ray spectroscopy analysis (Fig. 8). Such analysis shows that iron accumulated in the rhizodermis, exodermis and endodermis (white spots: Fig. 8A, B, D) whereas cortex and parenchyma regions exhibited a lower iron content (Fig. 8C–E). The concentration of iron in the cortical cells is presumably controlled to some extent by rhizodermal and exodermis cells which might prevent or at least diminish entry of iron into the cortex. Iron coating on the walls of exodermal cells was first reported by Armstrong & Boatman, 1967. Furthermore, the well developed aerenchyma and its role in oxygen diffusion protects the cells of cortex from the accretion of soluble Fe^{2+} (Mendels-

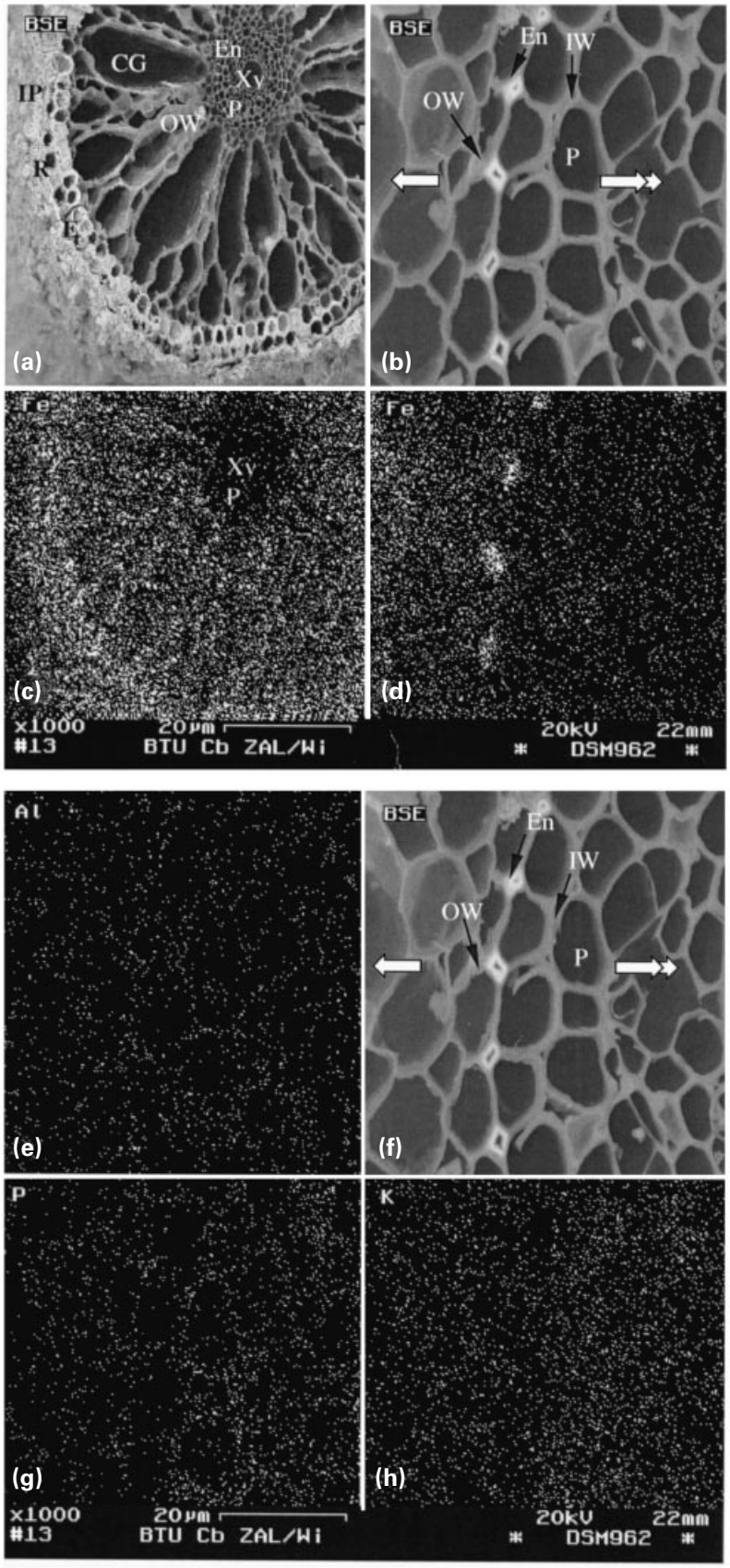

Fig. 7. Scanning electron micrographs (A, B, F) of section taken 10 mm from the apex of root of *Juncus bulbosus*. (C) Iron distribution in panel A; (D) iron distribution in panel B; (E) aluminium distribution in panel F; (G) phosphorus distribution in F; (H) potassium distribution in F. IP, iron plaque deposit; R, rhizodermis; E, exodermis (hypodermis); CG, cortical gas space (aerenchyma; En, endodermis (white spots); P, parenchyma; XY, xylem; OW, outer cell wall; IW, inner cell wall. Single arrow, direction of aerenchyma; double arrow, direction of xylem.

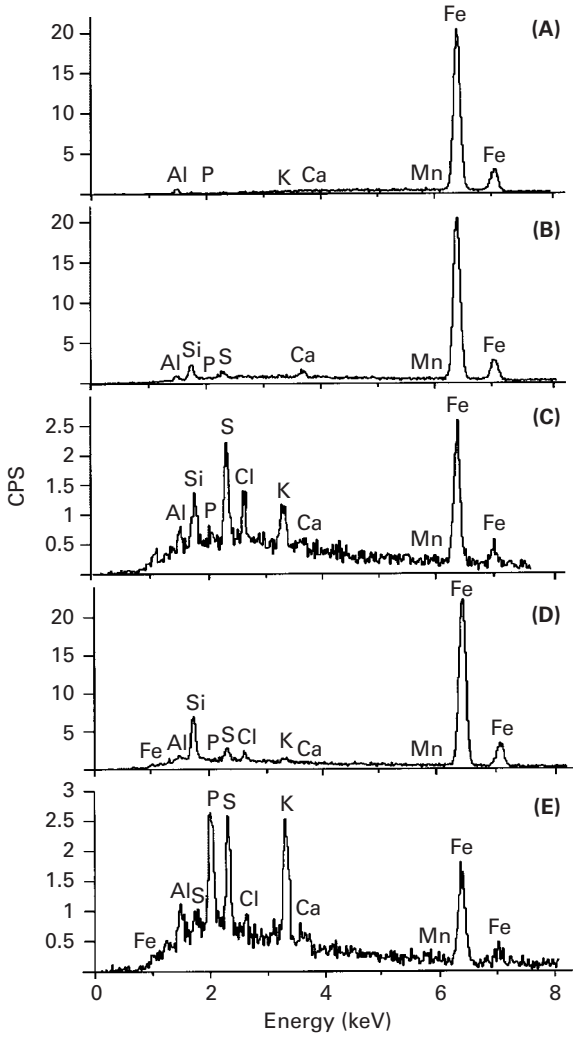

Fig. 8. Energy-dispersive X-ray (EDX) analysis showing internal elemental distribution in *Juncus bulbosus* as affected by iron plaque formation. (A) Rhizodermis; (B) exodermis; (C) cortex; (D) endodermis (white spot); (E) endodermis (parenchyma). Section taken 10 mm from apex.

sohn & Postek, 1982; Chabbi *et al*., 1998) and thereby avoids blockages in the aeration pathways, and severe consequences in the xylem system.

Further investigations indicated some organellar disturbance on the apical region (data not shown), suggesting that iron resistance induces changes in physiological traits, but how the plant controls this metal and its intercellular distribution and detoxification remains unclear (Kampfenkel, *et al*., 1995; I. Mendelssohn, personal communication). The plant might transport excess iron into the cell's waste disposal sites, the vacuoles, where it can be retained in complexes with organic acids and delayed from injuring the cell's fundamental biochemical equipment. No necrotic cells have been observed, which indicates that the plant has the full control of the excess iron. This observation is verified by the results of SEM and EDX analysis which show flow of nutrients, particularly phosphorus and potassium, to the xylem (Figs 7G, H, 8E). The measured total concentrations of phosphorus and potassium in the shoot of *J. bulbosus* were optimal (K: 0.66 ± 0.14 and 0.95 ± 0.13 mmol g^{-1} d. wt; P: 0.02 ± 0.01 and 0.03 ± 0.001 mmol g^{-1} d. wt; data not shown) and verify the functioning of root cells and translocation mechanism. It seems that an excess of iron in the environment did not cause any decrease in the absorption of potassium and phosphorus as it has, by contrast, been demonstrated to do in rice plants (Yoshida, 1981; Benckiser *et al*., 1984) and in *Lobelia dortmanna* (Christensen & Wigand, 1998). Such a conclusion raises the question whether or not *J. bulbosus* employs molecules known as phytochelatins which can bind metal iron in forms less toxic or whether the active uptake of potassium and phosphorus could be important in hindering iron toxicity.

In conclusion, ROL increases E_h and induces iron plaque formation (e.g. micro-space) which might foster a favourable zone for rhizobacterial establishment and a C source for plant metabolism as well as some alleviation of soil acidity. The REM and EDX analyses demonstrated that iron plaque formation *by J. bulbosus* in acid lignite mining sediment can immobilize iron but does not prevent adequate iron uptake and translocation into the root. The endodermis (white spots) and perhaps compartmentation of iron in subcellular structures (e. g. vacuoles) seem to be a second effective strategy for the avoidance of iron toxicity.

Survival of *J. bulbosus* in acid lignite mine sediment is not a single mechanism but a complex of interactions constituting morphological, physiological and biochemical adaptation. These interactions may help explain why *J. bulbosus* initiates plant colonization in acid lignite mining lakes in spite of extreme conditions.

ACKNOWLEDGEMENTS

I thank Prof. U. Schwertmann, TU of Munich, for providing powder X-ray diffraction, Dr W. Wiehe, central analytical laboratory ,TU of Cottbus for providing the SEM and TEM, Mrs N. Hendgen, R. Müller and G. Franke, TU of Cottbus for technical assistance. I acknowledge the helpful and valuable comments of Prof. U. Schwertmann of TU of Munich, Prof. Robert J. Kremer of the University of Missouri and two anonymous referees for their thorough reviews which improved the manuscript. This work was supported by the German Ministry of Research and Education (BMBF, FKZ 0339648) and LMBV GmbH.

REFERENCES

Ahmad AR, Nye PH. 1990. Couple diffusion and oxidation of ferrous iron in soils. I. Kinetics of oxidation of ferrous iron in soil suspension. *Journal of Soil Science* **41**: 395–409

Alam SM. 1981. Effects of solution pH on the growth and chemical composition of rice plants. *Journal of Plant Nutrition* **4**: 247–260.

Armstrong W. 1967. The oxidising activity of roots in waterlogged soils. *Physiologia Plantarum* **20**: 920–926.

Armstrong W. 1975. Waterlogged soils. In: Etherington JR, ed.

Environment and Plant Ecology. London, UK: John Wiley and Sons, 181–218.

Armstrong W. 1979. Aeration in higher plants. *Advances in Botanical Research* **7**: 226–332.

Armstrong J, Armstrong W, Beckett P. 1992. *Phragmites australis*: Venturi-and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation. *New Phytologist* **120**: 197–207.

Armstrong W, Boatman DJ. 1967. Some field observations relating the growth of bog plants to conditions of soil aeration. *Journal of Ecology* **55**: 101–110.

Bedford BL, Bouldin DR, Beliveau BD. 1991. Net oxygen and carbon-dioxide balances in solutions bathing roots of wetland plants. *Journal of Ecology* **79**: 943–959.

Begg CBM, Kirk GJD, Mackenzie AF Neue HU. 1994. Root-induced iron oxidation and pH changes in the lowland rice rhizosphere. *New Phytologist* **128**: 469–477.

Benckiser G, Santiago S, Neue HU, Watanabe I, Ottow JCG. 1984. Effect of fertilisation on exudation, dehydrogenase activity, iron-reducing populations and Fe^{+2} formation in the rhizosphere of rice (*Oryza sativa* L.) in relation to iron toxicity. *Plant and Soil*. **79**: 305–316.

Bigham JM, Schwertmann U, Carlson L, Murad E. 1990. A poorly crystallised oxyhydroxysulfate of iron formed by bacterial oxidation of Fe(II) in acid waters. *Geochymica and Cosmochymica Acta* **54**: 2743–2758.

Boone CM, JM Bristow, van Loon GW. 1983. The relative efficiency of ionic iron (III) and iron (II) utilisation by the rice plant. *Journal of Plant Nutrition* **6**: 202–218.

Carlson PR, Forest J. 1982. Uptake of dissolved sulfide by *Spartina alterniflora*: evidence from natural sulfur isotope abundance ratios. *Science* **216**: 633–635.

Carlson L, Schwertmann U. 1990. The effect of CO_2 and oxidation rate on the formation of goethite versus lepidocrocite from an Fe(II) system at pH 6 and 7. *Clay Minerals* **25**: 65–71.

Chabbi A, W Pietsch, Hüttl RF. 1997. Iron plaque formation by *Juncus bulbosus*: a habitat for microbial activity in acid mining lakes. 3*rd International BIOGEOMO Symposium on Ecosystem Behaviour*, *Villanova University*, *PA*, *USA*, *June* 21–25, 1997. Cambridge Publication. *Journal of Conference Abstracts* **2 (2)**: 153.

Chabbi A, W Pietsch, W Wiehe, Hüttl RF. 1998. *Juncus bulbosus* L.: Strategies of survival under extreme phytotoxic conditions in acid mine lakes in the Lusatian mining district, Germany. *International Journal of Ecology and Environmental Sciences* **24**: 271–292.

Chen CC, JB Dixon, Turner FT. 1980. Iron coatings on rice roots: mineralogy and quantity influencing factors. *Soil Science Society of America Journal* **44**: 635–639.

Christensen KK, Wigand C. 1998. Formation of root plaques and their influence on tissue phosphorus content in *Lobelia dortmanna*. *Aquatic Botany* **61**:111–122.

Cornell RM, Schwertmann U. 1996. The iron oxides. structure, properties, reactions, occurrence and uses. New York, USA: VCH Publishers.

Curl EA, Trelove B. 1986. The rhizosphere. *Advanced Series of Agriculture Science* **15**: 9–54.

DeLaune RD, SR Pezeshki, Pardue JH. 1990. An oxidation-reduction buffer for evaluating physiological response of plants to root oxygen stress. *Environmental and Experimental Botany* **30(2)**: 243–247.

Goldman JC, Oswald WJ, Jenkins D. 1974. The kinetics of inorganic carbon-limited algal growth. *Journal of the Water Pollution Control Federation* **46**: 554–574.

Hale MG, Moore LD. 1979. Factors affecting root exudation. II: 1970–1978. *Advanced Agronomy* **31**: 93–124.

Hale MG, LD Moore, Griffin GJ. 1978. Roots exudates and exudation. In: Dommergues YR, Krupa SV, eds. *Interaction between non-pathogenic soil micro-organisms and plants*. Amsterdam, The Netherlands: Elsevier, 163–204.

Hinneri S. 1976. On the ecology and phenotypic plasticity of vascular hydrophytes in a sulphate-rich, acidotrophic freshwater reservoir, SW coast of Finland. *Annals of Botany Fennoscandia* **13**: 97–105.

Janiesch P. 1991. Ecophysiological adaptations of higher plants in natural communities to waterlogging. In: Rozema J, Verkleij JAC, eds. *Ecological responses to environmental stresses*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 50–60.

Justin SHFW, Armstrong W. 1987. The anatomical characteristics of roots and plant response to soil flooding. *New Phytologist* **106**: 465–495.

Kampfenkel K, Van Montagu M, Inzé D. 1995. Effects of iron excess on *Nicotiana plumbaginifolia* plants. Implications to oxidative stress. *Plant Physiology*. **107**: 725–735.

Kapfer M. 1998. Assessment of colonization and primary production of microphytobentos in the littoral of acidic mining lakes in Lusatia, Germany. *Water Air and Soil Pollution* **108**: 331–340.

Maessen M, Roelofs JGM, Bellmakers MJS, Verheggen GM. 1992. The effects of aluminium, aluminium/calcium ratios and pH on aquatic plants from poorly buffered environments. *Aquatic Botany* **43**: 115–127.

Mendelssohn IA, Postek MT. 1982. Elemental analysis of deposits on the roots of *Spartina alterniflora* Liosel. *American Journal of Botany* **69**: 904–912.

Nixdorf B, Wollmann, K Deneke R. 1998. Ecological potentials for planctonic development and food web interactions in extremely acidic mining lakes in Lusatian. In: Geller W, Klapper H, Salomons W, eds. *Acidic mining lakes*. Berlin, Germany: Springer Verlag, 147–167.

Pietsch W. 1973. Vegetationsentwicklung und Gewässergenese in den Tagebauseen des Lausitzer Braunkohlen-Revieres. *Archiv für Naturschutz und Landschaftsforschung* **13**: 187–217.

Roelofs JGM. 1983. Impact of acidification and eutrophication on macrophyte communities in soft waters in The Netherlands. I. Field observations. *Aquatic Botany* **17**: 139–155.

Roelofs JGM, Schuurkes JAAR, Smits AJM. 1984. Impact of acidification and eutrophication on macrophyte communities in soft waters. II. Experimental studies. *Aquatic Botany* **18**: 389–411.

Schwertmann U. 1959. Über die Synthese definierter Eisenoxyde unter verschiedenen Bedingungen. *Zeitschrift anorganische allgemeine Chemie* **298**: 337–348.

Schwertmann U, Fitzpatrick RW. 1977. Occurrence of lepidocrocite and its association with goethite in Natal soils. *Soil Science Society of America Journal* **41**: 1013–1018.

Snowden (*née* Cook) RED, Wheeler BD. 1995. Chemical changes in selected wetland plant species with increasing Fe supply, with specific reference to root precipitates and Fe tolerance. *New Phytologist* **131**: 503–520.

Sondergaard K, Sand Jensen K. 1979. Carbon uptake by the leaves and roots of *Littorella uniflora* L. Aschers. *Aquatic Botany* **6**: 1–12.

Sorrell BK, Armstrong W. 1994. On the difficulties of measuring oxygen release by root systems of wetland plants. *Journal of Ecology* **82**: 177–183.

Sorrell BK, H Brix, Ott PT. 1993. Oxygen exchange by entire root systems of *Cyperus involucratus* and *Eleocharis sphacelata*. *Journal of Aquatic Plant Management* **31**: 24–28.

Spurr AR. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* **26**: 31–43.

Svedäng MU. 1992. Carbon dioxide as a factor regulating the growth dynamics of *Juncus bulbosus*. *Aquatic Botany* **42**: 231–240.

Trolldenier G. 1988. Visualisation of oxidising power of rice roots and possible participation of bacteria in iron deposition. *Zeitschrift für Pflanzenernährung und Bodenkunde* **151**: 117–121

Van Damm H. 1988. Acidification of three moorland pools in The Netherlands by acid precipitation and extreme drought periods over seven decades. *Freshwater Biology* **20**: 157–176.

Wetzel RG, Brammer ES, Forsberg C. 1984. Photosynthesis of submerged macrophytes in acidified lakes. I. Carbon fluxes and recycling of CO_2 by *Juncus bulbosus* L. *Aquatic Botany* **19**: 329–342.

Wigand C, Stevenson JC, Cornwell JC. 1997. Effects of different submersed macrophytes on sediment biogeochemistry. *Aquatic Botany*. **56**: 233–244.

Wortelboer F. G. 1990. A model on the competition between two macrophyte species in acidifying shallow soft water lakes in The Netherlands. *Hydrobiological Bulletin*. **24**: 91–107.

Yoshida S. 1981. *Fundamentals of rice crop science*. Manila, Philippines: International Rice Research Institute.

Zehnder AJB, Wuhrmann K. 1976. Titanium (III) citrate as non-toxic oxidation-reduction buffering system for the culture of obligate anaerobes. *Sciences* **194**: 1165–1166.