

The relative roles of internal aeration, radial oxygen losses, iron exclusion and nutrient balances in flood-tolerance of *Rumex* species

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SUMMARY

Radial oxygen losses (ROL) from the roots of three *Rumex* species, that occur in the river ecosystem in The Netherlands and show a differential response towards flooding, were compared. Oxygen loss from whole root systems was demonstrated and the ROL of single roots was quantified. Radial oxygen losses were higher in the flood-tolerant *R. maritimus* and *R. crispus* than in the intolerant *R. thyrsiflorus*. In all species oxygen loss occurred over the whole root surface between the base and the apex, but the rates differed as well as root wall permeabilities to oxygen. High oxygen losses in *R. maritimus* and *R. crispus* were correlated with high internal oxygen pressures near the root apex, consistent with prolonged root growth under anaerobic conditions in these species. On a flooded clay soil, the more tolerant species showed soil penetration and iron oxidation to greater depths, but all species developed an iron plaque on the roots. Shoot iron content was highest in the flood-tolerant *R. maritimus*. Upon flooding of the flood-intolerant *R. thyrsiflorus*, however, there was a substantial decrease in shoot dry weight and tissue nutrient levels. This was attributed to restricted root development rather than to iron toxicity.

Key-words: flood-tolerance, iron toxicity, nutrient stress, oxygen loss, *Rumex*.

INTRODUCTION

The process of rhizosphere oxidation by plant roots has been known for very many years (Molisch 1887; Groenewege 1922). This oxidation and the release of oxygen *per se* are now described as important characteristics of flood-tolerance (van Raalte 1944; Yoshida & Tadano 1978; Armstrong 1967, 1971, 1979; Ottow *et al.* 1982). It is generally accepted that the primary beneficial effect of radial oxygen loss (ROL) is the fact that potential soil toxins, such as reduced iron, manganese and hydrogen sulphide, which can increase to toxic concentrations under anaerobic conditions, can be immobilized or detoxified (Bartlett 1961; Armstrong 1967, 1978, 1979; Armstrong & Boatman 1967; Jones & Etherington 1970; van Breemen & Moormann 1978; Ottow *et al.* 1982). Most striking and of considerable importance is the accumulation of ferrous iron in the soil solution

(Ponnamperuma 1972). The subsequent oxidation creates clearly visible iron oxide and hydroxide precipitations on the roots (Groenewege 1922; Molisch 1926; van Raalte 1944; Armstrong 1967). A high and efficient ROL is said to slow down iron uptake, and to help prevent iron toxicity in the shoot (Tanaka *et al.* 1966; Martin 1968; Armstrong 1978, 1979; van Breemen & Moormann 1978; Yoshida & Tadano 1978; Ottow *et al.* 1982; Rozema *et al.* 1985; Iremonger & Kelly 1988). It has also been suggested, however, that iron plaque formation and iron oxide precipitations in the rhizosphere might hinder the uptake of essential nutrients (Howeler 1973; Benckiser *et al.* 1984; Wheeler *et al.* 1985; Otte *et al.* 1989), and in this respect ROL could be a disadvantage for the nutrient supply of the plant. In addition some doubts have been expressed recently concerning the significance of 'direct' iron toxicity as a determinant of flood tolerance and/or species distribution (Benckiser *et al.* 1984; Etherington 1984; Schat 1984).

Oxygen loss from roots can be demonstrated or quantified by several techniques. Dye techniques can easily be performed to show the location of oxygen loss in whole root systems (e.g. van Raalte 1941; Armstrong 1967; Trolldenier 1988). The ROL of single roots can be quantified accurately by polarographic measurement; the so-called 'cylindrical platinum electrode' method (Armstrong 1964, 1979) seems to be the most suitable. With this method ROL can be quantified at different positions along the root. It also allows the calculation of the internal oxygen concentration near the root apex, which is the final outcome of such root characteristics as the magnitude and distribution of respiratory demand, porosity and root wall permeability, and is closely correlated to root growth (Armstrong 1979).

In this study we compare the ROL of three *Rumex* species, which occur in the river ecosystem in The Netherlands and show a differential response towards flooding (Laan *et al.* 1989). One of the most obvious responses of these plants is the formation of a new root system upon flooding; the new roots of the tolerant species *R. maritimus* and *R. crispus* contain aerenchyma, but those of the intolerant *R. thyrsiflorus* do not. The significance of aerenchyma formation, and concomitant oxygen loss for the differential flood-tolerance of the species, was tested by growth of the plants in a flooded clay substrate. Attention was paid to the possible role of iron toxicity as a determinant for the flood-tolerance of the *Rumex* species, and the effects of flooding on the nutrient status were examined.

MATERIALS AND METHODS

Root oxidation of leuco-methylene blue

Plant growth. Seeds of *Rumex thyrsiflorus* Fingerh., *R. crispus* L. and *R. maritimus* L., collected from natural populations in the river area near Nijmegen (The Netherlands), were sown in trays containing black polyethylene grains (low density grains, BP Grangemouth, UK), which were totally submerged in 25% full strength modified Hoagland's solution (Hoagland & Arnon 1950). The trays were covered with a glass plate and placed in a germination cell for 1–2 weeks (25°C (day), 15°C (night); 16 h fluorescent light (Philips TL 33) at 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 8 h dark). After germination the young plants and young tillers of rice plants (*Oryza sativa* L.) were allowed to grow for 2–3 weeks in a growth room (temperature 25°C, RH 70%; 16 h fluorescent light at 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (LI-COR photometer), 8 h dark). They were then carefully transplanted to containers (13 $\times 10^{-3} \text{ m}^3$), filled with 25% Hoagland's solution and allowed to grow for another week. Some of these young plants were grown aerobically by bubbling air through the nutrient solution, while

another batch was transferred to containers, filled with a stagnant anaerobic 0.1% (w:v) agar in 25% Hoagland's solution; the latter were kept there until they had formed new lateral (*Rumex*), or adventitious (rice) roots. The water level of all the containers was maintained at the original level and nutrient solutions were changed twice a week. The pH of the unadjusted nutrient solution was 5.5 and remained constant during the experiment.

Plants were carefully transferred to a flat glass box (40 × 30 × 1 cm) with the shoot protruding through a small recess at the side of the box. The root system was spread over the box and the location of the separate roots was fixed with small glass rods (diameter 3 mm). The box was then closed by carefully sealing the upper glass plate with vaseline, while the recess containing the lower shoot parts was sealed with plasticine. The inner part of the box was flushed with nitrogen gas for 15 min through tubing placed inside the box before closure. During this nitrogen flush a leuco-methylene blue solution (25 mg l⁻¹), reduced via titration with sodium dithionite and maintained under anaerobic conditions by a nitrogen flow, was added to the box through a glass union fixed to the upper glass plate. The union was then closed and the nitrogen flow stopped. From this starting point, oxidation of leuco-methylene blue by roots of comparable length was followed by photographing the box in a time series.

Quantification of radial oxygen loss with the 'cylindrical platinum electrode' method

Plant growth. Seeds of all three *Rumex* species were sown on vermiculite in a greenhouse (temperature 15–27°C; 16 h light at 60–100 μmol m⁻² s⁻¹, 8 h dark). After germination and growth for 2 weeks, the plants were transferred to the modified 25% Hoagland's solution, and allowed to grow for another 5–6 weeks in a growth room (temperature 23°C, 16 h light at 150 μmol m⁻² s⁻¹, 8 h dark). They were then transferred to a 25% Hoagland's 0.05% (w:v) anaerobic agar solution and within 1 (*R. crispus* and *R. maritimus*) or 2 (*R. thyrsoiflorus*) weeks new laterals had developed with a length of 3–10 cm.

Experimental assembly and determination of radial oxygen loss (ROL). A plant was placed in a rectangular perspex vessel, which was completely filled with 0.05% (w:v) anaerobic agar solution (at least 24 h bubbling with oxygen-free nitrogen). The shoot base was sealed with wet cotton wool in a drilled rubber stopper. A cylindrical platinum cathode ($h = 5.0$ mm, diameter 2.25 mm) was drawn into position to ensleeve a newly formed lateral, and two small holes at the edge of the rubber stopper facilitated the positioning of the electrode at 0.2 cm from the root tip using a bicycle spoke. Saturated Ag/AgCl half-cells were used as anodes. Polarograms and determination of the ROL at a different position along the root were performed as described by Armstrong (1979) and Webb & Armstrong (1983). After determining a ROL profile, the position and length of the lateral on the tap-root were recorded. The diameter of the lateral was measured at the same positions where the ROL was determined using a travelling Vernier microscope (accuracy 0.01 mm, Precision Tools & Instruments, UK).

From the ROL data the root surface oxygen concentrations in the apical region were calculated according to Armstrong (1979). In this apical region, where root wall permeability is usually high, these root surface concentrations give a close approximation of the internal cortical gas-phase oxygen concentrations (Armstrong & Wright 1975; Armstrong & Webb 1985).

Growth of plants on a clay substrate

Experimental assembly. Plants germinated and pregrown for 2 weeks (as described in 'Root Oxidation of Leuco-Methylene Blue'), were transferred to vertical PVC tubes (diameter 12 cm, height 40 cm) and filled with a 1:1 (v:v) clay:sand mixture [river sand and clay from a beet-field (pH (H₂O) 6.9; weight percentage organic matter 5.4 ± 0.1)] which was enriched with some homogeneously distributed plant material. A PVC bottom containing four holes (diameter 6 mm) allowed free contact between the outer environment and the substrate. The plants were allowed to grow for 4 weeks in a greenhouse (c. 19°C, RH 70%, 16 h light, consisting of daylight filtered by greenhouse glass (120–1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and supplementary light (high-pressure sodium lamp, Osram Vialox, 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) when light intensity decreased to values lower than 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 8 h dark), after which half were flooded with tap-water to 2 cm above the soil surface. The other plants were used as controls; these tubes were checked daily and, when necessary, watered to maintain the original water content (drainage). During the flooding treatment the redox potential was recorded at different depths and the soil solution was sampled at the same depths to determine the 'free'-iron concentration.

After 4 weeks of flooding the plants were harvested by carefully pushing out the soil core complete with root system. The depth of oxidation of the soil, as indicated by its brownish colour, and clearly distinguishable from the reduced black soil, was recorded. Shoots were cut off and fresh weight and dry weight (24 h, 70°C) were determined; the root system was carefully washed out in a white container; the maximal root penetration and location of iron plaques and so-called micropedotubuli (Brewer 1964) were recorded during removal of the soil. Representative, randomly chosen living roots were sampled and used for the determination of iron content of the plaques and of the total amount of extracellular iron.

Redox potential measurements. Platinum wire electrodes were placed at different depths. The readings were stable after 1 or 2 days and these redox potentials were recorded (Pt cathode, saturated Ag/AgCl-anode, $E_0 = 199 \text{ mV}$).

Free iron concentration in the soil solution. Several times during the flooding treatment the soil solution was sampled at different depths with 2-ml pipettes. A piece of cotton-wool was pushed into the tip to prevent the entry of clay particles. The free-iron concentration was determined by adding 1 ml anaerobic 1 mM 2,2'-bipyridyl solution to 1-ml samples of soil solution, and after vigorous shaking A_{520} was determined (1 mM Fe[bipyridyl]₃ = 8.650). A few grains of sodium dithionite were then added to the samples and again the A_{520} was determined. When the difference between the absorbances exceeded 10%, the data were not taken into account, because this indicated a contamination of the samples with clay particles. The method did not allow sampling of the drained (control) soil.

Iron content of plaque on the roots. The apical 10–15 cm of the roots of the drained plants were very carefully extracted, and the roots of the flooded plants were separated into segments from the reduced and from the oxidized soil layers. One-gram fresh roots of drained plants and of root segments from the oxidized soil layers and 0.25 g of root segments from reduced soil layers were rinsed in demineralized water and the total amount of extracellular iron was determined after Bienfait *et al.* (1984); because of the expectedly high iron content, 4.5 instead of a 1.5 mM bipyridyl solution was used.

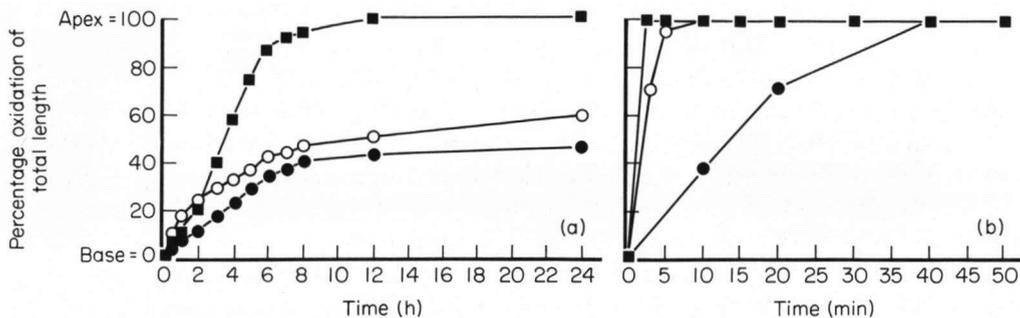


Fig. 1. Time course of readily perceived oxidation of leuco-methylene blue as blue coloration rate of primary (a) and newly formed (b) lateral roots of *Rumex thyrsoiflorus* (●), *R. crispus* (○) and *R. maritimus* (■). Data as a percentage coloration of the total length of the root; base = 0%, apex = 100%; means of four replicates. Mean length of primary roots \pm SD, *R. thyrsoiflorus* 19.3 ± 4.4 , *R. crispus* 22.0 ± 7.1 , *R. maritimus* 24.4 ± 2.5 cm; newly formed roots: *R. thyrsoiflorus* 5.0 ± 0.6 , *R. crispus* 12.8 ± 2.5 , *R. maritimus* 12.5 ± 2.0 cm.

Foliar nutrient levels. All dry leaves of the shoot system were ground in a sample mill (Tecator Cyclotec 1093) and broken down in a mixture of H_2SO_4 and salicylic acid (100 ml conc. H_2SO_4 in 18 ml H_2O + 6 g salicylic acid; overnight at room temperature for 1 h at $180^\circ C$). The destruction was completed by adding 400 μ l 30% H_2O_2 (v:v), raising the temperature to $230^\circ C$ (10 min), cooling down, adding another 400 μ l H_2O_2 , raising the temperature, etc. until the samples were colourless. Samples were analysed for calcium, magnesium, phosphorus and potassium on an inductive-coupled-plasma emission spectrophotometer (ICP); total nitrogen content was determined colorimetrically (Hampson 1977).

The total iron content of the leaves was determined on 50–100 mg dry leaf material ashed at $650^\circ C$, using the method of Scott (1944).

RESULTS

Root oxidation of leuco-methylene blue

Blue coloration at the root periphery could be observed in all the roots under investigation, indicating the presence of an oxidizing agent at or within the root surface. The time course of colour development along the root was recorded to compare the efficiency of the gas transport systems. Clear-cut differences between both species and root types were observed. The primary laterals of *R. thyrsoiflorus* and *R. crispus* showed a similar pattern: a slow progress of surface blue coloration from base to apex, with respective maxima of 58% and 46% of the total length after 24 h (Fig. 1a). In *R. maritimus* blue coloration also proceeded from base to apex, but took place much faster: after 12 h, all the roots were completely blue (100%, Fig. 1a).

In the newly formed laterals of all *Rumex* species, leuco-methylene blue oxidation was much faster than in the primary laterals. Here again, coloration took place from base to apex, and was slowest in the intolerant species *R. thyrsoiflorus* (100% coloration after 42 min, Fig. 1b). The tolerant species *R. crispus* and *R. maritimus* showed a much higher oxidation rate: the surfaces were completely blue after 10 and 2.5 min respectively (Fig. 1b). We used rice as a reference plant to check the method used, and to compare its responses with earlier results (Armstrong 1967, 1971; Trolldenier 1988). In rice, the blue coloration took place even faster than in *R. maritimus* and was complete within 1.5 min

(data not shown). It was impossible to observe whether the coloration started at the apical or the basal ends of the roots.

In addition to blue coloration, halo-formation took place, i.e. oxidation of leuco-methylene blue at a distance from the root. This halo-formation was most clearly visible in the newly formed laterals of the three *Rumex* species and in rice, and is indicative of rapid oxygen loss from the roots. In the primary laterals of the *Rumex* species some halo-formation also took place; in *R. crispus* and *R. maritimus* this was very poor and only visible after a long time. In the primary laterals of *R. thyrsiflorus* halo-formation started much earlier, but was restricted to the most basal parts of the root (data not shown).

The newly formed laterals of the *Rumex* species all showed progressive halo extension from base to apex (Fig. 2). In *R. maritimus*, halo-formation took place immediately after the start of the experiment and extended to 71% of the total root after 12 h; in both other species halo-formation became visible much later (> 2 h after the start, Fig. 2c). While after 12 h *R. crispus* had reached a value similar to that shown by *R. maritimus*, halo-formation remained very poor in *R. thyrsiflorus* (33% of total length, Fig. 2a), even though the length of the roots was three times shorter than in *R. crispus*. Halo-formation in rice proceeded from apex to base and was complete after 3 h (100%, Fig. 2d). In the oxidation course from apex to base, there was a small subapical zone which showed a delayed halo-formation in all investigated roots.

Radial oxygen loss from single roots. Oxygen loss from single newly formed roots was quantified along the root with the cylindrical Pt-electrode method (Armstrong 1979). Because the amount of oxygen lost from lateral roots appeared to be determined both by the length of the lateral and by the originating point from the tap-root, the ROL profiles are presented as a function of the total apparent diffusion path length, i.e. the distance between the base of the tap-root and the originating point of the lateral on the tap-root plus the distance from the originating point of the lateral to the electrode (Fig. 3).

Roots of comparable length of *R. maritimus* and *R. crispus* showed much higher oxygen losses than those of *R. thyrsiflorus*; while roots of *R. thyrsiflorus* did not show any apical ROL at a length of 4.5–5 cm, in both *R. maritimus* and *R. crispus* a considerable oxygen loss could be recorded at root lengths of 7.5–8 cm (Fig. 3b and c). With some exceptions there was a decrease in apical ROL with an increase in diffusion path length, and this was particularly evident in the *R. maritimus* profiles. The root surface oxygen concentrations were calculated (Armstrong 1979) from the ROL datasets. This revealed the enormous differences in the apical oxygen pressure between roots of comparable length of the species (Table 1), and indicates that root growth of *R. thyrsiflorus* will be restricted at a short length.

Furthermore, in *R. maritimus* the roots showed an increase in ROL from base to apex (Fig. 3c), indicating a partial loss in permeability in the wall layers of the subapical and basal root parts. This effect was clear in the shorter roots, but in longer roots and in *R. crispus* the oxygen profiles were much flatter. A reverse pattern could be observed in the roots of *R. thyrsiflorus*, where in most cases there was a decrease in ROL from base to apex (Fig. 3a). In *R. crispus* there were some exceptional oxygen profiles in short roots, beginning at the apical end of the tap-root (Fig. 3b).

Oxygen loss as related to growth and iron toxicity in plants grown on a flooded clay substrate

In the clay/sand soil used, the concentration of soluble ferrous iron in the soil solution was enhanced upon flooding to c. 0.2 mM in the absence of any plants (Table 2).

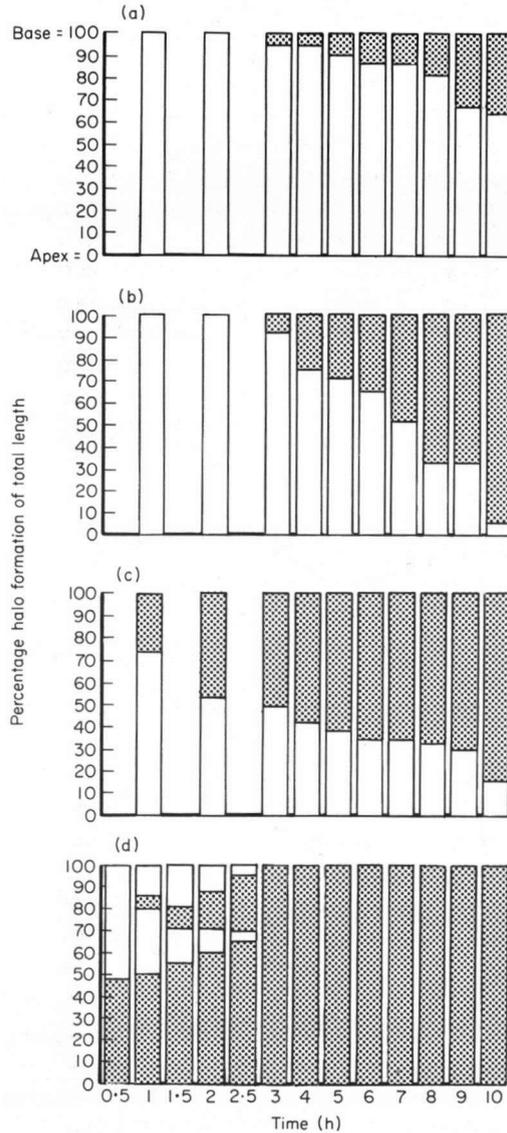


Fig. 2. Time course of halo-formation around newly formed lateral roots of *Rumex thyrsoiflorus* (a), *R. crispus* (b), *R. maritimus* (c) and *Oryza sativa* (d). Data as a percentage of the total length of the root; root apex = 0%, root base = 100%; length of *Rumex* roots as in Fig. 1, length of roots \pm SD of *Oryza sativa* 15.3 ± 0.7 cm.

Eight weeks after the start of the flooding treatment, the plants were harvested. The large number of new roots in *R. maritimus* had caused almost complete iron oxidation in the upper 30 cm of the soil (Table 3), clearly distinguishable because of the sharp transition zone between the oxidized (brownish) and the reduced (black) soil, and quantified by measurement of E_h and the amount of ferrous iron in the soil solution. E_h was higher in the oxidized zone (Table 3), and the amount of ferrous iron had decreased to values lower than 0.01 mM (Table 2). It should be noted that the redox potentials, whilst typical of soils showing iron oxidation, nevertheless are still indicative of mild reducing conditions and a

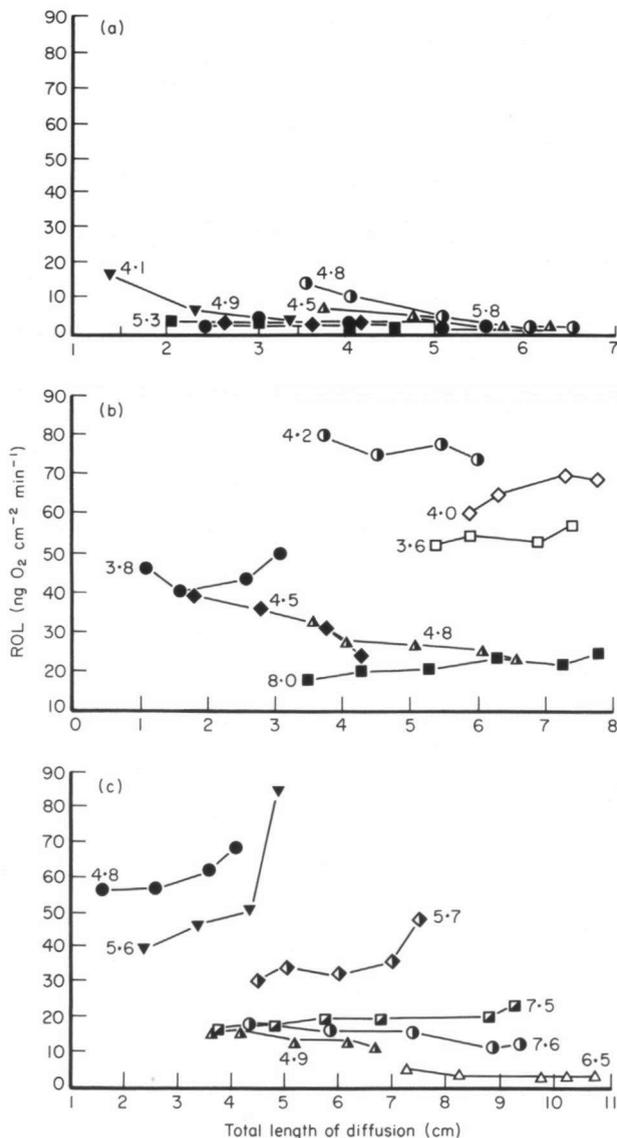


Fig. 3. Radial oxygen loss profiles of individual newly formed lateral roots of *Rumex* species with different length and originating at different positions on the tap-root as a function of the total calculated diffusion path length (a) *R. thrysiflorus*, (b) *R. crispus*, (c) *R. maritimus*. Profiles from base (left side) to apex (right side) of individual lateral roots of several plants indicated with different symbols. The length of each lateral root is indicated at profile; mean length of tap-roots = 5 cm. The origin of the lateral roots on the tap-root: at the base (closed symbols), halfway base and apex (half open symbols) or at the apex (open symbols). Horizontal axis: total calculated diffusion path length from base of tap-root to point of measurement on lateral root.

lack of significant amounts of free oxygen. In the *R. crispus* tubes the oxidation zone was c. 15 cm deep, and in the tubes with *R. thrysiflorus* only the upper 4–5 cm were oxidized (Table 3). These results illustrate the differences in soil profile oxidizing power between the *Rumex* species.

Closely connected to oxidizing power was maximal root penetration which was reached after 8 weeks. In the reduced soil layer the ROL is apparently not high enough to

Table 1. Internal oxygen pressure at 0.75 cm behind the apex of newly formed lateral roots of *Rumex* species

	Mean root length (cm)	Internal oxygen pressure (kPa)
<i>R. thyrsiflorus</i> (n=5)	5.1 ± 0.5	0.13 ± 0.04
<i>R. crispus</i> (n=7)	4.7 ± 1.5	3.2 ± 1.5
<i>R. maritimus</i> (n=4)	5.3 ± 0.5	3.7 ± 2.1

Mean of 4–7 replicates plus SD; data calculated from the ROL data as shown in Fig. 3.

Table 2. Effect of enhanced ferrous iron concentration in the soil solution on the amount of extracellular iron, precipitated on the roots and on leaf iron content of *Rumex* species, grown under drained or flooded conditions in a clay/sand soil for 8 weeks

Species/ treatment	Maximal [Fe ²⁺] in soil solution (mM)	Extracellular iron precipitated on root (µg [g fresh wt] ⁻¹)	Iron content of leaves (µmol [g dry wt] ⁻¹)
Control (no plants)			
Drained	ND*	—	—
Flooded	0.20 ± 0.04	—	—
<i>R. thyrsiflorus</i>			
Drained	ND	19 ± 4	3.6 ± 0.1
Flooded			
ox. †	<0.01	390 ± 70	4.4 ± 0.2
red. †	0.20 ± 0.04	7470 ± 1180	
<i>R. crispus</i>			
Drained	ND	13 ± 4	6.2 ± 0.4
Flooded			
ox.	<0.01	80 ± 15	4.1 ± 0.1
red.	0.23 ± 0.05	4640 ± 740	
<i>R. maritimus</i>			
Drained	ND	16 ± 4	5.7 ± 0.2
Flooded			
ox.	<0.01	45 ± 14	19.9 ± 0.4
red.	0.21 ± 0.05	4660 ± 650	

Means of five replicates ± SD.

*ND, not determined; †ox. = oxidized, red. = reduced soil layer.

oxidize the soil, and some deeper root growth stops completely, after which the tips turn black indicating death. The differences between the species result from the differences in the development of an aerenchyma system, the balance between internal diffusive resistance, distribution and the degree of respiration and oxygen leakage (Laan *et al.* 1989; P. Laan unpublished results). As a consequence, the apical oxygen concentration differs significantly for any given length of root (Table 1), and hence root penetration of

Table 3. Differential effect of internal aeration and concomitant oxidizing power of newly formed laterals of *Rumex* species on root penetration and the redox state of a clay/sand soil after 8 weeks of flooding

Species	Depth of oxidized soil layer (cm)	Maximal root depth (cm)	E_h (mV)	
			Oxidized soil layer	Reduced soil layer
Control (no plants)	0.0	—	—	-203 ± 2
<i>R. thyrsoiflorus</i>	4.6 ± 0.5	11.8 ± 2.5	55 ± 5	-183 ± 6
<i>R. crispus</i>	15.4 ± 1.8	29.0 ± 1.6	173 ± 6	-183 ± 15
<i>R. maritimus</i>	29.6 ± 2.5	36.6 ± 1.7	183 ± 15	-163 ± 6

Means of five replicates \pm SD.

R. maritimus was almost maximal (Table 3), while in *R. thyrsoiflorus* root penetration was poor and only a few roots could be found at a depth of 10–12 cm (Table 3).

Just below or above the transition zone between the oxidized and the reduced soil, iron plaque formation could be observed on the roots (Fig. 4). In *R. thyrsoiflorus*, where there is only poor oxygen loss, the rhizoplane was oxidized predominantly. The iron plaque contained 7470 μg iron per gram fresh roots, a higher value per unit fresh weight of roots than in *R. crispus* and *R. maritimus* (Table 2). It should be emphasized, however, that the diameter of the *R. crispus* and *R. maritimus* roots in most cases was twice as high as those of *R. thyrsoiflorus*. Therefore, these figures do not indicate a greater degree of plaque per unit length between the species, but serve to illustrate the ability of even *R. thyrsoiflorus* to bring about significant iron oxidation. In *R. maritimus* and to a lesser extent also in *R. crispus*, which both showed high radial oxygen losses, in addition to plaque formation, an obvious remote oxidation around individual roots was readily observed in the oxidized zone close to the transition zone. Just above the iron plaques and sometimes overlapping the plaque, micropodotubules were formed (Brewer 1964): cylinders of iron precipitations plus sand particles at a few millimetres distance from the root surface, leaving the root itself fairly clear from iron precipitates. It is this remote oxidation which must ultimately have led to the generalized oxidation of the profiles, and since the upper 4–5 cm of *R. thyrsoiflorus* sediment was visibly oxidized throughout, this also indicates a long-term remote oxidation, although signs of this could not be discovered around individual roots.

Roots from the oxidized soil layer, which did not show visible plaque formation, were also analysed. In all investigated species an increase in iron content of the flooded versus the drained plants was recorded (Table 2), probably due to precipitation in extracellular (apoplastic) space (Armstrong & Boatman 1967; Green & Etherington 1977). This increase was highest in *R. thyrsoiflorus* and much lower in both *R. crispus* and *R. maritimus*. It is interesting to note that the differential behaviour of the root systems, as stated above, did not result in differences of iron content of the leaves on the basis of dry weight in both *R. thyrsoiflorus* and *R. crispus*, which appeared to be as low as in the drained controls (Table 2). In *R. maritimus*, however, a fourfold increase was recorded.

Presumably as a consequence of the differences in root growth and concomitant soil oxidation, the biomass production and nutrient content of the shoots was variably

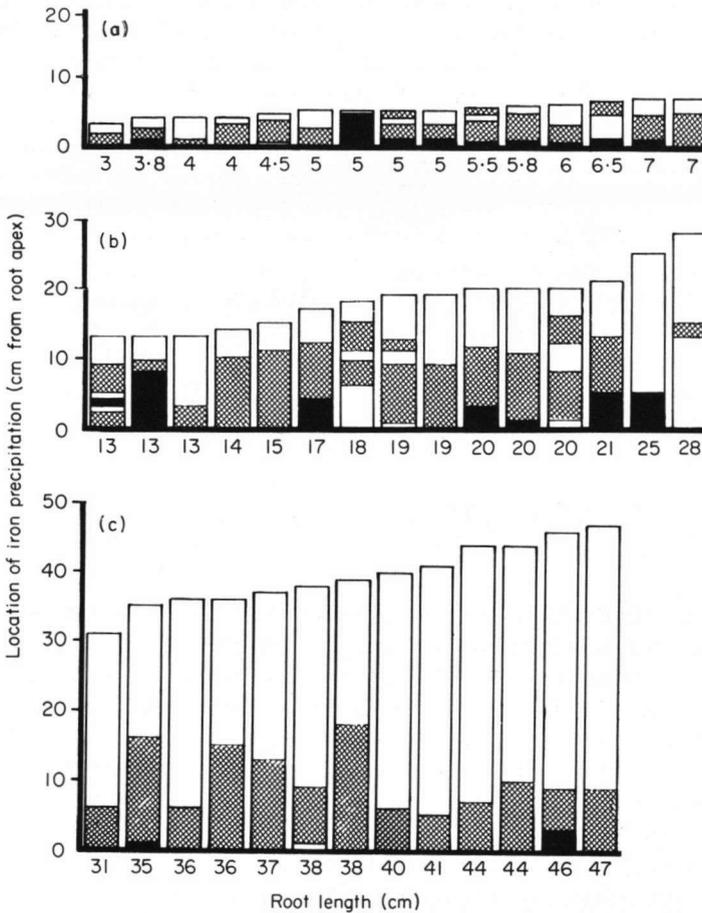


Fig. 4. Location of iron precipitation on newly formed roots of *Rumex thyrsiflorus* (a), *R. crispus* (b) and *R. maritimus* (c) with different length. (□) No iron precipitation, (▨) iron oxide plaque formed, (■) ferrous sulphide blackening. Plants grown on a flooded clay for 8 weeks in tubes with a length of 40 cm.

affected (Table 4). In *R. thyrsiflorus* and *R. crispus*, there was a general decrease in nutrient content upon flooding. However, shoot biomass production of *R. thyrsiflorus* was halved upon flooding, whilst the biomass production of *R. crispus* was hardly affected (Table 4). Thus, the lower nutrient content of *R. crispus* shoots did not result in a decrease of biomass production, as it did in *R. thyrsiflorus*. In *R. maritimus*, shoot biomass production was very high and not affected by flooding; also the nutrient content of the leaves, although already low in the drained plants, was not affected, except for potassium, which was halved.

DISCUSSION

The results show that roots of the three investigated *Rumex* species differ in oxidative power. All the species were able to oxidize methylene blue in solution and iron in flooded soil, but the roots developed in the anaerobic conditions were better able to effect these

Table 4. Effect of flooding on biomass production and on the nutrient content of leaves of *Rumex* species

Species/ treatment	Shoot dry weight (g)	Leaf nutrient content ($\mu\text{mol [g dry wt]}^{-1}$)				
		Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
<i>R. thyrsiflorus</i>						
Drained	15.2 ± 1.4	1878 ± 45	143 ± 10	1238 ± 37	323 ± 11	435 ± 22
Flooded	7.2 ± 1.4	1006 ± 64	44 ± 2	355 ± 15	190 ± 2	218 ± 3
<i>R. crispus</i>						
Drained	13.6 ± 1.4	1372 ± 54	90 ± 5	857 ± 39	532 ± 27	311 ± 7
Flooded	12.7 ± 2.5	702 ± 35	58 ± 10	347 ± 23	315 ± 13	160 ± 8
<i>R. maritimus</i>						
Drained	24.8 ± 2.6	1018 ± 91	59 ± 2	478 ± 11	615 ± 19	401 ± 5
Flooded	25.4 ± 3.9	1052 ± 52	59 ± 2	272 ± 10	761 ± 40	398 ± 11

Means of five replicates plus SD; plants grown in a clay/sand mixture for 8 weeks.

oxidations. The results with methylene blue (Fig. 2) indicate that there is significant permeability to oxygen along the whole length of the *Rumex* roots. This contrasts with patterns detected in rice here and elsewhere (Armstrong 1971).

As in the methylene blue experiments, the ROL profiles showed differences in oxygen losses between the species (Fig. 3); they are consistent with the differences in porosity, aerenchyma formation and respiratory demand along the roots (Laan *et al.* 1989; P. Laan unpublished results). Oxygen losses at the apices were high in *R. maritimus* and *R. crispus* and low in *R. thyrsiflorus*. It must be emphasized, however, that the rate of the ROL at the apices is very dependent upon the length of the root and its position on the tap-root. In general, the ROL decreased with increasing length, but the ROL patterns along the roots varied considerably among the species. In *R. maritimus*, the ROL tended to increase towards the apex, in *R. crispus* it was constant over the whole length of the root. In *R. thyrsiflorus* oxygen loss tended to decrease towards the apex. Apparently, in *R. maritimus* there was an obvious basipetal decrease in root wall permeability in the aging roots, in *R. crispus* this was less pronounced; in *R. thyrsiflorus* a decrease in root wall permeability could only be deduced by calculation.

The best correlation between the ROL and root length was obtained when root length was taken as the total diffusion path length between the base of the tap-root and the place of measurement (Fig. 3). Thus, in *R. maritimus* the basally attached roots showed high oxygen losses, and the more apical ones had much lower values. In *R. crispus* some exceptions were observed: some short laterals, originated at the apical ends of the tap-root, showed higher oxygen losses. This suggests that the tap-root of *R. crispus* did not show much diffusive resistance.

The differences described above explain, at least partly, the differential capacity of the root systems to oxidize the soil. Apical oxygen concentration is causally correlated to root growth (Armstrong 1979; Armstrong & Webb 1985), and differences in aerenchyma formation and subsequent internal aeration should result in a differential root growth and development. As oxygen loss was correlated to aerenchyma formation, the major effect observed is a differential soil iron oxidation depth.

With respect to iron toxicity, *R. thyrsiflorus* does not oxidize deeper soil layers, but the long-term oxidation of the shallower depth is apparently sufficient to detoxify that region in terms of iron. On the other hand, in *R. maritimus* shoot iron levels rose fourfold upon flooding, while biomass production was unaffected. Leaves of rice that clearly showed iron toxicity symptoms contained 8–20 $\mu\text{mol (g dry wt)}^{-1}$ (Yoshida & Tadano 1978; Ottow *et al.* 1982); *R. maritimus*, with healthy growing shoots and with leaves, containing 20 $\mu\text{mol (g dry wt)}^{-1}$, is apparently less sensitive to high iron levels in the leaves under these conditions. It is therefore unlikely that iron toxicity is a determinant of flood-tolerance in the investigated *Rumex* species; even if the soil solution iron levels had been higher, iron toxicity might have been more likely to occur in the flood-tolerant *R. maritimus* than in the relatively flood-intolerant *R. thyrsiflorus*.

It may seem surprising to find that the highest leaf iron content was recorded in the most flood-resistant *R. maritimus*, i.e. a high oxygen loss does not automatically lead to a low iron uptake. It should be taken into consideration, however, that iron uptake and iron exclusion are probably closely related to the growth rate of the roots. The concentration of ferrous at the site of uptake at the root is the result of, on the one hand, the flux of ferrous to those sites, and on the other of the oxidation rate by micro-organisms and the root itself (Bienfait 1989). The high growth rate of *R. maritimus* will only be maintained when water uptake, needed to counteract transpiratory loss, is high, and the flux of ferrous to the roots is therefore high. The half-life of ferrous in the presence of atmospheric oxygen at pH 7 is in the order of 30–60 min (Davison & Seed 1983). Moreover, at a fast growing root tip, microbial growth and activity are not yet fully developed, so that a bacterial contribution to ferrous oxidation is low. Thus, the net oxidation rate of ferrous at and just behind the tips of fast growing roots may be well below the ferrous supply rate, which then leads to high iron concentrations in the shoots.

In *R. thyrsiflorus*, the opposite situation is found: root growth is slow, soil oxidation is poor, but the ROL is sufficient to form iron plaques over the whole root surface (Fig. 4), where growth and activity of microbes have had time to establish, so that they can increase the local ferrous oxidation capacity. Thus, iron toxicity is not a problem. But in this case it is likely that soil exploitation is restricted and nutrient uptake strongly diminished. Moreover, the iron plaque formed on the root surface (Fig. 4) can bind considerable amounts of nutrients (Otte *et al.* 1989). A general nutrient decrease in the leaves was indeed recorded (Table 4), and a decrease in potassium, calcium and phosphorus content was obvious.

Finally, in *R. crispus* a general decrease in nutrient content was also observed, but this did not result in a decrease in biomass production. Apparently, soil exploitation was not significantly restricted and nutrient levels were not low enough to decrease the yield. Since the leaf nutrient content in the drained treatments differed significantly between the species, it may be assumed that nutrient deficiency levels in *R. thyrsiflorus* are reached at higher concentrations than in *R. crispus* and *R. maritimus*.

We propose that the relative flood-intolerance of *R. thyrsiflorus* is predominantly caused by a lack of root growth and concomitant nutrient deficiency. Experiments with higher soluble iron concentrations will determine whether the growth rate of *R. maritimus* may be affected ultimately by the soil solution iron concentration.

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