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The relative importance of anaerobiosis and high iron levels in the flood tolerance of *Rumex* species

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Abstract

In both hydroculture experiments and a greenhouse trial the combined effects of flooding and high iron levels on the growth and occurrence of iron toxicity were investigated in three *Rumex* species having different flood tolerance.

In a hydroculture experiment the plants were subjected to different FeCl$_2$ concentrations and anaerobiosis. At solution iron concentrations exceeding 750 $\mu$M, the growth rate of the flood-intolerant *R. thyrsiflorus* was sharply decreased. The root system was most negatively affected. Differences between the investigated species could be most likely explained from differences in root porosity and are thus closely related to a differential internal oxygen supply to the root systems.

In a greenhouse experiment soil flooding was combined with the addition of different ferrous iron concentrations to the soil solution. Flooding in combination with the addition of 5 mM ferrous iron did not result in a significant decrease in biomass production of any of the investigated *Rumex* species, in spite of the fact that several types of shoot iron toxicity were perceived. Especially at high iron levels significant amounts of ‘bronzing’ spots on the leaves of all species were observed. Petiole iron toxicity symptoms, which result in a sagging of the petioles, was most clearly observed in the flood-intolerant *R. thyrsiflorus*.

Although the hydroculture experiments revealed a severe effect of anaerobiosis and high iron levels on the root development and plant growth rate of especially the flood-intolerant *R. thyrsiflorus*, no such adverse effects were registered in the greenhouse experiments in neither of the species. This is most probably due to the fact that under greenhouse conditions the *Rumex* species are able to locally immobilize iron by oxidation, thereby avoiding the actual iron stress. Since biomass production was hardly affected under greenhouse conditions, it is concluded that high iron levels in the soil solution are of minor importance in the different flood tolerance of the *Rumex* species. It also indicates that great care has to be taken in the interpretation of hydroculture experiments to the actual effect of the suggested stress conditions under greenhouse or natural conditions.

Introduction

In waterlogged soils the available iron concentration is increased by the reduction of insoluble Fe(III) oxides to Fe$^{2+}$ (Ponnamperuma, 1972, 1984; Yoshida and Tadano, 1978). As a consequence, high internal iron concentrations in plant tissues may occur and thereby reduce plant yield (Fageria et al., 1987; Kuraev, 1966). In flood-tolerant plants, aerenchyma formation provides an efficient means to allow radial oxygen loss (ROL). This can help prevent high internal
iron levels in the shoot by reoxidation and concomitant precipitation of iron in the rhizosphere (Benckiser et al., 1984; Chen et al., 1980; Laan et al., 1989b; Ottow et al., 1982). Nevertheless, high iron levels and symptoms of iron toxicity are not restricted to flood-intolerant plants, but have been almost exclusively described for flood-tolerant species, including rice (Armstrong and Boatman, 1967; Howeler, 1973; Yoshida and Tadano, 1978).

The amount of iron accumulating in the plant will be the result of the oxidizing activity of the roots on the one hand, and of the transpiration rate of the shoot plus the iron concentration in the soil solution on the other (Bienfait, 1989; Jones, 1971; Laan et al., 1989b). In addition, the nutrient status of the plant can play a role in preventing iron toxicity. A low nutrient status may result in increased root exudation and concomitant rhizoflora activity, and thus a high ‘soil oxygen demand’. This can ultimately lead to the breakdown of iron-excluding mechanisms such as the dissolution of iron precipitates, which might be followed by an uncontrolled iron influx (Benckiser et al., 1984; Ottow et al., 1982). Therefore iron toxicity is most likely to occur in flood-intolerant plants, as well as in flood-tolerant plants growing on iron-rich, yet nutrient-poor soils.

Although iron toxicity symptoms may occur in both the shoot and root system, they have been most frequently described for the shoot. A well-known example is the so-called ‘bronzing disease’ in rice plants, characterized by the development of reddish-brown spots on older leaves (Ponnamperuma et al., 1955; Tanaka et al., 1966; Yoshida and Tadano, 1978). Iron toxicity in the roots in most cases is characterized by root blackening and root flaccidity (Kuraev, 1966; Tadano, 1975; Wheeler et al., 1985). According to Kuraev (1966) the inhibition of root growth is initially caused by high Fe$^{2+}$ concentrations in the rooting medium. In many hydroculture studies, however, it is to be doubted whether root injury is caused by high Fe$^{2+}$ levels, rather than by sulphide toxicity. Experiments conducted on hydroculture in most cases are carried out with FeSO$_4$ (Wheeler et al., 1985; van Diggelen, 1988). As sulphide is highly toxic to roots (Tanaka et al., 1968; Yoshida and Tadano, 1978) root injury due to sulphide toxicity is more likely to occur than to iron toxicity.

In this study we investigated the influence of the combination of anaerobiosis and high iron concentrations on the growth of three Rumex species, which differ in oxidizing activity and vary in their flood tolerance (Laan et al., 1989a,b). In a greenhouse experiment the occurrence and the nature of shoot iron toxicity symptoms were studied for plants grown in flooded, iron-enriched soils. In a hydroculture experiment, growth rates as well as root and shoot iron toxicity symptoms were studied for three Rumex species growing in an anaerobic medium containing different FeCl$_2$ concentrations.

Materials and methods

Plant growth

Seeds of Rumex thyrsiflorus Fingerh., R. crispus L. and R. maritimus L. were collected from natural populations in the river area near Nijmegen (The Netherlands). They were sown in trays containing black polyethylene grains (Stamylan LD, DSM, The Netherlands), which were totally submerged with a 1/4-strength Hoagland solution (Hoagland and 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 µmol m$^{-2}$s$^{-1}$, 8 h dark). After germination the young plants were allowed to grow for 2–3 weeks in a growth chamber (temperature 25°C, RH 70%; 16 h fluorescent light at 200 µmol m$^{-2}$s$^{-1}$, 8 h dark).

Hydroculture experiments

Plant growth

Plants, grown as described above, were carefully transplanted to containers (13 × 10$^{-3}$ m$^3$), filled with 1/4-strength modified Hoagland solution (see Laan et al., 1990) and aerated. They were allowed to grow aerobically for a week in a
growth chamber, after which the solution was replaced by a stagnant, 0.05% (w:v) agar solution. Plants were then grown in this medium until they had reached a mean total fresh weight of 5 g. At that moment the larger part of the primary lateral roots had died away and new laterals had developed.

Experimental design
Plants were weighed and planted in airtight plastic pots filled with a stagnant anaerobic 0.1% (w:v) agar in 1/4-strength iron-free Hoagland solution. Six Fe²⁺ concentrations of 8 replicates each, were obtained by adding different amounts of an anaerobic FeCl₂ stock solution to the pots. On alternate days the solutions were changed to prevent a decrease in free-iron concentration by oxidation. Before changing the solution, the Fe²⁺ concentration was checked using the ‘bipyridyl method’ (Laan et al., 1989b). The maximum decrease after two days did not exceed 25% of the original concentration.

Plants were harvested when the mean total fresh weight had doubled. Fresh weights of the total plant, shoot and lateral roots and the maximum length of the laterals were measured. Relative growth rates of separate plants were determined from the total fresh weights of the plants at the start and end of the experiment. The number of leaves with the visible iron toxicity symptoms of ‘bronzing spots’ were counted. All plant parts were dried separately (24 h, 70°C) and dry weights were recorded. Leaves were divided into two classes, (i) leaves without iron toxicity symptoms and (ii) leaves with iron toxicity symptoms. The iron concentrations of all leaves were determined.

Greenhouse experiments

Experimental design
Plants, pregrown as described above were transferred to vertical PVC tubes (diameter 120 mm, height 400 mm). These were filled with a 1:1 (v:v) clay/sand mixture of pH in H₂O 6.9 and weight percentage organic matter 5.4, which was enriched with some homogeneously distributed plant material. The plants were allowed to grow for 4 weeks in a greenhouse (temperature 19°C, RH 70%, 16 h light, consisting of daylight filtered by greenhouse glass (120–1000 μmol m⁻² s⁻¹) and supplementary light (high pressure sodium lamp, Osram Vialox), when light intensity decreased to values lower than 200 μmol m⁻² s⁻¹; 8 h dark).

After another four weeks the tubes were flooded either with tap water, or with tap water enriched with different FeCl₂ concentrations. Prior to the flooding treatment, air in the free spaces of the soil was replaced by nitrogen gas by forcing nitrogen gas for 25 min through glass pipettes (diameter 3 mm; length 350 mm), positioned down to the bottom of the soil column. Then, an anaerobic FeCl₂ solution was led through the pipettes into the soil columns. In this way the oxygen-free soil columns were gradually saturated with the anaerobic iron solution. Oxidation was prevented, thus assuring a more or less equal distribution of Fe(III) throughout the soil columns. The water level was kept at about 30 mm above the soil by adding tap water twice a day. The ‘free’ iron concentration was determined several times during the flooding treatment, using the ‘bipyridyl-method’ (Laan et al., 1989b).

After three weeks, the plants were harvested by carefully pushing out the soil core. The soil’s oxidation depth, as indicated by a brownish colour, was clearly distinguishable from the reduced black soil. This was recorded and the maximum root penetration was determined during removal of the soil. Shoots were cut off, and fresh weight was determined. The root system was then carefully washed out and the fresh weight of the tap root and lateral roots were determined separately. The dry weights of the several plant parts were determined after 24 h (70°C) and the leaf iron content was also determined.

Leaf iron concentration
Total iron content of the leaves was determined on 50–100 mg dry leaf material ashed at 650°C, then dissolved in 5 mL 5% (v:v) HNO₃ and 0.5 mL 6 N HCl and analyzed on an inductive-coupled-plasma emission spectrophotometer (ICP).
Results

Hydroculture experiment

Growth of *Rumex* species in anaerobic hydroculture was inhibited by Fe$^{2+}$ ions (Fig. 1). This effect was strongest in *R. thyrsiflorus*. Typical symptoms of iron toxicity were root flaccidity and root blackening at the higher iron concentrations. In the shoot, the oldest leaves showed brown necrotic spots. These ‘bronzing spots’ are considered to be typical of leaf iron toxicity (Foy et al., 1978; Wheeler et al., 1985; Yoshida and Tadano, 1978). The number of injured leaves increased with increasing ferrous iron levels in the nutrient solution (Table 1). At intermediate concentrations (750 $\mu$M) the total number of leaves affected was about 20% in all species. In contrast with the small effect of ferrous iron on RGR and root growth, injury was strongest in *R. maritimus* at high ferrous iron concentrations (40% of the total number of leaves at 1500 $\mu$M Fe, Table 1).

Old leaves with visible injury contained iron concentrations twice that of leaves without symptoms, irrespective of species and concentration used (Table 1). However, although RGR and root growth of *R. thyrsiflorus* were most severely affected at high iron levels, iron concentration of the leaves was lower than of the other two species.

Greenhouse experiment

According to earlier results (Laan et al., 1989b), the depth of oxidation differed between the species and was correlated with oxidizing activity of the roots due to aerenchyma formation. This was about 300 mm in *R. maritimus*, 150 mm in *R. crispus*, yet only 70 mm in *R. thyrsiflorus* (Table 2).

Sink activity increases with increasing ferrous iron concentration. Thus, we expected the oxidation depth to be severely restricted upon addition of ferrous iron to the soil solution. Indeed, oxidation depth decreased, but to a much lesser extent than expected (reduction of 29% (*R. thyrsiflorus*), 12% (*R. crispus*) and 21% (*R. maritimus*) at 5 mM Fe, Table 2). No significant differences were observed between the species.

This minor effect can be partly explained from the interaction between root oxidizing activity and ‘free’ iron concentration. The ‘free’ ferrous iron concentration had decreased significantly after 18 days of treatment, and could hardly be
Table 1. Effect of anaerobiosis plus different FeCl₃ concentrations in the nutrient solution on visible iron toxicity symptoms and on leaf iron concentration of Rumex species (means of 8 (% injury) or 4 replicates ±SE; data after growth for 10 (R. crispus and R. maritimus) or 15 (R. thyrsiflorus) days in hydroculture)

<table>
<thead>
<tr>
<th>Species/Treatment</th>
<th>Number of leaves with visible injury due to iron toxicity (% of total)</th>
<th>Leaf iron concentration (μmol g⁻¹ dw)</th>
<th>Leaves with injury</th>
<th>Leaves without injury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>R. thyrsiflorus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 μM Fe</td>
<td>0</td>
<td>-</td>
<td>6 ± 1</td>
<td></td>
</tr>
<tr>
<td>750 μM Fe</td>
<td>18</td>
<td>26 ± 5</td>
<td>13 ± 3</td>
<td></td>
</tr>
<tr>
<td>1500 μM Fe</td>
<td>27</td>
<td>31 ± 4</td>
<td>18 ± 4</td>
<td></td>
</tr>
<tr>
<td><strong>R. crispus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 μM Fe</td>
<td>0</td>
<td>-</td>
<td>4 ± 1</td>
<td></td>
</tr>
<tr>
<td>750 μM Fe</td>
<td>22</td>
<td>32 ± 8</td>
<td>12 ± 3</td>
<td></td>
</tr>
<tr>
<td>1500 μM Fe</td>
<td>18</td>
<td>42 ± 5</td>
<td>21 ± 4</td>
<td></td>
</tr>
<tr>
<td><strong>R. maritimus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 μM Fe</td>
<td>0</td>
<td>-</td>
<td>4 ± 1</td>
<td></td>
</tr>
<tr>
<td>750 μM Fe</td>
<td>21</td>
<td>24 ± 3</td>
<td>14 ± 3</td>
<td></td>
</tr>
<tr>
<td>1500 μM Fe</td>
<td>40</td>
<td>45 ± 6</td>
<td>27 ± 5</td>
<td></td>
</tr>
</tbody>
</table>

*SE maximal 7%.

detected in the oxidized soil layer (<0.01 μM). In the reduced soil, flooding itself gave rise to an [Fe²⁺] of about 0.24 mM (Table 2), but the extra addition of iron to the calculated concentration of 1 and 5 mM resulted in actual concentrations of 0.33 mM and 1.15 mM, respectively, after 18 days (Table 2). Moreover, even the highest ferrous iron concentration applied did not affect biomass production significantly (Table 2). Typical root iron toxicity symptoms (root flaccidity and root blackening) could only be observed in the reduced soil layer.

The shoots showed typical forms of iron toxicity at both petioles and leaves, which was especially clear at higher iron concentrations (Table 3, Fig. 2). Necrotic spots (‘bronzing spots’) appeared on the leaves of all species under study (up to about 20% of the total number of leaves,

Table 2. Biomass production, iron-oxidation depth and ferrous iron concentration in the soil solution after 3 weeks of flooding of Rumex species, subjected to different iron levels under soil-flooded conditions (means of 8 replicates ±SE; growth in a clay:sand (1:1 v/v) soil, and ferrous iron concentration determined in the reduced soil layer; actual [Fe²⁺] in soil solution after 18 d in control, 1 mM and 5 mM Fe-treatment: 0.24 ± 0.05, 0.33 ± 0.09 and 1.15 ± 0.18 mM, respectively)

<table>
<thead>
<tr>
<th>Species/Treatment</th>
<th>Biomass production (g)</th>
<th>Oxidation depth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Roots</td>
</tr>
<tr>
<td>R. thyrsiflorus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>2.2 ± 0.2</td>
<td>0.63 ± 0.04</td>
</tr>
<tr>
<td>1 mM Fe</td>
<td>2.1 ± 0.2</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>5 mM Fe</td>
<td>2.3 ± 0.2</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td>R. crispus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>2.6 ± 0.1</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>1 mM Fe</td>
<td>2.4 ± 0.1</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>5 mM Fe</td>
<td>2.5 ± 0.2</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>R. maritimus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>3.8 ± 0.6</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>1 mM Fe</td>
<td>4.4 ± 0.3</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>5 mM Fe</td>
<td>4.9 ± 0.4</td>
<td>2.7 ± 0.4</td>
</tr>
</tbody>
</table>
Table 3. Visible injury of leaves and petioles of *Rumex* species, after 3 weeks of soil-flooding in the absence or presence of additional ferrous iron (data are means of 8 plants ±SE and represent percentage of total number of leaves or petioles with injury).

<table>
<thead>
<tr>
<th>Species/Treatment</th>
<th>Injury on petioles (%)</th>
<th>Injury on leaves (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weak*</td>
<td>Severe*</td>
</tr>
<tr>
<td><em>R. thyrsiflorus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 mM Fe</td>
<td>30 ± 7</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>5 mM Fe</td>
<td>30 ± 7</td>
<td>18 ± 3</td>
</tr>
<tr>
<td><em>R. crispus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 mM Fe</td>
<td>24 ± 9</td>
<td>19 ± 7</td>
</tr>
<tr>
<td>5 mM Fe</td>
<td>24 ± 9</td>
<td>19 ± 7</td>
</tr>
<tr>
<td><em>R. maritimus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 mM Fe</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>5 mM Fe</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
</tr>
</tbody>
</table>

*a Weak injury refers to stadia 1 and 2, severe injury to stadium 3 (Fig. 2).

Fig. 2. Appearance of different iron toxicity symptoms on leaves and petioles of *Rumex* species after a 3-week growth period in a flooded clay:sand soil, enriched with 5 mM FeCl₂. Leaf iron toxicity (‘bronzing’) in *R. crispus* (a) and several stadia of petiole iron toxicity in *R. crispus*, indicated with an arrow: overview (b) and magnifications of different stadia (c–e); (c) stadium 1, (d) stadium 2, (e) stadium 3.
Table 4. Iron concentration of leaves and petioles of *Rumex* species with petiole injury (plants grown under soil-flooded conditions plus 5 mM FeCl₂; means of 3 replicates ±SE; data in parentheses represent iron concentration of comparable leaves without petiole injury)

<table>
<thead>
<tr>
<th>Iron concentration (µmol g⁻¹ DW)</th>
<th><em>R. thyrsiflorus</em></th>
<th><em>R. crispus</em></th>
<th><em>R. maritimus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>6.1 ± 0.9</td>
<td>8.4 ± 1.6</td>
<td>6.8 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>(13.9 ± 2.5)</td>
<td>(14.4 ± 0.8)</td>
<td>(18.7 ± 1.5)</td>
</tr>
<tr>
<td>Upper petiole</td>
<td>3.0 ± 0.4</td>
<td>7.6 ± 0.7</td>
<td>12.3 ± 1.3</td>
</tr>
<tr>
<td>Injured parts of petiole</td>
<td>14.4 ± 0.3</td>
<td>24.1 ± 10.2</td>
<td>25.6 ± 3.5</td>
</tr>
<tr>
<td>Lower petiole</td>
<td>4.6 ± 0.3</td>
<td>10.1 ± 1.1</td>
<td>19.1 ± 3.7</td>
</tr>
</tbody>
</table>

Table 2). Next to this, just above the water surface different stages of petiole injury developed (Fig. 2). This type of injury was characterized by a die-back of the cortical tissue, and finally resulted in a sagging of the petioles (Fig. 2e). This bending was especially found with *R. thyrsiflorus* and *R. crispus*, and only with a few leaves of *R. maritimus* (Table 3). Wilting of leaves containing petiole toxicity could only be observed at the latest stages of this type of injury, namely after bending down. The injured parts of the petioles (Fig. 2) contained high amounts of iron, while the apical petiole parts beyond, and the leaves, had 2–5 times lower iron contents (Table 4). On a dry-weight basis, the injured petiole parts of *R. thyrsiflorus* contained significantly lower iron concentrations than those of *R. crispus* and *R. maritimus* (14 against 25 µmol (g DW)⁻¹, respectively, Table 4).

Typically, the appearance of petiole injury in no case agreed with the occurrence of leaf injury, i.e. ‘bronzing spots’.

**Discussion**

**Hydroculture experiment**

In the hydroculture experiment two factors have played a role in affecting the growth of the plants, namely anaerobiosis and high ferrous iron concentrations. At low iron concentrations (100 µM) anaerobiosis led to a growth reduction. This was most severe in *R. thyrsiflorus* (Fig. 1) and, in accordance with earlier results (Laan et al., 1989a,b), is mainly due to differences in root porosity of newly formed laterals of the species (Laan et al., 1989a). Since a lack of aerenchyma formation in *R. thyrsiflorus* roots leads to a poor internal aeration of the root apices (Laan et al., 1989b, 1990), this also explains the poor root development and the restricted root length (Fig. 1).

With increasing ferrous iron concentration in the nutrient solution, growth rates of all species under study was further reduced. This was accompanied by a corresponding decrease in root development. Again the effect was strongest in *R. thyrsiflorus* (Fig. 1). As already suggested by Kuraev (1966), the initial effect of anaerobiosis in combination with high iron levels is an inhibition or cessation of root growth and development. This can probably be explained by the ‘sink activity’ of ferrous iron in solution. Ferrous iron will easily react with oxygen diffusing from the roots. Thus the higher the [Fe²⁺], the higher the oxygen demand of the solution. Since root porosity and the concomitant internal oxygen transport is poorly developed in *R. thyrsiflorus* (Laan et al., 1989a, 1990), this must ultimately have led to apical oxygen concentrations below the critical oxygen pressure for root extension (Armstrong and Webb, 1985), which will result in limited root growth or even death of the roots.

In contrast with the small effect of high iron levels on the growth rate (Fig. 1), in terms of leaf iron concentration and the resulting degree of leaf injury, *R. maritimus* suffered most (Table 1). The appearance of shoot iron toxicity symptoms is highly dependent on shoot iron concentration (Tanaka et al., 1966; Wheeler et al., 1985; Yoshida and Tadano, 1978). Since iron uptake rate depends on water uptake (Jones, 1971), this is most likely to occur in plants with a high transpiration rate and a well developed root system, i.e. *R. maritimus*. 
Greenhouse experiment

Under flooded conditions, all *Rumex* species investigated were able to oxidize the upper part of the soil (Table 2), which resulted in very low 'free' iron concentrations there (<0.01 mM, Laan et al., 1989b). Thus, although high iron concentrations were recorded in the reduced soil layer (Table 2), the bulk of root mass could always be found in the oxidized layer containing low iron concentrations. As a result, no significant growth reduction of either roots or shoot could be observed (Table 2). The sole effect of the increasing iron concentration in the soil solution was a slight decrease in oxidation depth (Table 2).

In spite of the occurrence of this 'avoidance' strategy, clear symptoms of iron toxicity could be detected in the shoot parts of all *Rumex* species (Table 3, Fig. 2). Typical 'bronzing spots', as observed in hydroculture, could be detected (Fig. 2a). The initial stages of this 'bronzing' damage were small, brown necrotic spots either at the leaf edges or near the leaf tip. With prolonged exposure to iron, this necrosis expanded over the leaf, and especially in *R. thyrsiflorus* covered large parts of the older leaves.

Interestingly, the iron concentration of leaves of *R. maritimus* appeared to be higher than that of the other two species (Tables 3). Nevertheless, the percentage of leaves showing 'bronzing' damage did not differ between the *Rumex* species, either in the 1 or 5 mM treatment (Table 3). It remains therefore unknown whether *R. maritimus* has additional mechanisms to avoid leaf injury in spite of the higher iron accumulation e.g. ferritin induction (Bienfait 1989).

Iron toxicity leading to 'petiole bending' (Fig. 2e) was mainly observed a few centimeters above the water surface. Obviously, ferrous iron, transported via the xylem, will be oxidized when it comes into contact with oxygen. This oxidation leads to the formation of $\text{O}_2^-$ and subsequently to other oxygen radicals which destroy the cell membrane (Bienfait, 1989). The process may take place both in the apoplast and in the cell, after uptake of iron. The ferric iron will precipitate on the spot, indicating whether oxidation takes place. Of course, the amount of iron precipitated is a function of both the rate of iron transported, which is determined by the transpiration rate, and the diffusion rate of oxygen, which will be determined by porosity. This is illustrated by the fact that *R. maritimus*, of which the petioles have a very low diffusional resistance to oxygen (Laan et al., 1990), showed the highest iron contents, also in the zone below the water level, just below the petiole bend. *R. thyrsiflorus*, of which the petioles have a high diffusional resistance, contained relatively low iron concentrations in and below the bend (Table 4).

The leaves with bended petioles apparently avoided wilting symptoms until late stages of injury. This can be explained by assuming the transport tissue of the petioles to remain intact, in this way enabling a continued water and nutrient transport to the leaves. Remarkably, iron accumulation in petiole-injured leaves was prevented (Table 4). From a physiological point of view, petiole injury can have advantages over leaf injury, as a significant loss of photosynthetic capacity can be avoided for some time.

In conclusion, although anaerobiosis in combination with high iron levels may result in iron toxicity and severe growth reduction, these effects could only be induced in hydroculture (Table 1, Fig. 1). Under greenhouse conditions, a decrease in biomass production was mainly due to anaerobiosis and could hardly be attributed to high iron levels (Table 2, Laan et al., 1989b). These responses must be explained on the basis of the ability of the *Rumex* species to change the soil conditions and thereby to avoid the actual stress. Under hydroculture conditions, oxygen deficiency can be avoided to an extent that is related to the ability of a species to allow internal aeration (Laan et al., 1989b, 1990). Iron stress, however cannot be avoided in hydroculture. Under greenhouse conditions, the same ability to allow oxygen diffusion as under hydroculture conditions must be assumed, but here the plant can decrease the free ferrous concentration locally by radial oxygen loss.

In the field, iron toxicity will therefore be of minor importance in the flood tolerance of *Rumex* species. The results also indicate that great care has to be taken with the interpretation of hydroculture experiments to the actual effect of the suggested stress conditions.
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References

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