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Growth and development of *Rumex* roots as affected by hypoxic and anoxic conditions

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Abstract

The growth characteristics of three *Rumex* species were determined under different solution oxygen concentrations in hydroculture. These species all occur in a river foreland ecosystem and they were found to differ in their flood tolerance. The flood-tolerant *R. maritimus* and *R. crispus* developed a large number of new, aerenchymatous roots within a short period under low solution oxygen concentrations. Biomass production was not affected. In the flood-intolerant *R. thyrsiflorus*, however, only few slow-growing new roots were developed and biomass production was significantly reduced at solution oxygen concentrations below 2% (v:v). These different responses could be partly explained by a differential aerenchyma formation in new roots of the flood-tolerant species. Aerenchyma can relieve the oxygen stress of the root systems via internal aeration.

The fast development of new roots of the flood-tolerant *R. maritimus* and *R. crispus* after the onset of anaerobiosis coincided with the reduction or cessation of growth of the primary roots. Notwithstanding the cessation of growth, however, primary roots of both species were able to recover following restoration of aerobic conditions after a 13-day anaerobic period. However, the roots of *R. thyrsiflorus* ceased growing very soon after the onset of anaerobiosis. All had died within 10 days.

The balance between the growth rates of the primary and the newly formed root system are discussed and related to the differential tolerance of the *Rumex* species to transient flooding.

Introduction

Under hypoxic conditions root growth ceases, as the oxygen concentration near the root apex rapidly reaches values below the Critical Oxygen Pressure for Extension growth (COPE) (Armstrong and Webb 1985). In addition, ion uptake is strongly reduced (DeDatta 1985; Drew and Sisworo, 1979; John et al., 1974; Thomson et al., 1989; Trought and Drew, 1980b). These processes result in the reduction of plant growth rate (Buwalda et al., 1988; Drew and Sisworo, 1979; Trought and Drew, 1980a). Plants can overcome these adverse effects by formation of aerenchyma that can restore oxygen supply (Armstrong, 1979; Laan et al., 1989a, 1990).

*Rumex* species occur in the river foreland ecosystem, where they infrequently encounter transient flooding (Blom et al., 1990). In their response to flooding, *Rumex* species develop new roots (Laan et al., 1989a, b). The flood-tolerant species develop aerenchyma in new roots that enable the plants to facilitate the diffusion of oxygen to relieve the oxygen deficiency of the root system (Laan et al., 1990; Laan and Blom, 1990). Thus, the extension rate of the new root system ultimately determines the flood tolerance of the particular species.
The development of new roots takes some time, so the plant must rely upon the primary root system during the initial period after flooding. Also primary roots of flood-tolerant species are better adapted towards hypoxic conditions than those of intolerant species (Laan et al., 1991).

In the present study we investigated the growth responses of three Rumex species to different solution oxygen concentrations. The growth rates of primary and newly formed roots were determined under alternating aerobic and anaerobic hydroculture conditions. In this way a transient flooding situation was simulated. In doing so it is possible to investigate whether the regrowth capacity of the primary roots, and the ‘turnover-efficiency’ from the old to the new root system were related to the flood tolerance of the species.

Materials and methods

Plant growth at different solution oxygen concentrations

Seeds of R. maritimus L., R. crispus L. and R. thyrsiflorus Fingerh. were collected from plants in the river forelands near Nijmegen, The Netherlands. These were sown in trays containing black polyethylene grains (Stamylan LD, DSM, The Netherlands). The trays were filled with a 5-strength Hoagland’s solution (Hoagland and Arnon, 1950) until total submergence of the grains. After germination (16 h light (Philips TL-33) at 60 µmol m⁻² s⁻¹, 25°C and 8 h dark, 15°C), the trays were transferred to a constant-temperature room (16 h fluorescent light (Sylvania F36W-GRO, 150 µmol m⁻² s⁻¹), 8 h dark; temperature 25°C, R.H. 70%). The plants were allowed to grow for another 1–2 weeks. Then the seedlings were carefully transferred to black polyethylene pots (volume 1.5 L, 4 plants per pot), filled with nutrient solution, which was kept air-saturated by bubbling with air. Each plant was sealed into the air-tight lid with modelling clay. The nutrient solution was restored to the original level every two days, and replaced once a week.

After one week the root system was completely covered with active charcoal powder to be able to distinguish old roots from those newly formed. The plants were grown under different solution oxygen concentrations, using gas blenders (HI-TEC model E55N3). After 10 days, the length of the longest new root and the total length of newly formed root material were measured. Dry weights of newly formed root material, and of the remainder of the plants were determined after 24 h (70°C).

Growth of roots during alternating aerobic and anaerobic conditions

Plant growth

Plants were grown as described above in a modified ¼-strength Hoagland’s solution containing 1.0 mM KNO₃, 1.0 mM NaN₃ and 0.5 mM KH₂PO₄ instead of 1.5 mM KNO₃ and 0.75 mM NH₄H₂PO₄ in a temperature room (16 h light (Sylvania F36W-GRO, 150 µmol m⁻² s⁻¹), 8 h dark; temperature 25°C, R.H. 70%).

Experimental setup

A two- to three-week-old plant was carefully transferred to a cuvette consisting of two glass plates of width 0.2 m and height 0.6 m. These were fixed together with 4-mm-thick glass strips. The space between the plates was filled with glass beads (diameter 4 mm). The cuvette was closed at the top with glass rods to minimize air contact. The system was placed at an angle of about 20° to allow the roots to grow towards the frontside. The bulk nutrient solution (10L container), which could be kept aerobic or anaerobic by bubbling through it either air or nitrogen gas, was circulated from bottom to top through the cuvette by an aquarium pump (Eheim 1016, 1 L h⁻¹). The whole system was blackened to prevent growth of algae. Measurements were started after about one week, when the longest roots had reached a length of 70–100 mm.

Growth measurements

Root growth was measured by marking the positions of the apices twice a day on a transparent sheet fixed to the frontside of the cuvette. Growth rates were checked by measuring individual roots during a few hours with a travelling Vernier microscope (accuracy 0.01 mm, Precision Tools and Instruments, UK). Growth of
newly formed roots was recorded as soon as detectable. The time of root initiation was then calculated by assuming the growth rate of these laterals to be constant until then. Roots were registered dead when growth rate had reduced to zero, and the apex had turned brownish or black. Average growth rates were calculated by measuring the distances between the markings on the sheet of 5 to 7 representative roots.

Results

Plant growth at different solution oxygen concentrations

At low solution oxygen concentrations, root growth of all species was restricted, but to different extents. A sharp decrease in root length was recorded below concentrations of 2% (Table 1). R. thyrsiflorus suffered most severely during hypoxic, or even anoxic conditions in the root environment: primary roots ceased growing and died, and only few, slow-growing new roots developed. Also plant biomass was lowest at solution oxygen concentrations of 0 and 1% in R. thyrsiflorus, but hardly affected in both R. maritimus and R. crispus. With these two latter species, highest biomass production was found at 2% O₂ (Table 1). In all cases root growth and biomass production was higher at 10% than at 21% O₂.

Within the experimental period, new root material was formed. At higher solution oxygen concentrations new root material was mainly produced because of the continued growth of the already existing roots. But with decreasing oxygen concentrations new roots developed (Fig. 1). This was especially true for R. crispus and to a lesser extent also for R. maritimus, but virtually no new roots were formed in R. thyrsiflorus. These differences are reflected in the total length of new root material formed after 10 days (Table 1). The largest increases were observed at low

<table>
<thead>
<tr>
<th>Species/ [O₂] (% v:v)</th>
<th>Root length</th>
<th>Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Longest new root</td>
<td>Total length of newly formed laterals</td>
</tr>
<tr>
<td>R. thyrsiflorus</td>
<td>198 ± 12 mm = 100%</td>
<td>314 ± 22 mm = 100%</td>
</tr>
<tr>
<td>21</td>
<td>154 ± 6</td>
<td>187 ± 17</td>
</tr>
<tr>
<td>10</td>
<td>120 ± 5</td>
<td>125 ± 12</td>
</tr>
<tr>
<td>4</td>
<td>86 ± 6</td>
<td>114 ± 9</td>
</tr>
<tr>
<td>2</td>
<td>9 ± 2</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>1</td>
<td>12 ± 1</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>R. crispus</td>
<td>294 ± 18 mm = 100%</td>
<td>355 ± 28 mm = 100%</td>
</tr>
<tr>
<td>21</td>
<td>102 ± 6</td>
<td>174 ± 12</td>
</tr>
<tr>
<td>10</td>
<td>91 ± 4</td>
<td>202 ± 14</td>
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<tr>
<td>4</td>
<td>84 ± 3</td>
<td>381 ± 2</td>
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<tr>
<td>2</td>
<td>43 ± 3</td>
<td>208 ± 16</td>
</tr>
<tr>
<td>1</td>
<td>37 ± 3</td>
<td>139 ± 11</td>
</tr>
<tr>
<td>R. maritimus</td>
<td>132 ± 8 mm = 100%</td>
<td>199 ± 12 mm = 100%</td>
</tr>
<tr>
<td>21</td>
<td>100 ± 3</td>
<td>109 ± 7</td>
</tr>
<tr>
<td>10</td>
<td>90 ± 4</td>
<td>117 ± 21</td>
</tr>
<tr>
<td>4</td>
<td>95 ± 5</td>
<td>114 ± 13</td>
</tr>
<tr>
<td>2</td>
<td>57 ± 2</td>
<td>136 ± 20</td>
</tr>
<tr>
<td>1</td>
<td>58 ± 3</td>
<td>98 ± 19</td>
</tr>
</tbody>
</table>
solution oxygen concentrations in R. crispus and R. maritimus, whereas a sharp decrease was recorded in R. thyrsiflorus below 2% O₂.

New roots, formed in response to low solution oxygen concentrations could easily be distinguished from the primary ones (Fig. 1). They were unbranched and due to the formation of aerenchyma (Laan et al., 1989a), their diameter was much higher (Table 2). Since the more efficient response of the flood-tolerant R. maritimus and R. crispus was mainly due to the development of large numbers of these newly formed lateral roots, they are expected to have a higher growth rate than the primary ones. The balance between the turnover from primary to new roots with respect to growth was, therefore, further investigated.

Table 2. Root diameter (in mm) of primary and newly formed lateral roots of Rumex species measured 20 mm behind the root apex (means of 15–20 replicates ±SD)

<table>
<thead>
<tr>
<th>Species</th>
<th>Primary laterals</th>
<th>Newly formed laterals</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. thyrsiflorus</td>
<td>0.38 ± 0.01</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td>R. crispus</td>
<td>0.65 ± 0.09</td>
<td>0.72 ± 0.02</td>
</tr>
<tr>
<td>R. maritimus</td>
<td>0.43 ± 0.06</td>
<td>0.84 ± 0.04</td>
</tr>
</tbody>
</table>

Growth of roots under alternating aerobic and anaerobic conditions

With all these Rumex species, anaerobic conditions in the rooting medium ultimately resulted in the cessation of the growth of the primary roots (Fig. 2). Growth rates of primary roots of R. thyrsiflorus and R. crispus were reduced

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Fig. 1. Build-up of the Rumex root system. **Left:** View of the root system of R. maritimus after 3 weeks of flooding in sand. **Right:** Schematic representation of the build-up of the root system after anaerobic hydroculture conditions. Root type 1, tap root; 2, primary lateral roots; 3, newly formed lateral roots, developed in response to hypoxic or anoxic conditions.

Fig. 2. Growth rates of primary (●) and newly formed (○) lateral roots of Rumex species upon alternating aerobic (□) and anaerobic (■) conditions: (A) R. thyrsiflorus, (B) R. crispus and (C) R. maritimus (means of 5–7 replicates ±SE; arrow indicates origin of new root formation, * indicates death of primary lateral roots; age of the plants at the start of the experiment 3–4 weeks).
rapidly after the onset of anaerobic root conditions and most roots had ceased growth completely after 48 and 56–80 h, respectively (Fig. 2). In *R. maritimus*, however, growth reduction could not be observed until 90–100 h after the onset of anaerobiosis and complete growth reduction of the primary lateral roots was reached after about 170 h. Growth of these primary laterals could not be recovered throughout the anaerobic treatment and in *R. thyrsiflorus* most roots had died after 240 h (Fig. 2).

With all species, new roots developed after growth of the primary roots was reduced, but their initiation time differed. In the case of *R. thyrsiflorus*, new, slow-growing roots could not be observed until the primary roots had ceased growing for 65 h. In *R. crispus*, new root formation coincided with complete growth reduction of most of the primary roots and their initial growth rate was also rather low (Fig. 2). With *R. maritimus*, however, new roots began to grow when primary roots showed a reduction in growth rate, but had not yet ceased to grow. This was about 14 h before the reduction to zero of the growth of the primary roots. Next to this, these roots showed higher initial growth rates than the primary ones.

With increasing length, new roots also showed growth reduction under these experimental conditions and 230 h after the onset of anaerobiosis the short, newly formed roots of *R. thyrsiflorus* had stopped growth completely. Growth reduction due to increasing length of *R. maritimus* roots coincided with the formation of new roots, again with a high initial growth rate.

Upon restoration of aerobic conditions, growth rates of the new roots were strongly increased (Fig. 2) to 2–2.5 mm h⁻¹ in *R. crispus* and *R. maritimus*. This was much higher than their initial growth rates and also than those of primary roots under aerobic conditions. Apparently, anaerobic conditions in the cuvette were so severe that even growth of the highly porous new lateral roots was inhibited. It is most likely that the high circulation rate of the nutrient solution withdrew oxygen from the roots, resulting in rather low apical oxygen concentrations. Although growth of new *R. thyrsiflorus* roots was increased upon a restoration of aerobiosis, the rate was still rather low (Fig. 2) and did not exceed values obtained under the initial aerobic conditions. Growth of primary roots of both *R. maritimus* and *R. crispus* recovered after renewed aerobic conditions. The primary roots of *R. thyrsiflorus* were dead, and no regrowth occurred.

**Discussion**

Although anaerobic conditions in the rooting medium finally resulted in a complete cessation of growth of primary laterals of all species, it took much longer to fully reduce growth rates of *R. maritimus* roots compared to the other two species (Fig. 2). Also, maximal root length reached under low solution oxygen concentrations was least affected in *R. maritimus* (Table 1). This clearly reflects the differential root porosities and the concomitant ability to use internal aeration for satisfying root oxygen demand (cf. Armstrong, 1979, Laan et al., 1989a, 1990). Since the porosity of the primary roots of *R. maritimus* is twice as high as that of *R. thyrsiflorus* and at least as high as that of *R. crispus* (Laan et al., 1989a, 1991), oxygen diffusion via internal aeration will be higher in *R. maritimus* compared to that in the other two species (Laan et al., 1990). Given that oxygen loss and respiratory characteristics of all three species is comparable (Laan et al., 1989b, 1991), the high porosity of *R. maritimus* will result in apical oxygen pressures that reach values below the critical oxygen pressure for extension growth (COPE, Armstrong and Webb 1985) at larger length, and hence primary roots of *R. maritimus* will get longer than those of the other two species (cf. Laan et al., 1989b).

The differences in internal aeration may also account for the differential growth rates of the newly formed roots of the species. Due to the formation of aerenchyma, the new roots of *R. crispus* and *R. maritimus*, will have much higher porosity than the primary roots (Laan et al., 1989a), thus providing oxygen conditions for high growth rates.

The total length of new roots formed after the onset of hypoxic or anoxic conditions was much higher in *R. crispus* and *R. maritimus* than in *R. thyrsiflorus* (Table 1). The high rate of new root
formation may counteract the reduced growth of the primary root system and may explain the fact that biomass production under low solution oxygen concentrations was less affected in *R. crispus* and *R. maritimus* than it was in *R. thyrsiflorus* (Table 1).

A facet which may distinguish between the tolerant and the intolerant *Rumex* species under situations of transient flooding, is the regrowth capacity of the primary roots. Roots of both *R. maritimus* and *R. crispus* were able to recover growth after a 13-day anaerobic period, while those of *R. thyrsiflorus* died after 240 h (Fig. 2). In *R. maritimus* this is most likely due to the high porosity of the roots (Laan et al., 1989a, 1991), which enables internal oxygen diffusion to take place continuously (Laan et al., 1990). This must have been the case for *R. crispus* too. Notwithstanding its low fractional root porosity, as measured by photography (Laan et al., 1989a), porosity of whole root systems, determined by pycnometry, was as high as of *R. maritimus* (Laan et al., 1991). Also young, aerobically grown *R. crispus* plants were able to satisfy root oxygen demand via internal aeration at low oxygen concentrations, whilst the oxygen demand of *R. thyrsiflorus* roots can not be satisfied via this process (Laan et al., 1990).

Since new root formation in both *R. crispus* and *R. maritimus* seemed to be somehow related to the functioning of the old root system, an intriguing question arises: What mechanism triggers new root formation following anaerobiosis? In *R. crispus* new roots started growing out immediately after most of the primary roots had ceased growth. In *R. maritimus* outgrowth of new roots had started even before the complete growth cessation of primary roots (14 h, Fig. 2). Obviously, no efficient turnover from old to new roots was found in *R. thyrsiflorus*. It took almost 3 days for the first roots to originate. The outgrowth of new roots may be a function of growth reduction or cessation of the major part of the primary roots, for in earlier experiments with *R. maritimus* we never observed an outgrowth of new, aerenchymatous roots until growth of the primary ones had reduced severely.

It may therefore be speculated that a trigger, inducting new root formation is hormonally mediated. A possibility is that cytokinins, synthesized in the root tips of the primary roots, act as a feedback inhibiting the growth of new roots. Such a mechanism was already suggested by Railton and Reid (1973) for tomato plants. In this option, the same response can be suggested for all three *Rumex* species, but with quantitative differences. Such a mechanism might more efficiently function in those species that form a large number of roots with restricted length and high root turnover (in the field *R. maritimus*) than in species with fewer and longer roots (in the field *R. thyrsiflorus*).

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