Growth and Survival Responses of *Rumex* Species to Flooded and Submerged Conditions: The Importance of Shoot Elongation, Underwater Photosynthesis and Reserve Carbohydrates

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Received 16 October 1989

ABSTRACT

Plants of *Rumex thyrsiflorus* Fingerh., *R. crispus* L. and *R. maritimus* L., which are zoned along a gradient of elevation in a river foreland ecosystem, and differ in their flood-tolerance, were subjected to different flooding levels. Under conditions of soil flooding, the growth rates of the flood-tolerant *R. crispus* and *R. maritimus* were as high as under drained conditions, but that of the flood-intolerant *R. thyrsiflorus* was halved. Upon submergence, the low elevation species *R. maritimus* showed rapid shoot elongation; when elongation resulted in a protrusion of leaves above the water surface, the plants survived. Alternatively, underwater photosynthesis also led to a 100% survival of submerged *R. maritimus* plants, provided that enough inorganic carbon was made available in the water. This could be attributed in part to the use of photosynthetically-derived oxygen for root respiration; in a hydroculture experiment, with 50 mM CO₂ in the water in the shoot environment, photosynthetically-derived oxygen contributed more than 50% to root oxygen consumption at low oxygen concentrations in the root environment.

The intermediately elevated species *R. crispus* appeared to be much more tolerant towards conditions of prolonged total submergence: older plants survived eight weeks submergence in the dark. This response was explicable in terms of a dormancy-strategy, which is characterized by a slow consumption of carbohydrates stored in the tap-root. The differential responses of *R. maritimus* and *R. crispus* to total submergence reveal the limitations of flood-tolerance and reflect the different life-histories of the species.

Key words: Photosynthesis, *Rumex*, submergence, carbohydrates, growth rate, shoot elongation.

INTRODUCTION

Growth rate and biomass production of terrestrial plants under flooded conditions are normally reduced (Trought and Drew, 1980; Drew, 1983; Jackson and Drew, 1984). Wetland-species, however, are able to cope with such conditions because of the formation of an aerenchyma system, which provides an efficient means to maintain aerobiosis in the root system (Armstrong, 1979; Laan, Smolders, Blom, and Armstrong, 1989b; Laan, Tosserams, Blom, and Veen, 1990), and as a consequence, growth can be maintained or restored.

In *Rumex* species, the development of aerenchyma determines to a large extent the degree of tolerance to flooding (Laan, Berrevoets, Lythe, Armstrong, and Blom, 1989a). However, under conditions of total submergence, a phenomenon which sometimes takes place during the growing season of flood-tolerant *R. maritimus* and *R. crispus* plants in their natural habitat, i.e. the river foreland ecosystem (Blom, 1990; Blom, Bøgemann, Laan, van der Sman, van de Steeg, and Voesenek, 1990), this ‘avoidance strategy’ fails, because the free diffusion or convection pathway for oxygen between air and root system is blocked.

Under such conditions a number of phenomena may take place to overcome or compensate for the lack of
oxygen: (i) petiole and leaf elongation by which leaves reach the water surface and after which aerobic conditions in the root system can be restored (Atwell, Waters, and Greenway, 1982; Barclay and Crawford, 1982; Keith, Raskin, and Kende, 1986; Brändle, 1990; Laan et al., 1990), (ii) underwater photosynthesis to restore both sugar and oxygen supply to the roots (Bowes, 1987; Gaynard and Armstrong, 1987; Setter, Waters, Greenway, Atwell, and Kupkanchanakul, 1987), (iii) dormancy of the plant (Barclay and Crawford, 1982, 1983; Brändle, 1985), (iv) anaerobic respiration, demanding a high sugar supply (Crawford, 1982; Jackson and Drew, 1984; Setter et al., 1987; Brändle, 1990).

In this study, growth and survival responses of Rumex maritimus, R. crispus and R. thyrsiflorus upon soil flooding and upon total submergence were examined. Growth rates after 3 and 6 weeks of soil flooding or total submergence of R. thyrsiflorus, R. crispus and R. maritimus were determined. Survival responses and mortality rates of the flood-tolerant R. maritimus and R. crispus after different periods of total submergence were related to factors that may determine their differential flood-tolerance, such as shoot elongation, underwater photosynthesis, and the content of carbohydrate reserves in the tap-root.

MATERIALS AND METHODS

Plant growth
Seeds of Rumex maritimus L., R. crispus L. and R. thyrsiflorus Fingerh., collected from natural populations in the river area near Nijmegen (The Netherlands), were sown on black polyethylene grains in a germination cell (16 h light at 60 μmol m⁻² s⁻¹ (PAR), 25°C; 8 h dark, 15°C). After germination, the seedlings were transplanted to PVC tubes (length 0.40 m, diameter 0.12 m), filled with a clay/sand mixture (1:1 (v:v) air-dried and homogenized clay from a beet-field (pH(H₂O) 6.9, weight percentage organic matter 54 ± 0.1) and cleaned, air-dried sand, and allowed to grow for several weeks in a greenhouse (16 h photoperiod at a minimum intensity of 200 μmol m⁻² s⁻¹ and a maximum of 1200 μmol m⁻² s⁻¹ (PAR), 15–22°C). The tubes were watered three times a week to field capacity. Plants of several different ages were used for the experiments. In the flooding treatments, the tubes were placed in an open container (1 × 1 × 1 m) and the water was raised to 1–2 cm above the soil surface. In the total submergence treatments, tubes were also placed in an open container, and the plants were inundated with tap-water to 0.55 m above the tubes. Fine wire-netting was placed on the top of the containers to prevent the leaves from protruding above the water surface. During the submergence treatment light intensity was measured at different depths (LI-COR photometer; LI-185B, Lambda Instr. Corp., USA) and every 5 or 6 d the water was gradually replaced. This prevented the plants from being exposed to the air. Control plants were watered daily to field capacity. Plants were harvested by pushing out the soil core with the complete root system. The root system was separated from the soil by rinsing the soil on a sieve. Shoots, tap-roots and laterals were separated and fresh weight was determined. Dry weights were determined after 48 h drying at 70°C.

Mortality was determined at the end of the experiment by placing the plants under drained conditions for two weeks. When growth of old leaves and/or formation of new leaves did not occur within this period, the plants were considered dead.

Starch content of tap-roots
Tap-roots were ground with a grinding mill (Cyclotec 1093 Sample Mill, Tecator, Sweden) and 50 mg of dry tap-root material was suspended in 2.0 cm³ of demineralized water, autoclaved (15 min, 121°C) and, after cooling to 37°C, incubated for 24 h with 2.0 cm³ amylglucosidasen-solution (1.5 cm³ amylglucosidase (E.C. 3.2.1.3) of specific activity 75 U mg⁻¹ protein (Sigma Chemical Co., USA) in 100 cm³ of a 100 mol m⁻³ acetate buffer, pH 4.5) at 37°C in a shaking water-bath. The suspension was carefully adjusted to exactly 50 cm³ and centrifuged (49 500 × g for 20 min). Glucose was determined in the supernatant with the Anthrone method (Yemm and Willis, 1954).

Photosynthesis and inorganic carbon content of the water
Photosynthetic activity of six totally submerged plants of each species was estimated by trapping gas escaping from the leaves via a funnel attached to a 25 cm³ pipette at least for 3 h at a constant light intensity at plant level of 160–175 μmol m⁻² s⁻¹ (20–23°C). The pH of the water varied with time and place from 8.0–9.5 (containers with tap-water), or from 8.3–8.9 (containers with tap-water, enriched with 10 mM NaHCO₃). The volume of the entrapped gas was recorded every 30 min. Gas release from plants under dark conditions was used as reference.

Total leaf area was determined with a leaf area meter (LICOR 3000, Lambda Instr. Corp., USA), or in case leaf area was less than 0.3 m², with a magnetic board-area-meter (MOP Kontron system, GMBH, Munchen, W. Germany, accuracy 5.0 mm²).

The bicarbonate content of the water was determined during photosynthetic measurements by titration of water-samples with 0.1 N HCl to pH 4.2.

Quantification of photosynthetic oxygen used for root respiration
Plants of R. maritimus were grown on aerated hydroculture for four weeks in a nutrient solution consisting of: Macro-nutrients: 1.0 mol m⁻³ KNO₃, 14.0 mol m⁻³ Ca(NO₃)₂, 14.0 mol m⁻³ NaNO₃, 0.5 mol m⁻³ K₂HPO₄, 0.25 mol m⁻³ MgSO₄; Micro-nutrients: 0.25 mol m⁻³ FeEDTA, 12.5 mmol m⁻³ KCl, 6.3 mmol m⁻³ H₂BO₃, 0.5 mmol m⁻³ MnSO₄, 0.5 mmol m⁻³ ZnSO₄, 0.1 mmol m⁻³ CuSO₄, 0.1 mmol m⁻³ H₂MoO₄, and transferred to a stagnant 0.05% (v:v) anaerobic agar in nutrient solution. After one week of anaerobiosis, a large number of new aerenchymatous roots had developed. These plants were used for root respiration measurements in a previously described system (Laan et al. 1990). This system consists of a thermostatted root compartment (volume 8 × 10⁻⁴ m³), and a perspex shoot compartment. The root compartment contained the above-mentioned nutrient solution; the shoot compartment was slowly filled with a 0.05 M NaH₂PO₄ buffer solution (pH 4.95), until total submergence of the shoot (approximately 3.5 × 10⁻³ m⁻³) and stirred with a magnetic stirrer. The depletion of oxygen was followed in the root compartment with the same plant under dark and under light conditions in the shoot compartment. First, a fully-aerated nutrient solution was circulated several times through the root vessel, until an oxygen concentration of c. 220 μM was reached. Then the root compartment was closed and the depletion of oxygen was followed. The first depletion curve was always made with the shoot in the dark
by enclosing the perspex tube with a black plastic cover. When oxygen was completely depleted from the root compartment, the nutrient solution was replaced by circulating fully-aerated nutrient solution through the root vessel; the black cover was removed and the shoot illuminated (150 W Leitz Wetzlar photolamps, one at the top, the other at the side of the perspex tube, giving a mean light intensity of \( 600 \mu \text{mol m}^{-2} \text{s}^{-1} \text{PAR} \); temperature of the water 24 °C). Sodium bicarbonate was added to the buffer solution in the shoot compartment, giving a final concentration of \( 5 \times 10^{-4} \text{ M} \) bicarbonate (at this pH almost completely as \( \text{CO}_2 \)). After 10–15 min the root vessel was closed again, the depletion of oxygen from the root compartment was followed. Following the same procedure, the third depletion curve was made after an extra addition of bicarbonate, giving a final concentration of \( 5 \times 10^{-3} \text{ M} \) in the shoot compartment. At the end of each series of measurements the shoot was cut off and sealed with a mixture of clay and plasticine, after which another depletion curve was made.

The contribution of photosynthetically-derived oxygen to root respiration was calculated by subtracting the root oxygen uptake rate of the plant with the shoot in the light plus additional bicarbonate from the root oxygen uptake rate (at the same oxygen concentration in the root environment) of the plant with the shoot in the dark. This was done at different root oxygen concentrations down to COPR (see for further details Laan et al., 1990).

**Photosynthesis of leaf discs**

The specific photosynthetic activity of leaf discs (diameter 3.5 cm) of *R. maritimus* and *R. crispus* was determined in the test described above. Instead of a Perspex tube, the cuvette was sealed with a double-walled transparent Perspex lid; the internal solution consisted of 0.05 M NaH$_2$PO$_4$ buffer in nutrient solution either at a pH of 8.0 or at pH 4.95. The solution was kept at 25 °C by a flow-through of thermostatted water via both the outer compartment of the cuvette and the cooling compartment of the Perspex lid. A Leitz Wetzlar photolamp was mounted above the cuvette, giving a light intensity at the discs of \( 600 \mu \text{mol m}^{-2} \text{s}^{-1} \). Discs were cut from leaves of the same age and mounted on a perforated table of the same dimensions with a fine copper wire. Via a central hole in the middle of the Perspex lid, different amounts of NaHCO$_3$ solution were added to the buffer solution. The production of oxygen at different bicarbonate (at pH 8.0) and at different CO$_2$-equivalents (at pH 4.95) was recorded with an oxygen electrode up to a CO$_2$ concentration at which saturation of photosynthesis took place. At the end of each measurement series, the cuvette was darkened with a black plastic cover and dark respiration was determined.

| Table 1. Growth of Rumex species under drained, flooded or totally-submerged conditions for 6 weeks and mortality after 3 and 6 weeks of total submersion (mean plant RGR of six replicates ± s.e.) |
|---|---|---|---|---|
| Species | Period (weeks) | Relative growth rate (mg g$^{-1}$ DW d$^{-1}$) | Mortality upon de-submersion (% of total) |
| | Drained | Flooded | Submerged | |
| *R. thyrsiflorus* | 0–3 | 115 ± 5 | 66 ± 5 | −22 ± 13 | 33 |
| | 3–6 | 67 ± 5 | 32 ± 7 | −57 ± 11 | 100 |
| *R. crispus* | 0–3 | 150 ± 6 | 83 ± 6 | −1 ± 3 | 0 |
| | 3–6 | 64 ± 3 | 54 ± 3 | −58 ± 9 | 33 |
| *R. maritimus* | 0–3 | 126 ± 3 | 111 ± 4 | −34 ± 6 | 100 |
| | 3–6 | 32 ± 2 | 41 ± 6 | −91 ± 7 | 100 |

The age of the plants at the start of the treatment was 4 weeks.
The typical response of *R. crispus* upon submergence seems, therefore, directed towards the maintenance of biomass, enabled by sugars stored in the tap-root under drained conditions on the one hand, and possibly by a low consumption rate of this stored starch on the other.

In contrast, *R. maritimus* showed a fast shoot elongation of leaves and petioles upon submergence. There was a strong tendency to concentrate biomass in the youngest leaves and to shed the older ones under both light and dark conditions. Lateral roots all died within 5 d (data not shown).

**Conditions conducive to survival of submerged plants**

The importance of shoot elongation in *Rumex maritimus*, which led to the protrusion of leaf tips above the water surface is shown in Fig. 1. When the water was lowered sufficiently for the leaf tips to protrude only 7.0 cm, all plants survived. Biomass production of both tap-root and laterals was restored; the protruded part of a flower-stalk, which was formed underwater, flowered within a few weeks. On the other hand, when the water surface was not reached, the starch content in the tap-root, biomass of root and shoot parts decreased rapidly, and all plants died within four weeks.

The importance of underwater photosynthesis for growth and survival of young *R. crispus* and *R. maritimus* plants during submergence was estimated by comparison of biomass production, mortality rates and shoot elongation under dark and light with and without inorganic carbon added to the water (Table 3).

In the dark, shoot elongation was very poor in both species. *R. maritimus* showed a significant biomass reduction of both shoot and root. Leaves were shed and only 17% of the initial shoot biomass was left after 20 d; no laterals could be detected, and the tap-root declined to 74% of its initial biomass. These plants could not restore growth under drained conditions and all died within 30 d (Table 3). In *R. crispus* there was also a biomass reduction, but this was less severe than in *R. maritimus*. After 20 d, most of the leaves remained viable, although a reduction of the initial shoot biomass was recorded. A reduction of initial biomass was also found for the root system. However, all plants survived 20 and 30 d submergence treatments (Table 3).

In the absence of additional bicarbonate, light stimulated shoot elongation in *R. maritimus* (Table 3). Elongation was not associated with an increase in dry weight, but increased water uptake fully accounted for the increase in fresh weight of the shoot. Although less severe than under dark, submerged conditions, the effect of light was to decrease the biomass of shoots and lateral roots (to 10% and 37% of initial biomass, respectively, Table 3); only the tap-roots increased their biomass. All plants survived a 20 d submergence period, but after another 10 d they were dead.

In the absence of bicarbonate, elongation of leaves and/or petioles in *R. crispus* was not stimulated by light. The specific photosynthetic activity was lower than in *R. maritimus* (0.13 versus 0.19 µmol O₂ m⁻² s⁻¹, Table 4), but this was compensated for by a larger leaf area (139 ± 22 cm² in *R. crispus* and 46 ± 8 cm² in *R. maritimus*). Initial biomass of all plant parts was maintained to within 90% of the starting mass, and mortality was 0% after 20 d and 30 d.

Addition of inorganic carbon (10 mM NaHCO₃) to the water significantly stimulated photosynthetic activity (Table 4) and elongation of the shoot in *R. maritimus* and of leaves in *R. crispus* (Table 3). With *R. maritimus* all plants survived a 30 d submergence treatment and growth in mass by roots and shoots was observed. Older shoot

### Table 2. Effect of 5 and 8 weeks of total submergence under light or dark conditions on leaf number, biomass production, starch content of the tap-root and recovery growth of *Rumex crispus* plants (means of seven replicates ± s.e.)

<table>
<thead>
<tr>
<th></th>
<th>5 weeks total submergence</th>
<th>8 weeks total submergence plus 2 weeks recovery growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light</td>
<td>Dark</td>
</tr>
<tr>
<td>Number of green leaves</td>
<td>9.7 ± 0.7</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>Biomass production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot FW (g)</td>
<td>68.2 ± 7.3</td>
<td>44.8 ± 4.3</td>
</tr>
<tr>
<td>DW (g)</td>
<td>40.0 ± 0.4</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Root dry weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tap-root (g)</td>
<td>4.3 ± 0.4</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>lateral roots (g)</td>
<td>1.4 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Tap-root starch content</td>
<td>693 ± 25</td>
<td>585 ± 43</td>
</tr>
<tr>
<td>(mg glucose equivalents)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (mg g⁻¹ DW)</td>
<td>3 068 ± 342</td>
<td>1 606 ± 271</td>
</tr>
</tbody>
</table>

* Newly formed shoot material only.

Plants were 14-weeks-old at the start of the experiment; light intensity was a minimum of 150 µmol m⁻² s⁻¹.
Contribution of photosynthetic oxygen to survival

Because growth of *R. crispus* and both growth and survival of *R. maritimus* plants were dependent on light and/or on the inorganic carbon content of the water (Table 3), underwater photosynthesis must have played an important role in the responses performed by the plants. Not only was there a significant release of oxygen into the water (Table 4), the beneficial effect for the plant is that photosynthetically-derived oxygen can diffuse downward, in this way recovering or maintaining aerobiosis of the root system.

Both *R. crispus* and *R. maritimus* exclusively used CO₂ as an inorganic carbon source (Fig. 2), although the specific photosynthetic rate of *R. maritimus* appeared to be almost twice as high than that of *R. crispus* (15 and 8.5 μmol m⁻² s⁻¹, respectively, Fig. 2).

Figure 3 shows the effect of light irradiation and the addition of inorganic carbon (as CO₂) on the contribution of photosynthetically-derived oxygen to root respiration ('internal aeration') in hydroculture. When 5-0 mM CO₂ and enough light were supplied to the submerged shoots, more than 50% of the total root oxygen consumption could be attributed to photosynthetically-derived oxygen (Fig. 3). At the lower CO₂-concentration (0.5 mM), more than 10% of root respiration was due to shoot-derived oxygen. At both high and low CO₂-concentration, the amount of photosynthetically-derived oxygen used by the roots increased with decreasing solution oxygen concentration (Fig. 3b). Since the oxygen uptake rate from the root environment of decapitated plants is higher than that of plants with their shoots in the dark (Fig. 3a), oxygen can either be taken up from the water by the shoot, transported downwards and used for root respiration, or decapitation leads to a stimulation of root respiration.

DISCUSSION

The results show that the flood-tolerance of the *Rumex* species is strongly dependent on the inundation level used in the experiments. Upon soil flooding, the flood-intolerant *R. thyrsiflorus* cannot cope with sustained periods of root anaerobiosis, while the flood-tolerant *R. crispus* and *R. maritimus* can (Table 1). These responses are in accordance with earlier results (Laan et al., 1989b) and can be explained on the basis of a differential capability to develop root aerenchyma (Laan et al., 1989c). In *R. maritimus* and *R. crispus*, the development of an aerenchyma system leads to the avoidance of anoxia in the root system by internal longitudinal oxygen transport (Laan et al., 1990).

Upon total submergence of the plants, responses are directed towards survival and the maintenance of biomass. Although a strong reduction in growth rate was recorded for all species, the 'flood-tolerant' *R. maritimus* suffered, especially, on submergence, because shoot elongation did not result in protrusion of leaves above the
Table 3. Effect of light and of addition of inorganic carbon to the water (light + C) on elongation and biomass production after 20 d, and mortality after 20 and 30 d of totally submerged *R. crispus* and *R. maritimus* plants

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>R. crispus</em></th>
<th><em>R. maritimus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark (cm)</td>
<td>Light (cm)</td>
</tr>
<tr>
<td>Shoot height</td>
<td>21.2 ± 0.4</td>
<td>22.5 ± 0.3</td>
</tr>
<tr>
<td>Biomass (%) of t = 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap-root</td>
<td>78 ± 11</td>
<td>125 ± 17</td>
</tr>
<tr>
<td>Lateral roots</td>
<td>65 ± 12</td>
<td>91 ± 21</td>
</tr>
<tr>
<td>Shoot</td>
<td>62 ± 5</td>
<td>89 ± 10</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after 20 d</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>after 30 d</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Age of the plants at the start of the experiment 4 weeks; means of 10–13 (shoot height) or 6 (biomass production and mortality) replicates ± s.e.; height of the shoot at t = 0 (n = 20 ± s.e.): R. crispus 17.7 ± 0.4, R. maritimus 18.7 ± 0.5 cm; biomass at the start of the experiment: R. crispus: shoot 439 ± 14, tap-root 36 ± 3, lateral roots 43 ± 6 mg DW; R. maritimus: shoot 611 ± 50, tap-root 23 ± 2, lateral roots 59 ± 8 mg DW; bicarbonate concentration: 1.35 mM (dark treatment), 0.98 mM (light treatment), and 10.9 mM (light + C).

Table 4. Effect of light intensity and of addition of sodium bicarbonate to the water on underwater photosynthetic activity of *R. crispus* and *R. maritimus* after 20 d of total submergence (means of six replicates ± s.e.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>R. crispus</em></th>
<th><em>R. maritimus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark (µmol m⁻² s⁻¹)</td>
<td>Light + C (µmol m⁻² s⁻¹)</td>
</tr>
<tr>
<td><em>R. crispus</em></td>
<td>0</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td><em>R. maritimus</em></td>
<td>0</td>
<td>0.19 ± 0.02</td>
</tr>
</tbody>
</table>

* Calculated from the volume of gas released from submerged plants, assuming the released bubbles to contain 21% oxygen (M. B. Jackson, pers. comm.).

Age of the plants, inorganic carbon concentration and treatments denotation as in Table 3.

water surface (Table 1; Fig. 1). This seems surprising, since *R. maritimus* occupies the lowest altitudinal level in the river foreland ecosystem, being confronted there with irregular periods of prolonged submergence during its growing season (Blom, 1990).

The responses of *R. crispus* and *R. maritimus* to total submergence, however, clearly reflect their different life-histories. Apparently, *R. maritimus* relies predominantly on a fast shoot growth and on the allocation of biomass towards lateral roots rather than to the tap-root. Consequently, this requires a high supply of oxygen and sugars. Therefore, shoot elongation, which can restore a free diffusion pathway between the air and the root system is of vital importance for this species (Fig. 1).

When the water surface is not reached, the plant may use underwater photosynthesis to satisfy its need for oxygen and sugars (Table 4; Fig. 3, Gaynard and Armstrong, 1987; Setter et al., 1987). Survival of *Rumex maritimus* plants resulted only when the inorganic carbon supply is sufficient to sustain a high photosynthetic activity (Tables 3 and 4); the concomitant transport of photosynthetically-derived oxygen (Fig. 3) enables the plant to maintain a high root respiration rate.

It should be noted that the submerged *Rumex* species predominantly use dissolved CO₂ as an inorganic carbon source (Fig. 2); hence, in the greenhouse experiments, where carbon was supplied as bicarbonate, an increase in
photosynthesis can, most probably, be explained by the fact that the absolute amount of dissolved CO₂ was constantly higher in the bicarbonate-enriched containers compared to those containers poor in carbon content. Addition of 10 mM bicarbonate to tap-water results in a final bicarbonate concentration of 11-2 mM and a pH of 8-35; according to Stumm and Morgan (1970), this gives rise to a free, dissolved CO₂-concentration of 16 μM. Next to this, significant amounts of free CO₂ can be supplied via soil respiration. Moreover, this high content of bicarbonate leads to a relatively high buffering capacity of the solution, and thus a pH drift is unlikely to take place. Alternatively, the plants may have generated CO₂ by a local acidification of the water around the leaves, as was shown for Potamogeton and Elodea (Prins, Snel, Zanstra, and Helder, 1982).

The impact of the carbon content of the water on survival could be observed mainly in R. maritimus. Since photosynthetic activity appeared to be very high (Fig. 2; Table 4), plus the fact that survival of this species seems to depend mainly on oxygen availability, a depletion of carbon and, thus, the death of the plants may easily occur. Comparable results were obtained by Setter et al. (1987) with totally submerged rice plants: by flushing 3% CO₂ through the water, growth rate was increased 5-fold. Gaynard and Armstrong (1987) found similar results with Eriophorum angustifolium and concluded that a significant improvement of root aeration and rhizosphere oxygenation could be attributed to photosynthetically-derived oxygen upon total submergence. They also showed that even at very low light intensities (15 μmol m⁻² s⁻¹) oxygen supply to the root apex was improved. Thus, if in R. maritimus the shoot does not reach the water surface, increased light capture due to shoot elongation can be useful, if enough bicarbonate is present in the water. Indeed, bicarbonate concentration in the river water can be high (2.5 mM, Rijkswaterstaat, 1968).

It is interesting that under totally submerged conditions, virtually no shoot elongation took place in the dark, while in light, leaves and petioles of R. maritimus especially showed significant increase in length (Table 3). A combination of both photosynthetic and photomorphogenetic aspects may explain these differences in elongation response. Since ethylene accumulation, in combination with the gibberellin GA₃ and/or with auxins, is of major importance in the elongation response of several plant species, including Rumex (Musgrave, Jackson, and Ling, 1972; Walters and Osborne, 1979; Horton and Samarakoon, 1982; Métraux and Kende, 1983; Ridge, 1987; Voesenek and Blom, 1989), it seems plausible that responses are, at least partly, acting via ethylene.

Light enables the plant to perform photosynthesis (Table 3), and thus an improved oxygen and/or sugar status throughout the plant is likely to occur. Since hypoxia stimulates ethylene synthesis (Jackson, 1982, 1985; Jackson, Dobson, Herman, and Merryweather, 1984; Raskin and Kende, 1984), photosynthesis leading to hypoxic conditions (3–5% O₂) in the plant would promote shoot elongation. In addition, an improved sugar status might lead to a continuation of osmotic water uptake and thus to an increased cell expansion.

In combination with this, light itself may stimulate shoot elongation, either via a decrease in photon flux density, as was shown for Hippuris vulgaris (Spence, Bartley, and Child, 1987), or via an altered light regime, as was shown for Sorghum vulgare (Craker, Abeles, and Shrophire, 1973); light, especially in the blue and far-red regions of the spectrum, induced ethylene production.
When neither the water surface is reached, nor enough carbon plus oxygen can be generated by photosynthesis, reverses are soon exhausted and *Rumex maritimus* plants die (Table 3; Fig. 1). For *R. crispus* photosynthesis is probably less important for survival (Tables 2, 3), but the amount of respirable sugars stored in the tap-root may be an important factor determining survival chances of the plants. It remains however, difficult to correlate the amount of reserve carbohydrates and their consumption rate by the roots with survival chances of the plants upon total submergence. In the experiments conducted, the occurrence of underwater photosynthesis does not allow a certain interpretation of this correlation and, in addition, older *R. crispus* plants perform differently from younger plants, because the respiratory demand of the root system strongly decreases with age, while the tap-root gets increasingly important as a sink for growth (Laan et al., 1990; P. Laan, unpublished results).

*R. crispus*, a perennial species, tends to allocate energy to the tap-root, while *R. maritimus*, the annual species, does not. The ratio tap-root: total biomass at the start of growth is 0.03 for the young plants of *R. maritimus* and 0.07 for *R. crispus* (Table 3). Thus, with regard to the availability of reserves, the survival changes of *R. crispus* are higher than those of *R. maritimus*.

In conclusion, for the annual species *R. maritimus* flood-tolerance seems to depend on oxygen availability, which is characterized by the inability to slow down metabolism. Upon total submergence, all responses are directed towards gaining oxygen, either by shoot elongation and protrusion of leaves above the water surface (Fig. 1), or by underwater photosynthesis (Table 4; Fig. 2). For the long-living perennial species *R. crispus*, oxygen availability is less important, and there may be a tendency to slow down metabolism upon submergence, contributing to the preservation of starch reserves in the tap-root and, consequently, to the capacity of regrowth on de-submergence (Tables 2, 3). This difference in life-history stresses the importance of sugar supply: with *R. maritimus* relying more on the generation of sugars from photosynthesis and *R. crispus*, relying more on the use of stored carbohydrates.

**ACKNOWLEDGEMENTS**

The authors wish to thank M. Mensink, L. de Haas, W. de Bruyn and M. Tossearms for their technical assistance, and Drs H. F. Bienfait, H. Konings, H. Schat, W. Armstrong, B. W. Veen, A. J. M. Smits and Mr H. M. van de Steeg for improvements to the manuscript.

**LITERATURE CITED**


Laan, P., Berrevoets, M. J., Lythe, S., Armstrong, W., and Blom, C. W. P. M., 1989a. Root morphology and aerenchyma...


