The role of flooding resistance in the establishment of Rumex seedlings in river flood plains

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Summary

1. This study deals with resistance to complete submergence of seedlings of Rumex acetosa, R. crispus and R. palustris.
2. Seedlings of R. crispus and R. palustris appeared to be more resistant to flooding than those of R. acetosa under both field and greenhouse conditions.
3. Younger seedlings of all species were able to survive a longer flooding period than older ones.
4. When completely submerged the three Rumex species showed a low, but significant, photosynthetic activity. In R. crispus and R. palustris photosynthetically produced oxygen is released by the roots. Excised Rumex root tips have a low hypoxia tolerance, but that of R. acetosa was the lowest. This correlates with a high rate of ethanolic fermentation in both R. crispus and R. palustris and a low rate in R. acetosa.
5. The overall resistance to flooding of Rumex seedlings established from field, greenhouse and laboratory experiments showed a relationship with their site distribution in a flooding gradient of river Rhine.

Key-words: Docks, ethanolic fermentation, field establishment, Polygonaceae, underwater photosynthesis

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Introduction

In many river areas Rumex acetosa L., R. crispus L. and R. palustris Sm. have a distinct vertical distribution range. Rumex acetosa is frequently found on elevated, seldom-flooded sites in the river area. Rumex palustris, on the other hand, occurs on sites characterized by frequent and prolonged inundation, whereas R. crispus occupies intermediate positions in this flooding gradient (Voesenek & Blom 1987; Blom et al. 1990). Floods in summer, mainly caused by an enhanced discharge of rain-water from the middle reaches of the Rhine, are unpredictable and may affect plants at different stages of the life cycle. In order to explain the distribution of these Rumex species it is essential to study the acclimatic responses of these species to flooding at all stages of their life cycle (Blom 1988). Characteristics of seed bank, germination, vegetative phase and generative phase in relation to flooding have been described previously (Voesenek & Blom 1989, 1992; Voesenek, Blom & Pouwels 1989; Voesenek 1990; Voesenek et al. 1990a; Van der Sman et al. 1991; Voesenek, de Graaf & Blom 1992). Seedling establishment in relation to flooding resistance will be discussed in this paper. According to Fenner (1987) the establishment phase of a plant can be defined as a period during which it still uses some of the nutrient reserves of the seed. Flooding during seedling establishment most often results in the complete submergence of the plant. It is known that both R. crispus and R. palustris can restore leaf-atmosphere contact by enhanced cell elongation in petioles, mediated by the gaseous hormone ethylene (Voesenek & Blom 1989; Voesenek et al. 1990b). However, small seedlings can only accommodate up to a few centimetres of water, thereby limiting the adaptive value of this response. It would be of great advantage for establishment of Rumex in frequently flooded areas if seedlings were able to survive the severe conditions of complete submergence.

In submerged shoots the diffusion of atmospheric oxygen via the shoot to the roots is restricted (Gaynard & Armstrong 1987). In terrestrial plants the photosynthetic rate of submerged shoots will decline dramatically, since aqueous solutions have a high diffusion resistance for CO2 (Bowes 1987). It has been reported that photosynthesis of some submerged aquatic macrophytes may result in oxygen release from the roots (Sand-Jensen, Prahl & Stokholm 1982; Roelofs, Schuurkes & Smits 1984; Smith, Dennison & Alberte 1984a). Recently it was
also shown that photosynthetic activity of terrestrial plants enhances root aeration during submergence (Gaynard & Armstrong 1987; Waters et al. 1989; Laan & Blom 1990). The use of photosynthetic oxygen for aerobic root respiration depends, however, on internal oxygen transport via aerenchyma (Armstrong 1979).

Inundation cuts off gas exchange between soil and atmosphere, which results, in combination with a continued respiration of roots and micro-organisms, in hypoxic or anoxic soil conditions (Ponnamperuma 1984; Drew 1990). Lack of oxygen is probably the most important factor limiting the growth and survival of higher plants in these habitats (ap Rees et al. 1987). If the transport of oxygen via aerenchymatous tissues is inadequate (Drew 1987) or impossible due to the lack of a connection between the submerged tissue and a source of oxygen, other adaptations, involving modifications of cell metabolism, become essential in the survival of hypoxic or anoxic conditions (ap Rees et al. 1987). There is ample evidence that ethanolic fermentation is the main mode of respiration under aerobic conditions (Smith & ap Rees 1979; Bertani, Brambilla & Menegus 1980; Monk, Crawford & Brändle 1984; ap Rees & Wilson 1984; Smith, Kalsi & Woolhouse 1984b; Tripepi & Mitchell 1984; Saglio, Drew & Pradet 1988). For example, Roberts and co-workers (1984) showed that differences in the rate of ethanol production between maize varieties are correlated with differences in survival of root tips under hypoxia. Relatively minor differences in the metabolism of flooding-tolerant and -intolerant plants may well occur and be of considerable ecological significance (Tripepi & Mitchell 1984; ap Rees et al. 1987).

The aim of the present work is to clarify the flooding resistance of Rumex seedlings in relation to the establishment of Rumex in river flood plains. A reciprocal establishment experiment was performed to study seedling establishment under field conditions, whereas the flooding resistance of whole seedlings was tested in the greenhouse. Since it was expected that adaptation to survive submerged conditions is important for establishment in frequently flooded sites, laboratory experiments were focused on differences in photosynthesis of leaves under water and the release of photosynthetic oxygen by the roots, on the in vitro root tip survival during hypoxia and on ethanolic fermentation of R. acetosa, R. crispus and R. palustris.

Materials and methods

RECIPROCAL ESTABLISHMENT OF SEEDLINGS UNDER FIELD CONDITIONS

The field experiment was performed at locations near the Waal, a major river of the Rhine delta. In three zones, each characterized by the specific occurrence of one of the Rumex species, 15 plots per zone were laid out with dimensions of 1.3 × 0.8m. The turf layer (2-3cm) was removed in order to create a gap suitable for seedling establishment. This was not necessary in the palustris zone (site with R. palustris) since the long-lasting spring flood of 1988 killed all established plants. In five plots per species per zone achenes were sown in a grid pattern. Each plot contained 50 sowing sites; per site two achenes were planted at a substrate depth of 1 cm. The plots were sown at the start of the growing season in every zone. In the acetosa zone this was on 3 April 1987. In both the crispus and palustris zones initiation of the growing season coincided with the subsidence of winter and spring floods (crispus zone, 24 April 1987; palustris zone, 2 August 1988). The achenes used for this experiment were collected in the field in 1986 and dry stored at room temperature in the dark. Control germination under optimal laboratory conditions was always higher than 90% for all species. Seeding emergence and survival in the experimental plots was recorded throughout the remaining growing season. When two Rumex seedlings emerged from one site, one of them was removed in order to create almost identical intraspecific interactions for all Rumex seedlings in one plot. One additional observation of seedling survival was carried out at the onset of the second growing season. In both the acetosa and crispus zones the surrounding vegetation in the plots was clipped at Rumex height (3-10 cm) to mimic natural grazing. Natural grazing was prevented by fencing the plots.

FLOODING RESISTANCE OF WHOLE SEEDLINGS UNDER GREENHOUSE CONDITIONS

After a 7-day germination period [for conditions see Voesenek & Blom (1989)] 400 seedlings per Rumex species were transferred individually into pots (height 50mm; diameter 70mm) filled with a sandy soil collected on the university campus. Two hundred seedlings per species were allowed to grow for 4 days in the greenhouse with a temperature of 15°C at night and approximately 25°C during the day (photoperiod 16h). Supplementary illumination by high pressure sodium lamps [400 W; photosynthetic photon flux density (PPFD) 115 μmol m⁻² s⁻¹] was provided in periods with low external light conditions. The soil in the pots was kept at field capacity by frequent watering. After this period of growth the two cotyledons were fully expanded and the onset of the first leaf was visible. The remaining 200 seedlings were grown for 12 days under identical greenhouse conditions. By that time the two first leaves had fully expanded, whereas the third was barely visible. Subsequently, these two groups of seedlings were completely submerged in plastic containers (w × l × h 50 × 70 × 45 cm; water column above pot 26 cm) filled with tap-water at a temperature of
Flooding resistance of Rumex seedlings

20°C which was maintained during the course of the experiment. Survival of 20 randomly a priori selected plants per species was determined after 4, 8, 12, 16, 20, 24, 28, 32, 36 and 40 days of complete submergence. If no regrowth occurred within a 2-week growing period under non-flooded conditions, the seedlings were assumed to be dead.

UNDERWATER PHOTOSYNTHESIS OF LEAF DISCS

Photosynthesis of submerged Rumex leaves was measured as O₂ evolution in a closed system with a Clark electrode (Delieu & Walker 1972). One-week-old seedlings were transferred to pots (height 50 mm; diameter 55 mm) filled with a mixture of sand and potting compost (1:1 v/v). Seedlings were grown for 25 days in a growth chamber at a temperature of 20°C and a photoperiod of 16 h (PPFD 200 μmol m⁻² s⁻¹). The soil in the pots was kept near field capacity by regular watering. Hereafter the seedlings were completely submerged in a 2 mol m⁻³ NaHCO₃ solution (pH 8.3) in the growth chamber. The inorganic carbon equilibrium in this water was very similar to the flood water in the field. After 3 days of submergence, two leaf discs (diameter 10 mm) of the youngest leaf were placed in a nylon mesh bag fixed in the experimental chamber (filled with 2.2 cm⁻³ 2 mol m⁻³ NaHCO₃) of the oxygen electrode apparatus (Delieu & Walker 1972). The temperature in the chamber was kept constant at 22°C. The leaf discs were illuminated by a halogen light source (Philips, 15 W) with a PPFD at leaf disc level of 600 μmol m⁻² s⁻¹. Before fixation of the leaf discs N₂ was bubbled through the NaHCO₃ solution in the experimental chamber for 2 min. This reduced the O₂ concentration considerably, thereby reducing photorespiration. Subsequently the chamber was closed and O₂ evolution was recorded for 10 min. Oxygen concentrations were determined according to Drew & Robertson (1974). All Rumex species were measured three times. As a reference the photosynthesis of underwater leaves of Nuphar lutea (L.) Sm., a macrophyte from habitats with a similar water quality to the water that submerges Rumex species (Smits et al. 1988), was also measured.

OXYGEN RELEASE FROM ROOTS

Respiration of aerobic and anaerobic soil organisms causes a rapid decline in the redox potential (E₉) of a flooded soil (Ponnamperuma 1984). Oxidation of the rhizosphere by completely submerged plants can occur if photosynthetic oxygen is released by the roots (Sand-Jensen, Prahl & Stokholm 1982). To study this process in submerged Rumex plants, oxygen release of roots in an agar solution and the decline in E₉ in densely rooted pots were studied in the light and in the dark.

Oxygen release of roots from completely submerged Rumex plants was determined qualitatively by oxidation of leuco-methylene blue (see Laan et al. 1989). The plants used in this experiment were grown in identical pots and under the same conditions as previously mentioned with the exception that the pots were filled with sand only and watered with 1/4 Hoaglands nutrient solution. This modification enabled easy separation of roots and soil before the plants were placed in glass cylinders to observe the oxygen release of roots. These 250-cm³ cylinders were half-filled with agar solution (10 kg m⁻³) to which a leuco-methylene blue solution (10 kg m⁻³) was added (0.5–1.0 dm⁻³). The solution was made colourless by the addition of sodium-dithionite. The roots of intact Rumex plants were placed in the agar solution. Small amounts of intruding oxygen during this procedure were reduced by sodium-dithionite. Subsequently the shoot was carefully submerged with 2 mol m⁻³ NaHCO₃ solution (pH 8.3) which was thoroughly bubbled with N₂ for several hours. The complete cylinder was illuminated for 5 h with a PPFD of 150 μmol m⁻² s⁻¹. This experiment was conducted with two plants per species.

In order to measure the decline in E₉ of a flooded soil, achenes were germinated and grown as in the greenhouse experiment. After a 45-day growth period the plants had developed a high root density and approximately six to seven leaves. Four plants per species and four pots filled with soil only, were submerged in the greenhouse with tap-water (photoperiod 16 h); replicates were submerged under dark conditions (temperature 18–23°C). Almost every day in a 7-day period the redox potential of the soil was measured at a depth of 25 mm in every pot by means of a fixed platinum electrode and a calomel reference electrode. The E₉ value was corrected for temperature and soil pH. At the termination of the experiment roots of all plants were separated from the soil and weighed after 48 h at 70°C. The redox potentials were statistically analysed with one-way Analyses of Variance, in which light and dark conditions were compared per plant species.

HYPOXIA TOLERANCE OF ROOT TIPS

Rumex achenes were allowed to germinate aerobically as described in Voesenek & Blom (1989) with the exception that R. palustris achenes germinated for 9 days instead of the usual 7 days. Hereafter per species five primary root tips were cut to a length of approximately 15–20 mm and placed in a 30-cm³ flask filled with a hypoxic solution (24 h bubbled with N₂) of 50 mol m⁻³ glucose, 0.1 mol m⁻³ CaSO₄ and 400 g m⁻³ chloramphenicol (O₂ concentration 0.20 ± 0.02 g m⁻³). Subsequently these flasks were sealed with serum-vial caps. The whole procedure was conducted in an anaerobic chamber. These flasks were placed in a temperature-controlled chamber in the dark (20°C)
Table 1. Flooding frequency, total duration and winter survival (per cent of seedlings alive at the onset of the winter) (census period: acetosa zone 18 November, 1987 – 5 May 1988; crispus zone: 13 November 1987 – 9 May 1988; palustris zone: 9 November 1988 – 25 May 1989) of *Rumex* seedlings germinated in the previous growing season

<table>
<thead>
<tr>
<th>Floods</th>
<th>Frequency</th>
<th>Duration (days)</th>
<th>Species</th>
<th>R. acetosa</th>
<th>R. crispus</th>
<th>R. palustris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetosa zone</td>
<td>1</td>
<td>22</td>
<td></td>
<td>84·6</td>
<td>42·4</td>
<td>14·3</td>
</tr>
<tr>
<td>Crispus zone</td>
<td>5</td>
<td>102</td>
<td></td>
<td>*</td>
<td>8·2</td>
<td>8·3</td>
</tr>
<tr>
<td>Palustris zone</td>
<td>4</td>
<td>125</td>
<td></td>
<td>0</td>
<td>39·8</td>
<td>19·0</td>
</tr>
</tbody>
</table>

* All seedlings had succumbed before winter.

for several hours. Ten flasks per species were incubated for every treatment hour (0, 1, 2, 3, . . . 24h). After a certain period of hypoxia, root tips were cut to a length of exactly 5 mm. These tips were placed in petri dishes on top of two layers of filter paper (Schleicher and Schüll), which were wetted with an aerated solution of glucose, CaSO4 and chloramphenicol (concentrations similar to hypoxic solution). After 7 days of aerobic incubation at 20°C in the dark the length of the root tips was measured. Root tips exhibiting no growth were classified as dead (Roberts 1987).

After a certain period of hypoxia, root tips were cut to a length of exactly 5 mm. These tips were placed in petri dishes on top of two layers of filter paper (Schleicher and Schüll), which were wetted with an aerated solution of glucose, CaSO4 and chloramphenicol (concentrations similar to hypoxic solution). After 7 days of aerobic incubation at 20°C in the dark the length of the root tips was measured. Root tips exhibiting no growth were classified as dead (Roberts et al. 1989). This experiment was repeated three times. For all three species this experiment was also performed with primary root tips in a continuously aerated incubation solution. This test provided information about the influence of experimental artefacts on the survival of root tips *in vitro*.

The whole procedure was repeated with the modification that 10-cm3 flasks were used with four root tips per species in every flask. Per species and per duration of the hypoxic treatment, five flasks were prepared. After the hypoxic treatment these 20 root tips per species were stained with a 10 kg m-3 tetrazoliumchloride solution (Sigma, Brussels, Belgium) (see Moore 1972) for 30min. The ability to stain and the intensity of the staining (light or dark red) were interpreted as indicators for root tip vitality (Bertani, Brambilla & Menegus 1981; Fagerstedt & Crawford 1987).

**ETHANOL AND CARBON DIOXIDE PRODUCTION OF ROOT TIPS UNDER HYPOXIC CONDITIONS**

Primary root tips (length 15-20mm; fresh weight 150-300mg) of the three *Rumex* species were incubated in a hypoxic solution (10-cm3 flasks) as described above. Every 3h during the first 24h, samples were taken from the flasks for ethanol and CO2 determinations. With a syringe 0-5cm3 of the solution was sampled from the flasks; 100mm3 was used for CO2 determination, whereas 0-3cm3 was needed for the enzymatical assay of ethanol (Bernt & Gutman 1963). Directly after sampling 0-5cm3 of a fresh hypoxic incubation solution was injected into the flask to restore the original solution volume. This procedure was conducted in an anaerobic chamber.

Carbon dioxide measurements were performed with an Oceanography International model 0525 HR Infrared Carbon Analyser (Oceanography International Corporation, Texas). This experiment was performed twice for all *Rumex* species.

**Results**

**ESTABLISHMENT UNDER FIELD CONDITIONS**

The acetosa zone was not flooded during the growing season of 1987; a short-lasting inundation was observed in the winter following seedling establishment (Fig. 1, Table 1). Both the crispus and palustris zones were flooded several times in winter and summer; noteworthy is the long-lasting summer flooding in the crispus zone (Fig. 1, Table 1). The small difference in flooding frequency and duration between the crispus and palustris zones must be interpreted with care since data are from 2 successive years (Table 1). The unpredictability of summer floods in the lower zones and the occurrence of germination flushes of especially *R. crispus* and *R. palustris* throughout the growing season, caused flooding of seedlings at all stages of development.

Floods were lethal for seedlings of *R. acetosa*. No seedling of this species survived the summer flood in the crispus zone (Fig. 1), whereas floods during the winter eliminated *R. acetosa* seedlings in the palustris zone (Table 1).

Survival of *R. crispus* seedlings in the acetosa zone was relatively low compared to *R. acetosa* in both the growing season and the winter. Floods in winter and summer resulted in a high mortality of *R. crispus* in its natural zone. Some seedlings, however, were able to survive long-lasting floods. The survival of *R. crispus* in the palustris zone was relatively high compared to *R. palustris*.

A relatively low seedling survival was observed for *R. palustris* in all *Rumex* zones. However, as for *R. crispus*, a few *R. palustris* seedlings were actually able to survive floods in both winter and summer.

In contrast to *R. acetosa*, both *R. crispus* and *R. palustris* showed additional germination and establishment shortly after subsidence of the summer flood in the crispus zone (Fig. 1).
Flooding resistance of Rumex seedlings

Flooding resistance of whole seedlings under greenhouse conditions

The flooding resistance under greenhouse conditions differed for the three Rumex species and the two seedling stages (Fig. 2). The two-cotyledon stage appeared to be the most flood resistant. Rumex acetosa appeared to be non-resistant at both seedling stages, whereas R. crispus and R. palustris were relatively resistant to flooding. Differences between both flood-resistant species were small.

Photosynthesis of submerged leaves

Leaves of all Rumex species were able to photosynthesize under submerged conditions (Table 2); no differences existed between the species. A higher photosynthetic activity was observed in the aquatic macrophyte N. lutea.

Only R. crispus and R. palustris were able to release oxygen from their roots to the reduced agar solution under submerged conditions (Table 2).

The redox potential in pots with R. crispus and R. palustris differed between light and dark conditions; the redox potentials in light were significantly higher than under dark conditions (Fig. 3). A fast decline of the redox potential was observed in R. acetosa under both light and dark conditions. No significant differences were observed in the redox potentials of the pots without plants submerged in light and dark. The root dry weights of the Rumex species showed no

<table>
<thead>
<tr>
<th>Species</th>
<th>Photosynthesis (µmol O₂ g FW⁻¹ h⁻¹)</th>
<th>Oxygen release by roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. acetosa</td>
<td>14-4 ± 0-3</td>
<td>—</td>
</tr>
<tr>
<td>R. crispus</td>
<td>13-8 ± 1-6</td>
<td>+</td>
</tr>
<tr>
<td>R. palustris</td>
<td>13-4 ± 0-6</td>
<td>+</td>
</tr>
<tr>
<td>N. lutea</td>
<td>64-4 ± 25-3</td>
<td>ND</td>
</tr>
</tbody>
</table>

FW, fresh weight. ND, not determined.
* +, Oxidation of leuco-methylene blue.
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Fig. 2. Survival of seedlings of *Rumex acetosa*, *R. crispus* and *R. palustris* in the course of time under submerged greenhouse conditions. Two stages of seedling development were incorporated in this study: (A) younger seedlings with only two cotyledons and (B) older seedlings with the first two real leaves.

significant differences after termination of the experiment (data not shown).

**HYPOXIA TOLERANCE OF ROOT TIPS: ETHANOL AND CO₂ PRODUCTION**

In a well-aerated incubation solution (24h) excised primary root tips of all *Rumex* species lost only 10–20% of their capacity to grow (data not shown). Therefore, it can be concluded that hardly any experimental artefacts interfered with the experiments considering the hypoxia tolerance of root tips; decline in growth and staining capacity of root tips is almost exclusively caused by the hypoxic treatment.

The hypoxia tolerance of root tips was low for all *Rumex* species (Fig. 4A, C, E). *Rumex acetosa* survived a hypoxia period for less than 10h. The two other species survived for periods up to 16h. It is noteworthy that both methods (staining and growth measurement) gave comparable results; a decreased growth was always accompanied by a reduced staining capacity.

Ethanol and CO₂ were produced in almost equal amounts in all species. However, distinct differences in ethanol and CO₂ production existed between the species. *Rumex acetosa* produced a limited amount of ethanol and CO₂, whereas *R. crispus* and *R. palustris* showed a considerable ethanolic fermentation under hypoxic conditions (Fig. 4B, D, F).

**Discussion**

Summer floods in Dutch river areas are unpredictable and may submerge seedlings at various stages of their establishment. It is therefore likely that these inundations have a strong impact on the success of seedling establishment in frequently flooded areas. However, other factors such as competitive interactions and herbivory may affect establishment in the river flood plains as well (see Fenner 1987; Harper 1977).

Under experimental field conditions seedlings of *R. crispus* and *R. palustris* were relatively resistant towards flooding when compared to *R. acetosa*. The latter species is therefore unable to establish in frequently flooded parts of the river area such as the natural habitats of *R. crispus* and *R. palustris*. Establishment of *R. acetosa* in gaps can occur in elevated parts of the river area such as dikes and river levees, which are only occasionally exposed to floods. High mortality rates were observed for seedlings of *R. palustris* and *R. crispus* when growing in the acetosa zone. Both competitive interference and herbivory may have been responsible for the high mortality of *R. palustris* and, to a somewhat lesser extent, *R. crispus* seedlings in the natural habitat of *R. acetosa* (Voesenek 1990).
Flooding resistance of Rumex seedlings

Seedlings still in the cotyledon stage were more resistant to flooding under greenhouse conditions than older seedlings. An adequate supply of carbohydrates can be critical in the survival of plant tissues at low O₂ concentrations (Setter et al. 1987; Crawford, Studer & Studer 1989; Brändle 1991). Carbohydrate levels are determined by the balance between consumption in respiration, access to stored reserves and production of carbohydrates in photosynthesis (Saglio 1985; Setter et al. 1987). Seedlings with only cotyledons probably have more stored reserves (Fenner 1987), whereas older seedlings with already shrunken cotyledons and two leaves not only possess less reserves, but also have a larger photosynthetic leaf area and a larger amount of respiring tissue. Further research is necessary to elucidate the role of a possible shift in the carbon budget in the differential survival of younger and older seedlings. It also stresses again the importance of studying various stages of plant development with respect to flooding resistance (see Fagerstedt & Crawford 1986). During both development stages seedlings of Rumex acetosa showed the lowest survival under submerged conditions. This correlates very well with the observations of the field experiment.

All three Rumex species were capable of a low, but significant photosynthetic activity under submerged circumstances. Literature concerning photosynthesis of terrestrial plants underwater is very scarce; it is known that Eriophorum angustifolium, a terrestrial plant from wet habitats, uses free CO₂ for photosynthetic activity under submerged conditions (Gaynard & Armstrong 1987). In submerged rice seedlings photosynthesis is essential to supply sufficient oxygen for root growth and ethanol consumption (Waters et al. 1989). Recently, Laan & Blom
L. A. C. J. Voesenek (1990) demonstrated that both underwater photosynthesis and restoration of shoot-atmosphere contact after submergence are of utmost importance for survival of *R. maritimus* (a species from very frequently flooded sites in river flood plains).

The lower photosynthetic rate of *Rumex* leaves when compared to the aquatic macrophyte *N. lutea*, may be related to the lack of an aquatic leaf morphology, which is in aquatic macrophytes an adaptation to the high aqueous diffusion resistances for CO$_2$ and O$_2$ (Bowes 1987).

While photosynthesizing underwater, only *R. crispus* and *R. palustris* were able to release oxygen from the roots, indicated by oxidation of leucomethylene blue and the relatively high soil redox potentials under light conditions. It is likely that in *R. acetosa* the aerenchymatic connection between leaves and roots is inadequate to support oxygen release. This is in agreement with observations on *R. thyrsiflorus*, a species closely related to *R. acetosa*, which lacks aerenchyma in both lateral roots and petioles (Laan et al. 1990).

Root tip survival under hypoxic *in vitro* conditions in *Rumex* was positively correlated with the ethanolic fermentation rate, measured as ethanol and CO$_2$ production. Root tips of *R. acetosa* showed the lowest tolerance of hypoxia accompanied by low ethanolic fermentation, whereas a higher tolerance and a high fermentation rate were observed in both *R. crispus* and *R. palustris*. These differences in ethanolic fermentation rate are not related to interspecific variation in basal aerobic root tip respiration (data not shown). The results obtained for these *Rumex* species distributed in a flooding gradient are rather analogous to results from a comparative study of three nympaheid macrophytes from organic sediments with low redox potentials and three macrophytes from mineral oxidative sediments; only the roots of the nympaheid macrophytes were vital after 49 h of anaerobiosis and showed a considerable ethanolic fermentation during the experimental conditions (Smits et al. 1990). The rapid fall in root tip vitality of *R. acetosa* forms no explanation for the overall low rate of ethanolic fermentation in this species. This is based on the following arguments: (1) during the first 3 h of the experiment no differences in root tip vitality were observed between the species, whereas the fermentation rate in *R. crispus* and *R. palustris* was approximately 10 times higher than in *R. acetosa*; (2) *R. acetosa*, *R. crispus* and *R. palustris* showed a 50% reduction in root tip vitality after 4-5, 7-8 and 10-11 h of hypoxia, respectively. This equal reduction in root tip survival was still accompanied by a three-fold higher fermentation rate in *R. crispus* and *R. palustris*, when compared to *R. acetosa*.

According to the theory of Roberts and co-workers (1984) it is assumed that the observed variation in hypoxia tolerance in excised *Rumex* root tips is related to differences in the rate of ethanolic fermentation. There is increasing evidence that anoxia or hypoxia tolerance in plants is positively correlated with ethanol production (John & Greenway 1976; Smith & ap Rees 1979; Webb & Armstrong 1983; Roberts et al. 1984; Davies et al. 1987; Mendelssohn & McKee 1987). Proof for the important role of ethanol production during hypoxia comes from ADH null mutants of maize (Schwartz 1969; Roberts et al. 1984) and barley (Harberd & Edwards 1982); these mutants were relatively flood intolerant.

The hypoxia tolerance of *in vitro* primary root tips of *Rumex* species is positively correlated with flooding resistance of whole plants under greenhouse and field conditions. In conclusion the overall flooding resistance of *Rumex* seedlings in all experiments conducted, is also strongly positively correlated with the distribution of the three species in the river flood plain. It is assumed that morphological (aerenchyma channels connecting shoot and root) and metabolic (ethanolic fermentation) adaptations in *R. crispus* and *R. palustris* promote establishment of both species in river habitats with unpredictable floods. Lack of these adaptations restricts the establishment of *R. acetosa* to higher zones.

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