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Survival tactics of *Ranunculus* species in river floodplains

Abstract The flooding resistance of four *Ranunculus* species was studied under controlled conditions and related to the tactics used by these species to survive in their natural habitat in river floodplains. *R. bulbosus*, a species from seldom-flooded river levées, was relatively intolerant of both waterlogging and complete submergence, due to a constitutively low level of aerenchyma in the root system. This lack of gas spaces resulted in high mortality rates during flooding treatments and an inability to use photosynthetically derived oxygen for root respiration during complete submergence. The pioneer *R. sceleratus*, predominantly abundant in low lying mudflats, was very resistant to waterlogging and shallow floods. Due to its constitutively high root porosity and its ability to greatly increase the elongation rate of petioles under water this species can ameliorate flooding stress. However, when leaf blades of *R. sceleratus* were unable to reach the water surface, this species died as quickly as the flooding-intolerant *R. bulbosus*. This indicates that fast elongation of petioles under water competes for energy and respirable reserves with maintenance processes. *R. repens*, a species from lower, frequently inundated floodplains, was very tolerant of prolonged waterlogging and submergence. Its high resistance to complete submergence under continuous darkness indicates that this species tolerates hypoxic and/or anoxic tissue conditions via metabolic adjustments. Lysigenous aerenchyma was also induced in the primary root system and in newly developed laterals, and it was able to use oxygen generated by underwater photosynthesis, for root respiration. *R. acris*, a species from less frequently flooded areas, was as resistant to waterlogging and submergence in the light as *R. repens*. However, it has a lower resistance than *R. repens* to complete submergence in the dark. A submergence pretreatment increased the maximum net underwater photosynthetic rate in *R. bulbosus*, whereas a significant decrease of light compensation points was observed in *R. repens* when it had previously been submerged. This study shows that *Ranunculus* species exhibit various strategies to cope with different flooding conditions. *R. repens* responds to flooding by its tolerance mechanism and *R. sceleratus* by avoidance. *R. acris* ameliorates submergence and *R. bulbosus* was not able to adapt high water tables.

Key words Aerenchyma · *Ranunculus* · Shoot elongation · Photosynthesis · Survival of flooding

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Introduction

High water levels related to excessive rainfall, discharge of melting snow and ice or to impaired land drainage severely affect plant growth and survival (Blom et al. 1994; Blom and Voesenek 1996). Soil flooding or waterlogging hampers the gas exchange between roots and atmosphere, ultimately leading to a dramatic shift in the endogenous gas composition of roots. The strong reduction in the oxygen concentration particularly affects the energy status, and thus the chance of survival, of roots (Drew 1992). Plants can also be submerged completely; this not only restricts gas exchange between shoots and atmosphere, but also strongly reduces the amount of light available for photosynthesis (Voesenek et al. 1992; Setter et al. 1997). In order to cope with the stressful environmental conditions related to waterlogging and submergence many plant species from aquatic and semi-aquatic habitats possess traits that increase survival during flooding.

Fitter and Hay (1981) used the term *tactic* to distinguish between different mechanisms of stress resistance. Different tactics *sensu* Fitter and Hay (1981) may be included in a life-history tactic as "a set of co-adapted traits" according to Stearns (1976). Fitter and Hay (1981) recognised three primary tactics which may occur in

combination: avoidance, amelioration and tolerance. With respect to flooding stress, *avoiders* are plants that deal with predictable stresses (e.g. winter floods in Dutch river floodplains) by having dormant stages (e.g. seeds, tubers, rhizomes). Plants that *ameliorate* flooding stress are exposed to it, but they alleviate its adverse effects (low oxygen). Well known examples in this context are the stimulation of the shoot elongation response upon submergence, the process of underwater photosynthesis and the formation of adventitious roots during waterlogging (Voesenek et al. 1992). What these three adaptive traits have in common is that they improve the oxygen status of the flooded root system. *Tolerance* towards flooding stress is interpreted as real metabolic tolerance. Many flood-tolerant plants achieve this by continued and/or accelerated glycolysis and ethanolic fermentation. These processes are fuelled by large carbohydrate reserves in the perennating organs (Armstrong et al. 1994).

In this paper we attempt to define the tactics employed by four *Ranunculus* species upon exposure to flooding stress, and we try to relate this to the habitat preference of these species in Dutch river floodplains. We hypothesize that the contrasting flooding conditions force these phylogenetically related species to use various survival tactics reflecting adaptations to their specific environment. *Ranunculus* species were selected for this study because they are characterized by a specific pattern of species distribution in flooding gradients. In a ridge-and-furrow grassland, *R. repens* predominantly occurs in furrows subjected to winter floods. *R. acris* occurs in an intermediate position, whereas *R. bulbosus* occupies only the well-drained sites on the highest parts of the ridges (Sarukhan and Harper 1973). A comparable elevational distribution of these three *Ranunculus* species can be observed in the dynamic floodplains along the River Rhine. In a typical Rhine floodplain *R. repens* occurs in zones characterized by an avarage annual flooding period of more than 45 days, calculated over the years 1976–1985. *R. acris* is found in a zone with approximately 30 days of flooding per year, whereas *R. bulbosus* habitats are characterized by only 19 days of flooding per year (authors, unpublished work).

We performed an experimental study with *R. repens*, *R. acris*, *R. sceleratus*, and *R. bulbosus*. Survival, biomass dynamics, petiole elongation capacity and aerenchyma development were investigated during different flooding regimes. Based on the results of this study we selected two species contrasting in flooding resistance, *R. repens* and *R. bulbosus*, to measure underwater photosynthesis.

Materials and methods

Plant species and experimental growth conditions

All the *Ranunculus* species under study are abundant in Dutch river floodplains. However, their elevational distribution and thus the flooding frequency and duration in their habitats differ considerably. Both *R. repens* and *R. sceleratus* are found on the lower parts

of the floodplains. The floodplain habitats of *R. repens* are characterized by unpredictable, frequent and long-lasting floods. The pioneer *R. sceleratus* is restricted to mudflats. *R. acris* is in floodplain areas that are at higher elevations and thus less frequently flooded than the habitats of *R. repens*. *R. bulbosus* predominantly occurs on rarely flooded, sandy river levées. Plant material and seeds used in the experiments were collected in the river floodplains of the Rhine. Plant material was individually transplanted into small plastic containers (volume 0.6 dm^3) filled with floodplain soil with a composition of 25% clay, 35% silt and 40% sand and placed under greenhouse conditions (temperature: 20°C; minimum photosynthetic photon flux density: 60 μmol m^{-2} s^{-1} for 16 h). After 1 month the plants were transplanted into larger containers (volume 2.0 dm^3). Four weeks later the experiments started. The containers were watered daily to keep the soil at a moisture content of approximately 60% (w/w).

Experiments

In order to quantify survival, biomass dynamics and petiole elongation capacity in response to flooding stress, ten replicates of each species were exposed to 12 different treatments. They varied in flooding depth (drained, waterlogging, submergence), light intensity (normal day/night regime and complete darkness) and duration (2, 4 and 6 weeks). The light intensity was only varied in the submergence treatment, to mimic flood water with low and high turbidities. The treatments were performed in large tanks (height: 0.9 m; diameter: 1.8 m). Each flooding/light treatment (drained, waterlogging, submergence light and submergence dark) was randomly distributed over three tanks. During the submergence treatment a water depth of 50 cm was applied, which was deep enough to avoid restoration of leaf-atmosphere contact due to submergence-stimulated shoot elongation. Plants were assumed to be dead when no recovery occurred under well-drained conditions within 2 weeks. Root and shoot biomass was measured after drying for 48 h at 105°C.

To quantify petiole elongation capacity the lengths of the youngest and the youngest mature petiole were measured after 2 weeks of submergence in the light and compared to the lengths of these petioles under control conditions.

In order to quantify constitutive and induced aerenchyma after 2 weeks of waterlogging and submergence in the light we measured the porosity of the primary root system and of newly developed laterals with a pycnometer method modified from Jensen et al. (1969). This method quantifies porosity as the percentage of root volume occupied by air. Plant material was not macerated, but internal gas was removed by several stages of evacuation under low pressure. Results were compared with the root porosity of plants grown under control conditions.

To measure net underwater photosynthesis, intact plants of *R. repens* and *R. bulbosus* were placed in a two-compartment cuvette separating root and shoot. The shoot was placed in the upper compartment (volume 900–1200 ml), and washed roots were placed in a root compartment of 220 ml. Before the start of a measurement both compartments were filled with water that contained only 2–3 mg l^{-1} oxygen. Net oxygen evolution of the shoot was measured with the aid of a Clark-type oxygen electrode (Walker and Leegood 1993) in the shoot chamber (Hootsmans and Vermaat 1991). A Masterflex type MR-07553 tubing pump was used for circulation inside the shoot compartment with a flow of 750 ml min^{-1}. The light source was an Osram Super NAV 600 W metal halide lamp that produced a maximum light intensity at plant level of 640 μmol m^{-2} s^{-1}. A water layer of 5 cm was used to absorb the heat of the light source. In this way the temperature inside the shoot compartment was kept at 20 $\pm$ 0.5°C. To quantify underwater photosynthesis two types of experiments were performed. The first experiment was designed to determine which carbon source was used by the plants under study. Therefore, the experiment was performed under non-limiting light conditions (640 μmol m^{-2} s^{-1}) and the free CO_2 concentration was varied by adding aliquots of HCl and NaOH to tap water that contained

a total of 2 mmol l^{-1} dissolved inorganic carbon (DIC). This is approximately the DIC concentration found in flood waters of the river Rhine.

The second experiment aimed to study the effect of submergence pretreatment (2 and 4 weeks) on the photosynthetic capacity of *R. repens* and *R. bulbosus*. It was performed at a DIC concentration of 2 mmol l^{-1} and a pH of 6.5, resulting in a non-limiting concentration of free CO_2 (865 μmol l^{-1}). A light-response curve was obtained by using metal gauges in front of the light source to create a series of light intensities (640, 400, 250, 140, 90, 60 and 0 μmol m^{-2} s^{-1}). All photosynthetic experiments were performed with three replicates.

Data analyses and statistics

Root porosity data was analysed for young and old root systems separately by an ANOVA with independent variables treatment (control, waterlogging, submergence) and species, to determine main effects for species. Differences between the treatments were assessed for each level of species and root system with the Tukey post-test after an ANOVA with treatment as independent variable.

For analysis of underwater photosynthesis, curve fitting was performed with a non-linear regression procedure for each individual plant using a Michaelis-Menten equation: $P = P_{max} \cdot I/(K_m + I) - R$, in which P is the dependent variable net photosynthesis, I is the independent variable light, P_{max} is the maximum gross productivity, K_m the light/free carbon dioxide level where gross productivity is half maximum and R is dark respiration (Clevering et al. 1995). Resulting photosynthetic parameter estimates and calculated light compensation points were averaged for three replicates. Differences between species and treatments were further analysed by ANOVA with parameters and compensation points as dependent variables.

Differences in petiole elongation capacity upon submergence were analysed for each species and age group separately with ANOVA and Bonferroni multiple comparison post-tests. All statistical analyses used the SAS statistical package (SAS Institute 1989).

Results

Survival and biomass

Complete submergence in darkness was the most severe flooding stress for all the *Ranunculus* species studied, whereas waterlogging was less stressful (Fig. 1A–D). The species showed considerable differentiation in survival rates under the various flooding treatments. Of all the species only *R. bulbosus* showed reduced survival after 4 and 6 weeks of waterlogging. The lack of resistance of *R. bulbosus* to waterlogging was also seen in the severe reduction of its root and shoot biomass compared to drained controls (Fig. 2). *R. sceleratus* was very resistant to waterlogging, since both its shoot and root dry weights increased more in waterlogged than in drained plants. In *R. acris* and *R. repens* only shoot dry weight increased more under waterlogged conditions; their roots grew equally well under waterlogged and drained conditions. We can rank the species as follows according to their waterlogging resistance: *R. sceleratus* > *R. repens* = *R.acris* > *R. bulbosus*.

R. repens and *R. acris* were the most resistant to the submergence-light treatment; nearly all plants survived at least 6 weeks of submergence (Fig. 1A,B). Despite
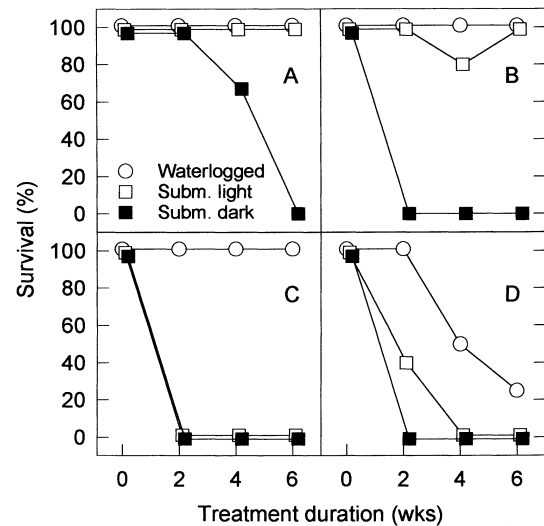

Fig. 1A–D Survival percentages of *Ranunculus* species under waterlogging, submergence in the light and submergence in the dark. Survival was determined after 2 weeks of recovery ($n = 10$). **A** *R. repens*, **B** *R. acris*, **C** *R. sceleratus*, **D** *R. bulbosus*

this high resistance both species showed a reduction in root and shoot dry weight when compared to drained control plants (Fig. 2). Based on the survival and biomass data *R. bulbosus* was slightly more resistant to the submergence-light treatment than *R. sceleratus*. Submergence with a day/night regime results in the following ranking: *R. repens* = *R. acris* > *R. bulbosus* > *R. sceleratus*.

R. repens was the only species that demonstrated some resistance towards complete submergence in the dark (Fig. 1A). All other species died within 2 weeks. The biomass data confirmed the differentiation between *R. repens* and the other species (Fig. 2). Ranking of the species resulted in: *R. repens* > *R. acris* = *R. sceleratus* = *R. bulbosus*.

Petiole elongation capacity

A slight, but statistically significant, stimulation of petiole elongation was observed in young and mature petioles of *R. repens* and in the young petioles of *R. acris*. A much bigger response was observed in *R. sceleratus* (Table 1). This species reached petiole lengths that were 2–3 times larger in submerged than in control plants. No significant submergence-induced petiole elongation was observed in *R. bulbosus*.

Aerenchyma

R. sceleratus was characterized by a constitutively high porosity, whereas *R. bulbosus* demonstrates a very low root porosity during all treatments and in both root types (Fig. 3). *R. repens* and *R. acris* were characterized by inducible aerenchyma in newly developed lateral
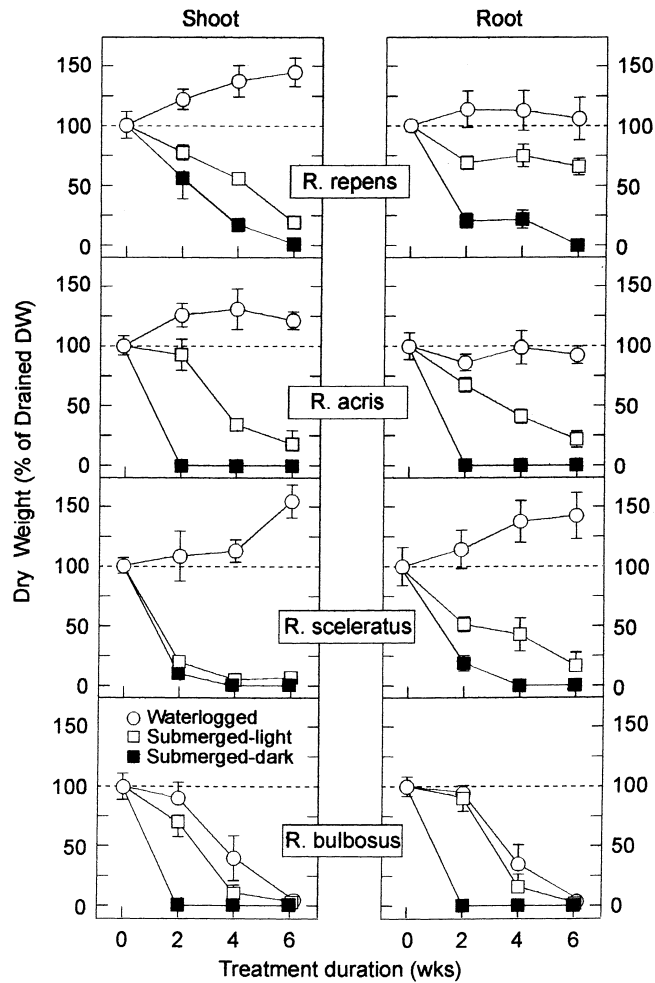
Fig. 2 Shoot and root percentage dry weights of *Ranunculus* species under waterlogging, submergence in the light and submergence in the dark. Dry weights for the treatments shown are relative to the dry weights of drained controls ($n = 10$; ±SE)

Fig. 3 Root porosity in newly formed laterals and primary root system of *Ranunculus* species under drained, waterlogged and light-submerged conditions. Porosity was determined as percentage porosity of total root volume ($n = 10$; ±SE). Experimental conditions lasted for 2 weeks. Different *letters* above each *bar* indicate significant differences between treatments within each species and root system subgroup

roots. Waterlogging also induced aerenchyma formation in the primary roots of *R. acris*. Plants of these two *Ranunculus* species that were waterlogged for 2 weeks had a significantly higher root porosity than control plants. Complete submergence also induced aerenchyma formation in the newly developed laterals of *R. acris*. Scanning electron microscopy photographs showed that all *Ranunculus* species under study had lysigenous aer-
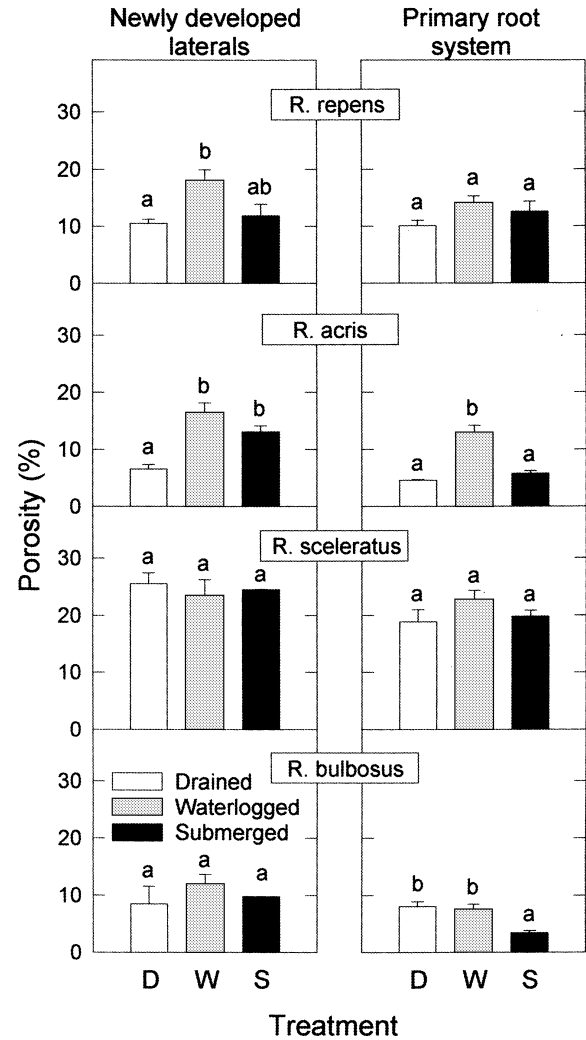
enchyma in the middle cortex region of the roots. In *R. repens* and *R. acris* this aerenchyma had a radial orientation, whereas a tendency to tangential lysigeny is found in *R. sceleratus* (Fig. 4).

Table 1 Petiole length (cm) of four *Ranunculus* species after 2 weeks of treatment ($n = 5$; ±SE). Differences between drained control and submergence treatment were analysed with Bonferroni multiple comparisons tests

Species	Mature petiole		Young petiole	
	Control	Submerged	Control	Submerged
R. repens	11.4 ± 0.4	15.3 ± 0.3*	12.9 ± 0.9	16.5 ± 0.6*
R. acris	14.0 ± 0.4	15.8 ± 0.7	15.3 ± 0.3	18.0 ± 0.3*
R. sceleratus	9.4 ± 0.4	25.8 ± 1.2*	7.9 ± 0.2	27.5 ± 1.7*
R. bulbosus	12.8 ± 0.7	14.9 ± 0.7	14.2 ± 0.4	17.0 ± 1.6

*Significant difference ($P < 0.05$) between control and submerged values within each species and petiole group
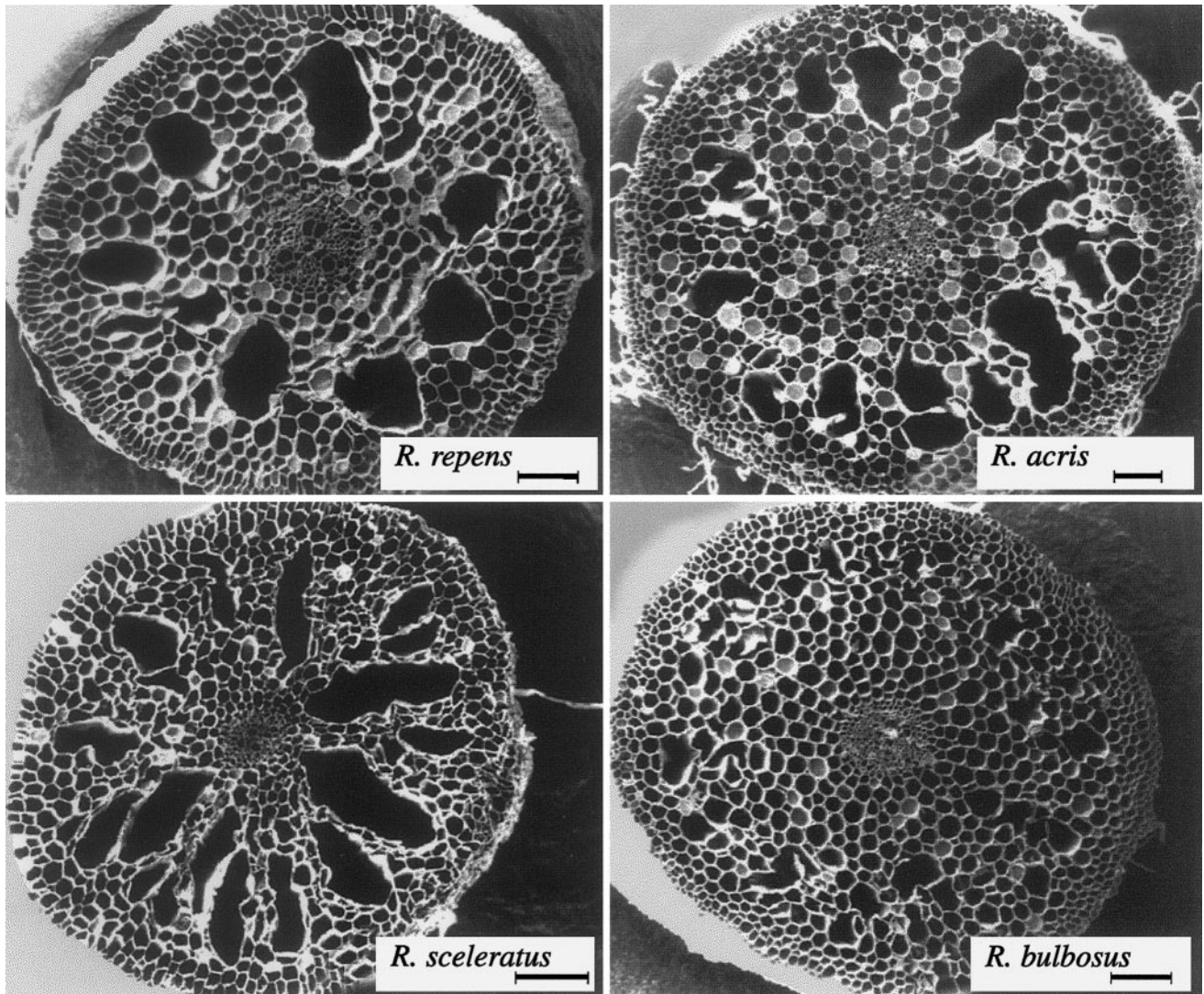
Fig. 4 Scanning electron microscopy images of newly developed lateral roots of *Ranunculus* species waterlogged for 2 weeks. The *horizontal bar* in each photograph illustrates a length of 100 μm

Underwater photosynthesis

An increase of the free CO_2 concentration stimulated net underwater photosynthesis in both *R. repens* and in *R. bulbosus*. *R. bulbosus* had a significantly lower K_m. No significant differences between the two species in CO_2 compensation point or P_{max} were observed (Fig. 5).

In *R. repens* submergence pre-treatments of 2 and 4 weeks did not significantly influence P_{max}, but the light compensation points of pre-treated plants were significantly lower than in drained controls (Fig. 6A). However, both submergence pre-treatments significantly enhanced light-saturated underwater photosynthesis in *R. bulbosus*, but had no significant effect on the light compensation points in this species (Fig. 6B).

Discussion

The survival and biomass data indicate that *R. acris* and especially *R. repens* are the *Ranunculus* species most resistant towards flooding. *R. bulbosus* is the most sensitive species. *R. sceleratus* can not be placed in this overall ranking of species since it turned out to be resistant to only one specific type of flooding: waterlogging. When *R. sceleratus* was submerged to a depth which did not allow restoration of leaf-atmosphere contact by enhanced petiole elongation, it died in our experiments within 2 weeks. For a species like *R. sceleratus* it is very important that under submerged conditions photosynthetic organs regain contact with the aerial environment. However, this elongation response is limited in the water depth to which it can accommodate. Our experiments demonstrated that *R. sceleratus,* with its rather opportunistic survival strategy based primarily on stimulated shoot elongation, is extremely sensitive to deep flooding compared to a dryland species like *R. bulbosus* with a more conservative survival strategy.
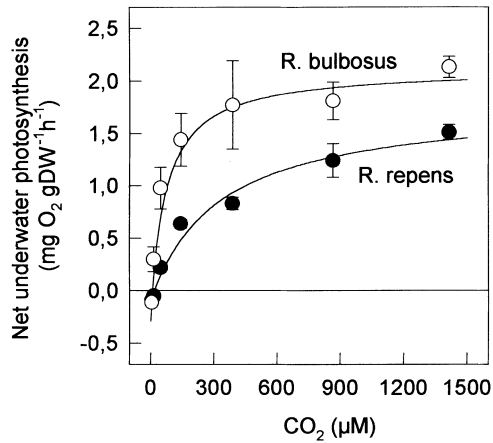
Fig. 5 Net underwater photosynthesis of *R. bulbosus* and *R. repens* at different free carbon dioxide concentrations. The dissolved in organic carbon (DIC) concentration was 2 mmol l^{-1}. Free carbon dioxide concentration was varied by altering the pH for each replicate ($n = 3$; $\pm SE$) of both species. Curves were fitted with non-linear regression using a Michaelis-Menten equation. Curve fit was significant for *R. bulbosus* and *R. repens*

This points to a trade-off between metabolic flood tolerance and stimulated shoot elongation, such as that previously demonstrated in a series of rice cultivars that differed in shoot elongation capacity when submerged. The highest survival of deep flooding was observed in the varieties with limited elongation capacity (Setter and Laureles 1996). The physiology of the stimulation of shoot elongation of *R. sceleratus* by submergence has been well studied, and is linked to entrapment of the gaseous plant hormone ethylene. Some leaf-borne auxin together with an enhanced endogenous level of ethylene is required to stimulate petiole elongation in this species (Samarakoon and Horton 1984; Rijnders et al. 1996). *R. repens* was by far the most tolerant *Ranunculus* species under submerged conditions in complete darkness.

R. bulbosus, *R. repens* and *R. acris* had a low root porosity under well drained conditions; waterlogged conditions only induced aerenchyma in the latter two species. Low constitutive root porosity, as observed in *R. bulbosus*, severely restricts the rooting depth of plants (Armstrong et al. 1994) and thus the ability to survive prolonged periods of flooding (Justin and Armstrong 1987; Visser et al. 1996). The low aerenchyma content of *R. bulbosus* roots explains its low resistance to waterlogging and the strong reduction of both root and shoot biomass during this treatment. The constitutively high level of aerenchyma in roots of *R. sceleratus,* and the inducible aerenchyma in roots of *R. repens* and *R. acris,* probably contribute to the high survival of these species under waterlogged conditions. All *Ranunculus* species under study have a type of aerenchyma that involves lysigenous collapse of cells. In maize, lysigenous aerenchyma formation is mediated by the phytohormone ethylene (Brailsford et al. 1993). Although very little is known about the involvement of ethylene in lysigenous aerenchyma formation in other plant species, it is very
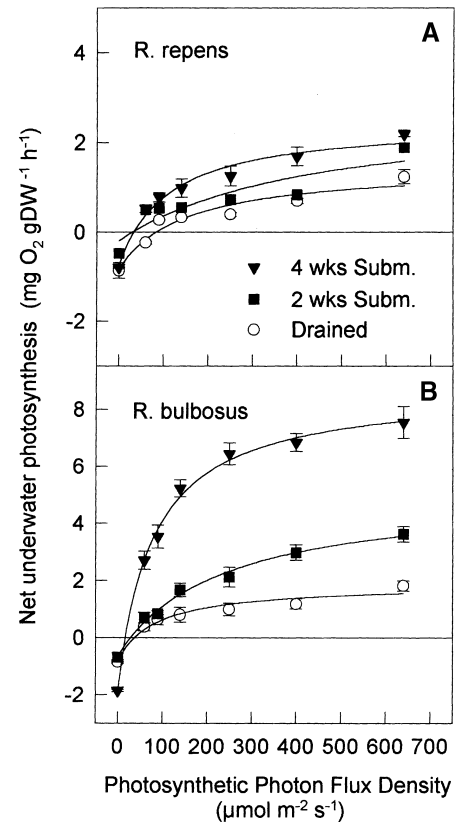

Fig. 6A-B Net underwater photosynthesis of *R. repens* and *R. bulbosus* at different photosynthetic photon flux densities after drained and two and four weeks submerged conditions ($n = 3$; $\pm SE$). Light intensity was reduced with metal gauges. DIC was 2 mmol l^{-1} at a pH of 6.5. All curves were fitted significantly by non-linear regression using a Michaelis-Menten equation

likely that ethylene action is a prerequisite for the signal-transduction chain leading to aerenchyma formation in both *R. repens* and *R. acris*. When they are waterlogged, ethylene will accumulate in roots of *Ranunculus* species due to the slow diffusion of this gas in water.

Complete submergence of terrestrial plants not only restricts aerobic metabolism, but also severely hampers photosynthesis. This is related to the reduction in incident light, the slow diffusion rate and the low availability of free CO_2, and the thick boundary layers around submerged plant surfaces (Maberly and Spence 1989; Sand-Jensen et al. 1992). Truly aquatic plants have several adaptations to ameliorate the carbon constraints of underwater photosynthesis. The mechanisms that concentrate CO_2 and thereby suppress photorespiration, such as polarization of leaves, HCO_3^- use, C_4 and CAM metabolism, are well characterized. Another way to cope with carbon stress is to exploit alternative inorganic carbon sources such as the sediment and the aerial CO_2 pool (Madsen and Sand-Jensen 1991). Terrestrial and amphibious plants are unable to use HCO_3^-; they are restricted to free CO_2 as carbon source (Sand-Jensen et al. 1992). Based on the relatively high CO_2 compensation points of *R. repens* and *R. bulbosus*, we conclude that both are strict CO_2 users. For *R. repens* this

conclusion is in accordance with the earlier work of Sand-Jensen et al. (1992) and Nielsen (1993).

R. bulbosus had a significantly higher maximum photosynthetic rate when it had previously been submerged, whereas a significant decrease in light compensation points was observed in *R. repens* when pretreated with submergence (Fig. 6A,B). From comparisons of previously submerged and emergent leaves in other studies, it is known that the submerged leaves of various plant species have a higher photosynthetic rate in relation to CO_2 concentration (Sand-Jensen et al. 1992) and light intensity (Clevering et al. 1995). The high photosynthetic rate of previously submerged leaves can be linked to an increased affinity for CO_2 due to changes in leaf anatomy and morphology that reduce transport resistance of CO_2, such as thinner leaves, thinner cuticle and epidermal chloroplasts (Sand-Jensen et al. 1992). We hypothesize that young leaves of *R. bulbosus* and *R. repens*, developed during the submergence pretreatment, have a different anatomy and/or morphology that explains the higher maximum photosynthetic rate and the decrease in the light compensation point.

Because *R. repens* is more frequently exposed to flooding than *R. bulbosus* in river floodplains, we expected higher photosynthetic rates (P_{max}), lower K_m values and lower compensation points in *R. repens*. This was not found. However, we should take into account that the measured differences between the species may also have been introduced by our experimental design. In order to answer the question of whether a positive carbon budget was possible in submerged terrestrial plants we deliberately used whole plants (shoot + root) in our photosynthesis experiments. *R. repens* contains more air spaces in roots (see Fig. 3), and probably also in shoot parts, than *R. bulbosus*. These air spaces might have caused a significant underestimation of the net underwater photosynthesis of *R. repens* since aerenchyma can store considerable amounts of oxygen (see Sorell and Dromgoole 1986) and facilitates oxygen diffusion to respiring tissue. Moreover, the high root to shoot ratio observed in *R. repens* (data not shown) probably further reduced the net photosynthesis of *R. repens* when compared to *R. bulbosus*.

Based on the aerenchyma data and the photosynthetic rates of *R. bulbosus* and *R. repens* it can be concluded that the higher flooding resistance of *R. repens* during submergence in the light is not related to a higher photosynthetic capacity. It is more likely that the very limited capacity of *R. bulbosus* to transport photosynthetic oxygen to oxygen-requiring tissues via longitudinally interconnected aerenchyma channels contributed to the low submergence resistance of this species. However, this conclusion is based on experiments performed under conditions optimized for underwater photosynthesis for CO_2 users (low pH, high light intensity). This raises the question: how important is underwater photosynthesis for the survival of *Ranunculus* species under field conditions? Flood water of the river Rhine has a pH of 7.5–8.0 and a DIC con-

centration of approximately 2 mmol l^{-1}. This results in a free CO_2 concentration of 142–47 μM. These low levels of free CO_2 can only result in a positive carbon budget at the whole-plant level when the underwater light intensity is high. We therefore conclude that underwater photosynthesis in water with low levels of free CO_2 is only of importance for strict CO_2 users when high light intensities reach the shoot. This can only be realized in very clear, non-turbid flood water.

The survival tactics of avoidance, amelioration and tolerance (Fitter and Hay 1981) can be interpreted as extremes in a continuum of stress resistance strategies. *R. sceleratus* colonizes mudflats in floodplains and needs to complete its life cycle between stressful floods. Unfavourable periods are survived as dormant seed. It is well equipped (aerenchyma and shoot elongation response) to grow vigorously under waterlogged conditions and to survive shallow floods. Its survival tactic according to Fitter and Hay (1981) is therefore intermediate between avoidance and amelioration. *R. acris* can resist both waterlogging and submergence in the light. This is achieved by mechanisms that ameliorate flooding stress such as induced aerenchyma channels and probably the ability to use photosynthetic oxygen to alleviate the oxygen shortage. In conclusion, this species uses an amelioration tactic to survive in grasslands subjected to high water levels. These amelioration tactics are also found in *R. repens*. In addition, this species probably possesses or develops a certain degree of metabolic flood tolerance. This allows survival for some time under the most severe flooding circumstances (submergence in the dark). Its tactic is intermediate between amelioration and tolerance. *R. bulbosus* is not included in this classification since it showed a high degree of intolerance towards waterlogging and submergence.

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