Physiological Ecology of Riverside Species: Adaptive Responses of Plants to Submergence


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In river floodplains, variation in flooding conditions results in successional stages in colonization ranging from annual pioneers to long-lived perennials. Reactions to submergence of species from the mid-successional zone are compared with adaptive responses of species from other zones. Presence and abundance are related to elevation and can be explained by characteristics of biomass production, and recovery in response to various submergence intensities.

Rumex species, from early to late successional stages, serve as models to elucidate, in more detail, mechanisms of adaptation. Flooding-resistant species develop large numbers of adventitious roots upon submergence and exposure to low oxygen conditions. Due to internal oxygen transport through aerenchyma, soil around these roots is re-oxidized, which stimulates bacterial nitrification. Ethylene and auxin promote adventitious rooting. Increased petiole elongation is also an adaptive feature of submergence-resistant Rumex species. Differences between species in submergence-induced growth are not only controlled by variation in endogenous levels of ethylene but also by different sensitivities to this hormone. Auxin does not affect Rumex petiole elongation, but a clear positive effect of gibberellin is demonstrated. Apparently, submergence induces a higher sensitivity to gibberellin and ethylene in the petioles of flooding-resistant Rumex. Many of the submergence reactions can also be induced by restricting the oxygen supply, suggesting that low-oxygen might be a triggering factor. The Rumex species we study represent various distinct communities. Thus, the ecophysiological phenomena observed in these model plants may explain processes and patterns in other species too and thus are interpretable at the riverside community level.

Key words: Ecophysiology, submergence, flooding, hormones, adaptation, nitrification, depth accommodation, adventitious rooting, Rumex.

INTRODUCTION

Anthropogenically induced floods occur worldwide. Cutting of woodland, drainage of agricultural areas, canalization of rivers and urbanization are among the main causes of flooding of river areas. The irregular excess of water not only threatens the food supply of many human populations but also affects the natural vegetation in river plains. In western Europe, the Rhine is one of the many rivers exhibiting strongly fluctuating water levels that are partly the consequences of man-made dikes constructed to restrain the extent of flooding. The combination of different flooding regimes varying in frequency, intensity, duration and elevation, characterize flood plains as highly dynamic areas full of clearly distinguishable hydrological patterns that in turn give rise to high biodiversity (Van der Valk, 1981; Van der Valk and Welling, 1988; Blom et al., 1990; Jongman, 1992; Boutin and Keddy, 1993).

Recording the frequencies and abundances of individual plant species provides insights into the process of vegetation succession that often is interrupted, especially close to the river bed. These records reveal a typical zonation within river plains ranging from early successional stages at the open sites situated along the edges of the river, via the mid successional stages of the less frequently flooded sites to the late successional habitats of the scarcely inundated parts of the area. Not only species composition but also life-history characteristics and physiology of the co-occurring species will change during flood-dependent succession (Blom, 1990; Voesenek, 1990; Van der Sman, 1992; Van Groenendael et al., 1993).

Our work aims to unravel the reactions of individual species to submergence, with the ultimate goal of gaining insight into the processes at the level of communities inhabiting the various zones in river plains. We use naturally occurring plants but results may also have relevance to responses of farm crops to flooding.

In this paper, two approaches are followed: First, in the 'more species—one habitat' approach, a number of species were chosen to screen the effects of various flooding regimes on survival and growth characteristics. These species were selected from higher and lower elevated sites in the mid-successional zone. In the literature, species from such mid-successional zones have tended to be overlooked; authors assuming that their life-history traits are less variable than in pioneer or late successional species (cf. Brown, 1992). The second or 'one genus—more habitats' approach aims to study a group of closely related species in more detail, especially with respect to their physiological characteristics. These model species were selected not only from the mid, but also from the early and late successional zones. All species under study, and their locations in the various zones in the river area, are presented in Fig. 1. Species from the late and mid successional zone are located at high to
intermediate elevated habitats; plants from the early successional zones colonize the borders along the river.

Up to the present, the following series of adaptative responses to submergence are known and have been researched: 

(a) Variation in life-history strategies at the levels of avoidance and tolerance of oxygen deprivation (Menges and Waller, 1983; Van der Sman, Joosten and Blom, 1993a; Van der Sman, Blom and Barendse, 1993b).

(b) Changes in physiological and metabolic processes resulting in morphological adaptations leading to the avoidance of asphyxiation by submergence or soil flooding. These include the formation of new aerenchymatous roots during and after de-submergence or waterlogging of the soil (Justin and Armstrong, 1987; Laan et al., 1993a; Laan, Clement and Blom, 1993b) and the accelerated growth, underwater, of stems and petioles (Voesenek and Blom, 1989a; Perik et al., 1989; Voesenek et al., 1990, 1992, 1993b).

This paper examines mechanisms of these adaptative reactions in order to understand more about the contribution of these responses to distribution, maintenance and survival of plants during the adverse conditions of the submergence period in the field.

Although the role of hormones in submerged or flooded plants has been extensively studied (e.g. Jackson, 1982, 1985; Jackson et al., 1985, 1987; Voesenek et al., 1990, 1993a, b; Brailsford et al., 1993), attention will be paid to the many questions that are still open in ecological respects.

SURVIVAL, GROWTH AND RECOVERY OF PLANTS UNDER VARIOUS FLOODING REGIMES

Effects of submergence treatments on survival and growth were studied in Daucus carota, Arrhenatherum elatius, Plantago media, Rumex acetosa, and P. lanceolata. Each of these species occurs on the higher elevated, seldom flooded sites of the mid-successional zones. Their reactions were compared with those of Festuca arundinacea, Inula britannica and Rumex crispus which grow in more frequently submerged parts of these zones in the river plain (Fig. 1). Eight-week-old plants were submerged in containers (width 1.85 m and depth 0.85 m) under greenhouse conditions. Duration of submergence was 1, 2 or 3 weeks for the species thought to be sensitive to flooding, and 3, 6 or 9 weeks for the more tolerant ones of the group. Submergence treatments were given in light to mimic clear-water conditions without large quantities of sediment. Submergence in the dark simulated flooding by silt-rich water. Survival and biomass production have been determined 1 week after the end of the submergence treatments. Recovery capacity was determined four weeks after 3 weeks of submergence.

Table 1 shows that presence and abundance of the species at the various elevations in the wild are in accordance with survival characteristics in greenhouse experiments. In the light, species from the higher sites survived submergence poorly, whereas the plants from lower habitats were better able to overcome the conditions. Most species were killed by submergence in the dark. In these severe conditions, only Rumex crispus survived, although for no longer than 6 weeks. Dry weight of the species from the higher elevations was strongly decreased, often to zero by submergence for 3 weeks or more (Tables 2 and 3). Species typical of the lower elevations retained green shoots even after 9 weeks of submergence. By the end of 9 weeks of submergence both Plantago lanceolata and Inula britannica retained a small amount of shoot and root biomass. However in contrast to most other species, Inula showed no significant decrease in root weight during 9 weeks submergence. An increase in shoot biomass during the first 3 weeks of submergence in clear water was found in Rumex crispus. This was the only species exhibiting shoot elongation when under water.
Table 1. Occurrence of species in the mid-successional zone of the river plain at Nijmegen (The Netherlands) and survival characteristics upon various periods of submergence in light or darkness

<table>
<thead>
<tr>
<th>Species</th>
<th>Presence in % of samples (p) and mean cover (c in %) at different elevation levels</th>
<th>Survival % in light (l) and dark (d) at submergence in weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>high p c</td>
<td>medium p c</td>
</tr>
<tr>
<td>Daucus carota</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>Arrhenatherum elatius</td>
<td>100 16.5</td>
<td>100 16.5</td>
</tr>
<tr>
<td>Plantago media</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>Rumex acetosa</td>
<td>89 9.0</td>
<td>100 0</td>
</tr>
<tr>
<td>Plantago lanceolata</td>
<td>89 3.0</td>
<td>100 0</td>
</tr>
<tr>
<td>Festuca arundinacea</td>
<td>— —</td>
<td>100 0</td>
</tr>
<tr>
<td>Inula britannica</td>
<td>— —</td>
<td>100 0</td>
</tr>
<tr>
<td>Rumex crispus</td>
<td>11 0.5</td>
<td>100 0</td>
</tr>
</tbody>
</table>

*—, Not present.

Table 2. Root biomass (mg d. wt per plant) of several species selected from higher elevations (seldom flooded) and lower elevations (often flooded) in relation to submergence under greenhouse conditions in the light

<table>
<thead>
<tr>
<th>Species from higher elevations</th>
<th>n</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daucus carota</td>
<td>5</td>
<td>397a</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arrhenatherum elatius</td>
<td>4</td>
<td>247a</td>
<td>23b</td>
<td>0</td>
<td>0</td>
<td>736c</td>
</tr>
<tr>
<td>Plantago media</td>
<td>5</td>
<td>221a</td>
<td>117b</td>
<td>0</td>
<td>0</td>
<td>870b</td>
</tr>
<tr>
<td>Rumex acetosa</td>
<td>5</td>
<td>790a</td>
<td>324b</td>
<td>113bc</td>
<td>0</td>
<td>1058d</td>
</tr>
<tr>
<td>Plantago lanceolata</td>
<td>5</td>
<td>246a</td>
<td>124b</td>
<td>50bc</td>
<td>18c</td>
<td>1126d</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species from lower elevations</th>
<th>n</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inula britannica</td>
<td>4</td>
<td>298a</td>
<td>626a</td>
<td>351a</td>
<td>286a</td>
<td>2998b</td>
</tr>
<tr>
<td>Rumex crispus</td>
<td>5</td>
<td>2382a</td>
<td>1917b</td>
<td>1863b</td>
<td>1623b</td>
<td>5948c</td>
</tr>
<tr>
<td>Rumex crispus (dark)*</td>
<td>5</td>
<td>2382a</td>
<td>1584b</td>
<td>868ab</td>
<td>0</td>
<td>3685e</td>
</tr>
</tbody>
</table>

For Festuca arundinacea no data on roots available.
The right-hand column (Recovery) shows biomass after 4 weeks recovery from 3 weeks of submergence.
Means in each horizontal line are different (P < 0.01) if they carry different superscript letters (Bonferroni Test).
Analysis was made after log transformations.
* All other species failed to survive dark submergence.

Table 3. Shoot biomass (mg d. wt per plant) of several species selected from higher elevations (seldom flooded) and lower elevations (often flooded) in relation to submergence under greenhouse conditions in the light

<table>
<thead>
<tr>
<th>Species from higher elevations</th>
<th>n</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daucus carota</td>
<td>5</td>
<td>745a</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arrhenatherum elatius</td>
<td>4</td>
<td>514a</td>
<td>28b</td>
<td>0</td>
<td>0</td>
<td>1142e</td>
</tr>
<tr>
<td>Plantago media</td>
<td>5</td>
<td>674a</td>
<td>825a</td>
<td>0</td>
<td>0</td>
<td>2155b</td>
</tr>
<tr>
<td>Rumex acetosa</td>
<td>5</td>
<td>1192a</td>
<td>258b</td>
<td>19b</td>
<td>0</td>
<td>1329d</td>
</tr>
<tr>
<td>Plantago lanceolata</td>
<td>5</td>
<td>812a</td>
<td>636ab</td>
<td>262b</td>
<td>18b</td>
<td>1816d</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species from lower elevations</th>
<th>n</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Festuca arundinacea</td>
<td>5</td>
<td>1057a</td>
<td>1120a</td>
<td>391b</td>
<td>148b</td>
<td>2296d</td>
</tr>
<tr>
<td>Inula britannica</td>
<td>4</td>
<td>469a</td>
<td>872a</td>
<td>568b</td>
<td>462b</td>
<td>2093b</td>
</tr>
<tr>
<td>Rumex crispus</td>
<td>5</td>
<td>710a</td>
<td>1350b</td>
<td>1295b</td>
<td>1209b</td>
<td>2107e</td>
</tr>
<tr>
<td>Rumex crispus (dark)*</td>
<td>5</td>
<td>710a</td>
<td>433b</td>
<td>6e</td>
<td>0</td>
<td>1176d</td>
</tr>
</tbody>
</table>

The right-hand column (Recovery) shows biomass after 4 weeks recovery from 3 weeks of submergence.
Means in each horizontal line are different (P < 0.01) if they carry different superscript letters (Bonferroni Test).
Analysis was made after log transformations.
* All other species failed to survive dark submergence.
Surprisingly, root biomass significantly decreased during the treatments. A limited amount of growth by this species appeared also during the first 3 weeks of submergence in darkness. Capacity to recover was considerable for all species tested except Daucus carota (Tables 2 and 3). By and large, after 4 weeks of recovery, biomass was twice or more than twice that at the start of the treatments.

Rumex crispus exhibited the most pronounced adaptive responses to flooding of the nine species tested. In contrast, Daucus, Arrhenatherum and Plantago media especially, were extremely susceptible to injury from submergence. Among the species from the elevated zone, Rumex acetosa was the only species that resisted flooding to any great extent.

These screening experiments provide a first indication of the adaptive behaviour of species upon submergence. To elucidate in more detail the mechanisms of the adaptations in roots and shoots, Rumex acetosa and Rumex crispus were compared with others in the same genus that are found commonly in other successional stages. They each serve as model species for plant communities being exposed to different submergence conditions.

**ADVENTITIOUS ROOT FORMATION, FUNCTIONALITY OF THE RENEWED ROOT SYSTEM IN RUMEX AND RESTORATION OF PHYSIOLOGICAL PROCESSES**

An adaptation of many plant species to flooding is the accelerated and increased formation of adventitious roots. These roots, which develop particularly well when plants are submerged, often contain more aerenchyma than the primary roots (Justin and Armstrong, 1987; Laan et al., 1989a; Smirnoff and Crawford, 1983). The genus Rumex shows a wide variation in the extent of adventitious root formation. Species from low elevations along river banks [and thus often flooded for long periods of time (see Fig. 1)] are usually well adapted to these floods. Experiments showed they develop large numbers of adventitious roots when waterlogged (Table 4). In contrast, adventitious rooting of species that grow on seldom flooded river habitats was poor.

The soil redox potential can decrease within a few hours of waterlogging to values (less than 330 mV; Fig. 2) that come close to indicating a complete absence of oxygen. Therefore, the rooting response of Rumex plants to waterlogging must be fast to avoid strong negative effects on plant viability. Within 2 d of waterlogging the soil, adventitious roots were found at the base of the shoots of R. palustris, and the number increased markedly with time (Fig. 3). These later roots developed mainly from the basal parts of the shoot and from the upper part of the tap root. Adventitious rooting by Rumex can also be induced by transferring hydroponically grown plants to a stagnant hypoxic agar solution (Fig. 4; Laan et al., 1989a). Adventitious root formation seems to be triggered by low oxygen concentrations in the root system.

The large gas-filled spaces in adventitious roots provide a route for strong oxygen diffusion from the emerged shoot to the root tips. This so called ‘internal aeration’ can in some cases maintain maximum aerobic respiration for limited periods in roots growing in anoxic flooded soils (Armstrong, 1979; Gaynard and Armstrong, 1987; Laan et al., 1990). When the shoot is still in contact with the atmosphere, oxygen can reach the root via the shoot through such aerenchymatous tissue. When the plant is completely submerged, oxygen may become available from underwater photosynthesis (Laan and Blom, 1990; Voesenek et al., 1993a). In most plants showing internal aeration, not all oxygen reaches the root tip. Some of the oxygen is consumed by respiration en route. Some diffuses out of the root radially, thereby re-oxidizing the first thin layer around the roots in the otherwise anaerobic soil (Smits et al., 1990; Laan et al., 1989b). The benefit of this phenomenon to the plant is the immobilization of resulting metal ions such as Fe$^{2+}$ and Mn$^{2+}$, which become toxic for the plant when in its chemically reduced and thus insoluble form (Bienfait, 1989; Laan et al., 1989b; Ernst, 1990; Laan, Smolders and Blom, 1991a). Furthermore, processes such as ion uptake by root cells (Grosse and Meyer, 1992) and active bacterial nitrification (Blacquiere, 1986; Laanbroek, 1990; Both, Gerards and Laanbroek, 1992) may take place. In contrast, chemically reduced soils possess no oxygen for respiration (Ponnamperuma, 1984); important oxygen-dependent microbial processes are inhibited and toxic compounds from anaerobic metabolism may accumulate.

Nitrification is one of the most important microbial processes with regard to plant nutrition. It generates available nitrate for the plant and is dependent on oxygen. In theory, a plant with aerenchymatous roots showing radial oxygen loss should be able to maintain nitrifying activity in the soil surrounding its roots. This was tested in waterlogged and drained soils by monitoring Rumex thyrsiflorus, a species unable to produce aerenchymatous roots and R. palustris, a species that readily produces aerenchymatous roots (Table 4) and showing radial oxygen loss from these roots (Laan et al., 1989a). Plants were grown for 63 d in river sand with a small amount of pasture soil serving as an inoculum for nitrifying bacteria. Nitrogen was provided as NH$_4^+$. In drained pots of both species, redox potentials exceeded 450 mV throughout the experiment, indicating the presence of free oxygen. In the waterlogged pots with R. thyrsiflorus, the redox potential dropped upon waterlogging and remained stable at 250 mV. At this level virtually no free oxygen would be present. In waterlogged pots with R. palustris, an initial decrease in redox potential was followed by a stabilizing at 350–390 mV, indicating entry of oxygen into the soil. The status of the nitrifying population was checked by measuring the potential nitrifying activity of the soil. This was achieved by incubating soil in a medium containing N$_2$O for several hours and analysing the amounts of NO$_2^-$ and NO$_3^-$ produced. In the waterlogged pots with R. thyrsiflorus the potential nitrification activity decreased to only 3% of that in well-drained pots after 6 weeks of soil waterlogging (Table 5). At the same time waterlogged pots of R. palustris still showed a considerable potential nitrification activity, maintained at about 30% of the activity present in the drained soil. Therefore, we conclude that in the soil with R. palustris two vital ingredients for nitrification, oxygen and an active...
nitrifying population, were still present. The third factor, NH$_4^+$, was constantly provided with the nutrient solution. By measuring the nitrate reductase activity in the leaves, we checked that NO$_3^-$ had indeed become available to the plants of *R. palustris* in the waterlogged pots. This parameter has been found to be a good indicator for NO$_3^-$ availability in this species; nitrate reductase activity in the leaves of plants treated for 6 weeks with NO$_3^-$ or a mixture of NH$_4^+$ and NO$_3^-$ being higher compared to plants provided with only NH$_4^+$ (Table 6). In support of the idea that *R. palustris* was supplied with NO$_3^-$ when waterlogged, nitrate reductase

**Table 4. Adventitious root formation by six *Rumex* species after 4 and 16 d of soil waterlogging (mean of 3 replicates ± s.e.). The species occur in the field along a flooding-frequency gradient from seldom flooded (*R. thyrsiflorus*) to regularly waterlogged or submerged (*R. palustris*)**

<table>
<thead>
<tr>
<th>Natural habitat</th>
<th>Species</th>
<th>Number of adventitious roots per plant</th>
<th>after 4 d</th>
<th>after 16 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td><em>R. thyrsiflorus</em></td>
<td>8.0 ± 2.3</td>
<td>37.3 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>Wet</td>
<td><em>R. palustris</em></td>
<td>49.8 ± 1.7</td>
<td>130.5 ± 9.8</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5. Nitrifying capacities (nmol NO$_3^- + NO_2^- g^{-1} dry soil h^{-1})$ of drained and waterlogged soils with either *Rumex* thyrsiflorus or *R. palustris* after being provided with NH$_4^+$ over 9 weeks**

<table>
<thead>
<tr>
<th>Soil</th>
<th><em>R. thyrsiflorus</em></th>
<th><em>R. palustris</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Drained</td>
<td>95.28*</td>
<td>183.69*</td>
</tr>
<tr>
<td>Waterlogged</td>
<td>3.48</td>
<td>55.56</td>
</tr>
</tbody>
</table>

Means of five replicates.

* A significant difference from waterlogged treatments (*P < 0.05, Mann-Whitney U-test).*
activity in leaves of waterlogged plants was no less than that from freely drained plants. But, the nitrate reductase activity of waterlogged pots with *R. thyrsiflorus* were very much decreased, compared to well-drained plants and even when compared to plants provided only with NH\(_4\)\(_2\)O.

**THE ROLE OF HORMONES AND LOW OXYGEN IN THE ADAPTATIVE RESPONSES OF *RUMEX* SHOOTS**

The gaseous growth substance ethylene promotes not only adventitious rooting and aerenchyma formation (Drew, Jackson and Gifford, 1979) but also shoot elongation (Musgrave, Jackson and Ling, 1972), which is another well known adaptive response upon submergence in many wetland species (Yamamoto and Kozlowski, 1987a, b; Yamamoto, Kozlowski and Wolter, 1987; Ridge, 1987; Voesenek and Blom, 1989a, b; Laan and Blom, 1990; Schweger and Brändle, 1991; Van der Sman et al., 1991; Crawford, 1992). Figure 5 illustrates the elongation responses of differently aged leaves in two *Rumex* species. Enhanced leaf elongation was observed in both leaves of *R. palustris*. In *R. acetosella*, however, no differences in leaf elongation were detected between submerged and non-submerged plants.

Possibly, other plant hormones also play a key role in the chain of reactions upon submergence and hypoxia, leading to a differential response towards ethylene within plants (Yamamoto and Kozlowski, 1987c; Jackson, 1990). Involvement of indole-3-acetic acid (IAA), gibberellin (GA) and abscisic acid (ABA) in depth accommodation has been described for a number of amphibious species i.e. *Callitriche platycarpa*, *Ranunculus sceleratus* and *Oryza sativa* (Musgrave et al., 1972; Musgrave and Walters, 1973; Samarakoon and Horton, 1983; Suge, 1985; Hoffmann-Benning and Kende, 1992). However, for species of the genus *Rumex*, little is known of the role of other plant hormones other than ethylene in the process of depth accommodation. The next part of this section will describe the role of ethylene, low oxygen, auxin and gibberellin in the submergence response of the shoots of *R. acetosella*, *R. acetosa* and *R. palustris* (see Fig. 1 for position of the species in the flood gradient).

**Ethylene**

We assessed the question of whether or not the marked difference in the ability of the flood-tolerant *R. palustris* and the intolerant *R. acetosella* to elongate rapidly when submerged is caused by a difference in ethylene production or by a variation in internal concentration. Under experimental conditions, the relationships between ethylene entrapment during submergence and the release after de-submergence were studied in relation to the internal gas volumes of the plants. Whole plants just starting the development of their fifth leaf were used (*R. palustris*, 24 d-old; *R. acetosella*, 27 d-old; for growth conditions see Voesenek and Blom, 1989b). Ethylene release in the air phase, was measured in a continuous flow system using a laser-driven intracavity photoacoustic detection system (Voesenek et al., 1990). Ethylene in the air above submerged *R. acetosella* was below the detection limit of the ultra sensitive photoacoustic laser-driven ethylene detector (approx. 0.05 nl l\(^{-1}\)), indicating a very low ethylene production level in this species (Table 7). In contrast, the quantities found for *R. palustris* were somewhat greater. The amount of entrapped ethylene (nl) was measured by the same detection system. Immediately after de-submergence, ethylene accumulating during 24 h of total submergence is quickly released from the plants, and this can be measured by the photoacoustic method. Just before de-submergence, glass cuvettes each containing a plant were closed, wrapped in aluminium foil and connected to the continuous flow system of pure nitrogen. This procedure removed all oxygen from the atmosphere of the cuvettes and prevented the generation of oxygen in the plants by photosynthesis. Under these conditions ethylene production after de-submergence is completely prevented (see also Voesenek et al., 1993b). The internal air volume (ml) of the same individuals was determined using a modified pycnometer method (Jensen et al., 1969). All gas was removed from the plants by submersing them in water in a dessicator to which a vacuum of 10 kPa was applied. Endogenous ethylene concentration after 24 h of submergence (nl ml\(^{-1}\)) was obtained by dividing the amount of entrapped ethylene (nl) released by the internal gas volume (ml) of the individual plant. Due to rounding errors, dividing the mean amount of entrapped ethylene by the mean internal air volume does not give exactly the same value as the endogenous ethylene concentration given in Table 7.

In drained *R. palustris* and *R. acetosa* (very comparable to *R. acetosella*) an endogenous ethylene concentration of 0.05 nl ml\(^{-1}\) was found using gas chromatography and a refined vacuum gas extraction technique (Voesenek et al., 1993b). Results presented in Table 7 also show the extremely small amount of entrapped ethylene after 24 h of submergence in *R. acetosella*. However, submergence did result in a 90-fold increase in the endogenous ethylene concentration due to the very small internal gas volume of this species. In contrast *R. palustris* produced relatively more ethylene in terms of total amount (volume). However, this led to an internal ethylene concentration which was only twice as high as in *R. acetosella*, because of the much larger internal gas volume of this species.

**Low oxygen**

Submergence not only leads to inhibition of gas exchange between the open air and the soil but also to oxygen depletion. An experiment was designed in which two oxygen levels were applied to investigate to what extent low oxygen levels influence the adaptation by the shoot of the submerged plant. To gain insight into the combined action of low oxygen availability and increased ethylene concentrations, plants growing under hypoxic conditions were treated with ethylene, its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) and an inhibitor of ethylene production aminoethoxyvinylglycine (AVG).
Table 6. Nitrate reductase activity in leaves of Rumex thrysiflorus and R. palustris plants provided with NH$_4^+$, NO$_3^-$ or both together during 5 weeks (n = 3), and when grown in drained and waterlogged soil (n = 5) for 6 weeks

<table>
<thead>
<tr>
<th></th>
<th>R. thrysiflorus</th>
<th>R. palustris</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3^-$</td>
<td>12.14$^a$</td>
<td>15.43$^a$</td>
</tr>
<tr>
<td>NO$_2^-/NH_4^+$</td>
<td>10.00$^a$</td>
<td>11.47$^a$</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>2.86$^b$</td>
<td>2.17$^b$</td>
</tr>
<tr>
<td>Drained</td>
<td>6.02$^a$</td>
<td>8.08$^b$</td>
</tr>
<tr>
<td>Waterlogged</td>
<td>2.66$^b$</td>
<td>8.91$^b$</td>
</tr>
</tbody>
</table>

Means in each row with different superscript letters are significantly different (P ≤ 0.05, Mann–Whitney U-test).

Fig. 5. Leaf (lamina + petiole) elongation of intact plants of Rumex palustris (24-d-old) and R. acetosella (27-d-old) after 7 d complete submergence. Control plants (□) were kept drained. Fifth leaf was just emerging at the time treatments began (for growth conditions see Voesenek and Blom, 1989c). Means marked (*) are significantly different from their control (Tukey test; P < 0.05). Vertical bars show standard error above each mean (n = 10).

Table 7. Ethylene release and endogenous ethylene concentrations of two Rumex species after 24 h of total submergence

<table>
<thead>
<tr>
<th></th>
<th>R. acetosella</th>
<th>R. palustris</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_2$H$_4$-release (nl g d. wt h$^{-1}$)</td>
<td>0.13±0.03</td>
<td>4.74±0.75</td>
</tr>
<tr>
<td>Entrapped C$_2$H$_4$ (ml)</td>
<td>0.03±0.05</td>
<td>0.24±0.034</td>
</tr>
<tr>
<td>Internal gas volume (ml)</td>
<td>0.24±0.15</td>
<td>9.21±1.13</td>
</tr>
<tr>
<td>C$_2$H$_4$-concentration (nl ml$^{-1}$)</td>
<td>4.47±1.15</td>
<td>9.21±1.13</td>
</tr>
</tbody>
</table>

Means of three replicates, with standard errors.

In R. palustris, petiole elongation was enhanced under a sub-ambient oxygen concentration of 3% in both the absence and presence of ethylene (Fig. 6). This low-oxygen effect was apparently acting via ethylene since the addition of the ethylene biosynthesis inhibitor (AVG) prevented the promotion of petiole extension. Specificity of AVG was tested by addition of the immediate precursor of ethylene (ACC) which overcame the AVG effect and returned elongation growth back to that in 3% oxygen in the absence of AVG (Fig. 7).

 Auxiliary and gibberellin

As shown in Table 7, the observed differential shoot responses of various Rumex species upon inundation cannot be explained solely by an increase in endogenous ethylene. Both the flooding intolerant R. acetosa or R. acetosella and the tolerant R. palustris have elevated internal ethylene concentrations. However, upon submergence or external application of ethylene, shoot elongation by R. palustris is promoted while that of R. acetosa and R. acetosella becomes restricted (Table 7; Voesenek and Blom, 1989a, b; Voesenek et al., 1990). Apparently, growth promoters other than ethylene, possibly auxin and gibberellins, are involved in the adaptation reaction. To test the involvement of auxin, plants of R. palustris were pretreated with the auxin-transport inhibitor N-1-naphthylphthalamic acid (NPA) before submergence. However, NPA had no effect on petiole elongation. Comparable results were found for submergence of plants in the synthetic auxin l-naphthaleneacetic acid (NAA), or 2-NAA (Fig. 8). Detailed analyses of variance on treatments within groups showed no effects of NPA or NAA except for 1 mm NAA which strongly inhibited growth. This was accompanied by an
anomalously rigorous twisting of the petioles thus considered non physiological. Submergence in indole acetic acid (IAA) gave comparable results as NAA (results not shown). Data obtained with isolated petioles, with or without aging prior to treatments gave similar results to intact plants.

Paclobutrazol pretreatment of submerged plants caused an inhibition of petiole elongation (Table 8). This inhibition was partially overcome by the application of 0.01 mM GA₃. Dose-response curves obtained after paclobutrazol and GA₃ treatment of both drained and submerged plants differed markedly (Fig. 9). Submergence enhanced the response to exogenously applied GA₃ by steepening the slope of the response to concentrations between 0.01–0.0001 mM, and raised the maximal response.

**DISCUSSION AND CONCLUSIONS**

We believe that our attempts to bridge the gap between physiology and ecology will clarify relationships between adaptive responses of individual plants and species distribution in the real world. The use of model species that serve as indicators for larger groups of plants allows an extrapolation of results to the level of plant communities. Later screening of adaptation reactions in other than model species can then be expected to validate the results obtained

### Table 8. Effect of the gibberellin-biosynthesis inhibitor paclobutrazol, and of gibberellic acid (GA₃) on the extension of petioles of Rumex palustris

<table>
<thead>
<tr>
<th></th>
<th>Drained</th>
<th>Submerged</th>
<th>Submerged + GA₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>No paclobutrazol</td>
<td>18±1*</td>
<td>33±8c</td>
<td>33±5c</td>
</tr>
<tr>
<td>Paclobutrazol</td>
<td>—</td>
<td>17±2a</td>
<td>25±8b</td>
</tr>
</tbody>
</table>

Means of 10 replicates. Means with different superscript letters are significantly different from plants grown under drained conditions with no paclobutrazol and GA₃. (P < 0.01, Tukey’s test).
with the indicator plants. Plants from the early successional zones in the river area exhibit strategies characterized by fast turnover rates (Van der Sman, Van Tongeren and Blom, 1988; Blom et al., 1990; Blom, Voessenek and Van der Sman, 1993). During the relatively short periods of water deprivation between successive floods, plants of these species (e.g., Chenopodium rubrum) very quickly complete their life cycle and produce seeds that will survive the next flood (Van der Sman et al., 1991, 1993a, b). Plants from the late-successional zones (e.g. Rumex acetosa) grow in densely covered habitats, are highly competitive and perennial (Blom, 1990; Voessenek, 1990). Species from mid-successional zones may be exposed to both flooding and competition. These plants adopt a rather opportunistic strategy. Successful species here cope with the adverse conditions of submergence by retaining much root and shoot biomass that can re-grow vigorously when desubmerged (Tables 2 and 3). After only one period of submergence some species from the elevated habitats are still able to recover, but field observations have demonstrated that these species, in contrast to plants occurring in lower sites, totally collapse if submerged more than once during the growing season.

Resistant plants tolerate submergence by their abilities to accelerate shoot extension that brings the upper leaves back into contact with the aerial environment. These same plants also form new adventitious roots and an aerenchymatous channel system to provide oxygen to the roots and rhizosphere. Due to aerenchyma formation, species such as R. palustris, show radial oxygen loss and maintain an oxidized rhizosphere with an active nitrifying population of bacteria that provides the plant with nitrate that appears to be taken up as judged by the high nitrate reductase activity. There are some limitations to the preservation of nitrification in waterlogged soils. When NH\textsubscript{4} is in short supply the plant and other soil microorganisms have proven to be better competitors than plants for the NH\textsubscript{4}, thus suppressing the nitrifying population (Engelaar et al., 1991). Furthermore, when oxygen deprivation in the soil is combined with a small soil pore diameter (i.e., compacted soil), aerenchyma formation and radial oxygen loss are inhibited (Engelaar et al., 1993). Under these conditions no oxygen will be present in the soil and nitrification becomes inhibited.

It has been known for several years that ethylene is the most important hormone involved in acclimation and adaptation mechanisms towards submergence (Osborne, 1984). This reflects the intimate relationship between submergence and ethylene levels in plants. Yet, it is also clear that other growth substances must be involved. It is widely recognised that plant hormones work in combination. Applying auxin to the shoot of a R. palustris plant resulted in the same number of adventitious roots as hypoxia of the root system did (Fig. 4). Remarkably, the same response was evoked by supplying the primary root system with low concentrations of ethylene (Fig. 4). The oxygen status of the roots was in both cases sufficient for normal aerobic respiration. These promoting effects of auxin and ethylene are commonly found with respect to adventitious root formation in soft- and hard-wooded cuttings (e.g. Gurumurti, Gupta and Kumar, 1985; Robbins et al., 1985; Bollmark and Eliasson, 1990; Nordström and Eliasson, 1991; Selby, Kennedy and Harvey, 1992). In contrast to the rooting response, auxin seems not to be involved in petiole elongation of R. palustris. Application of NPA, or IAA and 2-NAA had little effect (Fig. 8). Thus, this species contrasts with Regnellidium diphyllum, Rumunculus sceleratus and Nymphoides peltata, in which auxin is required for ethylene action (Walters and Osborne, 1979; Horton and Samarakoon, 1982; Malone and Ridge, 1983). A combination of ethylene and auxin treatment of three aquatic plants, Regnellidium diphyllum, Hydrocharis morsus-ranae and Rumunculus sceleratus showed that ethylene and auxin effects are additive (Cookson and Osborne, 1978; Smulders and Horton, 1991) and both enhance elongation. Neither phenomenon was found in R. palustris. Apparently, not all ethylene mediated growth is auxin dependent. This emphasizes the need for comparative studies between R. palustris and, for example, Rumunculus sceleratus in order to achieve a more fundamental knowledge of the hormonal basis in the elongation reaction of amphibious species.

In contrast to auxin, a clear positive effect of gibberellin on submergence growth could be demonstrated in R. palustris (Table 8). The partial restoration of the paclobutrazol induced inhibition by GA\textsubscript{3} could indicate that paclobutrazol also acts on other, GA\textsubscript{3}-independent, growth. The remarkable differences between air-grown and submerged dose-response curves indicate that submergence leads to a higher responsiveness of the petioles for GA (Fig. 9). Increase of responsiveness of ethylene-treated shoots to gibberellin was mentioned by Kutschera and Kende (1988) for deepwater rice. Gibberellin might act via enhancement of tissue extensibility (c.f. increasing the cell wall yielding; Kutschera and Kende, 1988) by altering the direction of cellulose microfibrils (Sauter, Seagull and Kende, 1993).

To understand, in more detail, the role of ethylene, auxin and gibberellin in the adaptation reaction upon submergence, attention needs to be focused not only on the separate and combined actions of these hormones but also on processes at the molecular level. Differences in hormonal regulation might explain the different adventitious rooting capacity of the various Rumex species. Differences in the numbers of dormant root primordia between the species might well be important also. Extensive research on both anatomy and on regulatory phenomena of adventitious root formation is necessary to determine the value of each factor. Apparently, low oxygen is one of the triggering factors for the adaptation reactions of submerged plants (see also Tonutti and Ramina, 1991; Crawford, 1992). Not only rooting may be stimulated at low oxygen conditions (Fig. 4), but also shoot elongation (Fig. 6). Preliminary findings strongly indicate that sub-ambient oxygen concentrations not only directly stimulate growth but also positively affect both ethylene production and tissue sensitivity, which was also demonstrated in roots of Zea mays L. by Brailsford et al., 1993. Results presented in Table 7 clearly demonstrate that differences in submergence-induced growth between species are not only controlled by variation in endogenous ethylene levels, but also by different sensitivity towards this hormone.
The occurrence of phylogenetically related species on a gradient from lower to higher elevated sites in the river area offers an unique possibility to study the metabolic and physiological mechanisms underlying the ecological phenomena of plant distribution related to submergence and flooding. The integration of the two approaches taken in this study (the model or ‘one genus — more habitats’ approach and the screening or ‘more species — one habitat’ approach) are helping to elucidate adaptive processes at the level of individual organisms and also at the community level.

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LITERATURE CITED


