Growth responses of *Rumex* species in relation to submergence and ethylene

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Abstract. Submergence stimulates growth of the petioles of *Rumex palustris* and *Rumex crispus* under field, greenhouse and laboratory conditions. Growth of *Rumex acetosa* petioles was hardly influenced by submergence. These growth responses under flooded conditions can be partially mimicked by exposing non-submerged *Rumex* plants to ethylene-air mixtures. Submergence of intact plants in a solution of AgNO$_3$ inhibited the elongation of all petioles of *R. palustris* and the youngest petiole of *R. crispus* and stimulated growth of the youngest petiole of *R. acetosa*. The ethylene-air mixture experiments, the effect of AgNO$_3$ and observed increase of the endogenous ethylene concentration during submergence suggest that ethylene plays a regulatory role in the growth responses of these *Rumex* species under submerged conditions. The three *Rumex* species showed a gradient in elongation responses to submergence, which correlates with the field distribution of the three species in a flooding gradient.

Key-words: *Rumex*; Polygonaceae; docks; submergence; ethylene; elongation response.

Introduction

*Rumex* species are common in the river areas in the Netherlands. These areas are characterized by strongly changing water levels caused by irregular run off of melting snow and rain water. Summertime floodings greatly influence the distribution, population biology and physiological processes of plants (Blom, 1985). The zonation of *Rumex* species occurring in these areas is supposedly related to these floodings. *R. acetosa* is found on high, seldom flooded dykes and river levees, whereas *R. palustris* occurs in very low, frequently inundated banks of former riverbeds. *R. crispus* occupies an intermediate position in the flooding gradient (Voesenek & Blom, 1987).

*Rumex* species occurring in the interface regions between land and water must have developed adaptations to survive such a habitat. One of the mechanisms for amphibious plants to survive is 'depth accommodation', in which enhanced elongation of the submerged shoot restores contact of this organ with the water surface (Jackson, 1983, 1985a; Osborne, 1984; Ridge, 1987). This growth in response to submergence is attributed to accumulation and/or increased production of ethylene in the shoot tissue (Ridge, 1987). Evidence exists that other plant hormones (auxin, gibberellin) are also involved in shoot elongation in response to submergence (Musgrave, Jackson & Ling, 1972; Walters & Osborne, 1979; Raskin & Kende, 1984). In addition to other hormones, there is much evidence that decreased oxygen availability and enhanced carbon dioxide concentrations may also be involved (Osborne, 1984; Jackson, 1985b; Ridge, 1987).

In a project investigating the effects of irregular floodings on *Rumex* zonations, field, greenhouse and laboratory experiments were carried out to assess the causal relations between responses to submergence, endogenous ethylene levels during submergence and species specific petiole elongation in response to this plant hormone.

Material and methods

Plant material

Three *Rumex* species, growing in areas with different flooding regimes, were selected for this study. *Rumex acetosa* L. and *R. crispus* L. are perennial species, whereas *R. palustris* Sm. is an annual or biennial. In the river area, this last species has a biennial life history related to the late germination after subsidence of the flooding water late summer. In the dutch river areas, *R. acetosa* and *R. crispus* are species of grazed grasslands, whereas *R. palustris* is mainly found on mudflats.

During all experiments mixtures of achenes from several plants per species, each belonging to one population, were used as seed source. The achenes with perianth were collected in 1986 in the river area and stored at room temperature in the dark until used.

Field experiment

The three *Rumex* species were sown in May 1986 in plastic containers (height: 50 mm; diameter: 55 mm) in the greenhouse. The temperature in the greenhouse
 varied from 15 °C at night to 20–30 °C during daytime (photoperiod 16 h). During low external light intensities, the plants were supplementary illuminated by high pressure sodium lamps (400 W) with a photon flux density at plant level of 115 μmol m⁻² s⁻¹. After 6 weeks of growth and one week of hardening outside the greenhouse, the young Rumex plants (approximately four to seven leaves) were transplanted (July 1986) into six field plots. Three plots were located in a very low, frequently inundated part of the river area, while the remaining three plots were located in a higher part of this area. Each plot initially contained 36 plants per species, transplanted randomly in a grid pattern. The transplanted plants developed no flowering axis during the study period. The plots in the lower zone were flooded once during the experimental period, 7 weeks after transplantation (duration 4 d; maximal depth 0.60 m above the soil surface). Eight days before and 9 d after this flooding, the longest petiole of every plant was measured. Therefore, petioles measured at one plant during both visitations of the field are not necessarily the same. The plots in the higher zone were not flooded during the study period.

Greenhouse experiment

Plants were grown from seed in containers (height: 170 mm; diameter: 180 mm) filled with river sand. After seedling emergence, the number of plants per pot was randomly reduced to one. The pots were regularly watered to keep the soil moisture near field capacity. They were watered twice a week with 0.5-strength Hoagland nutrient solution. The greenhouse conditions were the same as described in the previous experiment. After 46 d of growth, the plant rosettes (seven to ten leaves) were exposed to different treatments. Eighteen containers per species were submerged in plastic basins with a water level of 15 cm above the sand surface, another 18 containers were submerged to a depth of 40 cm. Both flooding depths resulted in complete submergence of the plants. A water regime as mentioned before was maintained in the control plants (drained controls). The number of leaves reaching the water surface was counted during the first 17 d of treatment. Finally, the longest petiole was measured after three weeks of treatment.

Laboratory experiments

The seeds were sown on Petri dishes on moistened filter paper and incubated for 7 d in a germination cabinet with a temperature of 25 °C during the light period of 12 h (photon flux density 30 μmol m⁻² s⁻¹; light source: Philips TL 8W/33XE3 tubes) and 10 °C during the dark period. After one week, the seedlings were transplanted singly into small containers (height: 50 mm; diameter: 55 mm) filled with a mixture (1:1 v/v) of sand and potting compost (Prepacked flower soil Jongkind No. 5). All containers were placed in another growth chamber at 20 °C, a day/night regime of 16/8 h and a photon flux density of 350 μmol m⁻² s⁻¹. Plants were used after 19 d of growth under these conditions. At that time, the fourth leaf of R. acetosa and R. crispus was emerging; in R. palustris the fifth leaf was already visible. All submergence and gas mixture experiments lasted 4 d and were conducted with twelve replicates per treatment. No flowering axes developed during the experiments. Plants were submerged in glass containers with a water column of 30 cm. The air-ethylene gas mixture experiments were conducted in glass vacuum desiccators. The air-ethylene mixture was renewed every 24 h. Previous experiments indicated that the concentration decline in the desiccators was negligible during the first 24 h. Controls were grown in identical desiccators filled with medical air (ethylene concentration: 4 × 10⁻⁴ Pa). This gas was also renewed every 24 h. Experiments with the ethylene action inhibitor AgNO₃ (Beyer, 1976; Liebemann, 1979; Drew et al., 1981) were performed by submerging plants in glass containers filled with 10⁻³ mol m⁻³ AgNO₃ solution. A preliminary experiment showed that at this concentration no toxic effects could be detected on the Rumex plants. The submergence, air-ethylene gas mixtures and AgNO₃ experiments were performed in the before mentioned growth chamber.

The endogenous ethylene concentration was measured in detached petioles and laminae of the Rumex plants. All petioles and leaflets of one plant were cut in two parts and subsequently submerged in 0.1% (v/v) Tween 20. Hereafter, shoot parts of five to 10 plants were put in a collection flask in a saturated (NH₄)₂SO₄ solution. The exposure of plant parts to air between excision and extraction was limited to a maximum of ca. 5–8 s per plant part. The gases in the plant tissue were extracted by the vacuum method (2.30 min; 10 kPa) described by Beyer & Morgan (1970). After the vacuum extraction a gas sample (10⁻⁶ m⁻³), taken with a gas tight syringe from the collection flask, was injected directly into a Chrompack Packard gas chromatograph model 438 A with a packed Poropack Q column (length: 100 cm), filled at a density of 0.34 g cm⁻³, used at 60 °C. Differences between treatments were assessed using the least significant difference (LSD) after an analysis of variance.

Results

Responses of Rumex species to submergence under field conditions

Rumex crispus and R. palustris showed increased lengths of the longest petioles in response to submergence (Fig. 1). After subsidence of the flood water, the elongated petioles have a very restricted lifespan. Most of them are damaged by wind or
animal grazing within 3 weeks. The length of the longest petiole of *R. acetosa* decreased in both zones.

**Responses of Rumex species to submergence under greenhouse conditions**

Within a few hours after submergence, the prostrate nature of the rosettes of *R. crispus* and *R. palustris* changes into a more vertical orientation, resulting in a reduction of the distance between leaf tips and water surface. *Rumex acetosa* hardly changed its laminae and petiole orientation. However, some laminae of this species folded sharply during submerged conditions.

Both *R. crispus* and *R. palustris* petioles showed growth stimulation under submerged conditions. In *R. palustris*, this increase of growth is dependent on inundation depth, whereas *R. crispus* showed an increase of approximately 80% at both depths (Fig. 2).

The functional significance of both the observed growth increase of petioles (Jackson, 1985b) and probably also the vertical orientation of the rosette during submergence is restoring contact of leaf tips with the atmosphere. This can be quantified by the number of laminae reaching the water surface after flooding (Fig. 3A & B). All species are able to overcome 15-cm inundation, although differences between the species do exist. However, only one species, *R. palustris*, is able to 'accommodate' to both depths.

After the inundation period, when the water level was lowered, the laminae with elongated petioles showed, even in the greenhouse, a high mortality. Within a few days most of them desiccated and collapsed.

**Submergence, exogenous ethylene and AgNO₃ under laboratory conditions**

The effect of petiole age on the growth responses of *Rumex* species during submergence is presented in Fig. 4A. All petioles of *R. palustris* showed significantly increased growth during submergence. The greatest length increase, however, was achieved by the youngest petiole. In *R. crispus* only the youngest petiole significantly increased its growth.
Mean number of laminae per plant-reaching the water surface

**Figure 3.** Mean number of laminae per *Rumex* plant (± 1 SE; n = 18) reaching the water surface after submergence in the greenhouse with 15 (A) and 40 cm (B) of inundation depth.

during flooding. The oldest leaf of *R. acetosa* showed a very slightly increased petiole growth, whereas the others were not affected by submergence.

These growth characteristics of petioles during submergence could be partially mimicked by placing *Rumex* plants in gas-tight evacuation chambers with a gas mixture of 0.5 Pa ethylene in medical air (Fig. 4B). The growth of petioles of *R. acetosa* was not significantly affected by 0.5 Pa ethylene; whereas it caused less elongation than submergence in *R. palustris*. The exogenously applied ethylene also induced the vertical orientation of the rosettes of *R. crispus* and *R. palustris* and the folding responses

**Figure 4.** The length of *Rumex* petioles (± 1 SE; n = 12) of different ages after submergence (A), exposure to a ethylene-air gas mixture (0.5 Pa) (B) and submergence in a AgNO₃ solution (C) under laboratory conditions, expressed as percentages of air controls (1 = oldest petiole; 4/5 = youngest petiole; * significantly different from control level [LSD; P<0.05]; (*) significantly different from submergence [LSD; P<0.05]). Mean actual length (cm) control petioles:

- *Rumex acetosa*: 1 = 3.1; 2 = 5.0; 3 = 6.1; 4 = 7.6;
- *Rumex crispus*: 1 = 2.1; 2 = 2.2; 3 = 2.7; 4 = 2.5;
- *Rumex palustris*: 1 = 1.1; 2 = 1.2; 3 = 1.2; 4 = 1.5; 5 = 2.2.
of some laminae of *R. acetosa*. *Rumex* plants submerged in Milli-Q water (Millipore-Company) with a nontoxic concentration of AgNO₃ (10⁻³ mol m⁻³) showed dramatic changes in growth responses (Fig. 4C). The elongation of *R. palustris* petioles during submerged conditions was for all petioles significantly reduced in the AgNO₃ solution. In *R. crispus*, only the growth of the youngest petiole was significantly retarded. Finally, application of AgNO₃ to submerged *R. acetosa* plants significantly stimulated elongation of the youngest petiole.

The growth responses of the youngest petioles of the three *Rumex* species to different exogenous ethylene concentrations are illustrated in Fig. 5. In *R. crispus* and *R. palustris*, the response was saturated at 0.5 Pa ethylene. In *R. palustris*, however, the elongation of the youngest petiole under submerged conditions was significantly higher than the saturated growth in ethylene-air gas mixtures. *Rumex crispus* showed no significant difference between submergence and the higher exogenous ethylene concentrations. *Rumex acetosa* showed very different responses to exogenous ethylene. High concentrations significantly inhibited length increase of the youngest petiole.

**Endogenous ethylene**

Table 1 shows the ethylene concentrations in the shoot tissue (petioles and laminae). After 8 h of submergence, the endogenous ethylene concentration in all species exceeded the concentrations in control plants. The highest concentration measured in all species (after 12–16 h) was approximately 0.1 Pa. These experiments were repeated twice; similar results were obtained.

**Discussion**

In the field as well as under greenhouse and laboratory conditions, comparable differences between the three *Rumex* species were found in response to submergence. In *R. palustris*, all petioles, even the older ones, showed an elongation response during submergence of the shoot. However, growth of older petioles was limited and their elongation stopped before they reached the water surface. The youngest petioles and those developing during submergence were able to overcome relatively deep inundations. In *R. palustris*, submergence resulted also in a significant growth increase of the youngest laminae (Voesenek & Blom, in press). However, 80% of the difference in leaf (petiole + laminae) length

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**Figure 5.** The length of the youngest petiole (±1 SE; n = 12) of *Rumex acetosa* (A), *Rumex crispus* (B) and *Rumex palustris* (C) during exogenous application of different concentrations of ethylene (Pa) and submergence under laboratory conditions expressed as percentages of air controls (*significantly different from control level [LSD; P < 0.05]*). Mean actual length (cm) control petioles: *Rumex acetosa*: 7.6; *R. crispus*: 2.5; *R. palustris*: 2.2.
between controls and submerged plants is caused by enhanced petiole growth. In *R. crispus*, only the youngest petiole showed an elongation response. This growth is limited and only a low level flooding can be overcome. *Rumex acetosa* showed no elongation of the youngest petiole. Under greenhouse conditions *R. acetosa* petioles even showed growth inhibition in response to submergence. This inhibition could not be mimicked by the submergence experiments under laboratory conditions. Submergence has no significant influence on laminae growth of both *R. crispus* and *R. acetosa* (Voesenek & Blom, in press).

The concentration of endogenous ethylene increased in all *Rumex* species to approximately 0.1 Pa in response to submergence. However, exogenously applied ethylene at this concentration caused only ca. 50% of the submergence response in *R. crispus* and *R. palustris*. This difference may be related to the involvement of other gases in the elongation response (see Jackson, 1985b). Carbon dioxide concentrations are assumed to rise in submerged shoot tissue. Such an increase in the carbon dioxide concentration and its participation in the elongation response is not expected in plants grown in desiccators filled with air-ethylene mixtures. The dose-response curves to exogenous ethylene (Fig. 5) also indicate that other factors are involved in the elongation response. Submergence of *R. palustris* always results in a significantly greater elongation response than in air-ethylene mixtures. Furthermore, the inhibitor of ethylene action, AgNO₃, does not suppress the elongation completely.

Although the preparation of the plant material and the vacuum extraction technique were performed in such a way that ethylene loss from the plant material was minimalized, some loss to the atmosphere may have been possible. This could have caused an underestimation of the endogenous ethylene concentration; it may explain, at least to some extent, the differences in the level of response to endogenous and exogenous ethylene. Submergence under greenhouse conditions resulted in growth inhibition of petioles of *R. acetosa*. High ethylene concentrations in gas mixtures also significantly inhibited petiole growth. However, 4-d floodings in the laboratory had no significant influence on the growth of the youngest petiole. The present authors suggest that the lack of an inhibition response in the laboratory is related to the short duration of the submergence experiments. It is also possible that the ethylene production of the control plants was enhanced. This might explain the growth stimulation, above the control level, when an inhibitor of ethylene action was applied. In summary, it can be concluded that *R. crispus* and *R. palustris* elongate their youngest petioles in response to submergence and ethylene. Growth of the youngest petioles of *R. acetosa* is hardly influenced by submergence, although some data indicated that petiole growth is inhibited by submergence and high ethylene concentrations. Differences in growth responses between the three *Rumex* species may be attributed to differences in sensitivity to enhanced ethylene concentrations in the plant tissue and not to differences in endogenous ethylene levels (Table 1).

Floodling of amphibious plant species does not lead to a uniform elongation response (Ridge, 1987). A restricted number of species have a fast and strong elongation response (e.g. *Regnellidium diphyllum* [Musgrave & Walters, 1974]; *Ranunculus sceleratus* [Cookson & Osborne, 1978]). These species are characterized by elongation of all petioles. The greatest length increase, however, is attained by the youngest petiole. These species occur in habitats with long lasting and frequent floodings. The responses of *R. palustris* to submergence show a strong resemblance to those of this group of plants. *Rumex palustris* occurs in the lower parts of the river area, which are frequently flooded and sometimes for long

<table>
<thead>
<tr>
<th>Treatment and duration (h)</th>
<th><em>R. acetosa</em></th>
<th><em>R. crispus</em></th>
<th><em>R. palustris</em></th>
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<tr>
<td>Submerged</td>
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<tr>
<td>2</td>
<td>0.019 ± 0.001</td>
<td>—</td>
<td>0.030 ± 0.002</td>
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<tr>
<td>4</td>
<td>0.029 ± 0.004</td>
<td>—</td>
<td>0.038 ± 0.002</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>0.032 ± 0.001</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>0.056 ± 0.013</td>
<td>0.052 ± 0.003</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>0.048 ± 0.020</td>
<td>0.050 ± 0.006</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>0.122 ± 0.007</td>
<td>0.097 ± 0.019</td>
<td>0.071 ± 0.012</td>
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<tr>
<td>14</td>
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<tr>
<td>15</td>
<td>—</td>
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<td>0.070 ± 0.010</td>
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<tr>
<td>16</td>
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<td>—</td>
<td>0.088 ± 0.012</td>
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<tr>
<td>18</td>
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<tr>
<td>20</td>
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<td>Non-submerged</td>
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<td>8</td>
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<td>0.034 ± 0.005</td>
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<td>16</td>
<td>0.015 ± 0.002</td>
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periods (up to approximately 100 d in the growing season). A second group of species shows a relative small and delayed growth response (e.g. *Ranunculus repens* [Ridge, 1985]; *Caltha palustris* [Ridge, 1987]). Petioles of species belonging to this group soon lose the ability of elongation when they grow older. These species occur in marshes and habitats with shallow floodings. The responses of *R. crispus* to submergence suggest that this species belongs to this second group of species. Its distribution in the river area is restricted to zones with less frequent and relative short and shallow floodings.

The three *Rumex* species show a gradient of elongation responses during submergence, which correlates to their respective distribution in the flooding gradient of the river area. A species seldom flooded, showed no elongation of petioles in response to flooding, whereas species frequently inundated in their natural habitats can elongate their petioles in response to this environmental change.

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**References**