The role of ethylene in shoot elongation with respect to survival and seed output of flooded *Rumex maritimus* L. plants

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Abstract. *Rumex maritimus* L. occurs in frequently flooded sites of lowland flood plains. Upon submergence this species exhibited rapid elongation of shoots, but the response depended upon the developmental stage when flooding was initiated. In the rosette stage, petioles showed a fast and large response; during early stem elongation the response of petioles was less, but the extension of the lower internodes considerable; during flowering stem development, high internodes extended and the contribution to final stem length diminished. Ethylene production by an intact plant before, during and after submergence was measured with a laser-driven photoacoustic technique. Internal ethylene concentrations increased within 12 h of submergence. Ethylene accumulated in the submerged plant due to increased synthesis as well as a reduced diffusion from the plant to the water (instead of air). The elongation response could in part be mimicked by exogenous ethylene. Directly after submergence a further increase of ethylene synthesis was observed, which may be of vital importance in causing shoots to continue their rapid elongation even after the water surface is reached. The responses of shoots were related to fitness in experimental field plots. Survival of submerged *R. maritimus* depended on its ability to emerge above the water surface, while seed production was positively correlated with shoot height above the water.

Key-words: Ethylene, fitness, flooding, internode elongation, petiole elongation, *Rumex maritimus*, shoot elongation

Introduction

Downstream river systems in The Netherlands are frequently flooded, not only during winter high waters, but in the growing season as well (Van de Steeg, 1984; Brock, Van de Velde & Van de Steeg, 1987). Plant performance in these river areas depends upon mechanisms to survive and reproduce during fluctuating water levels, including periods of complete submergence (Blom et al., 1990). Several *Rumex* species are distributed along the river flood plain according to the elevation gradient. *Rumex maritimus* L. occurs in the most frequently flooded areas and encounters flooding in several stages of its life cycle.

Flooding imposes a severe stress on plants. Complete submergence of the shoot will result in reduced aeration of the plant tissues and an impaired photosynthesis (Ridge, 1987). Survival and sexual reproduction may depend on adaptations which restore contact between shoot and air. A commonly observed mechanism is the rapid elongation response of petioles or internodes, which is stimulated by ethylene accumulation following submergence. The occurrence of this so-called ‘depth accommodation’ and the role of ethylene in the process have been studied for amphibious plants since 1970 (reviewed by Osborne, 1984; Jackson, 1985; Ridge, 1987) and have been recently demonstrated for several *Rumex* species (Voesenek & Blom, 1989a,b; Voesenek et al., 1990a). Not many studies, however, have been undertaken to assess the impact of this response on fitness. Ridge (1987) discussed the survival value of several morphological and anatomical changes induced by flooding, including elongation responses. The importance of petiole elongation for the survival of *Rumex* species was demonstrated by Voesenek (1990) and Laan (1990).

In this study we investigated the impact of rapid shoot elongation on the fitness of flooded *R. maritimus* plants. An outdoor experiment was designed to address this question in plants at different stages of the life cycle. Survival and seed production were used to determine fitness. To
elucidate the mechanism behind the observed rapid elongation growth, the role of ethylene was studied in several laboratory experiments. To monitor production and accumulation of ethylene in submerged plants we used the recently developed laser-driven photoacoustic detection technique (Harren, 1988; Harren et al., 1990) that has been used previously for the measurement of ethylene production of waterlogged Rumex plants (Voesenek, 1990; Voesenek et al., 1990b).

Materials and methods

Plant material

*R. maritimus* is a tap-rooted rosette species with petiolate leaves, annual in a 16-h or longer photoperiod but otherwise biennial. The reproductive stage starts with the elongation of internodes (bolting); the first stretched internode appears between the sixth and seventh rosette leaf or even later, depending on external conditions. Under favourable conditions axillary shoots are initiated in all primary rosette and stem leaves. The shoots appearing from the rosette leaf axils can also elongate, producing secondary stems. These stems may be induced together with the primary stem or in the following growing season. Plants grown in small (10⁻³ m³) pots in the greenhouse do not initiate axillary shoots.

*R. maritimus* seeds were harvested in 1986 in the flood plains of the Rhine system. A mixture of seeds from five plants was preserved dry in the dark at room temperature until use. Germination was always achieved as follows: seeds without perianths were placed in petri dishes (diameter 90 mm) on two layers of filter paper (Schleicher and Schüll 595), moistened with demineralized water. The petri dishes were placed in a germination chamber under an alternating temperature and light regime (12 h light 25°C/12 h dark 10°C; photosynthetic photon flux density: 30 μmol m⁻² s⁻¹). As soon as radicles emerged, after approximately 4 days, seeds were planted in pots, filled with a 1:1 mixture by volume of a potting compost (Jongkind, no. 5) and river sand. Several seeds were planted per pot; eventually seedlings were thinned to one plant per pot.

Outdoor experiment

This experiment was conducted in 1988 in large basins (length: 8 m; width: 2.4 m; depth: 1 m). Plants were grown in PVC pots (depth: 50 cm; diameter: 16 cm). Four series of *R. maritimus* plants, 24 per series, were grown under drained conditions (i.e. the pots stood in the basins in approximately 5 cm of water). The first series was planted on 2 May, 1988; the following series 2 weeks later and so on, until the last series was planted on 13 June. After 11 weeks from the start of the experiment (18 July) a flooding period of 4 weeks was simulated by filling one of the basins, containing 12 plants from each series, with tap water. Submergence depth was 40 cm above the brim of the pots. After 4 weeks the water was pumped out of the basin. Filling as well as emptying the basin took approximately 8 h. Plant heights were measured before and after the flooding treatment and at the end of the growing season; generative development was recorded throughout the experiment. After the flooding period, the length of the internodes of primary stems and the first three secondary stems of individual plants were measured. At the end of the growing season, ripe seeds, including perianths, were harvested and seed production was determined per plant.

Mean daily temperatures were recorded throughout the experiment. These fluctuated around 16°C during the first 6 weeks of the experiment and thereafter gradually rose to 18°C at the end of the flooding period (15 August). Water temperatures ranged from 14/19°C (morning/evening) during the first week of flooding up to 20/26°C during the last week of flooding. Statistical analyses on data were performed with the general linear models (GLM) procedure of the SAS statistical package (SAS Institute Inc., 1985).

Ethylene experiments

All greenhouse and laboratory experiments were conducted in the period from 1988 to 1990 with plants in small pots (height: 6 cm; diameter: 5.5 cm). The experiments with exogenous ethylene were performed in a growth chamber with a photosynthetic photon flux density of 140 μmol m⁻² s⁻¹ (Philips TL8W/33XE3 tubes); the plants used for the measurements of internal ethylene concentrations and for the photoacoustic measurements of ethylene production were grown in the greenhouse with a photosynthetic photon flux density of at least 110 μmol m⁻² s⁻¹ (additional light source: high pressure sodium lamps, 400 W, Philips HPS/SonT). The photoperiod was 16 h in all cases. Temperature was kept constant at 20°C in the growth chamber. In the computer-controlled greenhouse, temperatures fluctuated between 20°C (daytime) and 16°C (night). Experiments were performed with *R.*
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A. J. M. van der Sman

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**maritimus** plants in three stages: the rosette stage with five leaves larger than 1 cm in length present; the bolting stage with eight leaves and three internodes larger than 1 cm; and the early flowering stage. These stages were reached after approximately 5, 7 and 11 weeks, respectively.

The effect of externally applied ethylene was studied in a desiccator (300 mm diameter) containing eight plants. The desiccator was daily flushed with an ethylene/air gas mixture (ethylene concentration $5 \times 10^{-6}$ m$^3$ m$^{-3}$) for 4 days. Daily flushing was proven sufficient to maintain a nearly constant ethylene concentration (Voesenek & Blom, 1989b). Results were compared with eight plants submerged in tap-water in a glass container and another eight plants in a desiccator flushed with medical air (ethylene concentration $4 \times 10^{-9}$ m$^3$ m$^{-3}$) to serve as the control. This experiment was carried out twice with essentially similar results; for each developmental stage the results of only one experiment are presented.

To determine endogenous ethylene levels we used the vacuum extraction method of Beyer & Morgan (1970) to extract gases from the plant material (see also Voesenek & Blom, 1989b). For each $10^{-6}$ m$^3$ gas sample we used excised leaf, petiole and internode parts of 3-4 cm length from three or four plants. Ethylene concentrations in these samples were measured with a Chrompack Packard gas chromatograph at 60°C oven temperature, using a packed Poropack Q column (100 cm) filled at a density of 0.34 g cm$^{-3}$. All measurements were repeated three or four times in the rosette and bolting stages after 12, 24 and 36 h of submergence and in the flowering stage after only 12 h of submergence. Results were compared with internal ethylene concentrations of drained plants.

Ethylene entrapment in and production of an intact plant before, during and after a 24-h period of submergence were measured with the recently developed laser-driven photoacoustic detection system (Harren, 1988) in combination with a flow-through system. This apparatus allows monitoring of ethylene in a continuous air flow along the plant tissue, thereby avoiding autocatalytical or autoinhibitory processes as well as possible interactions of ethylene with varying concentrations of O$_2$ and CO$_2$ (Woltering, Harren & Boerriger, 1988). This highly sensitive method has been refined (Hess, 1989; Harren et al., 1990) allowing measurement of ethylene concentrations in air as low as $6 \times 10^{-12}$ m$^3$ m$^{-3}$. Since the method for measurement of ethylene production by waterlogged *Rumex* plants is described in detail elsewhere (Voesenek, 1990; Voesenek et al., 1990b), we will only mention the underlying principles here.

The photoacoustic effect is based on the generation of acoustic waves due to the accumulation of heat in a sample. In our case the sample is a volume of ethylene/air gas and the ethylene molecules are excited by a powerful CO$_2$-laser beam. Excitation is followed by relaxation, resulting in an increase in temperature which in turn leads to a corresponding increase in pressure inside the resonator of the photoacoustic cell. These pressure changes can be detected by a microphone and the magnitude of the signal is proportional to the number of absorbing molecules. The photoacoustic equipment was used in connection with a flow-through system, consisting of three separate cuvettes ($3 \times 10^{-4}$ m$^3$), each containing an intact plant. Photosynthetic photon flux density at plant level was a constant 50 $\mu$mol m$^{-2}$ s$^{-1}$. A continuous air flow (flow rate: $3 \times 10^{-3}$ m$^3$ h$^{-1}$) from which all carbohydrates were removed was conducted through each cuvette. After passing the plant tissue, the air was conducted to a glass column filled with KOH grains to remove water and CO$_2$, and thereafter through the photoacoustic cell. Cuvettes were either connected to the photoacoustic cell, or the effluent was vented into the surrounding air.

Two different experiments were performed with *R. maritimus* plants in the stem elongation phase. In the first experiment three plants were kept drained in the cuvettes during an acclimatization period of at least 12 h. Thereafter one plant remained in drained condition as the control. The second plant was submerged for 24 h. During this time ethylene was measured in the air space above the water surface. After 24 h the water level was lowered to waterlogging conditions (1 cm above soil) to be able to measure the amount of accumulated ethylene. We prevented the infiltration of air from outside the system into the cuvettes in order not to disturb the measurement. The production level of this plant was compared with the production level of a third, waterlogged plant. The three cuvettes were measured in turn (five measurements per cuvette in approximately 30 min) until the end of the submergence period. After lowering the water level, only the cuvette containing the previously submerged plant was measured for a period of 12 h. Over the last 100 h, the three cuvettes were measured in turn. This experiment was repeated three times with essentially similar results. The results of only one experiment are presented. Separate measurements were taken to ensure that the production level of the waterlogged
Shoot elongation upon flooding

Fig. 1. Mean internode lengths of primary and secondary stems (sprouting from the axils of the first three rosette leaves) of Rumex maritimus plants at the end of the outdoor flooding experiment; black = drained; open = flooded for 4 weeks at the age of (a) 11 weeks, (b) 9 weeks and (c) 7 weeks (n = 12). Internodes are numbered in the order of appearance (the first stretched internode of the primary stem appeared between rosette leaves 7 and 8). *Significantly different (P < 0.05).

plant did not change during the 12 h when it was not measured in the former experiments and to assess the mean production level of drained plants, using an empty cuvette as a reference for zero ethylene production. In the second experiment the submergence treatment was repeated but this time in comparison with submergence in AVG (aminoethoxyvinylglycine; 0.1 mol m⁻³), an inhibitor of ACC (1-aminocyclopropane-1-carboxylic acid)-synthesis, to prevent ethylene production.

Results

Outdoor experiment

At the start of the flooding period the first three series of R. maritimus plants had reached the bolting stage: the first was already flowering (mean primary stem length: 46 cm); the second about to flower (mean primary stem length: 37 cm); and the third was in the early bolting stage (mean primary stem length: 10 cm). Only two plants from the first series were completely submerged, and these remained under water and died during the flooding treatment. The second and third series plants were completely submerged, but reached the water surface within 1 week. All plants that emerged from the water, although some with only a very small (<9 cm) top part of the primary stem, survived and were flowering by the end of the flooding period. The lag in development of flowers between drained and flooded plants ranged from 1 day (first series) up to 10 days (third series). The fourth series was still vegetative at the start of the flooding treatment. These plants possessed approximately eight primary leaves with a maximal leaf length of 10 cm. During submergence, leaves quickly elongated up to approximately 30 cm, but none of the plants was able to reach the water surface and all died within 2 weeks.

At the end of the growing season, drained plants from all series had attained equal stem length (Table 1). No additional primary stem extension due to flooding was measured in the already-flowering plants from the first series. The largest stems were observed in the plants from the third series that had been flooded in the early bolting stage. This was correlated with the increased length of the first five primary stem internodes in these plants, directly after flooding (Fig. 1). Further developed stems showed increasingly smaller extension of higher internodes upon flooding. In the first series the elongation response was only significant for the upper internodes of secondary stems, which in development always lagged behind primary stems by approximately 10 days.

All flowering plants produced at least some viable seeds (even the two plants from the first

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<thead>
<tr>
<th>Series</th>
<th>Drained</th>
<th>Flooded</th>
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<tr>
<td>I</td>
<td>52.2 ± 2.5a</td>
<td>52.3 ± 2.0a</td>
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<tr>
<td>II</td>
<td>51.8 ± 2.0a</td>
<td>59.5 ± 3.5b</td>
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<tr>
<td>III</td>
<td>53.4 ± 2.0a</td>
<td>73.3 ± 2.0c</td>
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<td>IV</td>
<td>51.5 ± 3.4a</td>
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Means with the same letter are not significantly different (P < 0.05).
Table 2. Mean seed production in g plant\(^{-1}\) (±1SE) per series and treatment (see Table 1 for description).

<table>
<thead>
<tr>
<th>Series</th>
<th>Drained</th>
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<tr>
<td>I</td>
<td>18.7 ± 0.6(^b)</td>
<td>8.1 ± 1.0(^a)</td>
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<td>II</td>
<td>19.4 ± 0.3(^b)</td>
<td>9.2 ± 1.7(^a)</td>
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<td>III</td>
<td>19.0 ± 0.8(^b)</td>
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<tr>
<td>IV</td>
<td>10.4 ± 0.7(^a)</td>
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Means with the same letter are not significantly different (\(P < 0.05\)).

A large increase of internal ethylene concentration was measured within 12 h of submergence in the rosette stage (Fig. 3a); thereafter the concentration decreased. In the bolting stage the largest increase was the same, but was reached only after 24 h (Fig. 3b); subsequently it declined as in the former stage. The internal ethylene concentration after 12 h of submergence in the flowering stage was low compared to the earlier stages (Fig. 3c). There was a slight daily fluctuation in the ethylene concentration of drained plants in all three stages, with the higher values being found at the end of the light period (Fig. 3).

The results of the measurements of ethylene entrapment and production with the continuously monitoring system are presented in Fig. 4. Upon submergence the ethylene evolving from a *R. maritimus* plant decreased at first, indicating entrapment by the water column, but increased after approximately 10 h. After 20 h more ethylene

Table 3. Mean seed production (seed) in g plant\(^{-1}\) (±1SE) per length class (primary stem length in cm) and number of plants in this class (n) within three series of *Rumex maritimus* plants (see Table 1 for description of the experiment).

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<th>Length class</th>
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<td>Drained</td>
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<td>&lt;50</td>
<td>18.6 ± 1.5(^a)</td>
<td>3.7 ± 0.2(^a)</td>
<td>18.9 ± 0.3(^a)</td>
<td>2.8 ± 1.1(^b)</td>
<td>17.6 ± 0.9(^a)</td>
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<td>50–54</td>
<td>18.3 ± 1.2(^a)</td>
<td>7.3 ± 0.4(^d)</td>
<td>19.4 ± 0.5(^a)</td>
<td>6.3</td>
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<td>&gt;54</td>
<td>19.2 ± 0.2(^a)</td>
<td>11.4 ± 1.0(^b)</td>
<td>20.0 ± 0.7(^a)</td>
<td>13.7 ± 0.7(^b)</td>
<td>18.9 ± 1.6(^a)</td>
<td>10.1 ± 0.8(^c)</td>
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Means with the same letter are not significantly different (\(P < 0.05\)).
Shoot elongation upon flooding

Fig. 2. Mean petiole and internode lengths (±1 SE; SEs < 1 mm are not indicated) of *Rumex maritimus* plants before and after 4 days of treatment in three stages: (a) rosette stage; (b) bolting stage; (c) flowering stage. Air: plants daily flushed with medical air; C₂H₄: plants daily flushed with 5 × 10⁻⁶ m⁻³ ethylene/air; subm.: plants submerged in tap-water (n = 8). Leaves and internodes are numbered in the order of appearance (the first stretched internode appeared between leaves 6 and 7).

Evolved from the submerged plant through the water column than from the waterlogged plant. At this point (35 h) 50% more ethylene (0.3 nmol g⁻¹ h⁻¹) was produced by the flooded plant than by the drained plant (0.2 nmol g⁻¹ h⁻¹). After lowering the water level from submergence to waterlogging conditions, two peaks in ethylene production were observed (Fig. 4, inlay). The first and smaller one appeared within 1 h. The top of the second peak, which lasted for about 15 h, was reached after approximately 3 h. Ethylene production remained higher for several days, compared with drained and waterlogged conditions. In spite of the continuous light regime, a diurnal rhythm in the ethylene production level was observed. Submergence in water containing AVG, an inhibitor of ethylene biosynthesis (data not shown), resulted in a considerably reduced ethylene release from the water, compared with submergence in water without AVG. Very little rise in ethylene occurred in 24 h and at the end of the submergence period, the ethylene evolving from the plant in AVG was 0.07 nmol g⁻¹ h⁻¹, thus even less than from drained plants. After lowering the water table, the first ethylene peak was only about three times that of the drained level. This increase is small in comparison with the 20-fold increase after submergence in water. The second peak was completely absent, and ethylene production fell to drained levels 2 h after the small first peak.

Discussion

A shift from extension of petioles to extension of internodes and a decrease of the rapid elongation response upon flooding in the course of the development of a flowering plant was observed in outdoor and laboratory experiments. Changing responsiveness of petioles or internodes at different stages of ontogeny is mentioned by Ridge (1987) but most studies have only considered the response of either petioles or internodes in a
Application of external ethylene, and by measuring internal ethylene concentrations. Externally applied ethylene did not wholly reproduce the extension response of submergence in our experiments (Fig. 2). Métraux & Kende (1984) found that only ethylene in combination with a gas mixture containing low oxygen and high carbon dioxide concentrations could entirely reproduce the submergence effect in the internodes of deepwater rice. An enhancement of the ethylene effect by a high CO₂ concentration, probably occurring in submerged tissue, was also found by Raskin & Kende (1984). Another factor that cannot be reproduced by exogenous ethylene is the buoyant tension of the water column, which may play an additional role in the submergence response (Jackson, 1985; Ridge, 1987).

Internal ethylene concentrations, measured in excised plant parts, increased within 12 h of submergence. Results in the rosette stage were quite similar to those obtained for other Rumex species (Voeselek & Blom, 1989a). Ethylene concentrations in the stem elongation stage are also in agreement with values obtained for other genera (e.g. Métraux & Kende, 1983). After 12 h of submergence in the flowering stage, the ethylene concentrations were lower than those of the previous stages. Differences in kinetics and/or amounts of accumulated ethylene could be important for the changing response of the elongating
Shoot elongation upon flooding

In the present study we also found a positive relationship between seed output and stem elongation in flooded *R. maritimus* plants. Flowering plants that remained under water for 4 weeks were dead afterwards and produced only a small amount of seeds (probably originating from flowers that were pollinated before the onset of flooding). Plants submerged prior to flowering were able to elongate under water. In general, it appeared that the plants which were the least developed after the rosette stage, could extend a larger part of the shoot above the water surface and produce more seeds.

The performance of *R. maritimus* in the river flood plains is dependent on flooding regimes (Van der Sman *et al.*, 1988), which are largely unpredictable (Blom *et al.*, 1990; Voesenek, 1990). Roberts & Boddrell (1985) studied the viability of seeds and time of emergence of seedlings for many grassland and ruderal species. They found for *R. maritimus* relatively short-lived seeds with main emergence in the first spring after sowing. Very little emergence occurred after the end of May. In several outdoor experiments in which we applied different flooding regimes (results to be published elsewhere), we found that *R. maritimus* flowered under drained conditions when germinated before the end of June, but plants germinated in April and May gave the largest seed output (e.g. series I–III in this experiment, Table 2). Flooding during the rosette stage either resulted in the death of the plants (as in this experiment) or in delayed flowering and sometimes even the postponement of flowering until the following growing season. Delayed flowering always resulted in a reduced seed output as in drained plants. In this study, a 10-day delay in flowering resulted in a slightly
reduced seed output for the third series flooded plants, compared with plants from the second series in the same length category, which flowered in a longer photoperiod (Table 3). Since flooding will also delay the germination of R. maritimus until after June in several years, it is clear that there are many occasions in which this species is forced by flooding to remain vegetative in the year of seedling emergence. In the outdoor experiments rosettes appeared to be much more able to survive winter submergence than summer submergence. Plants flooded in winter and early spring (the usual situation in the natural habitat) exhibited rapid stem elongation until a large part of the shoot emerged from the water surface. Eventually these plants produced more seeds in the second year of growth than non-winter flooded controls, a further indication of the importance of the rapid stem elongation response for the seed output of this species.

In conclusion, we suggest that both ethylene entrapment during submergence and the enhancement of ethylene production during and especially after submergence, are responsible for the rapid elongation of R. maritimus shoots under water, even after the water surface is reached. This process is not only important for survival but also determines the seed output of flowering plants under experimental field conditions. It may therefore be very important for the fitness of this species in its frequently flooded habitat.

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References
Shoot elongation upon flooding


