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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 (Voeselek & Blom, 1989a,b; Voeselek *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 Voeselek (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^{-4} 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: $30 \mu\text{mol m}^{-2} \text{s}^{-1}$). 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 $140 \mu\text{mol m}^{-2} \text{s}^{-1}$ (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 $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ (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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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 $5 \times 10^{-6} \text{ m}^3 \text{ m}^{-3}$) for 4 days. Daily flushing was proven sufficient to maintain a nearly constant ethylene concentration (Voeselek & 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 $4 \times 10^{-9} \text{ m}^3 \text{ m}^{-3}$) 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 Voeselek & 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 Poropak 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 & Boerrigter, 1988). This highly sensitive method has been refined (Hess, 1989; Harren *et al.*, 1990) allowing measurement of ethylene concentrations in air as low as $6 \times 10^{-12} \text{ m}^3 \text{ m}^{-3}$. Since the method for measurement of ethylene production by waterlogged *Rumex* plants is described in detail elsewhere

(Voeselek, 1990; Voeselek *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 ($3 \times 10^{-4} \text{ m}^3$), each containing an intact plant. Photosynthetic photon flux density at plant level was a constant $50 \mu\text{mol m}^{-2} \text{ s}^{-1}$. A continuous air flow (flow rate: $3 \times 10^{-3} \text{ m}^3 \text{ h}^{-1}$) 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

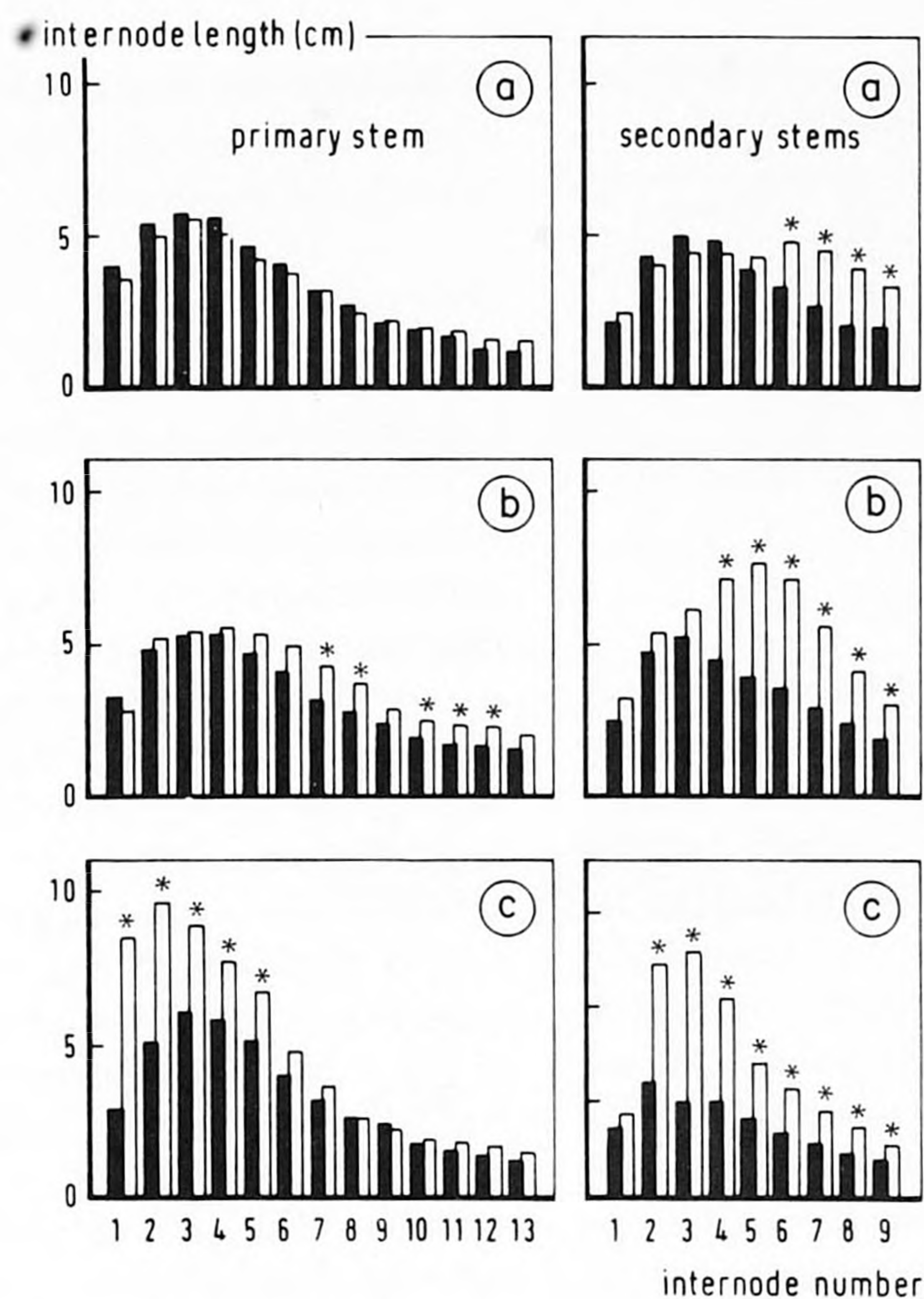


Fig. 1. Mean internode lengths of primary and secondary stems (sprouting from the axils of the first three rosette leaves) of *Rumex maritimus* plants at the end of the outdoor flooding experiment; ■ drained; □ flooded for 4 weeks at the age of (a) 11 weeks, (b) 9 weeks and (c) 7 weeks ($n = 12$). Internodes 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

Table 1. Mean stem length in cm (± 1 SE) of primary stems from drained and flooded *Rumex maritimus* plants at the end of the growing season in the outdoor experiment; the flooding treatment (water height: 40 cm above soil level) of 4 weeks' duration was started at the age of 11 (series I), 9 (II), 7 (III) and 5 (IV) weeks, with 12 plants per series and treatment. All flooded plants in series IV died during the flooding period.

	Drained	Flooded
Series I	52.2 \pm 2.5 ^a	52.3 \pm 2.0 ^a
Series II	51.8 \pm 2.0 ^a	59.5 \pm 3.5 ^b
Series III	53.4 \pm 2.0 ^a	73.3 \pm 2.0 ^c
Series IV	51.5 \pm 3.4 ^a	—

Means with the same letter are not significantly different ($P < 0.05$).

Table 2. Mean seed production in g plant⁻¹ (± 1 SE) per series and treatment (see Table 1 for description).

	Drained	Flooded
Series I	18.7 \pm 0.6 ^b	8.1 \pm 1.0 ^a
Series II	19.4 \pm 0.3 ^b	9.2 \pm 1.7 ^a
Series III	19.0 \pm 0.8 ^b	10.1 \pm 0.8 ^a
Series IV	10.4 \pm 0.7 ^a	—

Means with the same letter are not significantly different ($P < 0.05$).

series that were dead after the submergence period). There was no difference in seed output between the first three series of drained plants (Table 2). The fourth series produced only half the amount of the earlier series. Seed production of flooded plants was reduced, compared with drained plants. Differences between flooded series were not significant, but within each series a large variation existed which was associated with variation in stem length (Table 3). A very low seed production was recorded for plants that had exhibited very little or no extension growth and which appeared for less than 9 cm above the water surface (length class <50 cm). This class includes the two plants that remained submerged during the flooding period. An intermediate value was reached by plants in the category ranging from 10 to 14 cm above water level (50–54 cm). Most seeds were produced by plants that were larger than 54 cm. Drained plants from all three series were equally divided over these three length classes and showed no correlation between shoot height and seed production. Most of the flooded plants in the third length class, especially those from the third series, were much larger (up to 89 cm) than drained plants, which never exceeded 64 cm. The largest plants were always the ones with the least developed stems at the start of the flooding treatment (yet with the stem already initiated), which exhibited the fastest under-water elongation. The

plants from the third series produced slightly less seeds compared with those from the second series in the same length class.

Ethylene experiments

There appeared to be a remarkable difference between three stages of development with respect to the relative elongation response of petioles and internodes upon flooding. In the rosette stage (Fig. 2a) there was a very rapid extension of petioles (laminae are not presented since they showed only a very small [<1 cm] extension effect). In the bolting stage (Fig. 2b), the extension of petioles (from stem leaves) was much less, and in the flowering stage (Fig. 2c) was practically non-existent. Internode elongation was large in the bolting stage (Fig. 2b), but absent in the flowering stage (Fig. 2c). These results agree with the results of the outdoor experiment (Fig. 1). The process could be mimicked to a large degree by applying external ethylene (Fig. 2).

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 (± 1 SE) 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).

Length class	Series I		Series II		Series III	
	Drained Seed (n)	Flooded Seed (n)	Drained Seed (n)	Flooded Seed (n)	Drained Seed (n)	Flooded Seed (n)
<50	18.6 \pm 1.5 ^a (4)	3.7 \pm 0.2 ^e (3)	18.9 \pm 0.3 ^a (5)	2.8 \pm 1.1 ^e (4)	17.6 \pm 0.9 ^a (4)	
50–54	18.3 \pm 1.2 ^a (4)	7.3 \pm 0.4 ^d (4)	19.4 \pm 0.5 ^a (3)	6.3 (1)	18.6 \pm 2.1 ^a (3)	
>54	19.2 \pm 0.2 ^a (4)	11.4 \pm 1.0 ^{bc} (5)	20.0 \pm 0.7 ^a (4)	13.7 \pm 0.7 ^b (7)	18.9 \pm 1.6 ^a (5)	10.1 \pm 0.8 ^c (12)

Means with the same letter are not significantly different ($P < 0.05$).

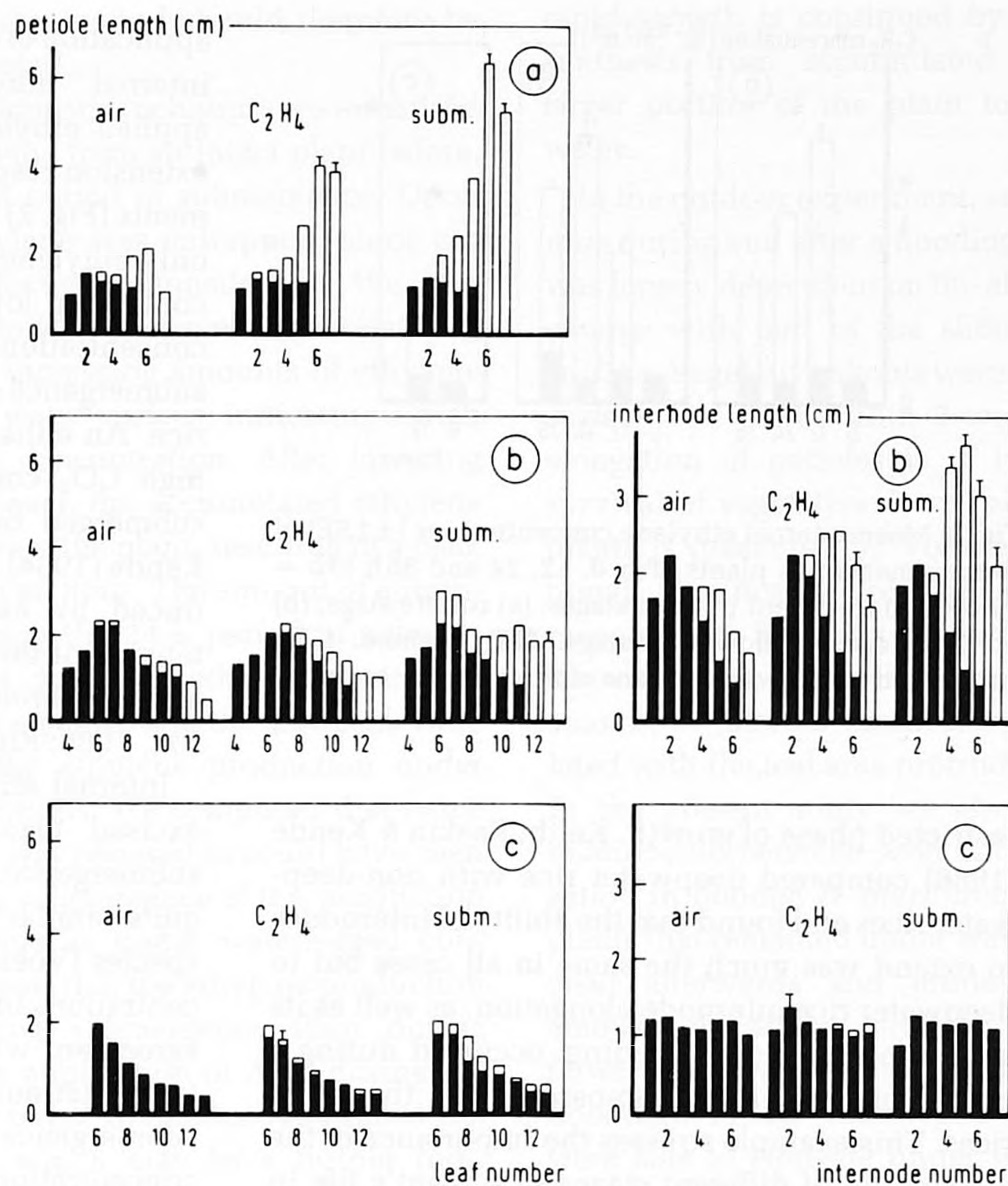


Fig. 2. Mean petiole and internode lengths (± 1 SE; SEs ≤ 1 mm are not indicated) of *Rumex maritimus* plants before \blacksquare and after \square 4 days of treatment in three stages: (a) rosette stage; (b) bolting stage; (c) flowering stage. Air: plants daily flushed with medical air; C_2H_4 : plants daily flushed with $5 \times 10^{-6} \text{ m}^3 \text{ m}^{-3}$ 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 ($0.3 \text{ nmol g}^{-1} \text{ h}^{-1}$) was produced by the flooded plant than by the drained plant ($0.2 \text{ nmol g}^{-1} \text{ h}^{-1}$). 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 $0.07 \text{ nmol g}^{-1} \text{ h}^{-1}$, 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

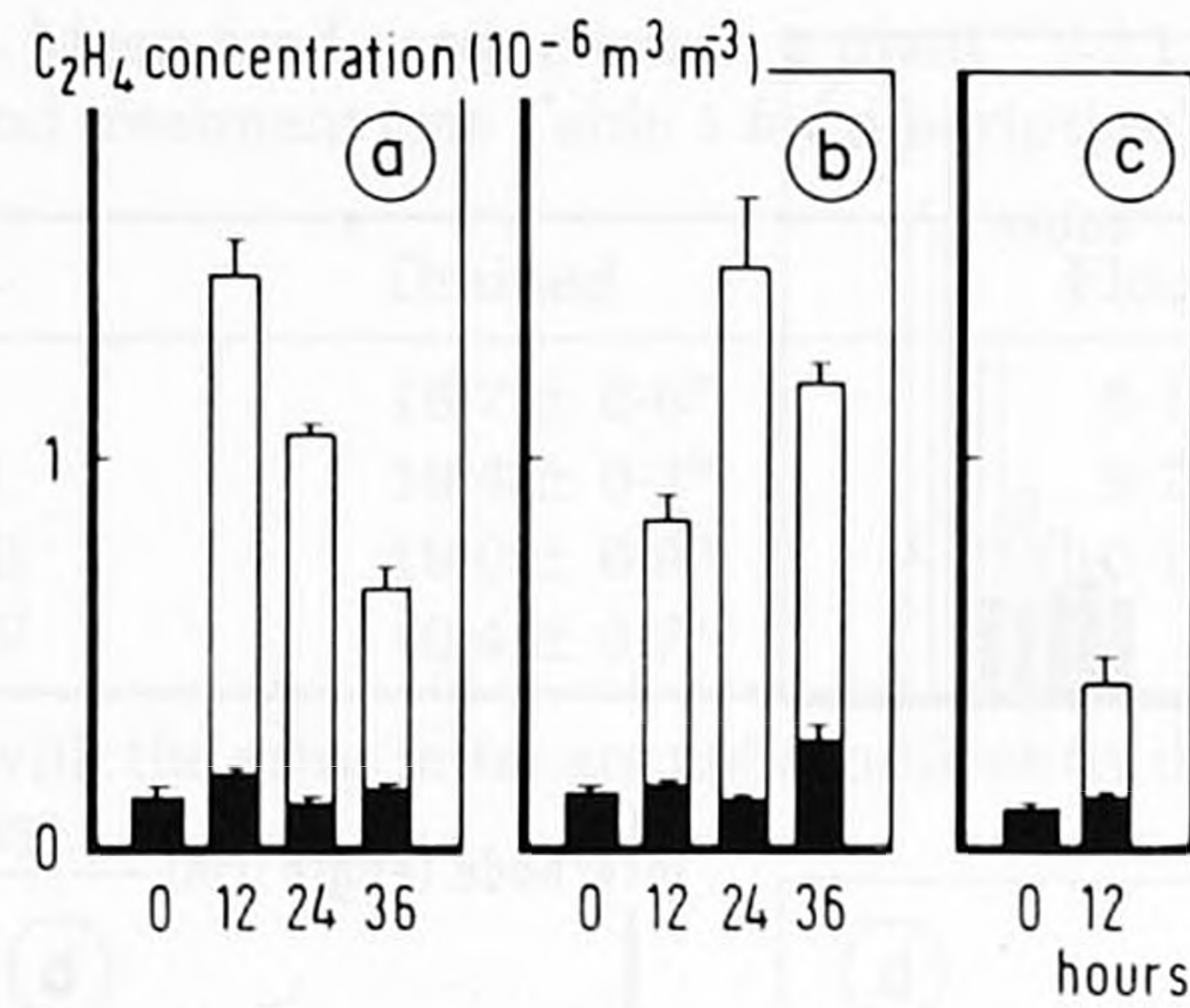


Fig. 3. Mean internal ethylene concentrations (± 1 SE) of *Rumex maritimus* plants after 0, 12, 24 and 36 h (0 h = 09.00 h) of treatment in three stages: (a) rosette stage; (b) bolting stage; (c) flowering stage. ■ drained; □ submerged in tap-water. Means of three or four samples.

restricted phase of growth. Keith, Raskin & Kende (1986) compared deepwater rice with non-deepwater rices and found that the ability of internodes to extend was much the same in all cases but in deepwater rice internode elongation, as well as its ability to respond to flooding, occurred during a much longer period, compared with the other rices. This example stresses the importance of the recognition of different stages in a plant's life in which its response to environmental conditions may vary. The role of ethylene was studied by mimicking the effect of submergence through

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 CO_2 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

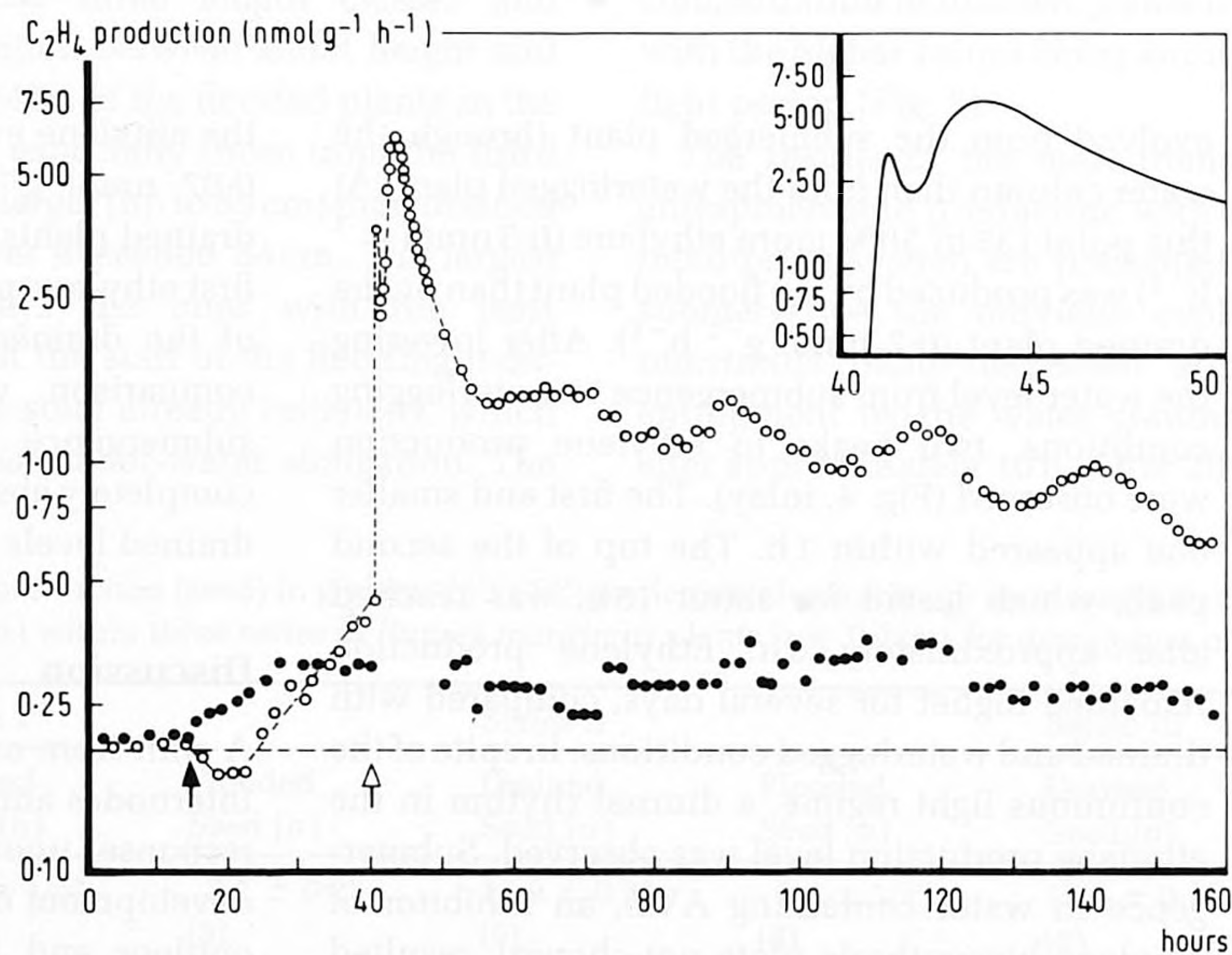


Fig. 4. Ethylene production (logarithmically presented) of *Rumex maritimus* plants in the bolting stage, monitored in a continuous flow system. — mean ethylene production of drained plants; ● plant waterlogged from 20 h onwards; ○ plant submerged from 10 h until 40 h and thereafter waterlogged. The inlay represents the two peaks in ethylene production occurring directly after submergence on a different scale. Results are presented in terms of the above-ground dry weight, determined at the end of the measurement.

tissues during ontogeny and should therefore be studied in more detail.

With the photoacoustic technique we measured the ethylene evolving from an intact plant before, during and after a period of submergence. Upon submergence ethylene was entrapped, since gas diffusion was effectively impeded by the surrounding water. However, even within a relatively short time (24 h), increasing amounts of ethylene evolved from the water surface, indicating a high internal ethylene concentration. After lowering the water to soil level, the accumulated ethylene diffused rapidly from the plant, resulting in a peak of ethylene within an hour. The amount of entrapped ethylene during the 24-h period of submergence, the amount of released ethylene during the last hours of submergence, and the first peak were compared with the ethylene production under waterlogged conditions. We computed that twice as much ethylene was released as could have been entrapped during submergence if the production level was the same as under waterlogged conditions. This suggests that the ethylene production was higher during submergence than during waterlogging. The application of AVG during the submergence treatment dramatically reduced the ethylene output, which may be a further indication of enhanced ethylene production during the 24-h submergence period. After lowering the water level a small amount of accumulated ethylene evolved from the plant treated with AVG. Thus, increased production as well as entrapment of ethylene appear to be important during submergence of *R. maritimus*.

A second, much larger ethylene peak appeared a few hours after the first, following submergence in water (Fig. 4). This second peak was absent following submergence in AVG. The time course of ethylene release we observed suggests that accumulated ethylene was released (first peak), while accumulated ACC was converted to ethylene directly after lowering the water level (second peak). Woltering & Harren (1989) applied ACC to orchid flowers and found a comparable large ethylene peak with the top appearing after approximately 4 h. Increase in ethylene biosynthesis in submerged tissue is due to enhanced ACC synthesis rather than increased conversion of ACC to ethylene (Métraux & Kende, 1983; Cohen & Kende, 1987). Thus probably only part of the extra synthesized ACC was converted into ethylene during the submergence period. The ecological advantage of this could be that even after restoration of shoot-atmosphere contact and the dissipation of entrapped ethylene into the atmosphere,

rapid growth is continued by an extra ethylene synthesis from accumulated ACC, allowing a larger portion of the plant to emerge from the water.

In the outdoor experiment, survival of *R. maritimus* during and after a flooding period of 4 weeks was largely dependent on the ability of the plant to emerge with part of the shoot above the water surface. Vegetative shoots were unable to reach the surface and died within 2 weeks. Evidence that elongation of petioles is of importance for the survival of vegetative *R. crispus* and *R. palustris* plants is presented by Voesenek (1990). A functional significance of the petiole elongation response was found for *R. maritimus* plants under laboratory conditions by Laan *et al.* (1990). The amount of internal aeration was positively correlated with the leaf area protruding from the water. 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.

Acknowledgments

We thank Rob Vergouwe, Bob Potter and Ingrid van Kessel for practical assistance, Dr Ir. G. Barendse for comments on the manuscript and the Foundation for Fundamental Research (BION), subsidized by the Netherlands Organisation for Scientific Research (NWO), for financial support.

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