Vegetative reproduction by species with different adaptations to shallow-flooded habitats

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SUMMARY

In shallow flooded parts of rich fens Mentha aquatica might thrive in deeper water than Epilobium hirsutum but previous experiments have provided no clear indication that the flooding tolerance of these species differs. In this study we investigated, by measuring growth, biomass allocation and vegetative reproduction, whether the impact of water level on vegetative reproduction might produce different lower boundaries on water level gradients. There was a striking contrast between biomass production at high water levels and the field distribution of both species. After 18 wk, the mean biomass of *E. hirsutum* grown in waterlogged and flooded conditions was 82% and 54%, respectively, of the mean biomass production of drained plants. Biomass of waterlogged and flooded *M. aquatica* was reduced to 57% and 37% in drained conditions. Waterlogged and flooded *E. hirsutum* had swollen stem bases and invested a high proportion of biomass in adventitious roots. Stems of *M. aquatica* did not swell, formed few adventitious roots and maintained an equal proportion of below-ground roots at all water levels. The effect of water level on vegetative reproduction corresponded well with the lower hydrological boundaries of both species. When waterlogged and flooded, most rhizomes of *E. hirsutum* emerged from above-ground parts of the stem base and were oriented in an upward direction. Plants in flooded soil allocated less biomass to rhizomes and also reduced the number and size of rhizomes. Rhizome formation of *M. aquatica* on the other hand was not directly affected by water level and only depended on plant size. These differences in vegetative reproduction are discussed in relation to the different abilities of both species to oxygenate their below-ground roots. It was concluded that the mode of adaptation to soil flooding might also affect vegetative reproduction and, therefore, a species’ ability for long-term persistence in soil-flooded habitats.

Key words: clonal growth, size-dependent reproduction, stem elongation, pseudoannuals, zonation.

INTRODUCTION

The elevational range on a water-level gradient inhabited by emergent macrophytes is usually determined by competitive interactions and the species’ physiological response to flooding. Interspecific competition restricts the upper boundary, whereas flooding tolerance determines a species’ lower boundary, i.e. the maximum water depth at which a species can persist (Grace & Wetzel, 1981; Studer-Ehrensberger et al., 1993; Blom & Voesenek, 1996). In shallow flooded habitats the lower limits might be determined during the recruitment phase by germination requirements or submergence tolerance of seedlings (Voesenek et al., 1993b; Clevering et al., 1996; Lenssen et al., 1998). During the established phase, ability for enhanced stem elongation might be necessary for survival of sudden rises in water level (Ridge, 1987; Voesenek et al., 1993a; Setter & Laureles, 1996). However, in shallow flooded habitats, where there is a small chance of plants becoming completely submerged, tolerance of anoxic substrate conditions is one of the most important factors determining a species’ lower boundary (Justin & Armstrong 1987; Armstrong et al., 1994). These characteristics, however, do not explain the different lower boundaries of two common species
from dry and shallow flooded sites, *Epilobium hirsutum* and *Mentha aquatica*. In rich fens, usually dominated by *Phragmites australis*, the lower limit of *M. aquatica* is usually deeper than that of *E. hirsutum* (Brock et al., 1987; Gryseels, 1989; Foij & Harding, 1995; Van de Rijt et al., 1996). In contrast to this field distribution, the germination requirements on the water level gradient and survival rate of submerged seedlings of both species are similar (Lenssen et al., 1998). Qualitative observations by Ridge (1987) suggest that both species are capable of enhanced stem elongation upon submergence and, in experimental conditions, both species may achieve optimal productivity in waterlogged soils (Lenssen et al., 1999).

A hitherto uninvestigated life-history phase of both species is vegetative reproduction, an important process for amphibious species since most are unable to germinate in flooded habitats (Van der Valk, 1981; Lenssen et al., 1998). In addition, vegetative reproductive organs usually also function as perennating organs. Therefore, the ability to form rhizomes and/or perennating organs is crucial for expansion and persistence at flooded sites. Indeed, many studies have shown a strong correlation between the maximum water depth at which a species can survive and the degree of rhizome formation at that depth (Grace & Wetzel, 1982; Grace, 1989; Coops et al., 1996; Clevering & Hundscheid, 1998).

The impact of flooding on vegetative reproduction might also be important for the lower limits of *E. hirsutum* and *M. aquatica*, since both species rely on rhizomes for overwintering and expansion. *E. hirsutum* is a pseudoannual (cf. Suzuki & Hutchings, 1997) forming rhizomes at below-ground parts of the shoot which become disconnected once the main shoot decays at the end of the growing season. At the apex of each rhizome a winter rosette is formed which produces one new shoot the next spring (Shamsi & Whitehead, 1973). *Mentha aquatica* forms long, horizontal runners from above-ground (and sometimes below-ground) parts of the stem. Connections between main shoot and runners are also lost at the end of the growing season. In contrast to *E. hirsutum*, each runner of *M. aquatica* may produce several daughter shoots, but these usually remain underdeveloped throughout the year in which the stolon was formed (personal observation).

We investigated whether the impact of flooding on vegetative reproduction explains the different lower boundaries of both species by growing vegetative shoots of both species at different shallow water levels and measuring growth, biomass allocation and rhizome formation. The hypothesis to be tested was that the response to water level treatment would only match the lower boundaries of both species with regard to vegetative reproduction. We therefore expected a similar response in both species to water level with regard to stem-elongation, biomass production and biomass allocation to stems, leaves and roots, but a greater decrease at higher water levels in number and weight of rhizomes of *E. hirsutum* than in those of *M. aquatica*.

**Materials and Methods**

**Plant material**

For the sake of simplicity we will hereafter refer to the stolons of *Mentha aquatica* L. as rhizomes. On 25 April 1996, rhizomes of *Epilobium hirsutum* L. and *M. aquatica* were collected from a shoreline of the River Nieuwe Merwede (lat 51°45′ N, long 4°45′ E). Winter rosettes of *E. hirsutum*, consisting of roots, a rhizome and the apical rosette, were dug up. From each rhizome of *M. aquatica* we separated one node from which both roots and a shoot with leaf-buds emerged. At each side of the node a 3.5-cm piece of rhizome was left attached. We maintained a minimum distance of 1 m between sites of collection of rhizomes of *M. aquatica* and *E. hirsutum* and used only one ramet from each rhizome of *M. aquatica* in order to incorporate some degree of genetic diversity. After collection, the vegetative material was planted in moist sand and stored at 5°C with a day/night regime of 11 h/13 h.

**Experimental procedure**

At the start of the experiment, on 8 May 1996, we selected 48 medium-sized ramets of each species and measured their fresh weight. A subset of ramets was weighed before and after drying for at least 48 h at 70°C to determine dry weight : fresh weight ratios. Each ramet was then planted in a separate plastic container (base diam. 55 cm; top diam. 65 cm; height 55 cm) filled with 10 l of a 1:1 (v/v) mixture of sand and river clay and 7.5 g Osmocote slow-release fertilizer (7.5% NH₄-N, 7.5% NO₃-N, 7.2% P₂O₅ and 12% K₂O) (Osmocote Plus, Grace Sierra International, Heerlen, The Netherlands). Containers were placed outdoors in the experimental garden of the Netherlands Institute of Ecology (Heteren, The Netherlands). At random positions within each of eight blocks we placed two replicates of each combination of species and water level (48 containers per species, 96 containers in total). Ramets were grown in moist soil for 1 wk, after which three water level treatments were imposed: drainage, waterlogging and flooding. Holes were made in the containers either in the base (drained treatment), 1 cm above the soil surface (waterlogged treatment) or 5 cm above the top of the shoot, equal to 10 cm above the soil surface (flooded treatment). Water level treatments were maintained manually by adding ground water, and water in the containers was replenished at least twice a week. In order to
restrict algal blooms, Daphnids were added to the containers with flooded plants, and filamentous algae were regularly removed manually from both waterlogged and flooded treatments. Redox measurements made 22 August 1996 at a depth of 5 cm in drained and waterlogged containers (one reading per container) indicated that flooding of the soil had caused a considerable decrease in soil redox potential (drained $303 \pm 8$ mV; waterlogged: $-97 \pm 29$ mV; both means $\pm$ SE, $n = 8$).

**Plant measurements**

On the same day as treatments were imposed, the shoot base of each ramet was tagged. On the first day, and 3, 6, 9 and 20 d after, shoot length was measured as the distance between shoot base and top.

After 10 wk, eight plants of each combination of species and water level were harvested. After the next 8 wk, stems of some plants had been broken by wind whereas others had suffered from insect attacks. These plants were excluded at the final harvest (week 18) so that only five replicates could be used for further analysis. At harvest, shoots were cut off at ground level and separated into leaves, stems (including main stem, branches and petioles), inflorescences, adventitious roots and rhizomes. Roots attached to the shoot above the soil surface were regarded as adventitious roots. Below-ground parts were separated into roots and rhizomes after the soil was carefully washed away. Rhizomes were counted and dry weights of all plant parts were determined after drying at 70°C for at least 48 h. The proportion of each plant organ to total biomass was calculated and will hereafter be referred to as the weight ratio. Mean rhizome weight of each plant was calculated as the ratio between total rhizome weight and number of rhizomes.

Many rhizomes of waterlogged and flooded *E. hirsutum* were oriented upwards and originated from above-ground parts of the stem base. At the first harvest these phenomena were quantified by measuring the angle between the stem base and the top of the rhizome. We also counted the number of rhizomes attached to above- and below-ground parts of the main stem.

**Data analysis**

The influence of water level on total shoot length was tested with ANOVA in SAS procedure GLM with the time of measurement as a within-plant repeated measurement. The GLM option Polynomial was used to compensate for differences in time intervals between length measurements (SAS, 1989). Influences of water level and harvest on total dry weight and weight ratios were tested with ANOVA according to a randomized block design. Prior to analysis, values of dry weight were log$_e$-transformed and weight ratios were arcsin-transformed. Total dry weights were analysed using initial dry weights as covariate. The influence of water level and harvest on biomass invested in inflorescences and rhizomes, and on mean rhizome weight and number of rhizomes, was examined by analysis of covariance with vegetative dry weight (= leaves + stems + roots + adventitious roots) as the covariate. Direct effects of water level and harvest could thus be distinguished from effects of plant size. Because of inequality of sample size, caused by difference in number of replicates between harvests 1 and 2, all $F$-values were calculated with type III Sums of Squares (Sokal & Rohlf, 1995). Means were compared by least significant difference.

Consequently, relationships between reproductive output ($R$) and vegetative dry weight ($V$) were investigated according to the allometrical relationship $R = aV^b$ which was analysed as $\log R = a + b \log V$ (with $a' = \log a$) by estimating parameters $a$ and $b$ with linear regression. Confidence intervals were used to determine whether $b$ was significantly different from 1, indicating that reproductive effort ($R/V$) either decreased ($b < 1$), remained constant ($b = 1$) or increased ($b > 1$) with plant size (Klinkhamer et al., 1992). If treatment effects in analysis of covariance were not significant, data from the different treatment levels were pooled before the precise relationships between vegetative plant dry weight and measures of reproductive output were investigated with linear regression.

**RESULTS**

Shoot lengths of both species were significantly influenced by interaction between time and water level (Table 1). Water level did not affect the shoot length of *Epilobium hirsutum* during the first 9 d, and flooded plants did not elongate faster than drained and waterlogged plants. After 20 d, however, drained and waterlogged plants were significantly taller than flooded plants (Fig. 1). By contrast, *Mentha aquatica* elongated faster when flooded and after 6 d, when most shoots had protruded through the water layer, they were significantly taller than those of drained

<table>
<thead>
<tr>
<th>Block (7)</th>
<th>W (2)</th>
<th>T (4)</th>
<th>W $\times$ T (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. hirsutum</em></td>
<td>0.86</td>
<td>2.27</td>
<td>290.93***</td>
</tr>
<tr>
<td><em>M. aquatica</em></td>
<td>3.74**</td>
<td>13.78***</td>
<td>659.22***</td>
</tr>
</tbody>
</table>

Lengths of plants were measured at the start of the experiment and 3, 6, 9 and 20 d thereafter on the same plant. Time (T) was therefore treated as a within-plant repeated measurement. Degrees of freedom are listed in parentheses. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. 

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**Table 1. F values of total shoot length of *Epilobium hirsutum* and *Mentha aquatica* under influence of block and water level (W)**
plants (Fig. 1). Waterlogged plants also elongated faster than drained plants but differences were significant only after 20 d (Fig. 1).

Total dry weights of both species were significantly affected by water level (Table 2). After both 10 and 18 wk total dry weight of *E. hirsutum* was reduced by waterlogging and more reduced by flooding. *Mentha aquatica* seemed to respond in a similar way, but its biomass was decreased more than that of *E. hirsutum* by soil flooding at the end of the experiment (Fig. 2).

The allocation of biomass to below-ground roots and adventitious roots of *E. hirsutum* was significantly affected by water level (Table 2). Flooded plants invested a significantly higher proportion of their biomass in adventitious roots than did waterlogged plants, whereas less biomass was allocated to below-ground roots (Fig. 2). These adventitious roots emerged from the flooded parts of the stem base, which was very swollen. Allocation to stems and leaves was not affected by water level but significantly differed between harvests (Table 2). At the second harvest a significantly lower proportion of biomass was present in stems and leaves whereas a higher proportion was allocated to inflorescences and rhizomes (Fig. 2).

*Mentha aquatica* also formed adventitious roots when flooded, but by contrast with *E. hirsutum*, it maintained an equal proportion of below-ground roots at all water levels (Table 2, Fig. 2). Allocation to stems was not affected by water level, but the proportion of biomass invested in leaves was significantly affected by the interaction between water level and harvest time (Table 2). This interaction was due to the relatively low leaf biomass of waterlogged and flooded plants (Fig. 2).

The impact of water level on vegetative reproduction differed greatly between both species. Water-
logging and flooding did not alter the orientation and position of *M. aquatica* rhizomes (results not shown). However, when waterlogged and flooded, the majority of *E. hirsutum* rhizomes were at a sharp angle with the stem base, indicating upward growth. At higher water levels, rhizomes also emerged from above-ground instead of from below-ground parts of the stem (Table 3).

The interaction between water level and harvest significantly affected allocation to rhizomes in *E. hirsutum* (Table 4). After 18 wk, the amount of dry weight allocated to rhizomes of drained plants was significantly related to plant size (results not shown). The slope of this regression did not differ significantly from 1, indicating that in all sizes of plant an equal proportion of biomass was allocated to vegetative reproductive organs (Klinkhamer et al., 1992). No relationship was found between total rhizome biomass and plant size for waterlogged and flooded plants (results not shown) and total rhizome biomass was always significantly smaller in both than when drained (Table 5). Mean rhizome mass and number of rhizomes of *E. hirsutum* were not size-dependent at any water level (results not shown) and both measurements were always significantly lower at higher water levels (Table 5).
Harvest 2 (at 18 wk) grown under drained rhizomes (ln-transformed data. means within the same row are indicated with different letters. Significance test for Tot RH d. wt based upon Degrees of freedom are listed in parentheses. *, P<0.05; **, P<0.01; ***, P<0.001; nd, not determined (insufficient data to allow statistical testing).

Table 4. F-values of ANCOVA for inflorescence dry weight, total rhizome dry weight, mean rhizome dry weight and number of rhizomes of Epilobium hirsutum and Mentha aquatica under influence of block, water level (W) and harvest (H) with vegetative dry weight as the covariable

<table>
<thead>
<tr>
<th></th>
<th>Block (7)</th>
<th>W (2)</th>
<th>H (1)</th>
<th>W×H (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. hirsutum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflorescence dry</td>
<td>0.39</td>
<td>0.68</td>
<td>0.90</td>
<td>0.15</td>
</tr>
<tr>
<td>weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total rhizome dry</td>
<td>0.43</td>
<td>111.77***</td>
<td>153.54***</td>
<td>101.7***</td>
</tr>
<tr>
<td>weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean rhizome dry</td>
<td>0.20</td>
<td>65.82***</td>
<td>146.65***</td>
<td>55.32***</td>
</tr>
<tr>
<td>weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of rhizomes</td>
<td>0.20</td>
<td>52.33***</td>
<td>0.63</td>
<td>6.94*</td>
</tr>
<tr>
<td><strong>M. aquatica</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflorescence dry</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total rhizome dry</td>
<td>0.95</td>
<td>1.83</td>
<td>17.00**</td>
<td>1.01</td>
</tr>
<tr>
<td>weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean rhizome dry</td>
<td>3.17</td>
<td>1.89</td>
<td>5.67</td>
<td>0.35</td>
</tr>
<tr>
<td>weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of rhizomes</td>
<td>1.06</td>
<td>0.25</td>
<td>1.07</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Degrees of freedom are listed in parentheses. *, P<0.05; **, P<0.01; ***, P<0.001; nd, not determined (insufficient data to allow statistical testing).

Table 5. Total rhizome dry weight (Tot Rh d.wt), mean rhizome dry weight (M Rh d. wt) and number of rhizomes (N rhizomes; mean±SE, n = 8, harvest 1, n = 5, harvest 2) of Epilobium hirsutum and Mentha aquatica grown under drained (Dr), waterlogged (Wl) or flooded (Fl) conditions

<table>
<thead>
<tr>
<th></th>
<th>Harvest 1 (at 10 wk)</th>
<th>Harvest 2 (at 18 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dr</td>
<td>Wl</td>
</tr>
<tr>
<td><strong>E. hirsutum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tot Rh d. wt (g)</td>
<td>18.5±1.3 c</td>
<td>2.9±0.5 d</td>
</tr>
<tr>
<td>M Rh d. wt (g)</td>
<td>1.12±0.1 c</td>
<td>0.5±0.09 d</td>
</tr>
<tr>
<td>N Rhizomes</td>
<td>16.8±0.6 a</td>
<td>7.3±0.9 c</td>
</tr>
<tr>
<td><strong>M. aquatica</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tot Rh d. wt (g)</td>
<td>5.4±1.1 c</td>
<td>2.9±0.5 c</td>
</tr>
<tr>
<td>M Rh d. wt (g)</td>
<td>0.60±0.14 c</td>
<td>0.40±0.07 c</td>
</tr>
<tr>
<td>N Rhizomes</td>
<td>9.8±1.0 abc</td>
<td>8.1±1.0 bc</td>
</tr>
</tbody>
</table>

Degrees of freedom are listed in parentheses. *, P<0.05; **, P<0.01; ***, P<0.001. Significant differences between means within the same row are indicated with different letters. Significance test for Tot RH d. wt based upon ln-transformed data.

Table 6. Results of linear regressions between vegetative dry weight of plants (independent variable) and vegetative reproductive characteristics of Mentha aquatica

<table>
<thead>
<tr>
<th></th>
<th>Total rhizome d. wt (at 10 wk)</th>
<th>Total rhizome d. wt (at 18 wk)</th>
<th>Mean rhizome d. wt (pooled data)</th>
<th>Number of rhizomes (pooled data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>0.815***</td>
<td>0.942***</td>
<td>1.59***</td>
<td>0.572***</td>
</tr>
<tr>
<td>R²</td>
<td>0.64</td>
<td>0.87</td>
<td>0.68</td>
<td>0.30</td>
</tr>
<tr>
<td>n</td>
<td>24</td>
<td>15</td>
<td>39</td>
<td>39</td>
</tr>
</tbody>
</table>

All regression are based on log,-transformed data. For total rhizome dry weight plants grown under different water levels were pooled per harvest. For mean rhizome dry weight and number of rhizomes all data were pooled. *P<0.05; **, P<0.01; ***, P<0.001; n, number of observations.
Sexual reproductive output of *E. hirsutum* was not affected by water level or time of harvest (Table 4), but varied only as a function of plant size. Regression analysis indicated the following relationship: 

$$
\log_e(\text{inflorescence d. wt}) = -13.86 + 3.5\log_e(\text{vegetative d. wt}) \quad (R^2 = 0.80; \ n = 38).
$$

The slope was significantly steeper than 1, indicating that larger *E. hirsutum* plants invested relatively more biomass into sexual reproduction than did smaller plants (Klinkhamer et al., 1992).

By contrast with *E. hirsutum*, water level did not directly affect any vegetative reproduction characteristic of *M. aquatica* (Table 4). Vegetative reproductive output was significantly related to plant size (Table 6) and differences in absolute amounts of total and mean rhizome biomass and number between different water levels (Table 5) must therefore be regarded as a consequence of the smaller plants growing at high water levels. The relationship between rhizome number and plant size was however rather weak and explained only 30% of the variance (Table 7). The equal number of rhizomes of drained and flooded plants (Table 6), which were markedly different in size (Fig. 2), might therefore have been caused by factors other than plant size.

**DISCUSSION**

By contrast with our hypothesis, both species differed in their ability for enhanced shoot elongation upon flooding. Only in *Mentha aquatica*, which might persist in deeper water than *Epilobium hirsutum*, was shoot elongation enhanced in submerged conditions. This response increases its survival after sudden rises in water level (Ridge, 1987; Voesenek et al., 1993a; Setter & Laureles, 1996) and might explain its frequent occurrence in water bodies with relatively strong fluctuations in water levels (Brock et al., 1987). However, the interspecific difference in stem elongation response is not particularly important in producing different lower boundaries in rich fens, where the two species co-occur. The vegetation in this habitat is dominated by tall, perennial species which indicate, apart from differences in ontogeny (Coleman et al., 1994), caused by growth-retarding effects of high water levels. The formation of inflorescences by *E. hirsutum* was also not affected by water level. Although investment in sexual structures might vary as a result of environmental conditions (Hartnett, 1990; Schmid & Weiner, 1993) no evidence was found that water level has a similar impact.

By contrast with the small impact of water level on biomass allocation on above-ground plant organs, marked effects were noticed on the morphology and size of the roots and rhizomes of *E. hirsutum* in particular. The swollen stem-base of *E. hirsutum* in waterlogged and flooded conditions results from callus formation (stem hypertrophy). This tissue is formed to enhance the surface for gas diffusion, thereby improving aeration from stem base to roots (Etherington, 1984; Armstrong et al., 1994). In addition, *E. hirsutum* replaced its primary, below-ground, root system to a large extent with adventitious roots. These adaptations, although very successful in maintaining productivity in soil-flooded conditions (Etherington, 1984), indicate that oxygen supply to the below-ground root system is not very efficient in *E. hirsutum* (Koncalova, 1990; Armstrong et al., 1994; Visser et al., 1996a). *Mentha aquatica*...
supplies its below-ground root system with oxygen by means of aerenchymatous gas-phase transport of either photosynthetically derived or atmospheric oxygen from shoot to root (Pedersen & Sand-Jensen, 1997), which is a more efficient means than through a hypertrophied stem base (Armstrong et al., 1994). This explains its maintenance of an equal proportion of below-ground roots at all water levels as well as its relatively small proportion of adventitious roots.

The precise physiological mechanism of the different abilities for vegetative reproduction in waterlogged and flooded conditions remains to be elucidated, but different degrees of efficiency of oxygen-supply to below-ground roots probably play an important role. The most conspicuous phenomenon is the change in position and orientation of rhizomes formed by waterlogged and flooded E. hirsutum. The mechanism of the emergence of rhizomes from the stem base might be similar to that leading to the emergence of adventitious roots from the stem base instead of from below-ground stem parts. This replacement of primary roots by adventitious roots is caused by a large accumulation of auxin and ethylene in the stem base, a consequence of the limited gas exchange between root and shoot. These increased levels of auxins and ethylene are not only responsible for adventitious root formation (Visser et al., 1995, 1996b) but also enhance responsiveness to gibberelins (Blom et al., 1994), which stimulate lateral bud growth and induce a more erect rather than horizontal rhizome orientation (Hutchings & Mogie, 1990), resulting in the vertically oriented rhizomes originating from the stem base observed in E. hirsutum. Mentha aquatica accumulates less auxin and ethylene at the stem base because internal ventilation by the primary root system (Pedersen & Sand-Jensen, 1997) allows unlimited transport of auxin to the roots and release of ethylene into the atmosphere (Visser et al., 1995).

In drained E. hirsutum, the amount of biomass allocated to rhizomes was size-dependent, a common phenomenon in pseudoannual species (Johansson, 1994; Verburg et al., 1996; Worley & Harder, 1996; Dong et al., 1997). However, by using plant size as a covariate we have shown that decreased total and mean rhizome biomass and rhizome numbers of waterlogged and flooded E. hirsutum were directly caused by higher water levels and were not a function of plant size. This indicates that a longer experimental period, allowing the plants at higher water levels to grow taller, would not have led to rhizome apparatus of a similar size to that of drained plants. Again, the limited effectiveness in E. hirsutum (in comparison with M. aquatica) of gas transport to the below-ground root system might be the cause. Hypoxic conditions in roots and rhizomes increase the sink for assimilates (Albrecht & Biemelt, 1998), thus decreasing the availability of carbohydrates for storage in rhizomes.

According to our hypothesis there was a strong correspondence between the impact of water level on vegetative reproduction and the lower limits of E. hirsutum and M. aquatica on water level gradients. Flooding-induced decrease in number and size of rhizomes is probably also the cause of the more limited water-depth penetration in E. hirsutum than in M. aquatica in many rich fens. Smaller rhizomes are a clear disadvantage in flooded environments since they contain less carbohydrates (Clevering et al., 1995), decreasing the likelihood of surviving burial in anaerobic mud during winter (Barclay & Crawford 1983; Brändle & Crawford, 1987) and the ability of shoots to penetrate water layers during spring growth (Clevering et al., 1995). Small rhizomes also produce smaller offspring which might be especially detrimental in the tall and dense vegetation of rich-fens (Jerling, 1988; Johansson, 1994).

The comparison of M. aquatica and E. hirsutum has indicated that the mode of adaptation to soil flooding has implications for vegetative reproduction. The results also indicate that in the short and long term the adaptive value of a certain strategy against soil flooding may vary. Judging by biomass production, M. aquatica was less well adapted to soil flooding than E. hirsutum. The latter species forms adventitious roots which can penetrate the soil as deeply as drained roots (Justin & Armstrong, 1987) and it is capable of maintaining high productivity in waterlogged soils (Etherington, 1984), at least in those which are iron-poor (Snowden & Wheeler, 1995). The rhizome formation of the apparently well adapted E. hirsutum was however seriously constrained, possibly restricting its lower limits on water-level gradients to shallower depths than those of M. aquatica. This species had stronger growth depression but unhampered vegetative reproduction in flooded soils, probably due to its internal aeration system allowing gas transport between shoot and root.

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