Ethylene enhances gibberellin levels and petiole sensitivity in flooding-tolerant \textit{Rumex palustris} but not in flooding-intolerant \textit{R. acetosa}

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\textbf{Abstract.} The role of gibberellin (GA) and ethylene in submergence-induced petiole elongation was studied in two species of the genus \textit{Rumex}. Analysis of endogenous GAs in the flooding-tolerant \textit{Rumex palustris} Sm. and the intolerant \textit{Rumex acetosa} L. by gas chromatography-mass spectrometry showed for both species the presence of GA\textsubscript{1}, GA\textsubscript{4}, GA\textsubscript{9}, GA\textsubscript{19}, GA\textsubscript{20} and GA\textsubscript{53}. Gas chromatography-mass spectrometry analysis of \textit{R. palustris} petiole tissue of submerged plants showed an increase in levels of 13-OH GAs, especially GA\textsubscript{1}, compared with drained plants. This effect could be mimicked by application of 5 \textmu L L\textsuperscript{-1} ethylene. In \textit{R. acetosa}, no differences between levels of GAs in drained or submerged plants were found. In \textit{R. palustris}, both submergence and ethylene treatment sensitized petioles to exogenous gibberellic acid (GA\textsubscript{3}). In \textit{R. acetosa} the effect was opposite, i.e. submergence and ethylene de-sensitized petioles to GA\textsubscript{3}. Our results demonstrate the dual effect of ethylene in the submergence response related to flooding tolerance, i.e. in the flooding-tolerant \textit{R. palustris} ethylene causes an increased concentration of and sensitivity to GA with respect to petiole elongation while in the intolerant \textit{R. acetosa} ethylene reduces growth independent of GAs.

\textbf{Key words:} Ethylene – Flooding – Gibberellin – Hormone analysis – \textit{Rumex}

Introduction

\textit{Rumex palustris} Sm. is a floodplain species capable of surviving prolonged periods of flooding. Enhanced elongation of petioles is an important adaptation in this species upon waterlogging (Voesenek et al. 1990a) and submergence (Voesenek et al. 1993). Stimulated petiole elongation is primarily caused by cell elongation (Voesenek et al. 1990b) and can be mimicked by application of high (1–10 \textmu L L\textsuperscript{-1}) ethylene levels (Voesenek and Blom 1989). To understand the underlying mechanisms of this process, physiological changes upon flooding in \textit{R. palustris} have been compared with those in the flooding-intolerant \textit{Rumex acetosa} L. Both species occur in river floodplains. In both species, endogenous ethylene concentration rises upon submergence due to physical entrapment of the gas (Voesenek et al. 1993; Banga et al. 1996). However, only in \textit{R. palustris} does this lead to petiole elongation while, in contrast, in \textit{R. acetosa} petiole elongation is inhibited (Voesenek and Blom 1989; Voesenek et al. 1993). Therefore, it appears that interspecific variation within the genus \textit{Rumex} with respect to stimulated petiole elongation is not related to the endogenous ethylene concentration during submergence but to opposite responsiveness of petiole tissue to ethylene.

The growth-promoting effect of ethylene has been demonstrated in a wide variety of aquatic and amphibious species of different taxonomical origin (Ku et al. 1970; Musgrave et al. 1972; Mètraux and Kende 1983). An important factor in the submergence-stimulated growth response to ethylene is the growth-promoting role of gibberellin (GA), as was demonstrated for \textit{Callitriche platycarpa} (Musgrave et al. 1972) and the deepwater variety of \textit{Oryza sativa} (Raskin and Kende 1984a; Suge 1985; Hoffmann-Benning and Kende 1992). The nature of GA involvement in enhancing growth upon submergence could be twofold: (i) a change in responsiveness towards GAs and (ii) a change in the endogenous GA concentration. In \textit{R. palustris}, an analysis of dose-response curves with gibberellic acid (GA\textsubscript{3}) under drained and submerged conditions indicated that submergence leads to a higher sensitivity to GA, i.e. petioles become more responsive to GA (Blom et al. 1994). To investigate the role of GA in relation to the
ethylene-mediated submergence response in *R. palustris* and *R. acetosa* the following hypothesis was formulated: submergence causes an increase in endogenous ethylene levels, which in turn enhance elongation growth in the flooding-tolerant *R. palustris* by increasing GA levels and/or sensitivity to GA, while in the intolerant *R. acetosa* elongation is inhibited by a decrease in GA concentration and/or sensitivity to GA. To this end, experiments were performed to (i) test whether the presence of GA is a prerequisite for stimulated shoot elongation in *R. palustris*, (ii) analyse the endogenous GAs upon submergence and under enriched ethylene conditions and (iii) determine the effect of ethylene on responsiveness towards GA. The last two experiments were performed for both species.

**Materials and methods**

*Plant material. Seeds (achenes) of *R. palustris* Sm. and *R. acetosa* L. were previously collected in river floodplains (Kekerdomsewaard and Doestewaard, river Waal, The Netherlands), were germinated on moistened filter paper in Petri dishes for 10 d in a germination cabinet [16 h photoperiod; day/night temperature of 25/10 °C; photosynthetic photon flux density (PPFD) of 30 μmol m −2 s −1 from fluorescent lamps (TL 8 W/33), Philips, Eindhoven, The Netherlands]. Seedlings were planted in plastic pots (60 mL) filled with a mixture (1:1, v/v) of sand and potting compost (Jongkind, Aalsmeer, The Netherlands) and grown for four to five weeks in a growth chamber (16 h photoperiod, day/night temperature of 21/15 °C, PPFD of 95 μmol m −2 s −1 from fluorescent lamps (Philips TLD 58 W/84). For GA analysis, plants were transferred to the greenhouse four weeks after germination and placed in 500-mL pots with a similar mixture of sand and potting compost for an additional period of two weeks. All plants were selected for homogeneity with respect to the developmental stage of the youngest leaf prior to the start of the experiments. Unless stated otherwise, elongation was recorded as increase in length of the petioles of the second-youngest leaf after 48 h of treatment.*

*Chemicals. Gibberellic acid was purchased from Sigma (Bornem, Belgium). Paclobutrazol was a gift from ICI-Agro (Ridderkerk, the Netherlands). Sumitomo Chemicals (Osaka, Japan) and prohexadione from Kumai (Tokyo, Japan), were gifts from Prof. Noburu Morofushi (University of Tokyo, Japan). Typicaly, GA3 (10 mL, 0–10−3 M) was applied to the soil 3 h prior to the start of submergence experiments. Paclobutrazol (10 mL, 10−3 M) was applied in a similar manner 4 d before the onset of experiments. Paclobutrazol and GA3 were first dissolved in a small volume of ethanol (2–3 mL, 96%). Controls received an equal amount of ethanol. Uniconazole (10 μL, 10−3 M) and prohexadione (10 μL, 10−4 M) were dissolved in acetonitrile (1:1, v/v) and applied on the apex 1 d before experiments. Gibberellins A9 and A4 (10 μL, 10−2 M) were dissolved and applied similarly 3 h before experiments. Controls received 10 μL acetonitrile (1:1, v/v). Ethylene (Hoecklos, Dieren, The Netherlands) was applied as pure ethylene for injection or as 5 μL L−1 mixture in medical air.*

*Extraction and purification for GA analysis. Because of the large number of plants necessary (>2000) for GA analysis, plants were submerged in large 200-L tanks filled with tapwater (22 °C) located in the greenhouse. Ethylene treatment was carried out in an air-tight growth cabinet with supplemental illumination from fluorescent lamps (80 μmol m −2 s −1) under ambient temperature. Enough pure ethylene was initially injected into the cabinet to attain a final concentration of 5 μL L−1 ethylene in air. Drained control plants were kept in the greenhouse under regular growing conditions. All treatments were deployed within 1 h on the same day and lasted 24 h. After this period, the petiole of the second youngest leaf was cut off, rinsed quickly in distilled water to remove soil particles, blotted dry and weighed. For each treatment, petioles of 200–400 plants were pooled and stored in 100% methanol at −23 °C until extraction. Total shoot samples of *R. acetosa* and *R. palustris* were obtained in a similar manner. All harvesting was done within 2 h to minimize diurnal endogenous GA variations.*

*Plant material used for GC-MS and GC-single ion monitoring (GC-SIM) was homogenized in 80% methanol containing deuterated GAs with 1 ng g−1 FW; pooled petiole FW was 25–30 g for each treatment) and twice gravity-filtered. After roto-evaporation polyvinylpyrrolidone was added to the aqueous residue as slurry and removed before acidification and partitioning. The sample was subsequently partitioned against (i) ethyl acetate, (ii) NaHCO3 and again (iii) ethyl acetate following the method of Endo et al. (1989). The final ethyl acetate fraction was led over a Na2SO4-anhydrous column to remove water, rotoevaporated to dryness and redissolved in 50% methanol. This fraction was loaded on a Sep-Pak (ODS) C18 column (Varian, Tokyo, Japan) and eluted three times with 80% acq. methanol. After rotoevaporation to dryness, the sample was redissolved in 100% methanol loaded on Sep-Pak (ODS) (analyticalchem, Tokyo, Japan) and eluted with 0.5% acetic acid in methanol. The eluate was evaporated to dryness and dissolved in 20% methanol for fractionation with HPLC (Waters 625 LC system with Waters 486 tunable UV absorbency detector at 210 nm; Waters, Tokyo, Japan). Conditions for HPLC were: column Capcell PAK C18-reversed phase (SG 21 nm, 5 μm, diam. 6 mm, length 250 mm; Shi-Seido, Tokyo, Japan), mobile phase, methanol with 0.1% acetic acid in Milli-Q H2O; 0-5 min 20% methanol isocratic, 5-40 min 20–80% methanol linear gradient, 40–55 min 80% methanol isocratic, flow rate 1.5 mL min−1. Eluates were collected at 1-min intervals. Plant material used for enzyme-linked immunosorbent assay (ELISA) was processed similarly with the exception of addition of deuterated GAs.*

*Enzyme-linked immunosorbent assay. The ELISA procedure was performed for the detection and semi-quantification of GA1, GA4, GA9 and GA30 in *R. acetosa* and *R. palustris* petiole and total shoot HPLC fractions, using an anti-GA1 (Nakajima et al. 1991) and an anti-GA30-methyl antibody (Yamaguchi et al. 1987). Gibberellin A19 was detected using an anti-GA34 antibody to which GA19 shows equal affinity (Yamaguchi et al. 1992). The ELISA procedure was as reported in Aizorn and Weiler (1983). Each assay was run in triplicate.*

*Gas chromatography-mass spectrometry and GC-SIM. After HPLC, the fractions of total shoot extracts of *R. acetosa* and *R. palustris* were collected, dried and dissolved in methanol. The appropriate fractions were combined in five groups of two to three fractions which were then methylated using ethereal diazomethane. The samples were trimethylsilylated by N-methyl-N-trimethylsilyl trifluoroacetamide. The derivatised samples were analysed using an AUTO MASS mass spectrometer (JEOl, Tokyo, Japan) equipped with a Hewlett Packard (Tokyo, Japan) gas chromatograph (0.25 mm i.d. × 15 m, film thickness 0.25 μm, DB-1, J&W). Operational conditions were the same as described in Gawronska et al. (1993). In short, the samples were co-injected with dissolved Paraffin (Gaskin et al. 1971) to establish retention times relative to n-alkane standards. After injection the oven temperature was kept at 80 °C for 1 min, increased to 245 °C at 30 °C min−1, followed by a further increase to 300 °C at 5 °C min−1. The He inlet pressure was 70 kPa and the injector, interface and source temperature were 250 °C, 250 °C and 200 °C respectively. Positive ion electron impact mass spectra at 70 eV were acquired scanning from 100 to 700 amu at 1 s per scan cycle.*

For GC-SIM, four to five prominent ions of each GA were monitored with dwell times of 50 ms. The contents of GA3, GA19, GA30, GA1, GA4 and GA9 were calculated from the peak area.
ratios 448/450, 434/436, 418/420, 506/508, 418/421 and 298/301, respectively, by reference to the identification of the compounds analysed.

*Experimental conditions for GA dose-response experiments.* Intact plants were completely submerged in tapwater (21°C) in 20-L aquaria located in the growth chamber and under the same conditions as mentioned above. Ethylene was applied in air-tight desiccators with a flow-through system (5 L h⁻¹). Air controls were also placed in desiccators and flushed with air at the same flow rate. All plants were pretreated with paclobutrazol (10 mL, 10⁻² M in tap water on soil) 3 d prior to the submergence experiments. Plants were selected based on the developmental stage of the youngest leaf and watered with the appropriate GA solution (10 mL, 0–10⁻⁴ M) 3–4 h before the experiment. Elongation was measured as difference of second youngest petiole length before and after the experiment. Experimental conditions lasted 48 h.

*Statistics.* Differences between treatments were assessed with the Tukey post-test after ANOVA (SAS Institute Inc. 1991). Curve fitting was performed with a non-linear regression procedure (SAS Institute Inc. 1991) using an adjusted Michaelis-Menten equation with Hill coefficient or interaction term (Weyers et al. 1987). To compare curves for different treatments, all curves were brought back to zero intercept, i.e. for each curve the response value at [GA] = 0 was subtracted from all other response values.

**Results**

**Involvement of GA in underwater growth of *R. palustris***. Paclobutrazol pretreatment of *R. palustris* plants 4 or 6 d prior to GA treatment resulted in an inhibition of petiole elongation in drained and submerged plants. This inhibition was partially restored by the addition of 10⁻⁵ M GA₃ (Table 1). However, neither the 4- nor 6-d pretreatment period completely reduced the submergence response to the same level of paclobutrazol-treated drained plants. Because of the apparent incomplete inhibition of GA biosynthesis by paclobutrazol, we also applied different GA biosynthesis inhibitors. However, both application of uniconazole (10 μL, 10⁻⁵ M on apex) and prohexadione (10 μL, 10⁻⁴ M on apex) resulted in a similar, incomplete growth reduction (Fig. 1). Following uniconazole treatment, elongation was completely restored with either application of GA₉ or GA₄. After prohexadione treatment, which is believed to inhibit the 3-β hydroxylations in the GA pathway (Nakayama et al. 1990), application of GA₄ was significantly more effective than GA₉ in restoring the submergence response. This suggests that in *R. palustris* 3-β hydroxylated gibberellins are the active GAs with respect to submergence-induced petiole elongation.

**Qualitative GA analysis.** In the genus *Rumex*, GAs have not yet been isolated and identified. Therefore, we first analysed shoot tissue of both *R. palustris* and *R. acetosa* qualitatively by ELISA (data not shown) and full-scan GC-MS analysis (Table 2). In both *Rumex* species, various GA members of the 13-hydroxylation (13-OH) and non-13-OH pathway were characterised. This analysis provided sufficient information to perform subsequent GC-SIM analysis of petiole tissue. Notwithstanding the absence of GA₉ in the GC-MS analysis, an internal standard for this GA was added for GC-SIM analysis to obtain information regarding changes in the biosynthetic GA pathway. Gibberellin A₉ is believed to be the direct precursor for GA₄, which was detected in the GC-MS analysis.

**Effect of flooding and ethylene on GA levels.** Introductory experiments, performed with the Tan-ginbozu dwarf rice bioassay (Nishijima and Katsura 1989) showed a clear increase in *R. palustris* petiole GA levels upon submergence (data not shown). These results were confirmed with an ELISA assay (data not shown) and GC-SIM. The GC-SIM analyses of *R. palustris* petiole tissue of submerged plants showed an increase in the GAs belonging to the 13-OH pathway compared with tissues of drained, well-aerated plants (Table 3). No effect of

![Fig. 1. Effect of uniconazole and prohexadione on GA₉ and GA₄ on petiole elongation of submerged *Rumex palustris* plants (n = 10). Plants were pretreated with uniconazole (10 μL, 10⁻⁵ M) and prohexadione (10 μL, 10⁻⁴ M) for 1 d before experimental treatments. Gibberellins A₉ and A₄ (10 μL, 10⁻⁵ M) were applied 3 h prior to the start of the experiment. Petiole elongation is expressed as the increase in length (mm ± SE) during 48 h treatment. All plants were submerged except for the aerated control group (drained). The experiment was repeated twice](image)
Table 2. Identification of endogenous GAs by full-scan GC-MS of the methylesters or MeTMSi derivatives in extracts from R. palustris (R. p.) and R. acetosa (R. a.)

<table>
<thead>
<tr>
<th>GA</th>
<th>Source</th>
<th>HPLC fractions</th>
<th>KRI&lt;br&gt;</th>
<th>Ion m/z (relative abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA3</td>
<td>R.p.</td>
<td>35–36</td>
<td>2505</td>
<td>448(62), 419(13), 416(20), 389(35), 251(25), 241(20)</td>
</tr>
<tr>
<td></td>
<td>R.a.</td>
<td>35–36</td>
<td>2505</td>
<td>448(60), 419(10), 416(20), 389(30), 251(25), 241(25)</td>
</tr>
<tr>
<td>GA20</td>
<td>R.p.</td>
<td>28–30</td>
<td>2490</td>
<td>418(100), 403(19), 375(70), 359(25), 345(8), 301(25)</td>
</tr>
<tr>
<td></td>
<td>R.a.</td>
<td>28–30</td>
<td>2490</td>
<td>418(100), 403(15), 375(80), 359(23), 345(10), 301(25)</td>
</tr>
<tr>
<td>GA19</td>
<td>R.p.</td>
<td>31–33</td>
<td>2598</td>
<td>462(5), 434(100), 402(30), 375(50), 374(50), 345(30)</td>
</tr>
<tr>
<td></td>
<td>R.a.</td>
<td>31–33</td>
<td>2598</td>
<td>462(8), 434(100), 402(37), 375(50), 374(60), 345(25)</td>
</tr>
<tr>
<td>GA4</td>
<td>R.p.</td>
<td>35–36</td>
<td>2510</td>
<td>418(18), 386(20), 358(10), 328(25), 289(40), 284(100)</td>
</tr>
<tr>
<td></td>
<td>R.a.</td>
<td>35–36</td>
<td>2510</td>
<td>418(15), 386(17), 358(10), 328(22), 289(30), 284(100)</td>
</tr>
<tr>
<td>GA1</td>
<td>R.p.</td>
<td>20–21</td>
<td>2679</td>
<td>506(100), 491(8), 448(12), 376(15), 313(12), 235(5)</td>
</tr>
<tr>
<td></td>
<td>R.a.</td>
<td>20–21</td>
<td>2679</td>
<td>506(100), 491(8), 448(12), 376(15), 313(12), 235(5)</td>
</tr>
</tbody>
</table>

*McTMSi, methylester trimethylsilyl ether
*KRI, Kovats Retention Index
m/z, mass/charge

submergence was noticed for GAs of the non-13-OH pathway. In R. palustris, the submergence effect could be mimicked by exposing the plants for 24 h to 5 μL L⁻¹ ethylene. In particular, the 3β-hydroxylated GA₃ and its immediate precursor GA₅₀, both belonging to the 13-OH pathway, showed pronounced increase following submergence or ethylene treatment. In the flooding-intolerant R. acetosa no substantial differences in levels of GAs were detected between petiole tissues of drained, submerged or ethylene-treated plants.

Effect of flooding and ethylene on GA responsiveness. Changes in responsiveness of petiole tissue upon submergence were investigated by dose-response experiments. To prevent bias from changes in endogenous GA levels, all plants were pretreated with paclobutrazol prior to the experiments. Analysis of the dose-response curves in R. acetosa clearly showed a decreased petiole responsiveness towards GA₃ upon submergence (Fig. 2A). In contrast, in R. palustris, submergence increased petiole responsiveness tenfold by steepening the slope of the dose-response curve from 3 logarithmic units to 2 and raising the maximal response level (Fig. 2B).

To find out whether the changes in responsiveness upon submergence could be mimicked by ethylene treatment, GA₃ dose-response experiments were performed at high ethylene concentrations. In R. acetosa, ethylene reduced the response of petioles to exogenous GA₃ (Fig. 3A). However, in the flooding-tolerant R. palustris,
Ethylene and GA in plants with contrasting flooding tolerance

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The growth inhibition by GA

3

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R. palustris.

directly on the apex, was not complete. This indicates that either the application of inhibitors was insufficient or that GAs are not the sole factor determining the submergence response. Application of AgNO3, an ethylene action inhibitor, suppressed the submergence response almost completely (Voesenek et al. 1990a). This suggests that ethylene at least partly contributes directly to the growth stimulation in the flooding-tolerant R. palustris. Furthermore, the incomplete reversal of the growth inhibition by GA3 after the 6-d paclobutrazol pretreatment indicates that either GA3 application was not fully effective or that paclobutrazol also acts on other growth-related processes. A paclobutrazol-induced inhibition of ethylene biosynthesis has been reported previously (Grossman et al. 1989). However, experiments with R. palustris petioles showed no effect of paclobutrazol on ethylene production (data not shown). A shorter paclobutrazol pretreatment of 4 d in combination with GA3 treatment did restore the growth response upon submergence completely, but in this case petiole growth was not reduced to air-grown levels by the pretreatment. A similar effect of the synthetic GA-biosynthesis inhibitors 2'-isopropyl-4'-(trimethyl ammonium chloride)-5'-methylphenyl piperidene-1-carboxylate (AMO-1618) and 2-chlorooethyltrimethyl-ammonium chloride (CCC) has been reported in Ranunculus sceleratus (Samarakoorn and Horton 1983). However, in Callitriche platycarpa (Musgrave et al. 1972) and deepwater rice (Raskin and Kende 1984a), inhibition of the submergence response with GA-biosynthesis inhibitors could be completely relieved by GA3. Results from the GC-SIM analysis show that in R. palustris only GAs from the 13-OH pathway increase upon submergence or ethylene treatment. Nevertheless, GAs from the non-13-OH pathway, i.e. GA3 or GA4, were found to be active as well in petiole elongation upon submergence (Fig. 1). Application of GA1 produced similar results to GA4 (data not shown). The increased level of endogenous GAs observed in submerged R. palustris agrees with findings for deepwater rice (Suge 1985; Hoffmann-Benning and Kende 1992). These authors hypothesize that in rice ethylene acts as trigger for the GA-mediated internode elongation upon submergence. However, no determination of endogenous GA levels after ethylene treatment was performed to substantiate this hypothesis. Our findings obtained with the flooding-tolerant R. palustris prove that ethylene is indeed responsible for the increase in endogenous GAs upon submergence. This effect is species specific as is shown by the unaltered endogenous GA levels upon submergence or ethylene treatment in the intolerant R. acetosa.

In the flooding-tolerant R. palustris the difference between dose-response curves for air and 5 µL L−1 ethylene treatment indicates that a high endogenous ethylene concentration causes an increased sensitivity to GA upon submergence with respect to petiole elongation. A comparable ethylene-mediated increase in responsiveness of cells towards GA has been shown for deepwater rice (Raskin and Kende 1984b). However, in the intolerant R. acetosa, the GA3 dose-response curves for air and ethylene-treated plants showed a response opposite to that of R. palustris. In this species ethylene clearly inhibited petiole elongation and this inhibition could not be counteracted by application of GA3. This may be explained by a simultaneous, but opposite effect of GA and ethylene on the pattern of cellulose microfibril orientation. Gibberellin has been demonstrated to cause transverse orientation of microfibrils and microtubules (Mita and Katsumi 1986; Sauter et al. 1993). Ethylene, however, is known to change microfibril orientation in the longitudinal direction (Roberts et al.

Discussion

The partial dependence of submergence-induced petiole elongation on endogenous GAs in R. palustris was clearly demonstrated. Application of the GA-biosynthesis inhibitors paclobutrazol and uniconazole decreased the submergence response in R. palustris and this reduction was counteracted by application of GA3 or GA4. However, the inhibition obtained with these biosynthesis inhibitors, either applied on the soil or directly on the apex, was not complete. This indicates that either the application of inhibitors was insufficient or that GAs are not the sole factor determining the submergence response. Application of AgNO3, an ethylene action inhibitor, suppressed the submergence response almost completely (Voesenek et al. 1990a). This suggests that ethylene at least partly contributes directly to the growth stimulation in the flooding-tolerant R. palustris. Furthermore, the incomplete reversal of the growth inhibition by GA3 after the 6-d paclobutrazol pretreatment indicates that either GA3 application was not fully effective or that paclobutrazol also acts on other growth-related processes. A paclobutrazol-induced inhibition of ethylene biosynthesis has been reported previously (Grossman et al. 1989). However, experiments with R. palustris petioles showed no effect of paclobutrazol on ethylene production (data not shown). A shorter paclobutrazol pretreatment of 4 d in combination with GA3 treatment did restore the growth response upon submergence completely, but in this case petiole growth was not reduced to air-grown levels by the pretreatment. A similar effect of the synthetic GA-biosynthesis inhibitors 2'-isopropyl-4'-(trimethyl ammonium chloride)-5'-methylphenyl piperidene-1-carboxylate (AMO-1618) and 2-chlorooethyltrimethyl-ammonium chloride (CCC) has been reported in Ranunculus sceleratus (Samarakoorn and Horton 1983). However, in Callitriche platycarpa (Musgrave et al. 1972) and deepwater rice (Raskin and Kende 1984a), inhibition of the submergence response with GA-biosynthesis inhibitors could be completely relieved by GA3. Results from the GC-SIM analysis show that in R. palustris only GAs from the 13-OH pathway increase upon submergence or ethylene treatment. Nevertheless, GAs from the non-13-OH pathway, i.e. GA3 or GA4, were found to be active as well in petiole elongation upon submergence (Fig. 1). Application of GA1 produced similar results to GA4 (data not shown). The increased level of endogenous GAs observed in submerged R. palustris agrees with findings for deepwater rice (Suge 1985; Hoffmann-Benning and Kende 1992). These authors hypothesize that in rice ethylene acts as trigger for the GA-mediated internode elongation upon submergence. However, no determination of endogenous GA levels after ethylene treatment was performed to substantiate this hypothesis. Our findings obtained with the flooding-tolerant R. palustris prove that ethylene is indeed responsible for the increase in endogenous GAs upon submergence. This effect is species specific as is shown by the unaltered endogenous GA levels upon submergence or ethylene treatment in the intolerant R. acetosa.

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Fig. 3A,B. Gibberellin dose-response curves of air- and ethylene-treated R. acetosa (A) and R. palustris (B) plants. Plants were pretreated with paclobutrazol (10 mL 10−3 M) and GA3 (10 mL 0−10−3 M) for 4 d and 3 h, respectively, prior to the start of the experiment. The treatments, drained air controls (O) and ethylene (5 µL L−1, ●), lasted for 48 h. Points represent the mean (± SE) of 10 plants. Curves were fitted on individual points with Michaelis-Menten equation and Hill coefficient after subtraction of response value at [GA] = 0. Insets represent original curves. The experiment was repeated three times.

GA3 dose-response experiments at high ethylene levels showed an opposite response, i.e. ethylene enhanced the responsiveness of petioles to GA3, compared with air controls (Fig. 3B).
1985) and this will thus counteract or prevent any GA-promoted elongation. Therefore, we hypothesize that in *R. palustris* ethylene has no effect on microfibril orientation, while in *R. acetosa* ethylene causes longitudinal microfibril orientation and growth inhibition.

In conclusion, we have shown that in the flooding-tolerant *R. palustris* the enhanced petiole elongation upon submergence can now be attributed to a combination of increased GA sensitivity and increased endogenous GA levels in the petiole tissue. Our results give no indication which of these factors is determinative in petiole elongation. More important, in *R. palustris* the observed changes in GA physiology can be mimicked by application of ethylene concentrations as encountered upon submergence. For the flooding-intolerant *R. acetosa*, no effect of submergence or ethylene on GA levels could be shown. Furthermore, submergence- or ethylene-induced reduction of GA sensitivity could not be alleviated by application of GA₃, showing that in *R. acetosa* GAs are not the factors limiting petiole elongation under submerged conditions. Therefore, it is clear that high levels of ethylene have a determining effect in the submergence response related to flooding tolerance of *Rumex* species, stimulating petiole growth in one case and inhibiting it in the other.

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### References


