Ethylene accumulation in waterlogged *Rumex* plants promotes formation of adventitious roots

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Abstract

Accumulation of the gaseous plant hormone ethylene is very important for the induction of several responses of plants to flooding. However, little is known about the role of this gas in the formation of flooding-induced adventitious roots. Formation of adventitious roots in *Rumex* species is an adaptation of these plants to flooded soil conditions. The large air-spaces in these roots enables diffusion of gases between shoot and roots.

Application of ethylene to non-flooded *Rumex* plants resulted in the formation of adventitious roots. In *R. palustris* Sm. shoot elongation and epinasty were also observed. The number of roots in *R. thyrsiflorus* Fingerh. was much lower than in *R. palustris*, which corresponds with the inherent difference in root forming capacity between these two species. Ethylene concentrations of 1.5–2 μL L⁻¹ induced a maximum number of roots in both species.

Quantification of ethylene escaping from root systems of *Rumex* plants that were de-submerged after a 24 h submergence period showed that average ethylene concentrations in submerged roots reached 1.8 and 9.1 μL L⁻¹ in *R. palustris* and *R. thyrsiflorus*, respectively. Inhibition of ethylene production in *R. palustris* by L-α-(2-aminoethoxyvinyl)-glycine (AVG) or α-aminobutyric acid (AIB) decreased the number of adventitious roots induced by flooding, indicating that high ethylene concentrations may be a prerequisite for the flooding-induced formation of adventitious roots in *Rumex* species.

Key words: Adventitious roots, epinasty, ethylene, flooding, *Rumex*, shoot elongation.

Introduction

Soil flooding or waterlogging causes major changes in the conditions for growth and function of plant roots (Jackson and Drew, 1984). Gas diffusion rates in flooded soil are extremely slow (Jackson, 1985), and respiration of micro-organisms and plant roots leads to a rapid exhaustion of soil oxygen. These oxygen-deficient circumstances result in a poor aerobic root metabolism, causing energy-dependent processes such as ion uptake, root growth and secondary metabolism to cease (Jackson and Drew, 1984). Also, anaerobiosis of the soil can lead to the production of toxic compounds like Fe²⁺, Mn²⁺, sulphide, and ammonia (Ernst, 1990).

A second important effect of slow gas diffusion rates is the accumulation of gases produced in the root system. High concentrations of carbon dioxide, methane and ethane may develop in waterlogged roots. In research, special attention has been paid to the accumulation of ethylene, a powerful plant growth substance (for reviews see Reid and Bradford, 1984; Jackson, 1985; Voesenek et al., 1992). Accumulation of ethylene in plants is responsible for at least two major adaptive plant responses to flooding. Shoot elongation in wetland plants, enabling a totally submerged plant to reach the water surface, is the first adaptation in which ethylene is an important regulator (Musgrave et al., 1972; Cookson and Osborne, 1978; Voesenek and Blom, 1989). The second ethylene-mediated response is the formation of aerenchyma. This type of tissue permits enhanced oxygen diffusion from the shoot to the submerged root system, and thus decreases the hazardous effects of soil anaerobiosis (Armstrong, 1979; Blom et al., 1990; Armstrong et al., 1994). In maize, formation of aerenchyma is initiated by increased ethylene concentrations during waterlogging, or by increased ethylene sensitivity during nutrient starvation (Konings and De Wolf, 1984; Atwell et al., 1988; He et al., 1992).

Much less certain is the role of ethylene in the formation of adventitious roots, a third major morphological accommodation to flooding (Jackson, 1955; Tsukuhara and Kozlowski, 1985; Laan et al., 1989; Drew, 1992). The

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literature is contradictory on this topic, as Wample and Reid (1979) and Yamamoto and Kozlowski (1985) attribute only a minor role to ethylene in the initiation of adventitious roots, whereas an ethylene-induced enhancement of root formation was found in maize (Drew et al., 1979, Jackson et al., 1981) and deepwater rice (Bleeker et al., 1987). Also studies on rooting in cuttings showed that ethylene might have either a stimulative (e.g. Picea abies; Bollmark and Eliasson, 1990) or negative (e.g. pea; Nordström and Eliasson, 1984) effect on the formation of adventitious roots. Jackson (1985) suggested that, in flooded plants, only the outgrowth of preformed primordia might be stimulated by ethylene, whereas de novo root formation needs other stimuli. In a previous publication it was shown that auxin is a strong enhancer of adventitious root formation in Rumex species. Application of auxin induced adventitious roots in non-flooded plants of both R. palustris, a species capable of developing large numbers of adventitious roots, and R. thrysiflorus, a poor-rooting species (Visser et al., 1995), although the difference in the number of adventitious roots between the species remained. It is well known that application of high concentrations of auxin increases ethylene production in many plants (Imaseki et al., 1977; Dubucq et al., 1978; Kelly and Bradford, 1990). Therefore, the response that was found in auxin-treated plants might be attributed to a higher endogenous ethylene level. Furthermore, Voesenek et al. (1990a) showed that ethylene production of waterlogged R. palustris plants increases dramatically. The high ethylene concentrations resulting from this increase in production had a distinct effect on the leaf and petiole elongation of this species, but the effects on the formation of adventitious roots are as yet unknown.

This study aimed to determine if ethylene plays a relevant role in the flooding-induced formation of adventitious roots. Two species of the genus Rumex were used for the experiments. Formation of adventitious roots enables survival of most Rumex species during soil flooding, although the extent of root formation greatly varies between species (Laan et al., 1989). Therefore, R. palustris, a wetland species that forms many adventitious roots, was compared with R. thrysiflorus, a species of rarely flooded sites that develops only a few roots upon waterlogging. The approach described by Jacobs (1959), who proposed a number of criteria for hormone-regulated processes, was followed. First, the effects of ethylene application to Rumex plants were compared with the effects of flooding on adventitious rooting and other morphological features of the plants. Then, ethylene concentrations in waterlogged root systems of Rumex species were measured to investigate if response-evoking concentrations could actually be found during flooded conditions. Finally, inhibitors of ethylene synthesis were used to determine if an increased ethylene concentration is a prerequisite for a complete rooting response during flooding.

Materials and methods

Plant growth

Seeds of Rumex palustris and Rumex thrysiflorus were collected in the river area near Nijmegen, The Netherlands. For soil culture, seeds were germinated on moist filter paper in a Petri dish (16 h light, 27°C, 20 μmol m⁻² s⁻¹ PPFD (Philips TL33); 8 h dark, 10°C). After 1 week, seedlings were transplanted to small plastic pots (diameter 55 mm) filled with a mixture of potting compost and sand (1:1; v/v), and raised in a growth room (20°C; 16 h light, 100 μmol m⁻² s⁻¹ PPFD (Philips TL84); 8 h dark) for 2 weeks. Plants were then transferred to larger pots (diameter 120 mm) and grown in the greenhouse (temperatures between 15°C and 25°C; 16 h light, minimum 200 μmol m⁻² s⁻¹ PPFD, maximum 1200 μmol m⁻² s⁻¹ PPFD; 8 h dark) for 5 weeks.

For hydroponic culture, seeds were germinated in trays filled with black polyethylene grains (Lacqten Low Density grains, Elf Atochem, France) soaked in nutrient solution (2 mM Ca(NO₃)₂, 1.25 mM K₂SO₄, 0.5 mM MgSO₄, 0.5 mM KH₂PO₄ and the micronutrients FeEDTA (90 μM), NaCl (50 μM), H₃BO₃ (25 μM), MnSO₄ (2 μM), ZnSO₄ (2 μM), CuSO₄ (0.5 μM) and H₂MoO₄ (0.5 μM)). The trays were placed in a growth cabinet (16 h light, 27°C, 20 μmol m⁻² s⁻¹ PPFD (Philips TL33); 8 h dark, 10°C) for 1 week, and then transferred to a growth room (22°C; 16 h light, 120 μmol m⁻² s⁻¹ PPFD (Philips TL84); 8 h dark).

Effects of ethylene on soil-grown plants

Uniform plants of R. palustris were selected and placed in glass containers (310 × 200 × 320 mm; control, submergence and waterlogging treatments) or in desiccators (diameter 260 mm; ethylene treatments). The water levels in the submergence and waterlogging treatments were kept at 300 mm from the bottom (plants totally submerged) and at the soil surface, respectively. In the ethylene and control treatments, a 20 mm layer of water in the containers, not reaching the bottom of the pots, provided a sufficiently high air humidity. All containers and desiccators were made airtight with silicon grease. Different ethylene concentrations in the desiccators were obtained by injecting appropriate amounts of pure ethylene (Hoekloos, Dieren, The Netherlands). Concentrations of ethylene were monitored throughout the duration of the experiment by taking gas samples, which were measured on a Chrompack 437A gas chromatograph (Chrompack, Bergen op Zoom, The Netherlands; 1.2 m × 2 mm-column Haysep QS). Ethylene was added to the desiccators if concentrations decreased more than 10% below the required level, which was usually once a day. After 6 d, plants were harvested and the length of the longest leaf, the number of epinastic leaves and the number of adventitious roots were determined.

Effects of ethylene on hydroponically-grown plants

Eight or ten plants of R. palustris (5-weeks-old) or R. thrysiflorus (7-weeks-old) were transferred to rafts of polystyrene foam on 10 l containers filled with nutrient solution. Air, or air mixed with ethylene, was flushed through the container using a bubble stone (flow rate 60 l h⁻¹). The required ethylene concentrations were obtained by mixing air and 50 μl l⁻¹ ethylene in air (Hoekloos, Dieren, The Netherlands) with a gas...
blender (HI-TEC type E55N3, Bronkhorst HIGH TECH, Ruurlo, The Netherlands). Concentrations of ethylene in the nutrient solution were monitored by placing a small Petri dish at the bottom of the containers with 2 ml of air trapped under it. After 1 h, gas samples were taken and analysed on the gas chromatograph; the concentration in this air bubble never deviated more than 5% from the ethylene concentration applied. Waterlogging was mimicked by placing a raft with plants on a container filled with stagnant liquid agar solution (0.1%, w/v; nutrient concentration as described before) that had been flushed with nitrogen 18 h prior to treatment (Visser et al., 1995). After 7 d, the number of adventitious roots was determined. These roots started to emerge after 2 d and were mainly found at the junction of the tap root and the shoot. Although the maximum number of roots (evoked by the stagnant agar treatment) differed between batches of plants, the relative differences between treatments remained similar through all experiments (Visser et al., 1995).

**Endogenous ethylene concentrations**

The root system of an intact plant of either *R. palustris* (5-weeks-old) or *R. thyrsiflorus* (7-weeks-old) was placed in a 0.5 l cuvette filled with a stagnant liquid agar solution (nutrient concentrations as described before), which had been flushed vigorously with nitrogen for 1 h prior to the experiment; the cuvette was made airtight at the root-shoot junction with plasticine. After 24 h, during which water losses resulting from transpiration were compensated for, the shoot was removed from the root system with a razor blade and the tap root was sealed with plasticine. The agar solution was then forced out through an outlet at the bottom of the cuvette by flushing the cuvette with a flow of nitrogen gas (101 h⁻¹) through an inlet at the top of the cuvette. At the moment that all agar had been driven out, the outlet flow was connected to a laser-driven photo-acoustic cell (details on this measuring method described in Harren et al., 1990). Ethylene entrapped in the root system during the stagnant hypoxic period of 24 h was released into the nitrogen stream and detected on the photo-acoustic cell, visualized by a large peak (Fig. 1). The surface under this peak was calculated, being the total amount of endogenous ethylene. Then, the air volume and total volume of the root system were determined with a pycnometer, using the method of Jensen et al. (1969).

The ethylene concentration in the internal air-spaces was calculated by dividing the released amount of ethylene by the internal air volume, and correcting for ethylene dissolved in the liquid fraction of the root tissue. For this correction the solubility of ethylene in plant tissue was assumed to be approximately equal to the solubility in water, and the concentration of ethylene dissolved in the plant tissue to be in equilibrium with the ethylene concentration in the air spaces. This means that at equal volumes, 9 times as much ethylene is present in air as in plant tissue. Ethylene production during the measurement was not likely to occur, since the nitrogen atmosphere would have prevented oxidation of ACC into ethylene (Vooseneke et al., 1993). Moreover, following the peak of entrapped ethylene (usually 1.5 h) no further ethylene release could be detected, and only when air instead of nitrogen was flushed through the cuvette, did ethylene production resume (insertion in Fig. 1).

**Inhibitor treatment**

Four-week-old plants of *R. palustris* were placed on polystyrene rafts (six plants per raft), floating in a 20 l container filled with either 101 (for AVG treatments) or 181 (for AIB treatments) aerated nutrient solution or stagnant liquid agar solution (de-oxygenated with nitrogen gas for 18 h prior to treatment; 0.1% agar, w/v; nutrient concentrations as in the nutrient solution). Various concentrations of the ethylene synthesis inhibitors AVG and AIB and the ethylene precursor ACC were dissolved in the nutrient or agar solution.

**Wound ethylene production**

Five-week-old *R. palustris* plants were treated for 18 h with different AIB or AVG concentrations (as described before). Then, 1 g samples of roots of treated and untreated plants were cut into pieces of approximately 2 mm and placed in sealed 10 ml serum vials with 0.5 ml water. After 24 h, gas samples were drawn from the vials and analysed on a gas chromatograph to determine wounding-induced ethylene production.

**Results**

The morphology of waterlogged and totally submerged *R. palustris* plants differed considerably from drained plants (Table 1). The length of the longest leaves was greater, and the number of adventitious roots increased substantially. Treatment of drained plants with various concentrations of ethylene partially (adventitious root formation) or fully (shoot elongation) mimicked the effects of flooding. A concentration of 1–3 µl 1⁻¹ ethylene evoked the maximum number of adventitious roots, whereas 10 µl 1⁻¹ was needed for the greatest response in leaf elongation. An additional effect of ethylene treatment was a downward bending of some of the leaf blades and

![Fig. 1. Time-course of ethylene release from the root system of *R. thyrsiflorus* (solid line) and *R. palustris* (dashed line) when de-submerged at (1) in a nitrogen atmosphere 24 h after transfer to stagnant de-oxygenated agar. Insert: De-submergence of a *R. thyrsiflorus* root system in a nitrogen atmosphere (1), and replacing the nitrogen by air at (2). Age of the plants 5 weeks (*R. palustris*) or 7 weeks (*R. thyrsiflorus*).](image-url)
Table 1. Morphological parameters of drained, waterlogged, totally submerged and ethylene-treated soil-grown R. palustris plants

Age of the plants 8 weeks, duration of the treatment 6 d; n = 6, SEs are given between brackets.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Length of longest leaf (cm)</th>
<th>Number of epinastic leaves</th>
<th>Number of adventitious roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (drained)</td>
<td>14.0 (0.9)</td>
<td>0.0 (0.0)</td>
<td>4.8 (0.6)</td>
</tr>
<tr>
<td>Waterlogging</td>
<td>17.0 (0.8)</td>
<td>0.0 (0.0)</td>
<td>20.8 (2.5)</td>
</tr>
<tr>
<td>Total submersion</td>
<td>19.9 (3.3)</td>
<td>0.0 (0.0)</td>
<td>23.2 (1.8)</td>
</tr>
<tr>
<td>0.5 μl Ethylene</td>
<td>15.3 (0.4)</td>
<td>0.8 (0.8)</td>
<td>8.8 (1.3)</td>
</tr>
<tr>
<td>1 μl Ethylene</td>
<td>16.2 (0.5)</td>
<td>2.8 (0.5)</td>
<td>12.3 (2.4)</td>
</tr>
<tr>
<td>3 μl Ethylene</td>
<td>18.8 (0.5)</td>
<td>2.5 (0.5)</td>
<td>16.0 (3.1)</td>
</tr>
<tr>
<td>10 μl Ethylene</td>
<td>21.1 (0.8)</td>
<td>4.0 (1.4)</td>
<td>12.5 (1.6)</td>
</tr>
</tbody>
</table>

Hydroponically grown R. palustris plants that were placed on a stagnant de-oxygenated agar solution for 1 week developed a great number of adventitious roots (Fig. 2), in contrast to normally aerated plants, which hardly formed any adventitious roots (Fig. 2; O. thysiflorus). Aerated plants placed on nutrient solution bubbled with various concentrations of ethylene also demonstrated increased formation of adventitious roots, with a maximum response at 2 μl L⁻¹ and higher concentrations. R. thyrsiflorus showed a similar pattern, although the maximum number of roots in this species was much lower than in R. palustris (Fig. 2). This difference in root formation was not due to a deficiency in ethylene, since in R. thyrsiflorus the response also appeared to be saturated at 2 μl L⁻¹ ethylene.

To examine whether the high response-saturating ethylene concentrations were realistic physiological concentrations in waterlogged root systems, ethylene was extracted from the roots of R. palustris and R. thyrsiflorus plants that had been placed on stagnant de-oxygenated agar. Conventional extraction procedures may cause unpredictable losses of ethylene (Voosenk et al., 1993), especially when applied to the fine lateral roots of Rumex species. Therefore, a new procedure based on a highly sensitive photo-acoustic cell was developed that allowed the measurement of the small amounts of ethylene present in the root system. The endogenous ethylene concentration could be derived from this total amount of ethylene and the volume of gas and tissue in the roots. Average ethylene concentrations up to 1.8 μl L⁻¹ (R. palustris) and 9.1 μl L⁻¹ (R. thyrsiflorus) were detected in the root systems of plants that had been placed on stagnant de-oxygenated agar for 24 h (Table 2). The variation between ethylene concentrations was only small, especially in R. palustris, although plants considerably differing in size and thus in internal air volume were used.

To separate the effect of high endogenous ethylene concentrations and other factors related to the stagnant oxygen-deficient conditions, the use of an effective inhibitor of ethylene biosynthesis was essential. Three commonly used inhibitors, AOA, propyl gallate and cobalt chloride, appeared to be toxic or without effect on ethylene production of Rumex leaf and root tissues (data not shown). However, when either AIB or AVG was applied to the nutrient solution of R. palustris plants, the production of wound ethylene by cut pieces of root tissue was greatly inhibited (Table 3).

Application of high concentrations of AIB to roots of R. palustris plants placed on stagnant de-oxygenated agar inhibited the formation of adventitious roots to about 50% of the initial difference between control and hypoxic plants (Fig. 3). Also AVG suppressed adventitious root formation of R. palustris in agar (Fig. 4). Inhibition by low AVG concentrations (10⁻⁶ M) was only limited, but this effect could be fully counteracted by application of

Table 2. Amounts of endogenous ethylene, air volumes and ethylene concentrations in individual root systems of hydroponically grown R. palustris and R. thyrsiflorus plants that were placed on a de-oxygenated liquid agar solution for 24 h

Age of the plants 5 weeks (R. palustris) or 7 weeks (R. thyrsiflorus).

<table>
<thead>
<tr>
<th>Species</th>
<th>Air volume (μl)</th>
<th>Amount of ethylene (μl)</th>
<th>Concentration of ethylene (μl L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. palustris</td>
<td>121</td>
<td>0.28</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>269</td>
<td>0.51</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>257</td>
<td>0.37</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>251</td>
<td>0.40</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>539</td>
<td>0.91</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Average (±SE)</td>
<td>1.8 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>R. thyrsiflorus</td>
<td>57</td>
<td>0.22</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>202</td>
<td>1.80</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>192</td>
<td>1.54</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>197</td>
<td>3.09</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>Average (±SE)</td>
<td>9.1 ± 2.5</td>
<td>9.1 ± 2.5</td>
</tr>
</tbody>
</table>
Table 3. Amounts of ethylene produced by wounded root tissue of hydroponically grown R. palustris plants treated with either AIB or AVG in the nutrient solution 18 h prior to cutting.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Amount of ethylene after 24 h (nl g(^{-1}) FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no inhibitor)</td>
<td>28.3 ± 4.6 (100%(a))</td>
</tr>
<tr>
<td>AIB 1.5 × 10(^{-3}) M</td>
<td>3.1 ± 0.2 (11%(a))</td>
</tr>
<tr>
<td>Control (no inhibitor)</td>
<td>13.5 ± 0.7 (100%(a))</td>
</tr>
<tr>
<td>AVG 10(^{-6}) M</td>
<td>0.9 ± 0.1 (6%(a))</td>
</tr>
<tr>
<td>AVG 10(^{-5}) M</td>
<td>0.9 ± 0.1 (6%(a))</td>
</tr>
</tbody>
</table>

Fig. 3. Number of adventitious roots in hydroponically grown R. palustris plants, 1 week after transfer to normally aerated conditions (control), stagnant de-oxygenated agar or stagnant de-oxygenated agar with various concentrations of AIB. Age of the plants 4 weeks; \(n=6\), bars indicate SEs.

Fig. 4. Number of adventitious roots in hydroponically grown R. palustris plants, 1 week after transfer to normally aerated conditions (control), stagnant de-oxygenated agar or stagnant de-oxygenated agar with or without various concentrations of AVG and 10\(^{-4}\) M ACC. Age of the plants 4 weeks; \(n=6\), bars indicate SEs.

The ethylene precursor ACC. When AVG concentrations were higher (10\(^{-5}\) M), inhibition of adventitious rooting increased considerably, but at these concentrations ACC could not completely overcome the inhibitory effects of AVG.

**Discussion**

Elongation of the shoot (Table 1) is an obvious and well-known effect of submergence on the morphology of the shoot of *R. palustris* (Voesenek and Blom, 1989; Banga et al., 1995). This effect has also been described for a number of other plant species, such as deepwater rice (Bleecker et al., 1987) and *Callitriche platycarpa* (Musgrave et al., 1972). Voesenek et al. (1990b) showed that the elongation response in *Rumex* is mainly caused by cell elongation in the petioles of the leaves. The benefit of this enhanced shoot growth is restoration of contact between the plant and the atmosphere during total submergence. In *Rumex*, petiole elongation is induced primarily by high ethylene concentrations in the shoot, which build up because of the low ethylene efflux from the shoot during totally submerged conditions. Application of high ethylene concentrations could, therefore, evoke the same elongation response in drained plants (Table 1). Waterlogging also caused enhancement of shoot elongation, not by entrapment of ethylene in the shoot, but probably by an increased diffusion of ethylene and transport of ACC, the direct precursor of ethylene, from the roots to the shoot (Bradford and Yang, 1980; Voesenek et al., 1990a). In tomato, this transport of ethylene and ACC from root to shoot is responsible for another morphological response of the plant to waterlogging, i.e. epinasty of the leaves (Jackson and Campbell, 1975). This downward bending of the leaves was found in *R. palustris* plants that were treated with high ethylene concentrations, but not in the totally submerged or waterlogged plants (Table 1). The reason why this ethylene-specific response does not occur in submerged plants is not clear yet, but unpublished work at our department indicates that other factors, such as low oxygen concentrations in the shoot, may interfere with this response.

The formation of adventitious roots was strongly enhanced by both flooding and ethylene treatment (Table 1). The effect of ethylene was even more clear in the larger hydroponically grown plants, in which ethylene application was restricted to the root system (Fig. 2). Both *R. palustris*, a species that develops many adventitious roots during waterlogging (Visser et al., 1995) and *R. thrysiflorus*, a poor-rooting species (Laan et al., 1989; Visser et al., 1995), showed increased numbers of adventitious roots at all applied ethylene concentrations. Even concentrations as low as approximately 0.5 \(\mu l l^{-1}\) induced more adventitious roots compared to non-treated plants, and concentrations of 2 \(\mu l l^{-1}\) caused initiation of the
same number of roots as stagnant oxygen-deficient conditions (Fig. 2), which are representative of waterlogged conditions (Visser et al., 1995). This agrees with results of Bleecker et al. (1987), who found a similar range of root-inducing ethylene concentrations in deepwater rice. Jackson et al. (1981) found that application of 5 \( \mu l \) \( l^{-1} \) ethylene promoted adventitious root formation in maize as well. Also consistent with this work is that, in contrast to this promoting effect on root number, ethylene appeared to retard the growth rate of adventitious roots (data not shown). This is a commonly observed phenomenon (Konings and Jackson, 1979; Etherington, 1983), but reduction of root growth was apparently not strong enough to prevent root development completely.

Although the active concentrations of ethylene were very small in an absolute sense, these levels are about two to three orders of magnitude higher than atmospheric ethylene concentrations \( (c. 0.005 \mu l \ l^{-1}) \). Therefore, it might be questioned whether these high concentrations actually occur in waterlogged plants. For submerged shoots, Voesenek et al. (1993) found concentrations up to 4.4 \( \mu l \) \( l^{-1} \), but as far as we know, no accurate estimates of endogenous ethylene concentrations in waterlogged root systems are yet available. This is mainly due to the practical problems that accompany such measurements, in particular, the loss of ethylene to the extraction solution or atmosphere, the production of ethylene during the measurements and the small amounts of ethylene per measurement. In our opinion, these problems were overcome by measuring in an airtight cuvette (no losses), under a nitrogen atmosphere (no production) and with a very sensitive detector (photo-acoustic cell; detection limit 0.05 nl \( l^{-1} \)). Twenty-four hours after the plants were placed on a stagnant agar solution, the concentrations of endogenous ethylene in both species were well within the range of concentrations that induced adventitious root formation \( (1.8 \mu l \ l^{-1} \ and \ 9.1 \mu l \ l^{-1} \) in \( R. palustris \) and \( R. thyrsiflorus \), respectively; Table 2). These high levels obviously result from entrapment of ethylene in the roots due to the low diffusion rate from the root tissues to the rhizosphere. Because the lateral roots of \( R. palustris \) are connected to the shoot by aerenchyma channels (Laan et al., 1989), a part of the ethylene produced in the roots might escape via this route. Aerenchyma in the primary lateral roots of \( R. thyrsiflorus \) is far less well-developed (Laan et al., 1989), which might explain the higher concentrations of ethylene in this species. On the other hand, the possibility can not be excluded that ethylene production in \( R. thyrsiflorus \) is higher than in \( R. palustris \), or that feedback inhibition of ethylene biosynthesis prevents further accumulation in \( R. palustris \).

Experiments with specific inhibitors of ethylene biosynthesis provided more insight into the role that ethylene plays in the induction of adventitious roots in \( R. palustris \) species. Both AIB, which is an inactive analogue of ACC and competes for binding to ACC oxidase, and AVG, which inactivates ACC synthase (Yang and Hoffman, 1984), inhibited the production of wound ethylene in damaged leaf tissue (Table 3). Since ethylene production rates due to wounding are much higher than during drained or submerged conditions, it was assumed that an inhibitor capable of suppressing this response would also be effective in decreasing ethylene production in intact plants. When applied to \( R. palustris \) plants in stagnant agar, both inhibitors decreased the number of adventitious roots that had been formed by 7 d (Figs 3, 4), indicating that ethylene production during waterlogging may well be a prerequisite for maximum induction of these roots. The inhibitory effect of AVG could be counteracted by simultaneous application of ACC, although this effect was only complete at AVG concentrations of \( 10^{-6} \) M. Higher concentrations of AVG gave a significantly stronger inhibitory effect on root formation. Unfortunately, at these concentrations some unspecific effects on root development occurred, as the inhibitory effect on rooting could not be fully alleviated by ACC (Fig. 4). Also, ACC could not be used to overrule the AIB-induced inhibition of ethylene biosynthesis, since it would require extremely high ACC-concentrations to compete effectively with the high \( (10^{-3} \) M) AIB-concentrations applied. These ACC-concentrations would definitely cause unspecific effects, as ACC appeared to cause stunted root growth at a concentration of \( 10^{-4} \) M already.

It is concluded that increased ethylene concentrations stimulate the induction of adventitious root formation in waterlogged \( R. palustris \) plants. Whether ethylene acts directly on the formation of adventitious roots, or has an effect on the levels or perception of other growth inhibitors is not clear yet. Earlier experiments with \( R. palustris \) showed that auxin, too, was a very effective promoter of adventitious root formation in intact plants, and that shoot-borne auxin appeared to be essential for adventitious rooting during waterlogged conditions (Visser et al., 1995). This role of auxin might be mediated by ethylene, since numerous papers have reported on stimulated ethylene production of plant tissue upon treatment with auxin (Imaseki et al., 1977; Dubucq et al., 1978; Kelly and Bradford, 1990). On the other hand, auxin transport (Suttle, 1988), metabolism (Beyer and Morgan, 1970) and the sensitivity of plant tissues to this hormone (Bertell et al., 1990) can be changed by high concentrations of ethylene. Each of these processes might explain the similar responses of \( R. palustris \) plants to auxin and ethylene treatments. Further investigations will, therefore, focus on the interactions between these two plant hormones.

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