Elongation by primary lateral roots and adventitious roots during conditions of hypoxia and high ethylene concentrations

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ABSTRACT

Soil flooding results in unusually low oxygen concentrations and high ethylene concentrations in the roots of plants. This gas composition had a strongly negative effect on root elongation of two Rumex species. The effect of low oxygen concentrations was less severe when roots contained aerenchymatous tissues, such as in R. palustris Sm. R. thyrsiflorus Fingerh., which has little root porosity, was much more affected. Ethylene had an even stronger effect on root elongation than hypoxia, since very small concentrations (0-1 cm$^3$ m$^{-3}$) reduced root extension in the two species, and higher concentrations inhibited elongation more severely than did anoxia in the culture medium. Thus, ethylene contributes strongly to the negative effects of flooding on root growth. An exception may be the highly aerenchymatous, adventitious roots of R. palustris. Aerenchyma in these roots provides a low-resistance diffusion pathway for both endogenously produced ethylene and shoot-derived oxygen. This paper shows that extension by roots of R. palustris in flooded soil depends almost completely on this shoot-derived oxygen, and that aerenchyma prevents accumulation of growth-inhibiting levels of ethylene in the root.

Key-words: Rumex palustris Sm.; Rumex thyrsiflorus Fingerh.; Polygonaceae; aeration; aerenchyma; diffusion; ethylene; flooding; oxygen; root elongation.

INTRODUCTION

Root growth is affected by many environmental factors and is usually very sensitive to conditions that stress the plant, such as oxygen shortage. In the field, hypoxic and anoxic conditions readily occur in roots that grow in water-saturated soil. The diffusion of gases from the atmosphere to the soil and vice versa is severely hampered during soil flooding because of the slow diffusion rate of gases in water. Under these conditions, aerobic respiration of plant roots and micro-organisms very quickly reduces soil oxygen concentrations (Drew 1992). Plant roots may survive flooding by the development of several morphological, anatomical and metabolic adaptations (Jackson & Drew 1984; Blom et al. 1994). These adaptations enable either anaerobic respiration of the roots or oxygen diffusion from non-submerged or photosynthesizing tissues to the root system, allowing the roots to grow in the hostile environment of an anaerobic soil. However, the primary lateral root system of most terrestrial plants cannot develop effective adaptations, and is thus very susceptible to long-term anaerobiosis (Laan, Clement & Blom 1991). In a variety of species, formation of new, adventitious roots can be found when the plants are soil flooded (reviewed by Jackson & Drew 1984). These adventitious roots often contain more aerenchyma than the primary lateral roots and are thus supposed to be less affected by anaerobic soil conditions.

In our study, we used two species differing greatly in resistance to flooding and in the extent of adventitious root formation under flooded conditions. Rumex thyrsiflorus is a non-tolerant species with a very low porosity (volume of air per volume of tissue) in the primary lateral roots. This species develops few adventitious roots in response to soil flooding, but these new roots contain almost no aerenchyma (Laan et al. 1989; Visser, Blom & Voesenek 1996a). In contrast, the closely related and flooding-resistant species Rumex palustris possesses primary lateral roots with a considerable amount of aerenchyma, and responds to soil flooding by forming adventitious roots with even higher porosity. The elongation by these adventitious roots appears to be almost undiminished by flooded soil conditions (Visser et al. 1996a).

The slow diffusion rate of gases in a flooded soil not only reduces the availability of oxygen, but also causes entrapment of gases that are produced in roots (Jackson 1985). The gaseous hormone ethylene is biosynthesized in very small amounts, but endogenous concentrations can increase 500-fold in soil-flooded conditions (Visser et al. 1996b). This hormone is known to have a negative effect on root elongation, even at relatively low concentrations (Konings & Jackson 1979). We therefore hypothesized that accumulation of ethylene (and possibly also accumulation of other gases, such as carbon dioxide) in a flooded root system may be as injurious to root growth as are low oxygen concentrations. The following experiments were performed to test this hypothesis and to find an explanation for the relatively high elongation rates of adventitious roots of R. palustris in flooded soil. To compare the negative effects of low oxygen concentrations and high ethylene or carbon dioxide concentrations, the elongation rate of...
primary lateral roots of *R. thyrsiflorus* and *R. palustris* was measured under various concentrations of oxygen, carbon dioxide and ethylene. The effect of exogenously applied ethylene on the elongation by adventitious roots of *R. palustris* was compared with the effect on primary lateral root growth. This revealed that the high resistance of these adventitious roots to flooding was not caused by an inherent insensitivity of the tissue to high ethylene concentrations. Therefore, we determined the extent to which diffusion of gases such as oxygen and ethylene was facilitated by the aerenchyma channels in adventitious roots, thus preventing oxygen deficiency or ethylene accumulation. For these measurements, a cuvette was designed that enabled us to measure ethylene diffusion rates within a single root.

**MATERIALS AND METHODS**

**Plant growth**

Seeds of *R. palustris* Sm. and *R. thyrsiflorus* Fingerh., collected from natural populations in the floodplain of the river Waal near Nijmegen (the Netherlands), were sown on polyethylene grains (Lactene Low Density, Elf Atochem, France) that were soaked in nutrient solution [2 mol m\(^{-3}\) Ca(NO\(_3\))\(_2\), 1.25 mol m\(^{-3}\) K\(_2\)SO\(_4\), 0.5 mol m\(^{-3}\) MgSO\(_4\), 0.5 mol m\(^{-3}\) KH\(_2\)PO\(_4\) and the micronutrients FeEDTA (15 mmol m\(^{-3}\)), NaCl (50 mmol m\(^{-3}\)), H\(_2\)BO\(_3\) (25 mmol m\(^{-3}\)), MnSO\(_4\) (2 mmol m\(^{-3}\)), ZnSO\(_4\) (2 mmol m\(^{-3}\)), CuSO\(_4\) (0.5 mmol m\(^{-3}\)) and H\(_2\)MoO\(_4\) (0.5 mmol m\(^{-3}\))]. After germination [1 week at 16 h, 20 μmol m\(^{-2}\) s\(^{-1}\) PPFD (Philips TL33), 27 °C; 8 h dark, 10 °C], the seedlings were placed in a climate room for 2 weeks [16 h, 120 μmol m\(^{-2}\) s\(^{-1}\) PPFD (Philips TL84), 22 °C and 8 h dark, 20 °C; relative humidity 50%] and then transferred to hydroponic culture. The hydroponic system consisted either of three 20 dm\(^3\) containers connected to a 30 dm\(^3\) vessel, through which nutrient solution circulated at a rate of 13 dm\(^3\) h\(^{-1}\), or of single 20 dm\(^3\) containers filled with nutrient solution. In both systems, six to eight plants were mounted in polystyrene rafts, and the nutrient solution was aerated by flushing air through bubble stones at a rate of 60 dm\(^3\) h\(^{-1}\).

**Measurement of root elongation rates**

One or two plants of either *R. palustris* or *R. thyrsiflorus* were placed on a small glass cuvette (10 × 10 × 15 cm), which was placed on top of a 1.5 dm\(^3\) reservoir. All sides except the front of the cuvette were covered with black tape. Up to eight lateral roots were led into capillary glass tubes (internal diameter 1 mm for primary lateral roots, 2 mm for adventitious roots) that were mounted at the inner side of the transparent front of the cuvette. These tubes protruded through the bottom of the cuvette, thus permitting nutrient solution, which was pumped from the reservoir into the cuvette at a rate of 1 dm\(^3\) min\(^{-1}\), to flow back into the reservoir. The surplus of nutrient solution left the cuvette via an overflow outlet that was connected to the reservoir. This system allowed elongation rates of a number of intact roots on each plant to be determined. During each experiment, a continuous flow of nutrient solution was flushed along the roots in the capillaries, and various concentrations of oxygen or ethylene could be applied to the roots by flushing the desired gas concentration through the nutrient solution in the reservoir. At the start of an experiment, the nutrient solution was flushed with air through a bubble stone. After an acclimatization period of 1 h, the exact vertical position of the root tip was determined at intervals of 15–30 min using a Vernier travelling microscope (accuracy ± 0.02 mm). The front of the cuvette was covered with a black plastic sheet in the intervals between measurements. The rate of elongation by each individual root was calculated from regression of at least six measurements (Fig. 1). Series of measurements with a regression coefficient of 0.95 or lower were not used in the data presented. After determining these ‘control elongation rates’, aeration was switched to flushing with either a mixture of air with ethylene, carbon dioxide or nitrogen, or pure nitrogen gas at a rate of 1 dm\(^3\) min\(^{-1}\). The concentrations (or indications such as ‘anoxic’) referred to in the text apply to the gas concentrations measured in the nutrient solution. Gas mixtures were either prepared with a gas blender (model E55N3, Hi Tec, Ruurlo, The Netherlands), using pressurized air, nitrogen and 50 cm\(^3\) m\(^{-3}\) ethylene in air from a gas cylinder (Hoekloos, Dieren, The Netherlands), or were obtained pre-mixed from a commercial source (Hoekloos, Dieren, The Netherlands). Measurements with a Consort oxygen-sensitive electrode showed that, within less than 30 min, oxygen concentrations in the nutrient solution equalled those of the gas mixtures that were applied. Therefore, measurements of the vertical position of the root tips resumed 30–60 min after switching gas concentrations (Fig. 1). Again, at least six measurements per root were used for calculating extension rates. For determining the effect of internal aeration on root elongation, a glass 5 dm\(^3\) compartment, wrapped in a black plastic sheet and covering the shoot, was placed on top of the cuvette and flushed
with various concentrations of oxygen. The effect of all treatments was expressed as a percentage of the control elongation rate of each individual root.

**Measurements of ethylene diffusion rates**

Segments with a length of either 50 or 95 mm were cut from the basal part of adventitious roots with a minimum length of 120 mm (diameter = 1.4 mm). A segment was placed in a glass cuvette, consisting of four compartments (8 cm$^3$ each) that were connected to each other by a small aperture (diameter 2 mm; see Fig. 2A). Either three (50 mm segments) or four (95 mm segments) compartments were used, with the root segments protruding for no more than 2 mm into the outermost compartments. These two outermost compartments were closed with a rubber septum, while warm agar (3% w/v, temperature 40 °C) was poured into the interstices between the compartments to prevent gas diffusion other than that within the root. After the agar had set, the middle compartments were filled with water. An inlet syringe needle, connected to a flow of ethylene-free air, and an outlet syringe needle, connected to a photoacoustic ethylene detector (Voesenek et al. 1990), were pushed through the septum of one of the outermost compartments. The basal production of ethylene by the root was measured for 1.5 h (Fig. 2b), whereafter an exact volume of 50-60 cm$^3$ m$^{-3}$ ethylene was injected into the opposite outermost compartment of the cuvette. At the same moment, an equal volume of gas was carefully withdrawn from this compartment to prevent an increase in pressure. After measuring the release of ethylene from the root segment for 1.5 h, the segment was squeezed in the middle with a pair of tweezers to block diffusion through the aerenchyma of the root. Ethylene release was then measured for another 0.5 h (Fig. 2b) to check whether any ethylene had leaked between the compartments. The ethylene diffusion rate was defined as the difference between the basal ethylene production (0–1.5 h) and the maximum ethylene release rate following application of ethylene to the opposite compartment (1.5–3 h). To confirm that ethylene release was due to diffusion through the root and not due to mass flow induced by differences in pressure between the compartments of the cuvette, we evaluated the kinetics of such a pressure-induced release of ethylene (Fig. 2c). The increase in pressure caused by injecting 1 cm$^3$ of air, without withdrawing a similar volume of gas from the compartment, resulted in a fast increase in ethylene release at the other side of the root segment. However, this increase was only temporary, and within 0.5 h ethylene release slowed down to the initial level. Consequently, such pressure differences cannot be responsible for the steady, increased ethylene release observed during regular diffusion measurements.

**RESULTS**

Exogenous oxygen concentrations below 5% severely decreased the rate of elongation by primary lateral roots of both *R. palustris* and *R. thyrsiflorus* (Fig. 3). However, roots of *R. thyrsiflorus* were much more strongly affected by these oxygen-deficient conditions than roots of *R. palustris*. Total absence of oxygen in the nutrient solution did not completely inhibit root elongation in these species; average root extension rates were 46 and 22% of those in 21% oxygen for *R. palustris* and *R. thyrsiflorus*, respectively. Oxygen concentrations between 5 and 10% had only a minor negative effect on root elongation.
A carbon dioxide concentration 300 times that of ambient air slowed elongation by primary lateral roots to a small extent (Table 1). When carbon dioxide-free air was flushed through the nutrient solution, a slight enhancement of growth was observed compared to the elongation rates of roots grown in nutrient solution flushed with normal air (0-03% carbon dioxide).

In contrast, ethylene retarded elongation by primary roots in both R. palustris and R. thyrsiflorus at concentrations as low as 0-1–0-3 cm−3 m−3 (Fig. 4). Remarkably, the growth inhibition of R. palustris roots at ethylene concentrations up to 1 cm−3 m−3 was greater than that of R. thyrsiflorus roots. The greatest inhibitory effects were found at high ethylene concentrations (c. 5 cm−3 m−3), which decreased root elongation of both species to about 15–25% of control elongation rates. These measurements were usually made during the first 2h of ethylene treatment. The ‘long-term’ effects of high ethylene concentrations were even stronger. Elongation by R. palustris primary roots that were treated with 25 cm−3 m−3 ethylene decreased to an average of 20-8% (SE ± 2-5%; n = 7) of the control elongation rate during the first 2h, but declined further to 12-1% (SE ± 1-7%; n = 5) of control rates during the following 2h.

The strong inhibition of high ethylene concentrations was little changed when combined with low oxygen concentrations (Table 2). Clearly, oxygen concentrations as low as 0-5% did not slow root elongation rates above that achieved by ethylene alone (compare to Fig. 4).

To determine the role of internal aeration in the growth of roots in oxygen-deficient medium, elongation rates of primary lateral roots of R. palustris were measured under various oxygen concentrations while, simultaneously, oxygen diffusion from the shoot to the roots was diminished. When nutrient solution in which the roots were grown was flushed with low oxygen concentrations while shoots were in air, root elongation continued at more than half the rate under aerated conditions (Table 3). However, additional flushing of the shoot compartment with the same oxygen concentration as in the root compartment led to a further decline of root elongation rates. Nevertheless, flushing of only pure nitrogen through both root and shoot compartments almost completely inhibited root extension.

Although oxygen shortage and increased ethylene concentrations reduced primary root elongation, we observed adventitious roots with a maximum length of up to 21 cm in plants grown in de-oxygenated stagnant agar solution (simulated soil flooding) for 7 d (unpublished results). If the roots had elongated at rates similar to those observed during oxygen deficiency (Fig. 4) or ethylene application (Fig. 5), these lengths would not have been achieved in 7 d. Therefore, the susceptibility to ethylene of adventitious roots compared to primary roots was tested. This revealed that, as with primary lateral roots, the elongation by adventitious roots of R. palustris was severely inhibited over a wide range of ethylene concentrations (Fig. 5).

The previous experiment, indicating a faster-than-expected elongation by adventitious roots, suggests that the aerenchyma channels in adventitious roots play an important role in removing high ethylene concentrations from the root tips. Measurements were carried out to esti-

![Figure 3](image-url)  
**Figure 3.** Effect of various oxygen concentrations on root elongation by primary lateral roots of R. palustris (○) and R. thyrsiflorus (□) [% of elongation during aerated (control) conditions]. Error bars indicate SEs, n = 4–8. Average elongation rates of primary lateral roots were 1-6 mm h−1 (SE ± 0-4; n = 24) for R. palustris and 1-2 mm h−1 (SE ± 0-1; n = 24) for R. thyrsiflorus, respectively.

![Figure 4](image-url)  
**Figure 4.** Effect of various ethylene concentrations on root elongation by primary lateral roots of R. palustris (○) and R. thyrsiflorus (□) [% of elongation during aerated (control) conditions]. Error bars indicate SEs, n = 4–8. Average elongation rates of the primary lateral roots were 1-6 mm h−1 (SE ± 0-1; n = 24) for R. palustris and 0-9 mm h−1 (SE ± 0-1; n = 19) for R. thyrsiflorus, respectively.

### Table 1. Elongation by primary roots of R. palustris under conditions of high carbon dioxide concentrations (± SE; n = 6–7).

<table>
<thead>
<tr>
<th>Concentration CO₂ (21% O₂, rest N₂)</th>
<th>Elongation rate (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-03% CO₂ (air)</td>
<td>100</td>
</tr>
<tr>
<td>0% CO₂</td>
<td>108.5 ± 2.2</td>
</tr>
<tr>
<td>10% CO₂</td>
<td>79.9 ± 4.3</td>
</tr>
</tbody>
</table>

Table 2. Effect of combined low oxygen and high ethylene concentrations on elongation by primary lateral roots of *R. palustris* [% of elongation during aerated (control) conditions]; ± SE; *n* = 4–8. Control elongation rate was 1.8 (SE ± 0.1; *n* = 37) mm h⁻¹. Values for 21% oxygen were derived from Fig 4

<table>
<thead>
<tr>
<th>Concentration oxygen (%)</th>
<th>Concentration ethylene (cm³ m⁻³)</th>
<th>Root elongation (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>0.5</td>
<td>21.7 ± 2.4</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>18.6 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>27.2 ± 1.0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>19.4 ± 3.0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>20.7 ± 1.5</td>
</tr>
<tr>
<td>0.5</td>
<td>1</td>
<td>20.6 ± 1.6</td>
</tr>
</tbody>
</table>

Table 3. Effect of low oxygen concentrations in the root compartment and in the shoot compartment on elongation by primary lateral roots of *R. palustris* [% of elongation during aerated (control) conditions]; ± SE; *n* = 6–8. Control elongation rate was 1.5 (SE ± 0.1; *n* = 21) mm h⁻¹

<table>
<thead>
<tr>
<th>Oxygen concentration (%)</th>
<th>Root elongation (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low oxygen in root compartment</td>
</tr>
<tr>
<td>4</td>
<td>91.1 ± 1.5</td>
</tr>
<tr>
<td>2</td>
<td>71.2 ± 2.2</td>
</tr>
<tr>
<td>0</td>
<td>54.2 ± 2.6</td>
</tr>
</tbody>
</table>

mate the rate at which endogenously produced ethylene could diffuse from the root tip to the shoot or non-elongating root parts. Applying a concentration of 6 cm³ m⁻³ ethylene to one end of a 95-mm-long adventitious root segment of *R. palustris* resulted in a rapid release of considerable amounts of ethylene at the other end (Table 4). The use of shorter segments or application of a higher concentration gradient resulted in faster release rates of ethylene.

**DISCUSSION**

Diffusion of gases between the root system of plants and the soil is strongly hampered when the soil is flooded, since gas diffusion rates in water are ≈10 000 times slower than in air (Jackson 1985). Obviously, these conditions result in a rapid decrease of oxygen availability in the soil. Many studies have been conducted to estimate internal oxygen concentrations in flooded roots (see e.g. Armstrong 1979) and their effect on root development (Trought & Drew 1980; Bertani & Brambilla 1982; Laan et al. 1991; Waters et al. 1991). The method we used to measure root growth enabled us to draw conclusions on the short-term effects of flooding-related changes in the internal gas atmosphere on root extension. Growth of primary lateral roots of the two *Rumex* species studied was not inhibited by oxygen concentrations higher than 5%. However, smaller concentrations slowed root elongation, particularly in *R. thyrsiflorus* (Fig. 3). Even in anaerobic nutrient solution, the more aerenchymatous roots of *R. palustris* (Visser et al. 1996a) were still capable of extending at almost half the maximum rate.

Concentrations of gases other than oxygen also change during flooding. Carbon dioxide produced by respiratory activity accumulates in the flooded plant (Stünzi & Kende 1989). In non-photosynthesizing tissues such as the roots we expect carbon dioxide levels to increase under flooded conditions, especially when anaerobic metabolism is induced. In *R. palustris*, carbon dioxide concentrations as high as 10% did not greatly influence root elongation

**Figure 5.** Effect of various ethylene concentrations on root elongation by primary lateral (○) and adventitious (●) roots of *R. palustris* [% of elongation during aerated (control) conditions]. Error bars indicate SEs, *n* = 4–8. Average elongation rates were 0.9 mm h⁻¹ (SE ± 0.2; *n* = 38) and 1.1 mm h⁻¹ (SE ± 0.1; *n* = 31) for primary lateral roots and adventitious roots, respectively.

<table>
<thead>
<tr>
<th>Length of root segment (mm)</th>
<th>Concentration gradient (cm³ m⁻³)</th>
<th>Number of measurements</th>
<th>Diffusion rate (× 10⁻³ mm² ethylene h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>6.1 ± 0.1</td>
<td>3</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>50</td>
<td>6.2 ± 0.3</td>
<td>3</td>
<td>3.3 ± 0.9</td>
</tr>
<tr>
<td>50</td>
<td>11.7 ± 0.4</td>
<td>2</td>
<td>3.9 ± 0.9</td>
</tr>
</tbody>
</table>

Calculated diffusion rate:

|                      | 10 | 2.2 ± 0.3 |

can even support root growth in anoxic nutrient solution (Table 3; Laan et al. 1996b). Much lower concentrations than this (up to 0.1 cm$^3$ m$^{-3}$) may stimulate extension of roots (Konings & Jackson 1979), but similar levels (1-10 cm$^3$ m$^{-3}$) reduce growth (in rice, tomato and mustard; Konings & Jackson 1979; in Epilohium hirsutum and Chamerion angustifolium; Ethington 1983). We conclude from our experiments that high ethylene concentrations (higher than 0.5 cm$^3$ m$^{-3}$) have an even stronger negative effect on the elongation by roots of R. palustris than oxygen deficiency (compare Figs 3 & 4), especially in the long term. Also in R. thysiflorus, ethylene concentrations such as those found in flooded Rumex plants (Visser et al. 1996b) had a negative effect on the elongation by the primary lateral roots (Fig. 4). In this species, the effects of high ethylene concentrations were similar to those found when the nutrient solution was flushed with pure nitrogen. Until now, oxygen deficiency was thought to be the main factor slowing root growth in flooded soil, but our results show that ethylene may contribute greatly to the cessation of primary lateral root extension in soil flooded Rumex species.

Exogenously applied ethylene also slowed elongation by adventitious roots of R. palustris (Fig. 5), even though soil flooding induces large numbers of adventitious roots to emerge (Visser et al. 1996a), which can grow to a considerable length within a week. It is possible that adventitious roots have an inherently slow ethylene production rate, resulting in lower endogenous concentrations during flooding. Unfortunately, we could not determine the ethylene production of a single intact root tip, and measurements on severed root tips were obscured by the rapid production of wound ethylene (unpublished results). However, ACC concentrations (the direct precursor of ethylene) and mRNA levels of ACC-oxidase did not substantially differ from those in primary lateral roots during flooded conditions (data not shown), and thus we have no reason to expect different ethylene production rates. Therefore, we investigated whether the aerenchymatous structure of adventitious roots might explain the discrepancy in the observed root growth during soil flooding and the high sensitivity of these roots to ethylene. Aerenchyma formation is a well-studied anatomical adaptation to flooded conditions, and enables the ready diffusion of oxygen from the emerging shoot parts to the submerged root system (Armstrong 1979; Laan et al. 1990). This shoot-derived oxygen is responsible for the maintenance of aerobic root metabolism (Laan et al. 1990), and can even support root growth in anoxic nutrient solution (Table 3; Laan et al. 1991).

Measurements on isolated segments of adventitious roots revealed that considerable amounts of ethylene diffused through the root segments when a sufficiently large concentration gradient was present (Table 4). From our measurements, and assuming that diffusion rates are approximately proportional to the concentration gradient and inversely proportional to the length of the segment, we calculate that the average diffusion rate through a root with a length of 1 cm at a concentration gradient of 1 cm$^3$ m$^{-3}$ would be $2.2 \times 10^{-3}$ mm$^3$ ethylene h$^{-1}$. As mentioned before, we could not determine the ethylene production of a single intact root tip, but from the literature it is known that ethylene production of intact roots of seedlings of tomato, mustard and rice ranges between $1.5 \times 10^{-3}$ and $6.5 \times 10^{-3}$ mm$^3$ g$^{-1}$ FW h$^{-1}$ (Konings & Jackson 1979). The tips of maize roots produce $c. 0.017$ mm$^3$ ethylene g$^{-1}$ FW h$^{-1}$, which is considerably more than the production rate at root tissue at a greater distance from the root apex (Atwell, Drew & Jackson 1988). Therefore, we assumed that the apices of adventitious roots of R. palustris (weighing about 1 mg) had a maximum ethylene production rate of $1.5 \times 10^{-5}$ mm$^3$ h$^{-1}$ per root tip.

With increasing ethylene concentrations in the root apex due to flooding, ethylene diffusion from the root tip to the shoot will increase until diffusion equals ethylene production, and the endogenous concentration reaches its maximum. As shown in Table 4, the rate of ethylene diffusion within a root segment with a length of 1 cm over a concentration gradient of 1 cm$^3$ m$^{-3}$ will amount to $2.2 \times 10^{-5}$ mm$^3$ h$^{-1}$. Over a similar concentration gradient, the ethylene diffusion rate within an adventitious root with a length of 20 cm will thus amount to $2.2 \times 10^{-3}$ mm$^3$ h$^{-1}/20 = 0.011 \times 10^{-3}$ mm$^3$ h$^{-1}$. However, such a steep concentration gradient of 1 cm$^3$ m$^{-3}$ will not occur in an adventitious root, since diffusion of ethylene from the root tip will already, at a much smaller endogenous concentration, equal the relatively slow production rate of the apex (less than $1.5 \times 10^{-5}$ mm$^3$ h$^{-1}$; see above). Because the ethylene diffusion and production rates at this equilibrium concentration are about $1.1 \times 10^{-4}$ mm$^3$ h$^{-1}/1.5 \times 10^{-3}$ mm$^3$ h$^{-1} = 7$-fold lower than the calculated diffusion rate at a gradient of 1 cm$^3$ m$^{-3}$ ($1.1 \times 10^{-4}$ mm$^3$ h$^{-1}$; see above) and the endogenous concentration is proportional to the production and diffusion rate, the equilibrium concentration in the apex of a root of 20 cm will amount to $c. 1 \times 10^{-3}$ mm$^3$ m$^{-3}$. This concentration is probably below that required to influence elongation of adventitious roots (Fig. 5). Thus, internal aeration along aerenchyma is not only an efficient mechanism to provide shoot-derived oxygen for growing root tips during soil-flooded conditions, but may also maintain ethylene concentrations below those that reduce root extension rates. We are aware that our diffusion calculations are a simplification of the actual in vivo process of gas diffusion in plant roots. The schizogenous aerenchyma in adventitious roots of Rumex is first found at a distance of 3-5 mm from the apex (Laan et al. 1989), which means that gas diffusion resistance in the most apical millimetres
of the root is higher than in the segments that were used in our experiments. Also, other parts of the diffusion pathway, such as the tap root and petioles, might have different porosities (Visser et al. 1996a) and therefore different diffusion resistances from adventitious roots. None the less, as far as we know, the experiments presented are the first attempt to measure ethylene diffusion in root tissue and they add valuable information to the theoretical approaches of gas diffusion within plant roots as described by Armstrong and coworkers (e.g. Armstrong 1979; Gaynard & Armstrong 1987).

In summary, this paper shows that ethylene accumulation, a result of entrapment of endogenously produced ethylene, may have an equal or even greater contribution to flooding-related inhibition of elongation of primary lateral roots than has oxygen deficiency. Elongation by adventitious roots is less impaired by flooding, since these roots have extensive aerenchymatous tissues. It is generally accepted that aerenchyma enables oxygen diffusion from the shoot to the roots and prevents oxygen deficiency in the flooded root system. However, our data show that an equally important function may be the export of ethylene from the root tips, preventing the accumulation of growth-inhibiting concentrations during soil flooding.

ACKNOWLEDGMENTS

We wish to thank Wim H. Poels and Yvonne Wilms for technical assistance with some of the experiments, and Professor Dr W. Armstrong (University of Hull, UK) for his valuable comments on the theoretical assumptions in the discussion. Our departmental colleagues and the anonymous referees of this paper are acknowledged for their critical remarks on an earlier version of the manuscript.

REFERENCES


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Erratum


As a result of a typesetting error in the production of issue 12 (December) of volume 19 (1996), the wrong Fig. 2 appeared in the above paper (p. 1426). The figure that appeared was actually a duplicate of Fig. 1. The correct figure is printed opposite.

The publishers apologize for this error.