Inhibition of root elongation by ethylene in wetland and non-wetland plant species and the impact of longitudinal ventilation

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ABSTRACT

The slow gas diffusion rate in flooded soil not only causes oxygen deficiency, but also favours the accumulation of ethylene in root systems to concentrations that may strongly affect root elongation. Previously published experiments showed that root elongation in rice is much less strongly inhibited by ethylene than in some other species less well adapted to wet conditions. Rice roots have also been reported to produce abnormally little ethylene. We tested if these traits are typical of wetland species and are thus likely to be widespread adaptive traits. Comparisons using 14 species indicated that insensitivity to the inhibiting effects of ethylene on root elongation is unlikely to be a common feature of temperate wetland species. However, resistance to longitudinal gas diffusion within roots of wetland species, which largely depends on diameter and the presence of gas-filled channels, was found to be less than in non-wetland species. We show that this can help maintain low internal ethylene concentrations by venting accumulated gas to the shoot and atmosphere.

Key-words: ethylene production; gas diffusion; internal ventilation; root extension; root growth; sensitivity to ethylene; soil flooding; waterlogging.

INTRODUCTION

The gaseous hormone ethylene is produced by all parts of plants and may accumulate rapidly in tissues when it cannot diffuse freely into the atmosphere. The diffusion rate of gases is four magnitudes slower in water than in air (Jackson 1985a), and outward diffusion of ethylene from root systems that grow in flooded soil is therefore extremely slow, which leads to increased ethylene concentrations in the flooded roots (Visser et al. 1996b). Additionally, the reduced oxygen concentrations that are prevalent in such roots may enhance ethylene production (Brailsford et al. 1993). Consequently, internal ethylene concentrations may rise to more than 10 µL L−1 (Visser et al. 1996b) and may largely exceed the threshold at which elongation of roots is negatively affected (Drew, Jackson & Giffard 1979; Abeles, Morgan & Saltveit 1992; Visser et al. 1997). Such inhibitory effects of ethylene on root elongation are first described by Harvey & Rose (1915) and are used together with the typical ‘triple response’ to identify ethylene-insensitive mutants (Guzmán & Ecker 1990). Still, the effect of this potential stress factor on the growth and characteristics of wetland plant species has hardly received any attention yet (Visser et al. 1997).

An obvious trait that helps prevent ethylene accumulation is the formation of aerenchyma in the root cortex, which in itself is stimulated by ethylene (Jackson 1989; Drew, He & Morgan 2000). Roots with aerenchyma have a substantially smaller longitudinal resistance to gas diffusion, thus allowing shoot-derived oxygen to enter the oxygen-deficient root system (Armstrong 1979; Jackson & Armstrong 1999). Moreover, a build-up of ethylene (which is produced mainly in the root apex; Finlayson, Liu & Reid 1996) is counteracted by enhancing its diffusion towards the atmosphere (Visser et al. 1997).

However, other root traits may also prevent damage from high ethylene concentrations. We have recently suggested a generalized concept wherein plant sensitivity to ethylene, among others, may be related to habitat characteristics such as soil flooding, thereby helping to make ecological sense of between-species variation in ethylene responses (Pierik et al. 2006). This concept is based upon observations such as those in Smith & Robertson (1971) who demonstrated that substantial differences exist between species in their responsiveness to ethylene treatment, with some species showing faster elongation in low concentrations and less inhibition in higher ones. Konings & Jackson (1979) showed that the response characteristics of three selected species correlate with ethylene production rates in the roots. Jackson (1985b) suggested that species with high ethylene production rates in their roots, and therefore high internal ethylene concentration, need only a small amount of additional exogenous ethylene to exceed a threshold at which ethylene becomes inhibiting to elongation. It also seems that faster ethylene production is
associated with an inherently stronger inhibition of elongation at high external dose of the gas (> 1 µL L\(^{-1}\)).

Interestingly, these ethylene relationships correspond well with the habitat characteristics of the species used by Konings & Jackson (1979). White mustard (Sinapis alba L.), a species from well-aerated soils, shows both a high ethylene production rate and a strong inhibition of elongation by high external ethylene. Tomato cultivars (Solanum lycopersicum L.) display an intermediate ethylene production rate coupled with intermediate susceptibility to inhibition by external ethylene. Tomato is also known to be moderately tolerant to wet soil conditions (Jackson 2002). Possibly, the resistance of rice root elongation to inhibition by ethylene and the associated slow ethylene production rate are traits that, among others, enable this species to cope with its wetland habitat by minimizing deleterious effects of ethylene on root growth. Differences in ethylene sensitivity between river foreland species with respect to ethylene-induced shoot elongation (Voetsenek et al. 2004) encourage this view.

The previous observations have led us to the hypothesis that three strategies may prevent a harmful effect of ethylene on root elongation in wetland plant species. Firstly, wetland plants have superior gas transport ducts (aerenchyma) that lead ethylene away from the root apex when the soil is flooded. Secondly, the root apices produce less ethylene compared with non-wetland species, resulting in a slower accumulation rate of ethylene. Thirdly, the root elongation rate of wetland plant species is less susceptible to inhibition by the high ethylene concentrations that can accumulate in roots as a result of entrapment by flood water.

In order to test these contentions, we designed experiments in which we tested 14 plant species for their resistance to ethylene diffusion, their ethylene production rates and the response of their root elongation rate to application of a high ethylene concentration. The experiments were set up following a phylogenetically balanced design by using seven wetland species and seven closely related non-wetland species, to find out if the hypothesized strategies are absent in the latter group of species.

### MATERIALS AND METHODS

#### Plant material

Fourteen plant species were selected from seven genera: Achillea ptarmica L. and Achillea millefolium L., Carex remota L. and Carex arenaria L., Geum rivale L. and Geum urbanum L., Plantago major ssp. intermedia (Gilib.) Lange and Plantago media L., Ranunculus sceleratus L. and Ranunculus bulbosus L., Rumex palustris Sm. and Rumex thrysiflorus Fingerh., and Thalictrum flavidum L. and Thalictrum minus L. These species represent couples of wetland and non-wetland plants, respectively, and thereby contrast in soil moisture content of their natural habitat (as indicated by their indicator values according to Ellenberg et al. 1992, Table 1).

Eight species were grown from seeds, which were all collected from the river area near Nijmegen, the Netherlands: Achillea millefolium, Achillea ptarmica, Geum urbanum, Ranunculus sceleratus, Rumex palustris, Rumex thrysiflorus, Thalictrum flavum and Thalictrum minus. Seeds of Achillea, Geum, Ranunculus and Thalictrum were germinated in Petri dishes on moist filter paper in a growth cabinet with 16 h 20 µmol m\(^{-2}\) s\(^{-1}\) photosynthetically active radiation [PAR (Philips TL33)] at 25 °C and 8 h dark at 10 °C. Rumex seeds were germinated as described in Visser, Blom & Voetsenek (1996a). After germination, seedlings were first placed on polyethylene grains soaked with nutrient solution and placed in a climate room, and were then placed on hydroponic culture (Visser et al. 1996a). The seedlings of Geum were directly placed on hydroponics.

The other species were collected by taking rooted cuttings from plants at field sites near Nijmegen, the Netherlands (Plantago intermedia and Ranunculus bulbosus) or were originated from the Botanical Garden of the University of Nijmegen (Carex arenaria, Carex remota, Geum rivale and Plantago media). These cuttings were immediately placed on hydroponic culture (cf. Visser et al. 2000).

#### Root elongation at elevated ethylene

The effect of 1 µL L\(^{-1}\) ethylene on root elongation of each species was determined according to the method of Visser et al. (1997). In short, root systems of intact plants were placed on a box-shaped glass cuvette (0.10 × 0.10 × 0.15 m) that was flushed with aerated nutrient solution. Selected roots were mounted to the front of the cuvette by using 1.5 mm diameter glass capillaries, after which the vertical coordinates of each root apex were determined at regular time intervals with a horizontally positioned vernier traveling microscope. Sometime after root elongation reached steady rates (usually within 1 h), the air stream that aerated the nutrient solution was replaced by air containing 1 µL L\(^{-1}\)
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 ethylene. One hour after this switch, the measurements resumed for another 2–3 h. An example of the resulting graphs is given in Fig. 1.

When roots did not fit into the cuvette (because of lateral root development or because they were too short), the chemical dye neutral red (Sigma–Aldrich Chemie B.V., Zwijndrecht, the Netherlands) was used to mark the roots. This dye has no effect on root elongation rate (Schumacher et al. 1983; Visser & Pierik, unpublished data). Prior to marking, the plants were transferred to hydroponic containers as previously described, and were acclimatized for 24 h. Then, individual roots were stained dark red by placing them for 6 min in 0.15% (w/v) neutral red in water. The length of the new grown, white root tips was measured after 24 h of growth in air-bubbled nutrient solution. Then, the roots were stained again, and the plants were placed on nutrient solution that was bubbled with 1 µL L⁻¹ ethylene in air [produced by mixing 61 µL L⁻¹ ethylene in nitrogen (Hoekloos, Dieren, the Netherlands) with air, using a gas blender (type F201EA; Hi-Tec, Ruurlo, the Netherlands)] for another 24 h. The inhibitory effect of ethylene was calculated from the respective elongation rates in air-bubbled and ethylene-bubbled nutrient solution.

By measuring control elongation rates and rates as affected by ethylene on the same roots, the relatively high variation of elongation rates among individual roots was not affecting the data. For comparison, both methods (microscope and neutral red staining) were applied to roots of Rumex palustris, which showed that the two methods yielded identical root elongation rates and identical effects of ethylene treatment (data not shown).

Longitudinal ethylene diffusion

The standard method to characterize the capacity of plant tissues to allow diffusion of gases is to measure the tissue porosity (Visser & Bögemann 2003). However, such measurements do not show if the air spaces in a tissue are connected to allow diffusion over the entire length of the organ. As this is essential to the current analyses, we decided to measure gas diffusion capacity in a direct manner. Diffusion capacities of primary lateral roots of all species were measured according to the method described in Visser et al. (1997). The cuvette used for the measurements was modified to facilitate the insertion of root segments (Fig. 2). Root segments 35 mm long were cut with a razor blade from the unbranched part of the root at some distance (typically > 60 mm) from the root apex. The cut ends were carefully blotted with tissue paper, after which the segments were inserted in a 30 mm-long glass tube and fixed by introducing warm 3% (w/v) agar into the tube using a syringe, in such a way that the cut ends of the segments protruded from the agar. The agar set within 3 min and provided an airtight seal after the tube was connected to a glass vial containing high ethylene concentration in air (between 4.5 and 60.0 µL L⁻¹, depending on the expected diffusion capacity) at one side, and to a small compartment with a rubber septum at the other side (Fig. 2). The latter compartment was flushed with humid ethylene-free air (0.25–1.0 L h⁻¹) via syringe needles, which was led to a photoacoustic ethylene detector (lower detection limit < 0.05 nL L⁻¹). Firstly, ethylene release was

Figure 1. Root elongation of an individual root of Achillea millefolium (circles) and Rumex palustris (triangles) before and after treatment with ethylene. Roots were fixed in a custom-made cuvette and flushed with nutrient solution saturated with air (first 180 min, open symbols), followed by treatment with nutrient solution containing 1 µL L⁻¹ ethylene (filled symbols). Elongation rates of these individual roots were 0.270 (control) and 0.075 (ethylene-treated) mm h⁻¹ (27.8% inhibition) for the A. millefolium root, and 1.57 (control) and 0.320 (ethylene-treated) mm h⁻¹ (20.4% inhibition) for the R. palustris root, respectively. Root lengths were measured with a vernier travelling microscope according to Visser et al. (1997).

Figure 2. Schematic drawing of the cuvette used for measurements of longitudinal gas diffusion trough root segments. Root segments were embedded in solidified agar in a glass tube that connected a vial containing ethylene with a compartment that was flushed with ethylene-free air. The outflow of this compartment was connected to a sensitive photoacoustic ethylene detector (Visser et al. 1997), which allowed calculations of the diffusion rate of ethylene through the root segment.
measured for approximately 15 min with the valve of the ethylene vial closed, typically leading to recordings of ethylene release that were below the detection limit of the detector. Then, the valve was opened and ethylene release was measured until the recordings reached a maximum and stabilized. This maximum was used to calculate the ethylene diffusion rate, which was then normalized for root diameter and concentration gradient. Previous tests made clear that ethylene release was not driven by pressure differences between both ends of the root segments, which would lead to different release kinetics (as shown in Visser et al. 1997).

Root diameter

The diameters of 12 roots were measured by cutting the root tips with a razor blade at 20 mm behind the apex and inspecting them at the cut end with an Olympus BX-40F3 microscope (Olympus Optical Co., Hamburg, Germany) with internal calibrated ruler.

Ethylene production of roots

Two centimeter long root tips were excised with a razor blade and immediately placed on the inner wall of a glass vial (45 mL, 8–15 root tips per vial) that was first coated on the inside with a thin layer of solidified Gamborg G-5 root growth medium (pH 5.75, 2% agar, 100 µg L\(^{-1}\) biotin, 2% sucrose). The vial was then closed with a rubber stopper and wrapped in aluminium foil. The stopper was perforated by two syringe needles, of which one was connected to a flow of ethylene-free air (0.25–1.0 L h\(^{-1}\)), whereas the other served as an outlet connected to a photoacoustic ethylene detector (Voosenek et al. 1990). In this way, ethylene release from the root tips was recorded in the continuous gas flow during 50–70 h. The fresh weight (FW) of the roots was determined after the measurements of the ethylene production rates, and deviated less than 10% from the initial weight (data not shown).

Initial ethylene production rates were high as a result of wounding [comparable to the results with a similar set-up used by Brailsford et al. (1993)], but the ethylene release subsequently decreased and typically stabilized after 24 h. The period between 24 and 36 h was used to calculate the average ethylene production rate.

Calculations of ethylene concentration in the root apex

Using the data on ethylene production rates, longitudinal ethylene diffusion and root diameter, we were able to calculate internal ethylene concentration estimates in the root tips when these were flooded, according to Visser et al. (1997). These calculations assumed that the predominant site of ethylene production was the root apex, that ethylene was not metabolized into other compounds by the roots, and that only small amounts of ethylene would diffuse radially into the soil around a flooded root [particularly because ethylene may accumulate in the flooded soil because of microbial activity (Jackson 1985a), thereby decreasing the concentration gradient between root and soil]. The outcome of the calculation was an estimate of the concentration of internal ethylene that would be in equilibrium with the rate of biosynthesis in the root apex and the rate of basipetal longitudinal loss via internal gas-filled channels. Ethylene diffusion capacity per single root (in this case with a length of 100 mm) was calculated from the normalized diffusion rates (Fig. 4) and the diameters of the roots (Fig. 5), and from the production rates expressed per root tip (Fig. 6b).

Statistical analyses

The data were statistically analysed by two-way analysis of variance (ANOVA) with habitat and genus as independent variables, and with ln transformation of the data in case of large differences among the variances of means to meet the conditions for ANOVA.

RESULTS

Sensitivity of root elongation to ethylene

Because the sensitivity of root elongation to increased ethylene concentrations largely determines how strong roots are affected by accumulated ethylene, a 1 µL L\(^{-1}\) flow of ethylene in air was applied to intact roots to determine the effect on elongation rate. This resulted in a range of sometimes contrasting responses (Fig. 3). Some species, like C. remota, and, surprisingly, the non-wetland species Thalictrum minor, showed no significant inhibition of root elongation. Alternatively, species such as A. millefolium, G. rivale and R. palustris displayed severely decreased root growth, with elongation rates less than 30% of the original

**Table 1.** Root elongation of the 14 selected species (for list of species, see Table 1) as affected by the application of 1 µL L\(^{-1}\) ethylene. Columns represent the means of 4–10 replicates; error bars indicate SEs; dotted line indicates ‘no inhibition’. Significant differences between species within the same genus are indicated:

*P* < 0.05.
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No particular trend was found when comparing the responses of wetland versus non-wetland species within the genera \((P = 0.106)\); in some cases, the wetland species was more strongly inhibited \((\text{Rumex, Thalictrum})\). In most of the paired comparisons, there was no statistically significant difference between the effect of ethylene on the wetland species and on the non-wetland species of the same genus.

**Gas diffusion capacity**

The concentration of ethylene in the root apex in a flooded soil will largely depend on the gas diffusion resistance of the tissues adjacent to the apex. The more porous these tissues are, the more likely it is that ethylene can be vented away from the apical meristem. When comparing the 14 selected species, the diffusion capacity of ethylene through the roots varied considerably (Fig. 4). The slowest diffusion rates were found in the non-wetland species \(A. \text{millefolium} \text{ and } P. \text{media}\) (ca. 0.1 nL L\(^{-1}\)), whereas high diffusion rates exceeding 10 nL h\(^{-1}\) (if normalized for a root length of 1 mm, a cross sectional surface of 1 mm\(^2\) and an ethylene concentration gradient of 1 \(\mu\)L L\(^{-1}\)) were found in both \text{Carex} species, \text{R. palustris} and \text{R. bulbosus}. Unfortunately, it proved impossible to measure diffusion through the roots of the wetland species \text{R. sceleratus}, as these roots appeared too fragile to fix in the cuvette. Nevertheless, in four of the six genera, the roots of wetland species showed a significantly higher diffusion rate of ethylene, whereas no difference was found between the species of the remaining two genera.

**Figure 4.** Longitudinal diffusion capacity of ethylene through root segments of the 14 selected plant species (for list of species, see Table 1). Root segments were mounted airtight between two compartments, after which ethylene was applied to the compartment at one side of the segment, and ethylene release was recorded in the compartment at the other end. Data were normalized for diameter, segment length and diffusion gradient; roots of \text{Ranunculus sceleratus} were not compatible with this method (no data; see Materials and Methods section). Columns represent the means of three to four replicates; error bars indicate SEs. Significant differences between species within the same genus are indicated: *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\).

**Figure 5.** Diameter of the primary roots of the 14 selected plant species (for list of species, see Table 1). Root diameters were determined at 20 mm behind the apex. Columns represent the means of 12 replicates; error bars indicate SEs. Significant differences between species within the same genus are indicated: *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\).

**Figure 6.** Ethylene production rates by the apical 20 mm of roots of the 14 selected species (for list of species, see Table 1), expressed per gram fresh weight (FW) (a) or per root tip (b). Columns represent the means of three replicates (8–15 root tips per replicate); error bars indicate SEs. Significant differences between species within the same genus are indicated: *\(P < 0.001\).
A high diffusion capacity will not only be determined by the porosity and, thus, the structure of the tissue, but also by the diameter of the root in which the porous tissue is present. However, it seemed that there was no clear correlation between the habitat of a species and the average diameter of its primary roots (Fig. 5, \( P = 0.418 \)). In some genera, the wetland species showed a larger root diameter (Plantago, Ranunculus, Thalictrum), whereas in other genera, the opposite was the case (Carex, Rumex), or no significant difference was found (Achillea, Geum).

**Ethylene production**

Ethylene production rates of the root tips are important for this study, because they determine the amount of endogenous ethylene that potentially accumulates. However, the outcome of our data depended much on the units in which ethylene production was expressed. If related to the FW of the root tip (Fig. 6a), two wetland species (G. rivale and T. flavum) produced more ethylene than their non-wetland relatives (G. urbanum and T. minor). Species of all other genera released less ethylene and showed no significant differences between wetland and non-wetland species. Ethylene production can also be expressed per root tip, which may be more relevant, as it indicates how much ethylene needs to be vented to the more basal root parts. In this case, no significant differences between species within genera were found (Fig. 6b).

**Accumulation of ethylene in the root apex**

Using the data on ethylene production, diffusion capacity and root diameter, we estimated the internal ethylene concentration in the root apex of 100 mm-long roots. These estimates were derived from combining data from different individual root segments and root tips, and therefore do not allow statistical treatment, but still a clear pattern appeared. Although the individual traits that contribute to the internal ethylene concentration (root diameter, ethylene production rates and longitudinal diffusion rates) did not show unequivocal differences between wetland and non-wetland species (Figs 4–6), their concerted action led in all cases to non-wetland species being more likely to accumulate higher ethylene concentrations than did their wetland relatives (Fig. 7). Apparently, it is not just one of these traits but a suite of morphological and physiological characteristics that, in various combinations, prevent ethylene to accumulate to similar concentrations in wetland species as in their non-wetland counterparts.

**DISCUSSION**

In this study, we tested if wetland plants possess common traits that prevent damage by entrapment of ethylene in the root system during soil flooding. Even rather low ethylene concentrations have been proven to substantially inhibit root elongation in crop plants [e.g. 0.1 \( \mu \text{L} \cdot \text{L}^{-1} \) in barley (Hall et al. 1977) and maize (Abeles et al. 1992), 0.6 \( \mu \text{L} \cdot \text{L}^{-1} \) in tobacco (McDonald & Visser 2003)]. By contrast, data presented by Smith & Robertson (1971) and Konings & Jackson (1979) indicate that this was not the case for rice (O. sativa), a wetland species with root elongation that is less strongly inhibited by ethylene than many others. This weak inhibitory effect, possibly combined with a low ethylene production rate and/or an efficient venting system for gases (by means of aerenchymatous tissue), could prevent soil flooding-induced accumulation and action of ethylene in the roots, as suggested earlier by Jackson (1985a). Such a strategy would effectively prevent action not only by ethylene produced by the plant itself, but also by ethylene originating from the surrounding flooded soil (Smith & Scott-Russell 1969; Jackson & Campbell 1975). However, until this study, it was unknown if these traits (except aerenchyma formation) are commonly found among plant species from regularly soil-flooded habitats.

Some of the wetland species that were tested in this study, viz. C. remota and R. sceleratus, indeed showed low levels of inhibition when given ethylene (Fig. 3), but other wetland species showed a strong inhibition of root extension in ethylene rather than a weak one; similar results from earlier experiments (Visser et al. 1997) already indicated that the wetland species R. palustris showed high, rather than low, sensitivity of its root elongation to ethylene, and similar results were found in this study for this species and G. rivale (Fig. 3), a species from wet meadows and forests. The inhibitory effect of ethylene on root elongation of these sensitive species was fast [i.e. within 1 h after the start of ethylene treatment (Fig. 1)], which is comparable to the rapid effects ethylene may have on other growth processes, such as hypocotyl elongation (Binder et al. 2004).
Thalictrum minus was not inhibited by external ethylene (Fig. 3), showing that lack of an inhibitory response to the gas is not restricted to species from wetland habitats.

Our results show further that, when evaluating the two other potentially beneficial traits we studied, only a higher diffusion capacity of ethylene was more frequently present in wetland species (four out of six cases) when compared to their non-wetland counterparts (Fig. 4). Presumably, these faster diffusion rates are due to a greater amount of aerenchymatous tissue in the roots of these wetland species (Justin & Armstrong 1987), which decreases the diffusion resistance for gases considerably (Armstrong 1979).

A relatively low ethylene production rate was found in some of the species that were tested (Fig. 6). Ethylene production will be a principal factor in determining the internal concentrations of this plant hormone in the root system, as the gas is hardly degraded by the plant (Beyer 1985). Low production rates are therefore likely to prevent or at least delay accumulation of ethylene in the roots during soil flooding. However, although the rate of ethylene formed by isolated root tips varied substantially between the species, there is no clearly distinguishable trend indicating that production rates are related to the degree of soil flooding in the respective habitats (Figs 6a, b). Instead, in the two genera that did show a significant difference between the two species, the non-wetland species produced less ethylene than the wetland species (expressed per root biomass, Fig. 6a).

Combining the data on ethylene production per root tip (Fig. 6b) with those on diffusion capacity (Fig. 4) and taking into account the root diameters (Fig. 5) allowed us to estimate the internal ethylene concentration in roots with a given length (in this case 100 mm). These concentrations varied substantially between species (Fig. 7), but in all cases, wetland species show lower internal concentrations than do their relatives from less frequently flooded habitats. Clearly, care is needed when extrapolating these values to an experimental or even natural environment, given that the assumptions for the calculations are not necessarily met under these conditions. However, these calculations do show the relative effects on different species of a combination of traits that are each likely to reduce the internal ethylene concentration. As shown in the previous paragraphs, each of these traits alone did not satisfactorily correlate with the resistance of the selected species to flooding, whereas the culmination of their combined effects, expressed as the equilibrium ethylene concentration at which the longitudinal diffusion rate is equal to the production rate of ethylene in the root, matches well with the habitat characteristics of the species.

In conclusion, our data do not support the hypothesis that the relative insensitivity of rice roots to inhibitory concentrations of ethylene (Konings & Jackson 1979) would be common among wetland species and therefore a potentially adaptive trait that prevents damage by ethylene accumulation during soil flooding. The presence of an effective venting system, as provided by aerenchymatous root tissues, in some cases combined with low ethylene production rates, is more likely to play a key role in avoiding cessation of root growth by ethylene accumulation. As shown in this paper, the accumulation of ethylene in roots of wetland species is expected to be smaller than that in their non-wetland relatives (Fig. 7), and this is probably even more so when plants acclimate to soil-flooded conditions (Justin & Armstrong 1987). During soil flooding, many wetland species produce adventitious roots that have a larger diameter and contain greater amounts of aerenchyma than do normal roots, resulting in a high diffusion capacity (Visser & Voeseenek 2005). Non-wetland species do not show such responses to soil flooding. Ethylene is considered to induce these morphological and anatomical changes in soil-flooded wetland species (Jackson 1985a; Visser et al. 1996b), and thereby facilitates its diffusion out of the plant into the atmosphere. Because only roots from non-flooded root systems were used for the measurements, our experiments may even have underestimated the venting capacity of the wetland species, whereas this is probably not the case for the non-wetland species, because these are incapable of forming additional porous tissues. This ethylene-induced induction of aerenchyma and adventitious rooting in wetland plants helps prevent oxygen shortage during soil flooding, and this may further explain the differential habitat preferences of wetland versus non-wetland species.

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