Planta (1985) 165:486–492

Planta

Stimulation of ethylene production and gas-space (aerenchyma) formation in adventitious roots of *Zea mays* L. by small partial pressures of oxygen

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Abstract. Adventitious roots of two to four-week-old intact plants of *Zea mays* L. (cv. LG11) were shorter but less dense after extending into stagnant, non-aerated nutrient solution than into solution continuously aerated with air. Dissolved oxygen in the non-aerated solutions decreased from 21 kPa to 3–9 kPa within 24 h. When oxygen partial pressures similar to those found in non-aerated solutions (3, 5 and 12 kPa) were applied for 7 d to root systems growing in vigorously bubbled solutions, the volume of gas-space in the cortex (aerenchyma) was increased several fold. This stimulation of aerenchyma was associated with faster ethylene production by 45-mm-long apical root segments. When ethylene production by roots exposed to 5 kPa oxygen was inhibited by aminoethoxyvinylglycine (AVG) dissolved in the nutrient solution, aerenchyma formation was also retarded. The effect of AVG was reversible by concomitant applications of 1-aminocyclopropane-1-carboxylic acid, an immediate precursor of ethylene. Addition of silver nitrate, an inhibitor of ethylene action, to the nutrient solution also prevented the development of aerenchyma in roots given 5 kPa oxygen. Treating roots with only 1 kPa oxygen stimulated ethylene production but failed to promote gas-space formation. These severely oxygen-deficient roots seemed insensitive to the ethylene produced since a supplement of exogeneous ethylene that promoted aerenchyma development in nutrient solution aerated with air (21 kPa oxygen) failed to do so in nutrient solution supplied with 1 kPa oxygen. Both ethylene production and aerenchyma formation were almost completely halted when roots were exposed to nutrient solutions devoid of oxygen. Thus both processes require oxygen and are stimulated by oxygen-deficient surroundings in the 3- to 12-kPa range of oxygen partial pressures when compared with rates observed in air (21 kPa oxygen).

Key words: Adventitious root – Aerenchyma – Environmental stress – Ethylene and O_2 deficiency – Oxygen deficiency – Root (adventious) – *Zea* (aerenchyma, ethylene).

Introduction

The presence within roots of gas-filled channels, interconnected longitudinally (aerenchyma) is typical of many species that tolerate over-wet, poorly aerated soil conditions (Philipson and Coutts 1978; Kawase 1981 b; Smirnoff and Crawford 1983). Although aerenchyma is not unique to this group of plants there is much circumstantial and some experimental evidence indicating it is related causally to the ability of their roots to survive in oxygen-deficient surroundings. (Armstrong 1971, 1979; Saebø 1974). The relationship probably derives both from a decreased resistance to an internal flux of oxygen from the aerial environment and from a diminished oxygen demand proportional to the volume of the cell-free voids. These attributes are thought to decrease the dependence of roots on soil oxygen.

Without an alternative source of oxygen via aerenchyma, roots of most species may die in oxygen-deficient surroundings, possibly from acidosis of cytoplasm caused by leakage of protons from the vacuole (Roberts et al. 1984). Therefore, it is not surprising that aerenchyma has been found associated with a retention of intact mitochondria in the growing zone (Vartapetian 1973) and a large

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Abbreviations: ACC = 1-aminocyclopropane-1-carboxylic acid; AVG = aminoethoxyvinylglycine

ATP/ADP ratio (Saglio et al. 1983; Drew et al. 1985) despite the absence of oxygen in the external environment. Perhaps as a consequence, extension growth by roots of *Zea mays* is favoured by a well-developed aerenchyma. Conversely, elongation rates in oxygen-deficient surroundings are known to be retarded by chemical treatments that prevent aerenchyma formation (Drew et al. 1981).

In roots of certain non-wetland species, notably *Zea mays* (Norris 1913; McPherson 1939) aerenchyma develops more quickly and extensively in poorly aerated media such as wet soil or stagnant solution cultures. The phenomenon may therefore be an acclimatic response to conditions that could otherwise asphyxiate these roots. We have published evidence implicating an increased ethylene content as an inducing agency (Drew et al. 1979; 1981), a conclusion since supported by others (Kawase 1981a; Konings 1982; Konings and deWolf 1984). We now examine the cause of this ethylene enrichment and provide further evidence for its involvement in aerenchyma development.

Material and methods

Caryopses of *Zea mays* L. (cv. LG11) were surface-sterilised for 3 min in 10% (v/v) sodium hypochlorite solution and germinated in the dark on moist filter paper for 2 d. Seedlings of similar size were then suspended for 2 d on stainless-steel mesh over 0.1-strength nutrient solution before exposure to light (16 h photoperiod, 375–400 μmol m^{-2} s^{-1} photosynthetically active radiation at 20° C). After a further 5 d the root system and approx. 10 mm of the shoot base of each plant were sealed into a $0.9 \cdot 10^{-3}$ m^3 or $1.5 \cdot 10^{-3}$ m^3 glass jar containing a standard nutrient solution (Barber and Lee 1974) but with ten times the stated concentration of iron. Gas-tight sealing was obtained with 'Blue-Tack' adhesive rubber (Bostik, Leicester, UK) or cold-curing silicone rubber ('Silastomer 9161'; Dow Corning, Barry, South Clamorgan, UK). The nutrient solution was aerated with compressed air flowing at $0.15 \cdot 10^{-3}$ m^3 min^{-1}. Immediately before each experiment, when the first (oldest) or second whorl of adventitious roots began to emerge, they were marked 10 mm from the tip with a spot-application of charcoal slurry using a fine brush. At the end of the experiments the distance between this mark and the root tip gave an estimate of extension growth. Known oxygen partial pressures were applied by replacing the flow of air (21 kPa oxygen) with one of nitrogen gas containing 1, 3, 5 or 12 kPa oxygen from pressurised cylinders (British Oxygen, Special Gases, London, UK). Gas flows were regulated with electronic mass-flow controllers and valves (Precision Flow Devices Inc., San Jose, Cal., USA). Ethylene at a partial pressure of 10 Pa (100 ppm, v/v) was bled into the flow as required to produce 0.1 Pa ethylene in air or in 5 kPa oxygen. Stagnation treatments were imposed by stopping aeration and sealing the inlet and outlet ports to each jar for up to 13 d.

Dissolved oxygen in the nutrient solution was measured in 10-mm^3 samples by electron-capture gas chromatography as described by Hall (1978). Ethylene production by 45-mm-long apical root segments was estimated by gas chromatography sensitive to $5 \cdot 10^{-4}$ Pa in 10^{-6} m^3 of air (Jackson and Campbell 1975). Approximately 0.5 g fresh weight was placed into $10.7 \cdot 10^{-6}$ m^3 glass vials immediately after excision, sealed with 'Suba-Seal' puncture caps (W. Freeman & Co., Barnsley, Yorkshire, UK) and flushed for 60 s with the same gas mixture experienced by the roots when attached to the plant. After approx. 2 h, at 20° C in the dark, 10^{-6} m^3 was removed from each vial for ethylene analysis using a plastic disposable syringe. Immediately after sampling, the vials were flushed with the same gas mixture as before. This was followed by at least two further cycles of sampling, flushing and incubation.

Gas-space content of roots was estimated in one experiment by a weighing-water-displacement method. Approximately 0.5 g of root was accurately weighed and placed in a modified $10 \cdot 10^{-6}$ m^3 density bottle connected to a small reservoir of distilled water and to an 'Agla' micrometer syringe (Welcome Diagnostics, Dartford, Kent, UK). The difference in the positions of the micrometer before and after inserting the roots indicated the volume of displaced water and thus root volume. Assuming that excluding internal gas-space, 1 g of tissue occupied 10^{-6} m^3, gas-space as a percentage of the total root volume was calculated from 100 (volume-fresh weight)/volume. In the remaining experiments, aerenchyma was estimated indirectly from transverse sections of root prepared from 2-, 3-, or 4-d-old tissue. The position of such tissue along the root was calculated by (extension growth/d of growth) × age in d of the required tissue. Approximately 10 sections from each position were mounted in water on a microscope slide under a cover slip sealed at the edges with nail varnish and stored for up to 4 d in a refrigerator before examination. The extent of the aerenchyma did not change during this time. The epidermis, the cortex-stele boundary, the perimeters of cell-free voids in the cortex and on one occasion the extent of collapsed cells were traced onto translucent paper attached to a projection screen mounted on a microscope. The percentage of the cortex that comprised cell-free voids or collapsed cells was estimated electronically from the tracings using a digitizer drawing-board ('Versawriter'; Versa Computing Inc., San Jose, Cal., USA) attached to an Apple IIe computer (Apple Computer, Inc., Cupertino., Cal., USA). Three or four roots from each of up to eight plants were analysed.

Results

Root porosity and oxygen partial pressures in non-aerated nutrient solution. Adventitious roots of the second and third oldest whorls that emerged from the base of the shoot into non-aerated nutrient solution that had been sealed-off from the atmosphere for 13 d, contained considerably more gas-space than roots extending into solutions agitated and aerated by a fast flow of air (Table 1). The stagnant conditions also advanced by 2–3 d the emergence of the third whorl of adventitious roots. Consequently these were longer than the equivalent aerated roots at the time they were measured. Lack of aeration also inhibited shoot length (Table 1) and the development of red pigmentation ordinarily present in aerated roots.

Equilibrium partial pressures of oxygen in the nutrient solution were measured at three depths each day for the first 5 d. These decreased from approx. 21 kPa at the start of the experiment to

Table 1. Effect of non-aeration of nutrient solution on the extension and gas-space content of the second and third oldest whorls of adventitious roots of *Zea mays*. Shoot length is also shown. Means and SEs of four replicate plants. Plants were treated for 13 d when 16 d old

	Root extension (mm)		Gas space (% of root volume)		Shoot (mm)
	2nd whorl	3rd whorl	2nd whorl	3rd whorl	
Control	319±45	85±27	3.4±1.3	0	762±32
Non-aerated	168±58	124±25	12.3±6.4	30.2±0.8	399±27

between 3–9 kPa and were smallest after 24 h. Slightly more oxygen was detected 20 mm below the upper surface than at greater depths in the 150-mm-tall containers used.

Effect of small or zero oxygen partial pressures on aerenchyma formation. A range of oxygen partial pressures that included those measured in the non-aerated (stagnant) nutrient solution were tested for their effect on aerenchyma development. These tests were made independently of the increased gas-entrapping or excluding influence of the static nature of non-aerated nutrient solutions by bubbling them briskly with mixtures of oxygen in nitrogen gas to minimize the diffusive resistance to root gas exchange by an unstirred water covering. The amount of cortical gas-filled space and collapsed tissue as assessed in transverse sections was increased greatly by oxygen deficiency in the 3 to 12-kPa range. In contrast, complete exclusion of oxygen, or supplying only 1 kPa oxygen prevented cell collapse and the formation of gas-space (Fig. 1). The range of oxygen partial pressures which promoted aerenchyma encompassed those measured in non-aerated nutrient solutions, 3–5 kPa being especially active. The greatest effect was seen in tissue that comprised the root tip at the start of treatment.

Effect of small or zero oxygen partial pressures on ethylene production by roots. The oxygen partial pressures tested for their effect on aerenchyma formation were also examined for their effect on ethylene production. Root systems of intact plants were treated for 3 d. Apical segments 45 mm long were then excised and incubated in sealed vials containing the same oxygen partial pressures that were applied before excision. Compared with roots exposed to air (21 kPa oxygen) those in 1, 3, 5, 12 kPa oxygen formed much more ethylene. The greatest stimulation (approx. 18-fold) was with 3 or 5 kPa oxygen. In contrast, almost no ethylene was produced when oxygen was excluded (Fig. 2). These results were obtained after 3 d, but other experiments have shown comparable responses to anoxia or 3 and 5 kPa oxygen within a few hours of treatment. The same methodology was used except that imposition of oxygen shortage was delayed until the root segments were excised and placed in incubation vials. With oxygen excluded, ethylene production stopped almost totally within 2 h (Fig. 3). In contrast, 3–5 kPa oxygen strongly

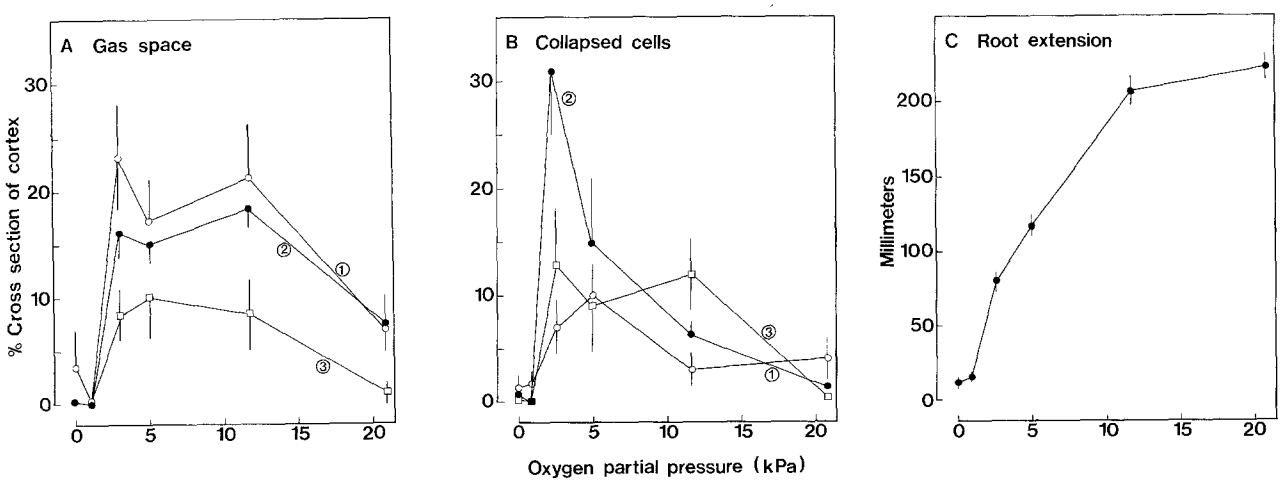

Fig. 1A–C. Effect of different partial pressures of oxygen in vigorously bubbled nutrient solution on the extent of **A** gas-space and **B** collapsed cells in adventitious roots of *Zea mays* taken from the oldest whorl expressed as a percentage of the cross-sectional area of the cortex at the following three stages of development: (*1*) tissue that was 15 mm behind the tip when treatment began, (*2*) tissue that comprised the root tip when treatment began, (*3*) tissue initiated 3.5 d after treatment began. Extension growth by these roots is given in **C**. Plants were 14 d old at the start of the experiment and treated for 7d. Means and SEs of six roots

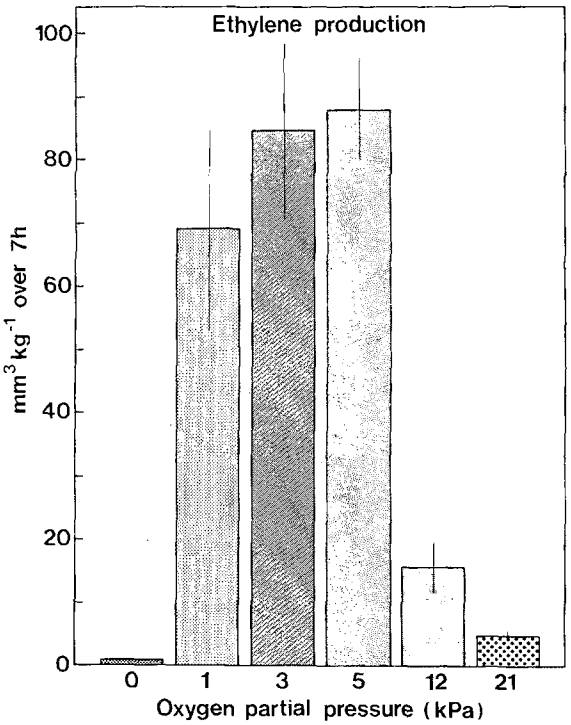

Fig. 2. Effect of different partial pressures of oxygen supplied for 3 d to the roots of intact plants of *Zea mays* on ethylene production by 45-mm-long apical root segment incubated for 7 h in the treatment oxygen tension. Means and SEs of three replicates

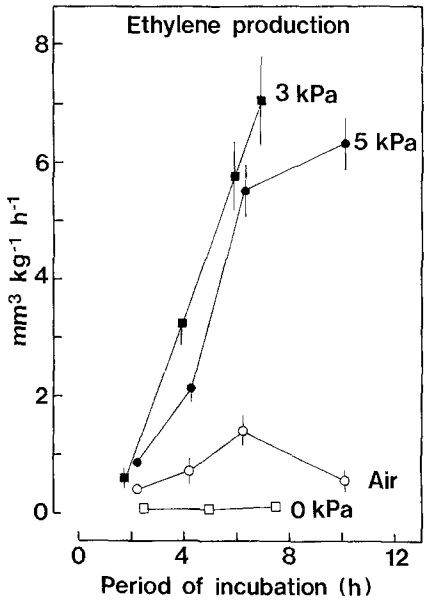

Fig. 3. Effect of different partial pressures of oxygen supplied for up to 10 h on ethylene production by 45-mm-long apical root segments of *Zea mays*. The treatment oxygen partial pressures commenced immediately after segment excision. Means and SEs of four or five replicates

stimulated ethylene production. The effect was evident during the 2–4 h following excision of the root apices, but full expression took longer (Fig. 3).

Effect of severe oxygen shortage (1 kPa oxygen) and exogenous ethylene on aerenchyma formation. The results so far indicate that the promotion of aerenchyma by small oxygen partial pressures is associated with faster production of ethylene. However an exception was found in 1 kPa oxygen. This last treatment stimulated ethylene production (Fig. 2) but not aerenchyma development (Fig. 1). An inability to respond to the extra ethylene-formed in 1 kPa oxygen could provide one explanation. In support of this we found no enhancement of aerenchyma formation when exogenous ethylene at 0.1 Pa was applied in 1 kPa oxygen as a supplement to that produced endogenously (Table 2).

Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) and inhibitors of ethylene production and action. Root segments excised from plants grown in aerated nutrient solution and then incubated for 2 h in vials containing moist air evolved ethylene faster in response to increasing concentrations of

Table 2. Effect of applying 1.0 kPa oxygen and 0.1 Pa ethylene separately and together for 6 d on the extent of cortical gas-space in adventitious roots of *Zea mays* (oldest whorl). Means and SEs of 5–10 roots. Plants were 15-d old at the start of the experiment

Treatment	Gas space (% of cortex) in root tissue of decreasing age			
	6 d	4.5 d	3.0 d	1.5 d
Air	5.9± 3.7	6.9±1.8	1.3±0.6	0
1 kPa oxygen	0	4.3±1.9	4.3±2.7	0
Air+0.1 Pa ethylene	23.1±11.3	15.6±2.8	11.6±2.5	3.4±1.9
1 kPa oxygen+ 0.1 Pa ethylene	5.2±4.3	4.1±2.5	1.8±1.8	0

ACC. Roots in 3–5 kPa oxygen also readily converted ACC to ethylene (results not shown). Conversely, when aminoethoxyvinylglycine (AVG), an inhibitor of ACC biosynthesis from methionine, was applied to roots in 5 kPa oxygen, ethylene production was halted almost completely. This response to a 1-d treatment with the inhibitor was probably accomplished without major interference from non-specific side effects since root extension was increased slightly rather than inhibited (Table 3). Inhibition of ethylene formation by AVG was associated with a marked decrease in the extent of aerenchyma in roots exposed to 5 kPa oxy-

Table 3. Effect of increasing concentrations of aminoethoxyvinylglycine (AVG) on ethylene production by 45-mm-long apical segments of adventitious roots of *Zea mays* excised after 16 h exposure to 5 kPa oxygen. Extension growth prior to excision is also shown. Means and SEs shown

	Concentration of AVG (mmol m^{-3})			
	0	0.5	1.0	10.0
Ethylene production[a] (mm^3 kg^{-1} h^{-1}) n=4	3.1 ±0.4	0.06 ±0.02	0	0.01 ±0.01
Extension growth (mm) n=11	11.0 ±1.0	11.0 ±1.0	13.0 ±1.0	16.0 ±1.0

[a] 1 mm^3 kg^{-1} h^{-1} is equivalent to 1 nl g^{-1} h^{-1}

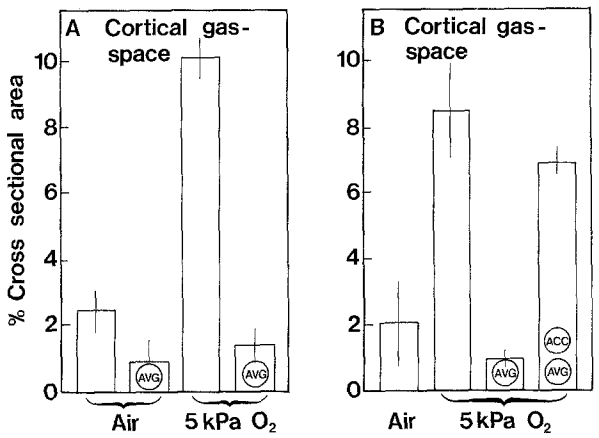

Fig. 4A, B. Effect of aminoethoxyvinylglycine (1.0 mmol m^{-3} AVG) and 1-aminocyclopropane-1-carboxylic acid (0.5 mmol m^{-3} ACC) on cortical gas-space in adventitious roots of *Zea mays*. **A** Effect on 3-d-old tissue of a 5-d exposure of the whole root system to AVG in air and in 5 kPa oxygen. **B** Effect on 3-d-old tissue of a 4-d exposure of the whole root system to air or to 5 kPa oxygen and AVG with and without ACC. Plants were 16 d old at the start of the experiment. Means and SEs of six roots from the second-oldest whorl

gen for 3–4 d. The small amount of gas-space in roots growing in nutrient solutions supplied with air was also decreased substantially by AVG (Fig. 4A). The effect of AVG on aerenchyma in 2-d-old tissue was almost completely reversed by applying ACC (Fig. 4B). The stimulation of aerenchyma formation by 5 kPa oxygen was nullified by the addition of small concentrations of silver nitrate (0.4 mmol m^{-3}), an inhibitor of ethylene action (result not shown).

Discussion

Adventitious roots that extend for several days into sealed and unstirred (stagnant) nutrient solution are more porous than similar roots growing in well-aerated solutions (Table 1). This confirms our earlier conclusion made from estimates of cell-free voids in the cortex of transverse sections of root viewed under a microscope (Drew et al. 1979). We had also found that gas extracted from these aerenchymatous roots by applying a partial vacuum contained increased concentrations of ethylene. Any explanation for an increased abundance of ethylene in roots under stagnant conditions must include a consideration of the much slower rate of gas diffusion in water compared with air (Burg and Burg 1965). Thus, submerged roots seem likely to retain more endogenous ethylene than roots in moist air or well-drained and structured soil (Konings and Jackson 1979). In stagnant rather than vigorously stirred and aerated nutrient solution, the entrapping effect of water will be exacerbated by the accumulation of dissolved ethylene originating from the roots.

But this alone is not the full explanation of how roots in unstirred nutrient solution become enriched with ethylene. A water covering that is sufficient to entrap ethylene will also partially exclude atmospheric oxygen. At temperatures warm enough for rapid root extension, the resulting slow entry of oxygen will be inadequate for the needs of respiration. In such circumstances, roots can be expected to deplete the surrounding solution of dissolved oxygen. In our experiments, oxygen content throughout the stagnant nutrient solution decreased by more than 50% within 24 h. These changes in oxygenation affected the rate of endogenous ethylene production. Without oxygen, little or no ethylene is produced by roots (Jackson et al. 1978; Bradford and Dilley 1978; Drew et al. 1979), fruit (Burg 1973), or mung-bean hypocotyls (Imaseki et al. 1977).

However, the effect of intermediate oxygen partial pressures such as those we measured in stagnant solutions is less clear. Much evidence in the literature indicates that a decrease might be expected. For example, the last step in the usual biosynthetic pathway for ethylene depends on oxygen. Also ATP (aerobically generated) is required to provide the 3,4-carbon moiety of S-adenosylmethionine (SAM) the penultimate precursor of ethylene. Molecular oxygen is also necessary earlier in the pathway where methionine is regenerated from methylthioribose (Yung et al. 1982). In accord with these biochemical findings, partial oxygen deficiency does indeed depress ethylene formation in mung-bean hypocotyls (Imaseki et al. 1977), rice coleoptiles (Raskin and Kende 1983a) and rice leaf-bases (Raskin and Kende 1983b). But roots of maize respond quite differently. Instead of inhibiting ethylene production, oxygen partial

pressures of 1, 3, 5, 12 kPa all stimulated it several-fold after 3 d treatment. We have recently reported a similar result with barley roots (Jackson et al. 1984). A stimulation of ethylene production of this kind probably contributes appreciably to the enrichment of roots with ethylene under stagnant conditions, much of the ethylene being entrapped within the roots by the unstirred, waterbound surroundings. The stimulation of ethylene production by 5 kPa oxygen commences within 2–4 h (Fig. 3) (see also Jackson 1982), but is expressed more sluggishly than the inhibiting effect of a total absence of oxygen, which is complete in less than 2 h (Fig. 3). Anoxia probably acts at the last step in the biosynthetic pathway and its effect is correspondingly rapid. The slower effect of subambient oxygen partial pressures in stimulating ethylene production indicates activity earlier in the pathway, perhaps at the step catalysed by the enzyme ACC synthase, the so-called 'pacemaker' reaction (Yang et al. 1980). An alternative explanation springs both from the proposal of Bradford and Yang (1981) that in some unexplained way the formation of ACC is stimulated by the absence of oxygen in root tissue, and from evidence of Bertani and Brambilla (1982) that a core of anoxic cells exists in roots exposed to partially oxygen-deficient environments. This anoxic core could be a source of additional ACC which might then diffuse out into better-aerated neighbouring cells that contain sufficient oxygen to allow its conversion to ethylene. In this way an overall increase in ethylene production would be effected. The problem merits closer biochemical study. It is not restricted to roots; Raskin and Kende (1983b) recently reported a stimulation of ethylene formation in internodes of deep-water rice treated with 3 kPa oxygen.

Our finding that oxygen partial pressures which favour ethylene production also promote aerenchyma formation (Figs. 1, 2, 3) is correlative evidence for implicating the additional ethylene in gas-space formation. An apparent discrepancy in our results is the failure of 1 kPa oxygen to accelerate aerenchyma formation even though extra ethylene was synthesised. Clearly, in these severely oxygen-deficient conditions the roots lacked a capacity to respond to the ethylene. Even when a supplement of exogenous ethylene was given, aerenchyma did not form if the oxygen supply was limited to 1 kPa (Table 2). Thus, the oxygen requirement for ethylene production is less than that needed for ethylene-promoted lysigenous breakdown of cortical cells (Campbell and Drew 1983) that forms aerenchyma.

In less severe oxygen shortage, the additional ethylene generated seems to promote gas-space development. The evidence rests not only on their joint occurrence but also on the effectiveness of inhibitors of ethylene production and action. Maize roots readily convert exogenous ACC to ethylene, so endogeneous ACC is probably the natural precursor of the gas. Accordingly, treatment with AVG, an inhibitor of pyridoxal-phosphate-mediated enzyme reactions such as the conversion of SAM to ACC (Yang et al. 1980), greatly depresses ethylene production by roots in 5 kPa oxygen. Roots treated with AVG are also almost totally devoid of aerenchyma (Fig. 4a). A part of this effect on gas-space could possibly be ascribed to non-specific toxicity unconnected with ethylene production. Certainly, AVG retards root extension by approx. 25% after 4 d indicating an effect of this kind. However such interference cannot account for much of the effect of AVG because its inhibiting effect on aerenchyma formation is overcome if ACC is supplied so as to by-pass the blockage of endogenous ACC production (Fig. 4B). Further evidence in favour of a mediating role for ethylene in aerenchyma formation in 5 kPa oxygen comes from the inhibiting influence of applying silver nitrate, an ethylene antagonist. The effect in preventing gas-space formation exceeds by far its slowing effect on extension growth indicating a useful degree of specificity of action. Konings (1982) reported that other inhibitors of ethylene production (cobalt chloride and amino-oxyacetic acid) also inhibit aerenchyma formation in maize roots in unstirred nutrient solutions. However, the effects of the inhibitors were not tested at known oxygen partial pressures. Furthermore, the specificity of their action was not examined by attempting to reverse the responses by concomitant applications of ACC or ethylene.

In summary, the stagnant nutrient solution that resulted in aerenchyma development in adventitious roots in *Z. mays* contained subambient partial pressures of oxygen (3–12 kPa) that stimulated ethylene production within 2–4 h. The production was sustained for several days and the ethylene seemed to accelerate cortical gas-space (aerenchyma) formation. This conclusion is supported by evidence of the joint occurrence of faster production of both ethylene and aerenchyma, and the efficacy of inhibitors of ethylene production or action in retarding the development of aerenchyma.

We thank Mrs. A. Izzard for technical help Mrs. Jean M. Nash for typing the manuscript and Dr. Peter F. Sorter of Hoffman-La Roche Inc. (Nutley, N.J., USA) for a gift of aminoethoxyvinylglycine.

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Received 6 November 1984; accepted 3 April 1985