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Ethylene-promoted Adventitious Rooting and Development of Cortical Air Spaces (Aerenchyma) in Roots May be Adaptive Responses to Flooding in *Zea mays* L.

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Abstract. The roots and stem base of intact, 10 day old maize (*Zea mays* L. cv. LG11) plants, grown in nutrient solution, were continuously aerated either with ethylene (5 μl l^{-1}) in air or with air alone. Ethylene treatment hastened the emergence of adventitious (nodal) roots from the base of the shoot, but slowed their subsequent extension. Ethylene also promoted the collapse of cells in the cortex of these roots, with lysigenous development of prominent air spaces (aerenchyma). Non-aeration of the nutrient solution caused endogenously produced ethylene to accumulate in the roots, and stimulated both the emergence of adventitious roots and the formation of cortical air spaces in them. With non-aeration the concentration of oxygen did not fall below 1% in the equilibrium gas phase (air = 20.8%). Complete deoxygenation of the nutrient solution, produced by passing oxygen-free nitrogen gas, prevented both air space formation and the evolution of ethylene by root segments.

These results suggest that adventitious rooting and cortical air space formation in nodal roots in *Zea mays* may be stimulated by enhanced concentrations of endogenous ethylene arising either from entrapment of the gas by unstirred water layers around the roots and/or by increased biosynthesis. These responses are considered conducive to survival in waterlogged soil.

Key words: Adventitious roots – Air spaces (aerenchyma) – Ethylene – Oxygen – *Zea*.

Introduction

Flooding the soil often leads to oxygen deficiency in the roots of dryland plants by impeding the replacement, by diffusion from the atmosphere, of oxygen used in respiration (Grable, 1966). Impaired gas-exchange with the environment can also cause trapping of endogenous ethylene within submerged tissues, increasing its concentration (Kawase, 1972; Musgrave et al., 1972; Konings and Jackson, 1979). Similarly, ethylene produced by micro-organisms accumulates in flooded soil, frequently attaining concentrations that exceed those which retard root extension (Smith and Russell, 1969). Furthermore, ethylene production by *shoot* tissue can be stimulated by exposing *roots* to anoxic contitions (Jackson and Campbell, 1976; Bradford and Dilley, 1978).

In this paper we examine the possibility that extra ethylene derived in one or more of these ways may have adaptive significance in *Zea mays* by stimulating adventitious rooting at the base of the stem and enhancing the formation of cortical air spaces (aerenchyma) within them. Such air spaces may improve the oxygen status of the newly formed roots by reducing the amount of respiring tissue and by providing a pathway of low resistance to the internal diffusion of oxygen from the well aerated shoot (Williams and Barber, 1961; Armstrong, 1971).

Materials and Methods

Plant Growth Conditions

Maize (*Zea mays* L. cv. LG 11) was germinated in the dark on moist filter paper at 20° C. After 2.5 days, plants were suspended over aerated nutrient solution on stainless steel gauze in the dark for 1 day before exposure to light in a controlled environment room. A 16 h photoperiod of 115 W m^{-2} (20 klx) was supplied by fluorescent tubes. The composition of the nutrient solution was a 1/10 dilution of the following (m mol l^{-1}): Mg^{2+}, 1.5; Ca^{2+}, 1.5; K^+, 6.0; Na^+, 2.0; $H_2PO_4^-$, 1.0; $NO^-{}_3$, 10.0; SO_4^{2-}, 1.5 with 5 mg l^{-1} Fe as Fe-EDTA and micro-nutrients. After a further 2 days, plants were supplied with the above solution at full strength.

Experimental Treatments

Ten days after germination, at the 4-leaf stage when the first whorl of adventitious or nodal roots was emerging, each plant was sealed

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Table 1. Effect of ethylene on the emergence and elongation of adventitious roots of *Zea mays*[a]

	Duration of treatment			
	7 days		14 days	
	Control	Ethylene	Control	Ethylene
1st whorl to emerge				
Number	4.0	5.2[b]	4.2	4.3
Extension per root	28.3	6.7[b]	45.0	13.3[b]
2nd whorl to emerge				
Number	3.5	4.5	3.5	4.0
Extension per root	5.6	4.4	31.6	11.3[b]
3rd whorl to emerge				
Number	0	4.0[b]	4.2	4.7
Extension per root	0	1.2[b]	14.9	9.5[b]
4th whorl to emerge				
Number	0	0	1.7	5.5[b]
Extension per root	0	0	1.8	7.5[b]
Total number of adventitious roots per plant	7.5	13.7[b]	13.6	18.5[b]
Total extension of adventitious roots per plant (cm)	133	58[b]	365	188[b]

[a] At the beginning of the treatments, only first whorl adventitious roots had emerged from the base of the shoot. The average number was 3.9 with a mean length of 1.4 cm
Values are means, $n=6$
[b] Significantly different from controls ($P<0.05$)

Table 2. Effect of non aeration on the emergence and elongation of adventitious roots of *Zea mays*

	Duration of treatment			
	7 days		11 days	
	Control	No air	Control	No air
1st whorl to emerge				
Number	3.8	4.0	4.0	4.3
Extension per root	23.0	7.0[a]	28.1	7.8[a]
2nd whorl to emerge				
Number	4.2	4.0	3.7	4.0
Extension per root	16.5	14.9	28.6	23.2
3rd whorl to emerge				
Number	2.5	3.5	5.0	5.0
Extension per root	2.9	5.9[a]	6.2	17.0
4th whorl to emerge				
Number	0	0	0	5.0[a]
Extension per root	0	0	0	2.2[a]
Total number of adventitious roots per plant	10.5	11.5	12.7	18.0[a]
Total extension of adventitious roots per plant (cm)	164	108[a]	249	222

[a] significantly different from controls ($P<0.05$)
Values are means, $n=6$

into a plastic lid fitted to a 1.5 l jar of nutrient solution, about 1 cm above the shoot base using a gas-tight silicone rubber gasket. During this operation, emerged adventitious roots were marked 1 cm behind the tip by a spot of charcoal dust applied with a fine brush. This charcoal mark allowed subsequent elongation of roots to be measured. Plants were grown in nutrient solution receiving the following treatments: (a) bubbled with charcoal-filtered air drawn from outside the laboratory; (b) bubbled with ethylene in air (5.0 ± 0.5 µl l^{-1}); (c) sealed, with no aeration; (d) bubbled with oxygen-free nitrogen gas. Gases were supplied to each jar at 100 ml min^{-1} and the effluent from the ethylene treatment was piped outdoors to prevent contamination of the growth room. Concentrations of ethylene (µl l^{-1}) measured during the experiment were: ethylene treatment, 5.3; air supplied to controls, 0.02; nitrogen gas, 0.01; air in the environment room, 0.02.

Measurement of Root Growth and Air Space Development

Adventitious (nodal) roots of *Zea mays* are grouped in whorls which emerge sequentially. The first whorl to emerge is designated number one, and the second oldest whorl, number two etc. The number of nodal roots that had emerged 1 mm or more from the stem base, and their extension, were recorded separately for each whorl. The air space in these roots was measured after 7 days in transverse sections of fresh material cut by hand at intervals along the length of the new growth that had occurred during the experiment. Control and ethylene-treated roots extended at different rates, and as it was necessary to compare zones of similar age, the following procedure was used. For the 1st whorl, sections were cut the same *fractional* distance along the new growth made during the treatment period, e.g. 0.2, 0.4, 0.6, 0.75 and 0.95, this last section being just behind the root tip. Likewise, for the 2nd whorl, zones of similar age could be compared from observation of the time at which roots emerged and their extension rates. Sections were stained in aqueous toluidine blue (5 g l^{-1}) and photographed under a microscope. From the photographic prints, areas comprising intact cells, well defined air spaces, and collapsed cells (areas where cells had lysed but no air spaces could be distinguished clearly) were measured.

Scanning Electron Microscopy

Transverse sections of fresh roots were cut using degreased, stainless steel razor blades and frozen in liquid nitrogen before freeze drying. Specimens were sputter coated with gold under vacuum and viewed using a Cambridge Stereoscan microscope.

Measurements of Ethylene Production

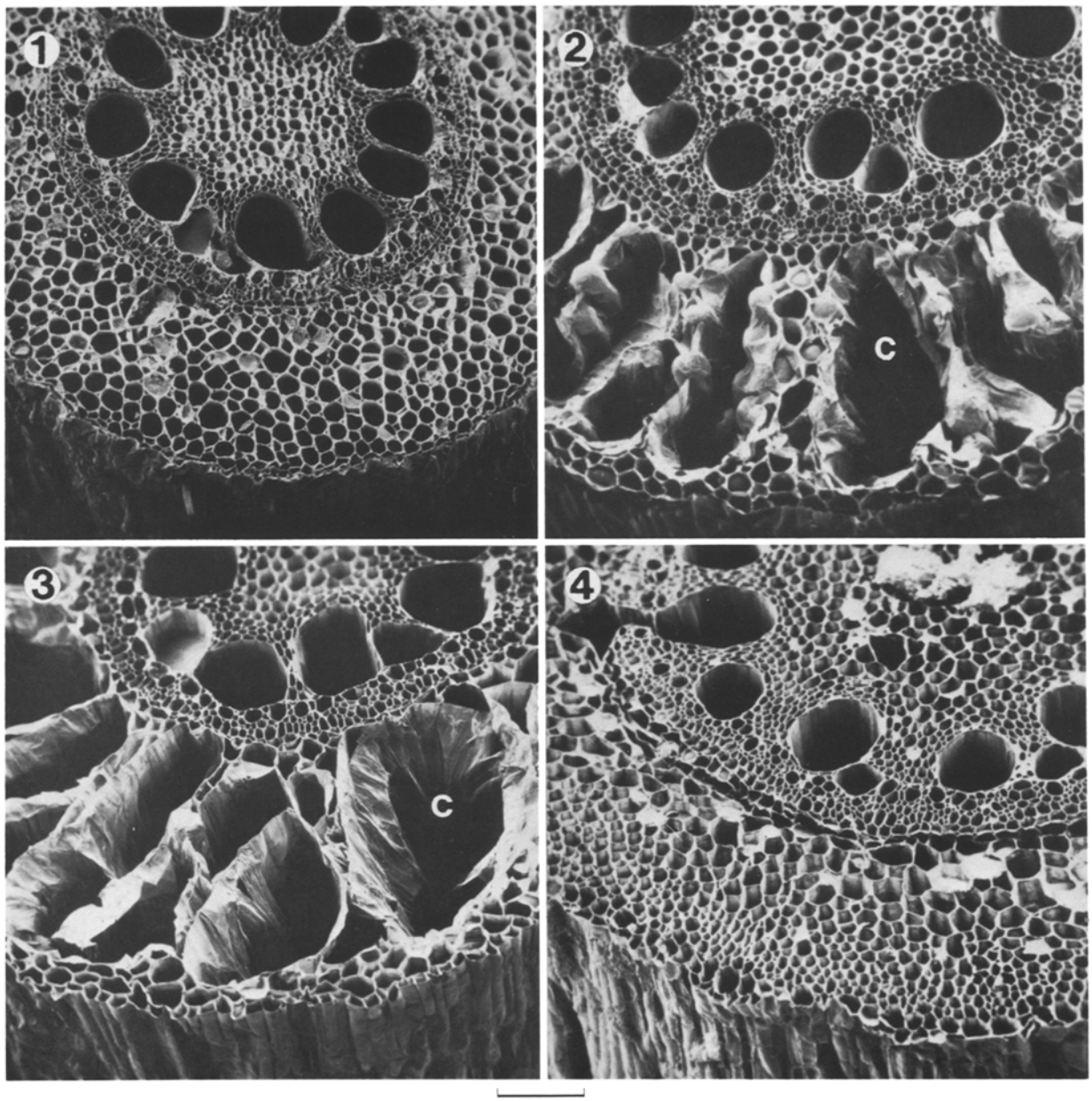
Gases were extracted under reduced pressure, approximately 60 kPa (0.6 atm) from batches of nodal roots from 4 plants (about 10 g fresh weight) using the method of Beyer and Morgan (1970). Ethylene in 0.3 to 0.5 ml gas samples was assayed using a gas chromatograph sensitive to 0.005 µl l^{-1} (Jackson and Campbell, 1975). Ethylene production rates by nodal roots were measured separately after placing 14 apical segments, 2 cm long, in 10 ml Erlenmeyer flasks. Each flask was sealed with a rubber "Suba-Seal" puncture cap and flushed for 1 min with air or oxygen-free nitrogen gas. The ethylene content of the flasks was estimated in 1 ml gas samples withdrawn with a hypodermic needle

and syringe. Immediately after each sampling, the flasks were flushed for 1 min with air or nitrogen as appropriate.

Results

Ethylene or Non-Aeration Promote the Emergence of Adventitious Roots

Ethylene added to air promoted the emergence of the 3rd whorl of adventitious roots at 7 d, and the 4th whorl at 14 d (Table 1), but the number of roots that finally emerged in whorls 1–3 were not affected. The elongation of adventitious roots was approximately constant throughout the 14 d period for both treatments, although ethylene slowed the rate to about 27 % of the controls. Non-aeration promoted the emergence of the 4th whorl of adventitious roots (Table 2) in a broadly similar way to ethylene, but the rates of extension of roots of the 2nd and 3rd



Figs. 1–4. Transverse sections of 2nd whorl adventitious roots in scanning electron microscopy. Sections were prepared from the zone approximately 0.6 along the length of the root. c; cortical air space. (×88); bar = 250 μm

Fig. 1. Control grown in well aerated solution

Fig. 2. Root receiving 5 μl l^{-1} ethylene in air

Fig. 3. Root from non-aerated solution

Fig. 4. Root receiving nitrogen gas (anoxic treatment)

Table 3. Effect of ethylene on cortical air space development in adventitious roots of *Zea mays*[a]

	Relative distance along root from base[c]	Per cent cross sectional area of cortex[b]							
		Air space + collapsed cells		Collapsed cells		Air space		Intact cells	
		Control	Ethylene	Control	Ethylene	Control	Ethylene	Control	Ethylene
1st whorl of adventitious roots to emerge	0.20 (base)	27	36[d]	10	17[d]	17	19	73	64[d]
	0.40	16	36[d]	5.6	18[d]	10	18	85	65[d]
	0.60	20	26	7.5	18	12	8.4	81	74
	0.75	10.7	12	3.9	7.3	6.8	4.5	92	88
	0.95 (apex)	0	0	0	0	0	0	100	100
2nd whorl of adventitious roots to emerge	0.20 (base)	0	34[d]	0	17[d]	0	17[d]	100	67[d]
	0.40	0.7	27[d]	0	14[d]	0.7	13[d]	99	73[d]
	0.60	0	17[d]	0	8.9[d]	0	7.6	100	84[d]
	0.75	0	2.5	0	0.7	0	1.8	100	98
	0.95 (apex)	0	0	0	0	0	0	100	100

[a] Plants were treated for 7 d. Ethylene concentration was 5 μl l^{-1} in air

[b] Measurements were made from photomicrographs of transverse sections

[c] Distance is expressed as a fraction of the new extension made by the root since the start of the treatment or for 2nd whorl roots, since the emergence of the controls

[d] Mean values, $n=6$ significantly different from controls ($P<0.05$)

Table 4. Concentration of ethylene extracted under reduced pressure from roots of *Zea mays* subjected to various aeration treatments[a]

Treatment	Ethylene concentration (μl l^{-1})
Control (aerated)	
adventitious roots	0.84 ± 0.08
seminal roots	0.88 ± 0.04
Non aerated	
adventitious roots	1.77 ± 0.19
seminal roots	1.22 ± 0.11
Ethylene (5 μl l^{-1} in air)	
adventitious + seminal roots	3.2 ± 0.84

Values are means ± SE, $n=4$. Treatment period, 7 d

[a] The concentrations of O_2 dissolved in the nutrient solutions in this experiment after 7 d (expressed as per cent in the equilibrium gas phase) were: Controls, 19.5; no air, 1.7; ethylene, 21.0

whorls were not appreciably slowed relative to controls. The 1st whorl roots, which were about 5 cm long at the start of the treatment extended only a short distance into the oxygen deficient solution between 7 d and 11 d presumably as a direct consequence of oxygen shortage in these preformed roots.

Ethylene or Non-Aeration Increases Cortical Air Space Formation

The influence of various aeration treatments on root structure after 7 d are illustrated in photomicrographs taken by scanning electron microscopy (Figs. 1–4). Compared with the relatively intact cortex of controls (Fig. 1), ethylene caused extensive formation of air spaces in the *new* root tissues produced during the treatment (Fig. 2). This appeared to be a consequence of cell dissolution after cell expansion was completed (lysigeny) as no air spaces were observed in the apical 2 cm. Similar responses were also obtained with the 1st, 2nd and 3rd whorl of adventitious roots. The effects of non-aeration were very similar to those induced by applying ethylene (Fig. 3). However, when oxygen-free nitrogen gas was passed through the solution to exclude oxygen completely from around the root system and stem base, development of air spaces was totally arrested (Fig. 4). Extension growth by the 1st whorl of nodal roots was stopped by this treatment, and that of the 2nd whorl was restricted from 32 cm (controls) to only 2 to 4 cm after 14 d. Emergence of the 3rd and 4th whorls of nodal roots was prevented by treatment with nitrogen gas.

The effects of ethylene on cell breakdown and development of air spaces at different distances from the root tip are given quantitatively in Table 3. In the basal (older) zones, ethylene increased the area of air spaces and collapsed cells. In control plants, air spaces were absent from the 2nd whorl roots, and in the 1st whorl were present only in the older zones. No air spaces occurred in the younger, apical tissue in any of the controls or ethylene treated roots.

Gas Analysis

Non-aeration of the nutrient solution resulted in greater concentrations of ethylene in the gases

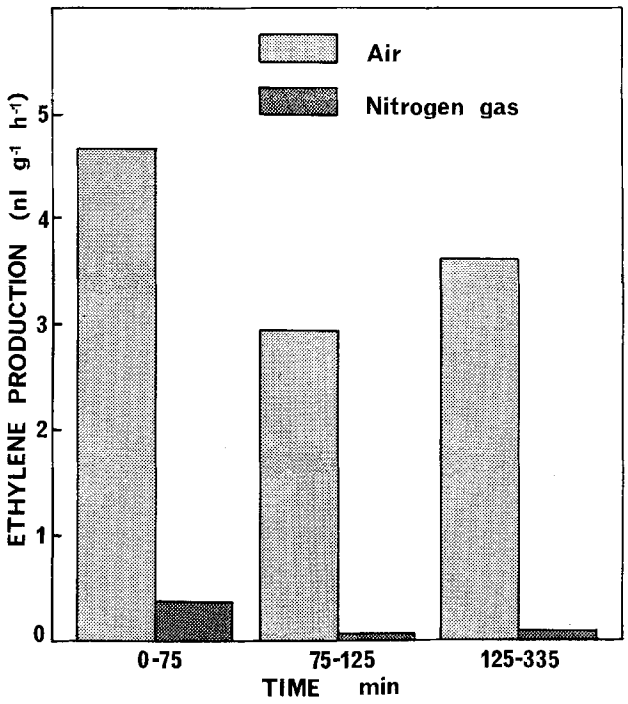


Fig. 5. Evolution of ethylene by excised, 2 cm apical segments of adventitious roots of maize. Plants were grown in well aerated nutrient solution before excision and enclosure in flasks flushed with air (controls) or nitrogen gas. Each value is the mean of 6 replicates. Nitrogen treated roots gave significantly lower values than controls ($P < 0.05$), throughout

extracted from roots, compared with controls (Table 4), the effect being more pronounced for adventitious than for seminal roots. The concentration of oxygen in the medium did not fall below 1%. The ethylene concentration in the gases extracted from ethylene-treated roots was reasonably close to that supplied exogenously in the treatment gas mixture. Treatment with nitrogen gas to exclude oxygen completely, which suppresses air space formation, was greatly inhibitory to ethylene formation (Fig. 5). Thus, aeration treatments that favoured the accumulation and/or production of ethylene also enhanced air space formation.

Discussion

The growth of adventitious roots and the formation of air spaces in them are recognised as adaptive responses to flooding in higher plants (Kramer, 1951; 1969; Armstrong, 1971; Coutts and Armstrong, 1978). Our results suggest that these responses in *Zea mays* could arise from increased ethylene concentrations in the responding tissues. In non-aerated, unstirred cultures, which however contain some oxygen, ethylene of endogenous origin may become trapped within the tissues. The presence of stationary water layers around submerged organs retards ethylene losses, the diffusion coefficient in water being some 10^4 times *less* than in air (Burg and Burg, 1965). Thus, increased concentrations of ethylene have been found with submerged roots and stems of intact chrysanthemum, sunflower, tomato and radish (Kawase, 1972) and the submerged shoots of aquatic plants (Musgrave et al., 1972).

Whether the oxygen deficiency associated with non-aeration caused increased synthesis of ethylene in the nodal roots of *Zea mays*, contributing further to elevated internal concentrations (Table 4), remains to be examined. Increased ethylene production was found to take place in sunflower stems subjected to oxygen stress (Kawase, 1978), but it is unlikely that the tissue was strictly anaerobic since free oxygen is usually required for ethylene biosynthesis in plants (Mapson, 1969). Anoxic tissue would not therefore be expected to produce much ethylene. Experiments in which nitrogen gas was used to exclude oxygen from roots of tomato (Jackson et al., 1978) and maize (Fig. 5) bear this out. However, ethylene production in basal parts of the shoot and in roots located near the water surface, experiencing low but appreciable oxygen supplies (either from the rooting medium or by internal diffusion from aerial tissues) may be stimulated by the presence of anoxic roots below. It is interesting to speculate that cell injury or degradation in anoxic roots might lead to increased levels of amino acids, including methionine, a likely precursor of ethylene, or to other substances stimulatory to ethylene biosynthesis, (see Jackson and Campbell, 1976 for further discussion).

Wample and Reid (1975) found that simply surrounding the roots and stem base with well oxygenated water can stimulate adventitious root initiation and emergence on the stems of sunflower, anaerobic conditions in the medium not being an essential factor. Entrapment of endogenous ethylene may explain these effects. The promotion of adventitious root formation on stems of tomato by low exogenous concentrations of ethylene in the rooting medium has been previously recorded (Jackson and Campbell, 1975). Similar effects can be induced in sunflower (Kawase, 1974) by treating roots with "Ethepon", which decomposes in plants to release ethylene. The stimulation of adventitious rooting in dicotyledons by ethylene has been known since the studies of Zimmerman and Hitchcock (1933). In our experiments with maize, ethylene did not seem to initiate primordia as the number of roots in each whorl was not affected (Table 1); rather it stimulated the emergence of roots through the tissues of the stem base. The primordia concerned were presumably initiated before the start of the treatment.

Our results suggest that the acceleration of air

space formation in the newly produced nodal roots of *Zea mays* has some features in common with the swelling of preformed stems in sunflower, induced by "Ethephon" or flooding. Evidence that ethylene may encourage cell separation and development of air spaces in these *stems* has recently been given by Kawase (1979), although he provided no information concerning roots. He suggested that ethylene may promote cell separation via an enhanced cellulase activity leading to wall fission. This proposal is reminiscent of increased cellulase activity in the separation zone during leaf abscission, a process stimulated by ethylene application or by ethylene produced by adjacent senescing cells (Jackson and Osborne, 1970, 1972).

The mechanism by which ethylene causes a distinctive pattern of cell collapse and lysis in the mid cortex in the roots of *Zea mays*, giving rise to aerenchyma formation, remains to be resolved. The gas cannot be regarded as toxic: if this were the case, a more indiscriminate degeneration of cells would have taken place. One possibility is that cells lyse under turgor following enzyme-mediated weakening of wall structure (Bateman, 1976) in thin walled, ethylene sensitive cells of the mid cortex. Alternatively, some cell-wall-degrading enzymes may directly affect the viability of protoplasts (Wood, 1976).

The promotion of the apparently adaptive phenomena in *Zea mays* by ethylene described here, accords with reports that in appropriate species the gas can induce other morphological changes that appear conducive to the survival of flooding, including: faster extension growth in submerged stems of aquatic plants (Ku et al., 1970; Musgrave et al., 1972); leaf epinasty (Jackson and Campbell, 1975; 1976); leaf abscission (Harvey, 1913) and enlargement of lenticels (Wallace, 1926).

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