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THE EFFECTS OF ETHYLENE IN THE ROOT ENVIRONMENT UPON THE DEVELOPMENT OF BARLEY

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SUMMARY

Following observations that ethylene can occur in anaerobic or partially anaerobic soils at concentrations which can affect plant growth, shoot and root growth of barley plants, maintained in solution culture, were examined after long-term exposure of the roots to ethylene in air; the subsequent growth on transfer to a similar but ethylene-free environment was also studied. Both root and shoot dry weights were reduced slightly by the ethylene treatment; seminal root extension was inhibited greatly while lateral root growth was stimulated; absorption of ions and their transport from root to shoot within the plant was not affected. On transfer to an ethylene free environment the extension rate of the seminal axes increased markedly and was the greater the shorter the period of the preceding ethylene treatment; the extension of laterals initiated during the ethylene treatment was stimulated greatly but the growth of those formed subsequently was inhibited.

Split root experiments showed that on any plant only those roots actually exposed to ethylene became modified by the gas while those remaining in an ethylene free environment were typical of roots of untreated plants.

INTRODUCTION

It has been shown that ethylene can occur in anaerobic or partially anaerobic soils at concentrations which can be injurious to plant roots [7 13 15]. Earlier studies of the effects of ethylene upon root growth [2 13 14] have been restricted to measurements of the extension and the increase in fresh weight over short periods ranging from a few hours to a few days. The possible importance of ethylene as a factor impairing the growth of plants in poorly drained soils has

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justified an examination of its effects upon both root and shoot growth in longer experiments. In the first experiments described here the barley root systems were treated with continuous exposure to ethylene in air. In further experiments the exposure to ethylene was brief and was followed by a period of growth in an ethylene-free environment to provide information on the permanence of any changes in the pattern of development caused by ethylene.

METHODS

Barley seeds (*Hordeum vulgare* L, variety Midas) were germinated on moist filter paper in the dark at 20°C. When 2 to 3 seminal roots had appeared the seedlings were transferred to rods suspended over tanks containing the following nutrient solution: K 1.5, Ca 0.2, Mg 0.4, NO_3 0.7, H_2PO_4 0.3, SO_4 0.7 milliequivalents $litre^{-1}$ together with minor nutrients as used by Hackett [8]. Plants were grown in controlled-environment cabinets, with an air temperature of 15°C and a light intensity of 24 kilolux and were selected for uniformity immediately before the start of experimental treatments.

Using a cold-curing silicone resin each seedling was sealed into the metal lid of a 1500-ml glass preserving jar. The experimental gas mixtures were bubbled through the jars of nutrient solution and the gas which accumulated in the space between the nutrient solution and the gas tight lid was collected and led to waste outside the laboratory.

Mixtures of ethylene in air used in the work were either obtained from the British Oxygen Company Ltd (Special Gases Department, London SW19) or prepared using the diffusion method described in another report [5]. Ethylene was measured with a gas chromatograph using the methods described by Smith and Restall [13].

The potassium and calcium contents of wet ashed plant material were determined by flame photometry, and phosphate contents by the vanadate method [17]. In some experiments the absorption of K^{42}, Sr^{85} and P^{32} was measured. The diameters of plant root members were measured under a microscope using a micrometer eye piece and their lengths were measured with a ruler; dry weights were recorded after desiccation overnight in an oven at 90°C.

RESULTS

(A) *The effects of exposure of the root system to ethylene for periods of up to three weeks*

In a number of experiments where the roots were exposed to between 1 and 10 parts per million ethylene in air for periods of up to 21 days there were small reductions in the final dry weight of

both roots and shoots but these reductions were not significant in even the largest single experiment. However, a linear regression obtained from pooled data of a number of experiments by plotting root and shoot dry weights of ethylene-treated plants against those of controls showed the dry weight reduction by ethylene to be statistically significant in both roots and shoots (Fig. 1).

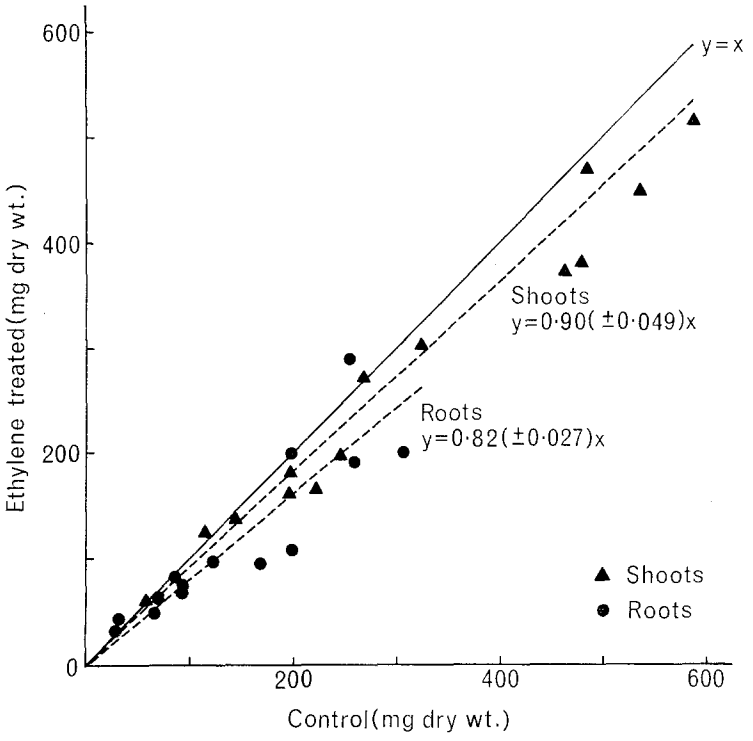

Fig. 1. The root and shoot dry weights of ethylene-treated barley plants plotted against those of untreated plants. Concentrations of 1–10 ppm ethylene in air were applied to the roots for periods varying between 7 and 21 days. The regression lines are significantly different from unity at the 1% level of probability.

Table 1 shows the effect of exposure to ethylene upon some dimensions of the root system. There was a marked reduction in the length of the seminal axes, but there was no effect upon the number of primary laterals per unit length of axis so that their total number per axis was reduced. Exposure to ethylene caused increases in the

diameters of the seminal axes, the lengths of primary laterals and in the numbers and lengths of secondary laterals; however there was a resultant reduction of approximately 35 per cent in the total length of laterals per axis.

TABLE 1

The effect of exposure to ethylene, for the period between 8 and 20 days after germination, on the seminal roots of 20-day-old barley plants

		Control		Ethylene-treated
Main axis				
	Mean length mm	506	***	230
	Mean diameter mm	0.18	**	0.24
Lateral members				
Primary	Mean length mm	20	**	28
	Mean number per cm axis bearing laterals	5.4	—	5.9
Secondary	Mean length, mm	2.6	***	5.2
	Mean number per cm axis bearing laterals	0.7	***	2.3
Total length of all laterals per axis, mm		7536	***	4872

*** A significant difference at $p < 0.1$.
** A significant difference at $p = 0.1$–1.0.

Exposure to the gas gave the root system a stunted appearance (Fig. 2). In the zone 0.5–2.5 cm behind the apex, the main axes and laterals were swollen, curved and covered densely with root hairs (Inset Fig. 2).

The development of nodal roots was not studied in detail but superficial examination revealed great variability between similarly treated plants both within and between experiments.

(B) *Growth during the period following an exposure to ethylene*

Table 2 shows the results of an experiment in which some root systems were exposed to 10 ppm ethylene for 3 days (between 9 and 12 days after germination) and others for 13 days (between 9 and 22 days after germination). The plants which received the shorter exposure to ethylene were sampled at the end of the gas treatment also 4, 10 and 16 days later, while those which received the longer

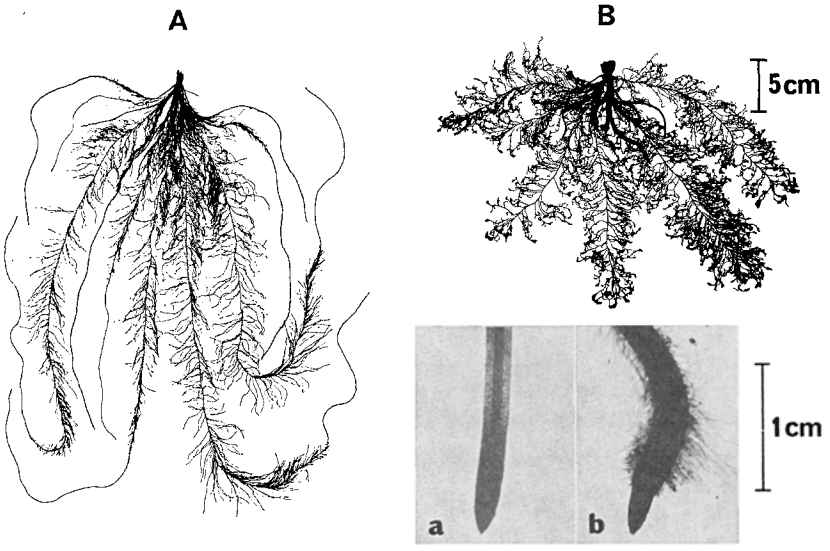

Fig. 2. The root systems of 20-day-old barley seedlings grown in solution culture. (**A**) Control. (**B**) Exposed to 10 ppm ethylene in air for the final 11 days of growth. *Inset*: Apices taken from (**a**) control and (**b**) ethylene treated seminal roots.

ethylene exposure were sampled at the end of treatment and 13 days later.

After ethylene was withdrawn the increase in root dry weight did not differ significantly from that of the control plants. As described in Section A, ethylene inhibited the extension of the seminal axes (Table 2) but growth was resumed on transfer to an ethylene free environment although the rate of recovery appeared to depend upon the duration of exposure. After the shorter exposure to ethylene the subsequent extension of treated axes was similar to that of controls, but after the longer exposure the subsequent extension of treated axes was only about half that of controls.

The shorter exposure had no significant effect upon the subsequent emergence and extension of the nodal axes in an ethylene free environment. During the longer exposure to ethylene the extension of nodal axes was inhibited but their emergence was stimulated. However, during the subsequent period in an ethylene free environment fewer additional nodal axes emerged on the treated

TABLE 2

The effect of exposure to ethylene on the subsequent growth of the roots of young barley plants after transfer to an ethylene-free environment

Period and duration of exposure	Age of plants, days	Treatment	Mean length seminal axes, mm	Mean length nodal axes, mm	Mean number nodal axes	Dry weight root system mg
Plants exposed to ethylene for 3 days	12	Control	224 ***	—	—	19
		Treated	169	—	—	21
(Days 9-12 after germination)	16	Control	341 ***	4.0 —	3.6 —	38 —
		Treated	294	3.4	4.1	36
	22	Control	524 —	7.2 —	8.3 —	89 —
		Treated	492	9.1	8.0	81
	28	Control	705 —	12.9 —	13.5 —	171 —
		Treated	651	13.6	15.1	179
Plants exposed to ethylene for 13 days	22	Control	524 ***	7.2 ***	8.3 **	89 —
		Treated	268	1.6	15.8	77
(Days 9–22 after germination)	35	Control	980 ***	9.95 **	36.7 *	441 —
		Treated	487	18.9	28.3	467

*** A significant difference at $p < .001$.
** A significant difference at $p = .01–.001$.
* A significant difference at $p = .05–.01$.

plants than on the controls although their extension was more rapid than that of controls (Table 2).

During the period which followed exposure to ethylene, the extension of the lateral members which were attached to part of the axis which had extended during exposure to the gas (zone A in Table 3) was almost 5 times greater than that of laterals in an equivalent position on the control roots. Exposure to ethylene considerably reduced subsequent extension of laterals born on that part of the axis which extended immediately after the end of exposure to the gas (zone B in Table 3). Figure 3 shows this effect 9 days after the end of the 13 day exposure to ethylene.

TABLE 3

The effect of exposing the root systems of barley plants to 10 ppm ethylene in air, for 3 days, on lateral growth during the subsequent 10 days in an ethylene-free environment

	Zone A†			Zone B††		
	Controls		Ethylene-treated	Controls		Ethylene-treated
Primary laterals						
Mean length mm	30	***	142	27.0	***	5.3
Mean number per cm of axis	5.7	—	5.4	6.0	—	6.0
Secondary laterals						
Mean length mm	3.5	**	6.3	2.4	*	1.7

*** A significant difference at $p < .001$.
** A significant difference at $p = .01$–$.001$.
* A significant difference at $p = .05$–$.01$.
† A 50 mm zone of the seminal axis immediately above the position of the apex at the end of the ethylene treatment.
†† A 50 mm zone below but adjacent to zone A and produced immediately after the ethylene treatment.

While exposure to the gas caused a marked change in the distribution of the extension growth of the laterals it had no effect upon their number per unit length of axis, even though the extension of axes had been inhibited during exposure to the gas.

In the experiment illustrated in Table 3 the mean extension rate per day of laterals in zone A, during the period 4–10 days after the end of the ethylene treatment was 14.0 mm in the treated plants and 2.6 mm in the controls; 10–16 days after treatment the equivalent figures were 8.6 and 2.0 mm per day. Thus although the rate of extension of laterals was declining with age in both treated and control plants the enhancement of extension which followed exposure to ethylene persisted for a considerable time after the end of the treatment.

(C) *The exposure of part of the root system to ethylene*

As has been pointed out elsewhere [6] the development of one part of a root system can be affected not only by its immediate environment, but also by the growth of the remainder of the root system. Since there can be considerable gradients in the concentration of

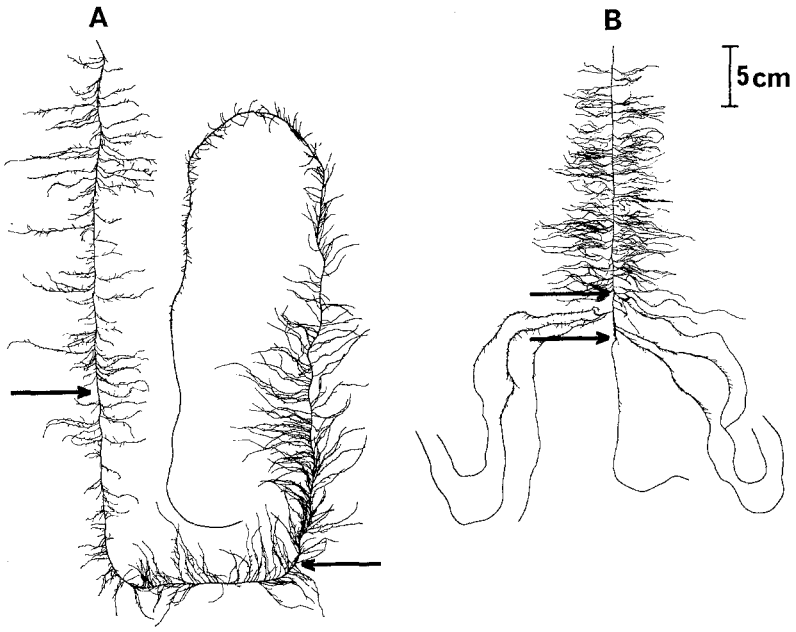

Fig. 3. Seminal axes of 35-day-old barley seedlings grown in solution culture. (**A**) Control. (**B**) The root system was exposed to 10 ppm ethylene in air for a 13-day period (9–22 days after germination), before transfer to an ethylene free environment for the final 13 days growth. The *arrows* indicate the position of the apex at the start and finish of the ethylene treatment.

ethylene in field soils [7], it was of interest to know how the exposure of a part of the root system to ethylene affected the growth of the remaining roots. An experiment was carried out in which, 7 days after germination, 3 of the 6 seminal axes of each plant were sealed into one jar of nutrient solution while the remaining axes were sealed into a separate, adjacent jar. All nodal axes were removed as they developed. Seven days later the plants were treated in one of 3 ways as follows:– half the root system exposed to 10 ppm ethylene in air, the remainder to air only; both halves of the root system exposed to air only; both halves of the root system exposed to 10 ppm ethylene in air.

Plants were removed from the jars and measured after 14 days of treatment. Exposure of part of the root system to ethylene had very little effect on the extension of the remaining axes exposed to air only and there was little effect on the partition of dry matter

TABLE 4

The effect on the seminal roots of 28-day-old barley plants of exposing half the axes to ethylene for the period between 14 and 28 days after germination

	Half the axes treated			No axes treated		All axes treated
	Untreated		Treated			
Mean length of axes mm	520	***	236	493	***	256
Mean dry weight of axes mg	16.7	—	17.3	15.8	—	16.7

*** A significant difference at $p = .01$–0.05.

between the parts of the root system exposed to the two gaseous atmospheres (Table 4). In this treatment those axes exposed to ethylene were similar to those of plants which had their entire root system treated with ethylene.

TABLE 5

The effect of exposing the root systems to ethylene for the period between 5 and 22 days after germination on the absorption and concentration of ions in 22-day-old barley plants

	Ion	Shoot		Root	
		Control	Ethylene-treated	Control	Ethylene-treated
Total concentration of ions, mole g^{-1}	K	852	991	1876	1663
	Ca	101	89	57	59
	H_2PO_4	304	275	288	318
Concentration of labelled ions absorbed during the 20 hours preceding harvest, mole g^{-1}	K	73	61	376	327
	Ca	2.2	2.2	21	20
	H_2PO_4	7.0	7.0	32	35

None of the differences between ethylene treated and control plants were significant at the 5% level of probability.

(D) *The effect of ethylene upon ion absorption*

In one experiment the effect of ethylene upon ion absorption was examined. Root systems were exposed to 10 ppm ethylene from the fifth day after germination until the end of the experiment, 17 days later.

A solution labelled with radioactive isotopes of phosphorus, potassium and strontium (the latter as a tracer for calcium) was used in place of the nutrient solution for the final 20 hours of the experiment. At the end of the experiment the concentrations of the stable and radioactive isotopes of the 3 elements within the plants were measured. Table 5 shows that ethylene had no significant effect upon either the concentration of the 3 elements in the plants or their absorption during the final 20 hours of the experiment, as measured by the radioactive tracers.

DISCUSSION

Ethylene is often an inhibitor of tissue extension [1] but there have also been reports of stimulation of extension by ethylene [11 12 14]. The inhibition of extension of the main root axes of barley plants and the apparently simultaneous stimulation of lateral root extension during exposure to ethylene is most simply explained as being primarily the inhibition of axis extension by ethylene; the enhanced extension of laterals could be a secondary effect due to the interference with root growth co-ordination mechanisms. The effect may thus be analogous to the stimulation of the extension of laterals which follows the removal of the root apex [6 9 16].

Exposure to ethylene caused only a small reduction in the dry weight of the root system; the increased diameter of root axes and the stimulated growth of lateral members compensated almost entirely for the reduction in the length of the main axes. Chadwick and Burg [2 3] found that in young plants, prior to lateral root initiation, a reduction in the weight of the root system accompanied the inhibition of the extension of axes by ethylene. Cornforth and Stevens [4] showed that when germinating barley seedlings were exposed to ethylene, reduced accumulation of dry matter in the root system was accompanied by its increased accumulation in the shoots. Therefore it appears that ethylene can interfere with the mechanisms governing the distribution of metabolites both between roots and shoots and between the various parts of the root system.

The extremely rapid extension of lateral members in an ethylene-free environment, following exposure to the gas, suggests that ethylene causes a persistent change in the behaviour of their meristems.

The experiments do not indicate whether these changes were a direct effect of ethylene upon the meristems at a particular stage of development, (John and Hall [10] have shown that ethylene affects the rate of cell extension and division) or an indirect effect caused by a disturbance of hormonal control mechanisms by the gas.

Concentrations of ethylene which have been shown to modify the form of barley root systems can occur in anaerobic soils [7]. In these experiments ethylene alone was varied whereas in anaerobic soils low oxygen levels alone can have a profound effect on plant growth. The extent of the contribution of the ethylene production to the overall effect of anaerobic soil conditions is therefore unknown. Further experiments are necessary to examine more fully the effects of ethylene on plant growth under a wider range of conditions.

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