

SOME EFFECTS OF HALOGENATED HYDROXYBENZOIC ACIDS ON SEEDLING GROWTH

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Summary Effects of 3,5-diiodo-4-hydroxybenzoic acid (DIHB) on seedlings growing with their roots (a) exposed to white light, (b) in compact soil, (c) in waterlogged compost or (d) in saline solutions, have been monitored. Treatment with DIHB improved certain aspects of seedling growth in each of these adverse cultural conditions. The growth of barley in compact soil was also promoted by 3,5-dichloro-4-hydroxybenzoic acid, but wheat seedlings were inhibited by this treatment.

Possible modes of action of 3,5-dihalogeno-4-hydroxybenzoic acids are discussed.

INTRODUCTION

Root growth of some plant species is inhibited on exposure to white light (see literature cited by Wilkins *et al.*, 1). Such inhibition of cress seedling roots can be eliminated by treatment with 3,5-diiodo, 3,5-dibromo, or 3,5-dichloro-4-hydroxybenzoic acid (DIHB, DBHB or DCHB), such that the roots grow to the same length as those of seedlings maintained in darkness (2, 3). The order of activity of the halogen substituent is I > Br > Cl (4). In darkness, however, cress root growth is not affected appreciably by DIHB, DBHB or DCHB (2). DIHB-induced promotion of cress root growth in the light results from enhanced cell size rather than cell numbers. Furthermore, this promotion is related to reductions in (a) endogenous ethylene production by the roots (5) and (b) growth-inhibiting effects of high concentrations of exogenous ethylene around the roots (6). DIHB also suppresses IAA-induced ethylene production by excised cress root segments (5). It is probable, therefore, that DIHB-induced promotion of cress seedling root growth in the light is related, at least in part, to decreases in the endogenous production of ethylene.

High concentrations of ethylene result in swelling of root apices and proliferation of root hairs (7), and both of these are morphological characteristics of light-exposed roots grown in hydroponic culture in the absence of DIHB (6). Stunting and swelling of root systems also occurs when growth is restricted by high mechanical resistances in the root growth medium (8, 9). Since (a) mechanical resistance to the growth of bean roots, pea epicotyls and potato sprouts stimulates endogenous ethylene production (10, 11, 12), and (b) compact soils contain more ethylene than less compacted soils (13, 14), it seems likely that growth inhibition in compact soil may be related to the effects of ethylene.

A waterlogged environment can have several effects on the endogenous growth-regulator status of roots (15, 16), one of which is an increase in ethylene content of the tissues (17, 18, 19). Waterlogged soil in which tomato seedlings are

growing contains increased concentrations of ethylene and it seems probable that gas moved from the roots to the shoots is responsible for stimulating epinastic curvature of leaf petioles (20, 21, 22).

Since DIHB-enhanced growth of light-exposed cress seedling roots is related to decreased ethylene production by the tissues, we have examined the possibility that DIHB might promote growth in other situations of physiological stress, such as compact soil and waterlogged conditions, in which ethylene might well be involved. Furthermore, we have extended our investigation to include the possible effects of DIHB on the growth of seedlings subjected to a saline environment, because this form of stress also results in stunting of seedling root and shoot growth (23, 24).

MATERIALS AND METHODS

General

Seeds of Pisum sativum L. cv. Meteor, Hordeum vulgare L. cv. Hassan or Sultan, Triticum aestivum L. cv. Eclipse or Zea mays L. cv. LG11 were germinated between sheets of Whatman No. 1 filter paper at $25 \pm 1^\circ\text{C}$ for 22 h in darkness. Seeds of Lycopersicon esculentum Mill. cv. Moneymaker were germinated for 4 days under the same conditions. Seedlings of the appropriate plant species were then selected under green light (510 - 550 nm) for radicles 1 mm in length. The chemicals 3,5-diiodo- and 3,5-dichloro-4-hydroxybenzoic acids (DIHB and DCHB) were supplied to the seedlings as aqueous solutions of their disodium salts. Treatment effects were assessed as (a) length of the primary root of pea and maize seedlings or length of the longest root in the case of barley and wheat seedlings, and/or (b) total root dry weight, and/or (c) shoot length and dry weight. Least significant differences between treatments (LSD) at the 5% level of probability ($P = 0.05$) are represented on the figures by vertical bars.

Soil Experiments

A silt loam soil of the Wood series (air dry) was brought to 20% moisture content using distilled water (control) or solutions of DIHB or DCHB, and was compressed into rigid plastic isodiametric pots to achieve mechanical resistances of 42.4 bar (compact soil) or 12.2 bar (loose soil) (25). Germinated pea, barley or wheat seeds with radicles 1 mm long were planted in small depressions made in the top layer of soil. The pots were then filled to capacity with appropriately treated soil firmed gently at a pressure of 0.15 kg cm^{-2} .

Pea seedlings were grown for 65 h and barley and wheat seedlings for 7 days in a growth room at $20 \pm 2^\circ\text{C}$ with a 16 h photoperiod and light intensity of 27.9 W m^{-2} (Mazda daylight fluorescent tubes). Bending of pea seedling roots, induced by the compact soil treatments, was assessed by calculating the percentage increase in root length on straightening. This procedure was not necessary for the comparatively straight roots of pea seedlings grown in loose soil or for the roots of barley and wheat seedlings grown in compact or loose soil.

Waterlogging Experiments

Tomato seedlings were transplanted into 'Eff' soil-less compost contained in plastic cups (205 mm deep, Mono containers Ltd.) with perforated bases. The cups did not release ethylene spontaneously. An open-ended glass tube (150 mm long, 12 mm diameter), wrapped in aluminium foil to exclude light, was inserted in each cup

to a depth of 50 mm and its top was sealed with a Suba-Seal puncture cap to facilitate sampling of the gas atmosphere.

Tomato seedlings were grown for 20 days (5-leaf stage) at $22 \pm 1^\circ\text{C}$ with a 14 h photoperiod and a light intensity of 27.9 Wm^{-2} . Waterlogging treatments were carried out under the same environmental conditions over a 3-day period, the perforated cups being placed in 1000 ml glass beakers containing 600 ml distilled water (pH 5.5) or DIHB solutions (pH 5.5) such that the surface of the compost was just submerged. Water lost during the experiment was replenished.

Epinastic growth of the petioles of leaves 1 and 2 (the oldest leaves) during the waterlogging treatment was assessed by measuring the angle formed by the main stem and the basal 20 mm of the petioles adaxial surfaces.

Salinity Experiments

The effect of saline conditions on barley seedling growth was determined by incubating seedlings in glass test tubes (145 mm long, 10 mm diameter) containing (a) a strip of filter paper (145 x 30 mm) and (b) distilled water, or solutions of DIHB and/or sodium chloride (NaCl) (1.25 ml). Tubes were either closed with a Suba-Seal puncture cap, so that air samples for ethylene determination could be withdrawn immediately prior to seedling harvest, or were left open to allow free gaseous exchange. Seedlings were incubated for 20 h at $25 \pm 1^\circ\text{C}$ in darkness on a clinostat (1 rpm).

The effect of saline conditions and/or DIHB on wheat coleoptile straight growth was determined using the techniques of Taylor & Burden (26). Subapical coleoptile segments (10 mm long) were excised from 70-h-old seedlings and were incubated for 18 h at $25 \pm 1^\circ\text{C}$ in darkness on a clinostat (1 rpm) in test tubes (97 mm long, 10 mm diameter), to each of which had been added 1 ml of a buffer solution containing, in some cases, DIHB and/or NaCl.

Light/Dark Experiments

After selection under green light for uniformity of root length (1 mm), barley or maize seedlings were placed in glass test tubes ('Quickfit', 150 mm long, 25 mm diameter with reduction adaptors, 24/29 : 14/23, and Suba-Seals fitted) containing a strip of filter paper (140 x 30 mm) and 3 ml distilled water or DIHB solution. Tubes containing seedlings to be kept in darkness were wrapped completely in 2 thicknesses of aluminium foil before being transferred together with unwrapped tubes to a growth room maintained at 25°C with continuous white light (Mazda daylight fluorescent tubes, 16.7 Wm^{-2}). A Grant Miniature Temperature Recorder (Model D) was used to check the internal temperature of the tubes. Ethylene concentrations within the sealed tubes and root growth were measured after incubation of the seedlings for 20 h at 25°C .

The effect of light on cress seedling root growth was determined by germinating seeds on rafts of polystyrene beads floating on 200 ml distilled water or DIHB solution in covered crystallising dishes (2). Root length was measured after the seedlings had been incubated for 72 h in light or darkness at 25°C . This method is referred to as the raft test.

Effect of Ethylene on Growth

Barley seedlings were transferred in green light to conical flasks ('Quickfit';

500 ml) containing 1 sheet of filter paper (90 mm diam.) and 4.5 ml distilled water or DIHB solution. Ethylene or air was injected into the internal atmosphere of the flasks after they had been closed with Suba-Seal puncture caps, and the actual concentration of ethylene in each flask was checked by gas-liquid chromatography (GLC). Root growth was assessed after incubation of the seedlings for 20 h at 25°C in darkness.

Ethylene Determination

Ethylene in 2 ml gas samples was estimated by GLC using a Pye 104 dual column chromatograph with flame ionisation detectors (5).

RESULTS

Effect of Light

Light-exposed roots of cress seedlings grown in the raft test and maize seedlings grown in tubes were shorter in length than those of seedlings grown in darkness at the same temperature (Fig. 1a,b). Treatment of cress seedlings with $10^{-5}M$ DIHB solution significantly promoted elongation so that the roots were the same length as those of seedlings grown in darkness. Roots of cress seedlings treated with DIHB in darkness, however, were not affected significantly (Fig. 1a). In contrast, maize seedling root length was enhanced by DIHB both in light and darkness (Fig. 1b).

Elongation of the roots of barley seedlings grown in closed tubes in light or darkness, monitored as the length of the longest root of each seedling, was promoted by $10^{-3}M$ DIHB solution (Fig. 1c). DIHB treatments effective in enhancing root growth in the light also significantly reduced the release of ethylene by the root tissues (Fig. 1d). The lengths of the longest root of light-exposed barley seedlings grown in glass petri dishes (90 mm diam.) were 14.9 ± 0.3 mm and 18.8 ± 0.6 mm in the presence of distilled water or $10^{-4}M$ DIHB solution (3.5 ml) respectively.

Soil Experiments

The effect of DIHB on pea seedling root elongation in compact soil was concentration dependent, with 10^{-5} - $10^{-7}M$ solutions being the treatments most effective in enhancing unstraightened and straightened lengths (Table 1A). Those concentrations of DIHB most effective in promoting root elongation also produced decreases in (a) root bending and (b) root diameters 1 mm and 5 mm behind the root apices. Shoot height was not influenced appreciably by DIHB-treatment (Table 1A). In loose soil, the only significant effects on pea seedling growth were small decreases in root diameter 1 mm and 5 mm behind the root apices in response to the $10^{-6}M$ treatment and an increase in diameter 5 mm behind the apex with the $10^{-3}M$ treatment (Table 1B).

Incorporation of DIHB in compact soil resulted in increases in (a) length of the longest root, (b) total root dry weight per seedling, (c) shoot height and (d) shoot dry weight of Hassan barley seedlings (Table 1C). The optimum concentration was $10^{-7}M$ DIHB ($0.005 \mu g$ DIHB/g dry soil), which gave a 47% increase in the length of the longest root and a 20% increase in shoot height. Similar effects were detected when seedlings were grown in loose soil containing DIHB, but in this case the $10^{-7}M$ treatment only induced 15% and 9% increases in longest root length and shoot height respectively (Table 1D).

Figure 1

Effect of light and/or DIHB on seedling root growth

Data for cress seedlings are the means of 4 independent replicates for each light (L) and dark (D) treatment, each consisting of 30 seedlings; those for barley are the means of 5 replicates each consisting of 20 seedlings; those for maize of 4 replicates each containing 10 seedlings.

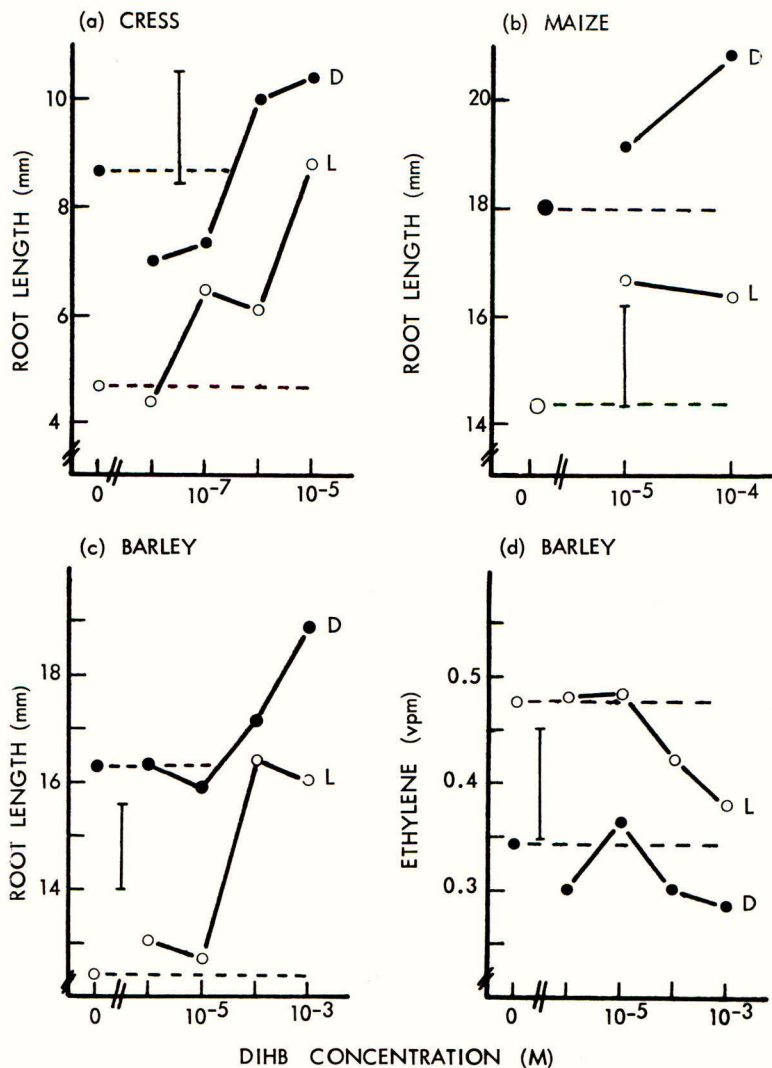


TABLE 1

Effect of DIHB or DCHB on seedling growth in compact and/or loose soil

For peas grown in compact soil, data for root lengths (mm) and shoot heights (mm) are means of at least 30 measurements per treatment, whilst root diameters (mm) are means of at least 72 observations. Results for barley and wheat seedlings are the means from at least 36 measurements per treatment; dry weights are presented as mg/seedling.

	DIHB or DCHB CONCENTRATION (M)						LSD (P=0.05)
	0	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³	
(A) METEOR PEAS IN COMPACT SOIL TREATED WITH DIHB							
Root length unstraightened	13.5	17.1	18.2	17.7	14.6	13.8	2.0
Root length straightened	17.3	22.4	22.3	21.5	17.5	17.7	2.8
% increase in root length on straightening	28.1	31.0	22.5	21.5	19.9	28.3	-
Root diam. 1 mm behind apex	1.20	1.17	1.08	1.07	1.07	1.14	0.09
Root diam. 5 mm behind apex	1.77	1.67	1.59	1.60	1.63	1.65	0.12
Shoot height	8.2	9.0	9.7	8.9	8.3	6.1	nsd
(B) METEOR PEAS IN LOOSE SOIL TREATED WITH DIHB							
Root length	62.9	61.7	63.0	62.8	61.9	60.9	nsd
Root diam. 1 mm behind apex	1.00	1.04	0.93	0.98	1.03	1.05	0.06
Root diam. 5 mm behind apex	1.32	1.37	1.18	1.27	1.32	1.68	0.07
Shoot height	19.8	18.0	17.8	18.4	17.6	17.0	nsd
(C) HASSAN BARLEY IN COMPACT SOIL TREATED WITH DIHB							
Length of longest root	49.7	73.2	65.4	61.8	57.1	59.2	6.3
Total root dry weight	5.9	7.6	6.5	6.7	6.5	5.0	1.3
Shoot height	70.6	84.9	82.4	83.7	84.0	71.2	6.2
Shoot dry weight	6.1	7.3	6.9	6.8	6.9	5.3	1.0
(D) HASSAN BARLEY IN LOOSE SOIL TREATED WITH DIHB							
Length of longest root	124.5	143.6	133.6	139.4	133.2	133.4	8.5
Total root dry weight	11.0	14.8	10.0	10.7	10.4	9.3	3.1
Shoot height	121.6	133.0	132.7	127.6	117.9	124.7	10.1
Shoot dry weight	11.6	12.8	12.5	11.6	11.0	11.4	nsd
(E) SULTAN BARLEY IN COMPACT SOIL TREATED WITH DCHB							
Length of longest root	73.1	91.6	-	96.3	83.8	-	14.5
Shoot height	105.5	122.0	-	123.2	117.9	-	11.4
(F) ECLIPSE WHEAT IN COMPACT SOIL TREATED WITH DCHB							
Length of longest root	70.3	65.2	-	62.0	-	51.6	10.5
Shoot height	109.0	107.9	-	104.1	-	76.8	10.8

Inclusion of DCHB in compact soil also resulted in significant promotion of barley seedling growth (Table 1E). In contrast, however, the growth of wheat seedlings in compact soil was inhibited by DCHB (Table 1F).

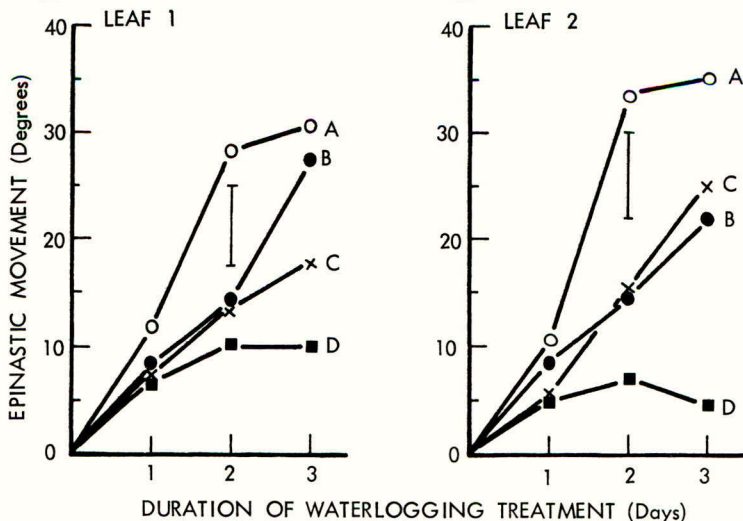
Waterlogging Experiments

Waterlogging of tomato seedlings growing in compost resulted in an epinastic movement of the petioles of leaves 1 and 2, which became more pronounced as the experiment proceeded (Fig. 2). A significant reduction in petiole movement was found when DIHB was included in the waterlogging solution but the degree of movement remained greater than that in non-waterlogged plants. The $10^{-5}M$ DIHB treatment resulted in ethylene, present in the gas-sampling tubes, being at a concentration of 0.19 vpm, whilst the concentrations of that in the waterlogged control and the waterlogged + $10^{-6}M$ DIHB treatments were 0.40 and 0.34 vpm ($LSD_{p=0.05}=0.18$). Ethylene was not detectable from the soil atmosphere of non-waterlogged plants.

Figure 2

Effect of DIHB on the epinastic response of leaf petioles of waterlogged tomato plants

Data for each treatment on each day are the means of petiole movement on 7 independent plants. Treatments were (A) waterlogged control, (B) waterlogged + $10^{-6}M$ DIHB, (C) waterlogged + $10^{-5}M$ DIHB and (D) non-waterlogged control.



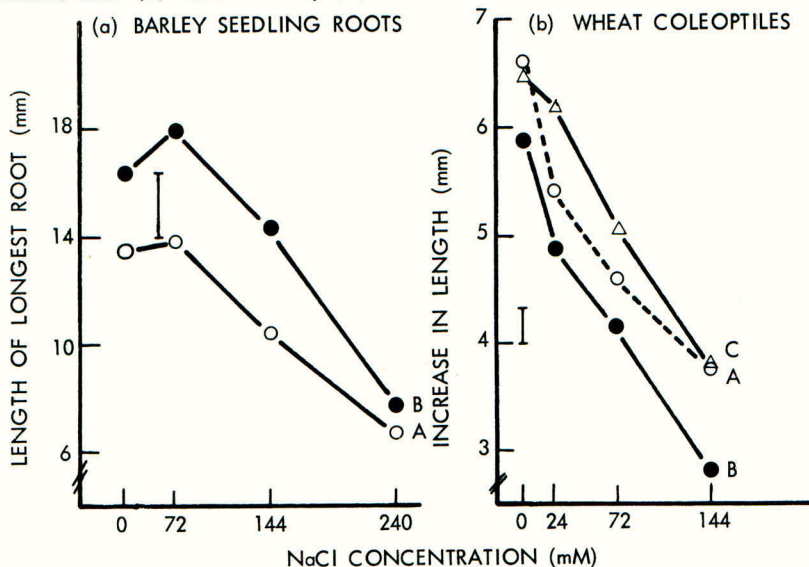
Salinity Experiments

Barley seedlings grown in darkness in closed glass test tubes showed progressive decreases in root growth i.e. in the length of the longest root, with increasing salt concentration (Fig. 3a). Treatment with $10^{-4}M$ DIHB solution significantly promoted root growth in the presence of 72 mM and 144 mM but not 240 mM NaCl solution. In fact compared with the water control, 144 mM NaCl inhibited growth by 25% whilst the 144 mM NaCl + $10^{-4}M$ DIHB treatment promoted growth by 9%. Total root dry weight was also increased by DIHB treatment. For example, the dry

Figure 3

Effect of NaCl and/or DIHB on the growth of barley seedling roots and excised wheat coleoptile segments

For each treatment, data for the length of the longest root of barley seedlings are the means of 8 replicates each consisting of 6 seedlings; those for wheat coleoptile segments are the means of 12 replicates each consisting of 10 segments. Treatments were (A) NaCl control, (B) NaCl + 10^{-4} M DIHB and (C) NaCl + 10^{-5} M DIHB.



weights for the 144 mM NaCl and the 144 mM NaCl + 10^{-4} M DIHB treatments were 0.38 and 1.14 mg per seedling respectively ($LSD_{p=0.05} = 0.23$).

Release of ethylene into the tubes by the barley seedlings decreased as the salt concentration was increased and at 72 mM and 144 mM NaCl significantly smaller amounts of ethylene were released in the presence of DIHB (Fig. 4a). When ethylene was expressed as concentration per gramme dry weight of root tissues (Fig. 4b), the general effects of the DIHB treatments were similar to those presented in Fig. 4a. However, the amounts of ethylene released in response to the 240 mM NaCl and the 240 mM NaCl + 10^{-4} M DIHB treatments were almost the same as for the water and 10^{-4} M DIHB controls.

In general, barley seedling root growth was better in open than in closed tubes (Table 2), except in the 144 mM NaCl treatments where was no difference. In the presence of 144 mM NaCl, DIHB produced greater enhancement of root growth in the open tubes but in the absence of NaCl it had greater effect in the closed tubes.

Straight growth of excised wheat seedling coleoptile segments incubated in a buffer solution containing NaCl, exhibited smaller increases in length as the salt concentration was increased (Fig. 3b). In the 24 mM and 72 mM salt treatments, 10^{-5} M DIHB solution promoted coleoptile extension, whilst the 10^{-4} M DIHB treatments inhibited growth compared with the salt-only controls.

Figure 4

Effect of NaCl and/or DIHB on the release of ethylene by barley seedlings

Data are the means of 8 replicate determinations of ethylene concentration per treatment. Treatments were (A) NaCl control and (B) NaCl + 10^{-4} M DIHB.

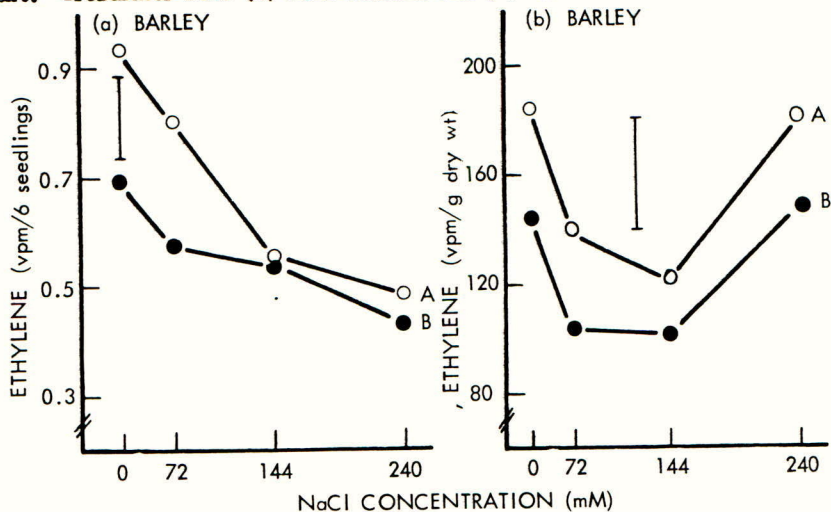


Table 2. Effect of DIHB and/or NaCl on growth of barley seedling roots in open or closed tubes

Data are the means of 72 observations per treatment of root length.

	Root Length (mm)	
	Closed Tube	Open Tube
Water control	12.7	18.3
10^{-4} M DIHB	15.1	20.3
144 mM NaCl	10.9	10.9
144 mM NaCl + DIHB	12.7	14.2
LSD (P=0.05)	1.1	

Table 3. Effect of ethylene and/or DIHB on root growth of barley and maize seedlings

Data for barley and maize are the means of 90 and 40 observations of root length per treatment respectively.

	Root Length (mm)	
	Barley	Maize
H ₂ O control	14.9	20.9
10^{-5} M DIHB	18.5	19.7
1 vpm ethylene + H ₂ O	10.6	18.4
1 vpm ethylene + 10^{-5} M DIHB	12.4	20.0
5 vpm ethylene + H ₂ O	8.5	12.9
5 vpm ethylene + 10^{-5} M DIHB	13.1	13.1
LSD (P=0.05)	1.1	2.2

Effect of Exogenous Ethylene on Seedling Growth

Growth of the roots of 1-day-old barley or maize seedlings in an atmosphere of 1 ppm or 5 ppm ethylene in darkness was significantly retarded compared with that of seedlings grown in air (Table 3). The length of the longest root of barley seedlings was enhanced by DIHB-treatment both in air and ethylene. However, DIHB had no significant effect on maize seedlings.

DISCUSSION

Light-induced inhibition of root elongation of cress, maize and barley seedlings was alleviated in the presence of DIHB, and in cress and barley seedlings it was eliminated completely (Fig. 1a,b,c). Maize and barley seedlings showed significant increases in root elongation in darkness in response to DIHB (Fig. 1b,c), an effect which has also been observed with dark-grown lettuce seedling roots (27). With barley, root growth promotion in light and darkness was related to decreases in the amount of ethylene released by the seedlings (Fig. 1d). These findings support those of Robert *et al.* (5), who found ethylene concentrations in hydroponic culture solutions in which cress seedling roots were growing to be (a) greater in the light than in the dark and (b) smaller in the light if DIHB was present.

Pea and barley seedlings were smaller when grown in compact soil than in loose soil, but root growth in compact soil was improved when DIHB was present (Table 1A, B,C,D). Although these increases in root length in response to DIHB were significant when compared with roots grown in untreated compact soil, they were less impressive when compared with the size of seedling roots grown in untreated loose soil. With peas grown in compact soil, DIHB-treatment resulted not only in increased root elongation but also in decreased root diameters and decreased root bending (Table 1A). Such changes in root morphology might indicate that DIHB was alleviating 'ethylene-type' growth reactions, a speculation supported by the finding that DIHB significantly reduced the growth-inhibiting effect of high concentrations of ethylene supplied exogenously to barley seedlings (Table 3). DIHB also reduced the amount of ethylene released by barley seedlings (Fig. 1d, 3a). Thus it is not surprising that DIHB should have some beneficial effect on seedling growth in compact soil because (a) compact soils contain higher concentrations of ethylene than loose soils (13, 14) and (b) plant tissues release increased amounts of ethylene when their growth is impeded (10, 11, 12).

If the action of DIHB on seedlings growing in compact soil was to reduce the production or the physiological effects of ethylene, then the fact that barley seedling growth was enhanced by DIHB in loose soil must be considered, for here only low levels of ethylene would be likely. However, barley is particularly sensitive to ethylene (28) so it might benefit from DIHB treatment if low concentrations of ethylene in loose soil exert a detrimental effect on root growth. A DIHB-induced reduction in the effects or production of ethylene might also provide a situation in which ethylene stimulated (29) rather than inhibited root growth.

Waterlogged tomato plants exhibit epinastic curvature of the leaf petioles, but inclusion of DIHB in the waterlogging solution reduced both epinasty and the concentration of ethylene in the soil solution (Fig. 2). Increased concentrations of ethylene in shoots seem to have an important role in the epinastic response, and this ethylene may be produced endogenously by the plant in response to low levels of oxygen in the soil or may be taken up from the soil by the plant (30, 21). Epinastic growth can also be suppressed by spraying waterlogged tomato plants with cytokinins or mixtures of gibberellic acid and cytokinin (21, 31, 32).

Saline conditions inhibited the growth of barley seedling roots and excised wheat coleoptiles, but 10^{-4} and 10^{-5} M DIHB respectively reduced the amount of inhibition (Fig. 3a,b). With barley seedlings, the amount of ethylene released

was inversely related to the salt concentration, and it was consistently lower in the presence of DIHB (Fig. 4a). However, when the data are expressed in terms of ethylene released per gramme dry weight of root tissue (roots were the only tissues to have emerged from the seed), the concentration of ethylene was higher in the 240 mM than in the 144 mM sodium chloride treatments (Fig. 4b). Such a difference might be related to the possibility that 240 mM salt solution was imposing an osmotic stress on the seedlings (33, 34). The leaves of citrus trees show enhanced ethylene production when the trees are subjected to phytotoxic saline treatments (35).

Larqué-Saavedra *et al.* (6) proposed that non-aerated waterlogged conditions increased the root growth-promoting activity of DIHB in the light because there was little effect of DIHB on cress seedling root growth on aeration of the hydroponic culture solutions in which they were growing. Anaerobic conditions would also be likely in the rhizosphere of waterlogged tomato plants (22) and roots growing in compact soil might also be affected by areas of low oxygen content (36). Thus in these two situations, the growth-promoting properties of DIHB might be enhanced if oxygen was depleted. Such a decrease in oxygen concentration would be likely in the closed tubes in which the effect of DIHB on the ethylene production of barley seedlings was being monitored (Figs. 1, 3, 4). Under these conditions, however, the action of DIHB did not seem to be particularly dependent upon an anaerobic environment. In the salinity experiments (Table 2), for example, a comparison of the effect of DIHB on barley seedling root growth in closed and open tubes revealed growth promotions of (a) 19% and 11% respectively in the non-saline controls and (b) 16% and 30% respectively in the presence of salt. Furthermore, DIHB-treatment of light-exposed barley seedlings resulted in root length being promoted by 32% and 26% when the experiments were carried out in sealed tubes (gaseous exchange limited) and glass petri dishes (unrestricted gaseous exchange) respectively.

Although it is probable that DIHB influenced either the production, release or physiological effects of ethylene in each of the experimental situations reported in this paper it is not yet clear whether this action is of primary importance in relation to growth. For this reason, it is important to consider other possible modes of action of DIHB. For example, DIHB is known to interfere with the growth-inhibiting effects of exogenously applied abscisic acid (ABA) and xanthoxin (1, 27, 37). Since the endogenous concentration of ABA is known to increase in (a) light-exposed root tissues (38, 39) and (b) shoots of plants subjected to saline or waterlogged conditions (40, 41) it is possible that any action of DIHB on plants subjected to these treatments (Figs. 1, 2, 3) could be related to its interaction with this potent growth inhibitor. Another possible mode of action of the 3,5-dihalo-*geno*-4-hydroxybenzoic acids is decarboxylation within the plant to form the corresponding 2,6-dihalo-*genophenol*. These phenols are known to possess appreciable growth-promoting properties and they have also been shown to be relatively inactive in wheat tissues whilst having considerable activity in peas (42).

In conclusion, the data presented in this paper demonstrate that it is possible to use a chemical treatment to improve the growth of seedlings subjected to certain adverse environmental factors. Further research is necessary, however, to determine whether treatment with the 3,5-dihalo-*geno*-4-hydroxybenzoic acids is likely to have long-lasting effects on the growth of crop plants and also to determine the precise mode of action of these chemicals.

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CAN STRESS EXPERIENCED BY ROOT SYSTEMS BE ALLEVIATED BY EXOGENOUS GROWTH REGULATORS?

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Summary Waterlogged or compacted soil conditions can induce a wide variety of growth responses. Many effects may be mediated by endogenous plant hormones since the content of each of the main groups of growth regulators has been reported to change in plants subjected to short periods of waterlogging. Little is known of hormonal changes in mechanically impeded roots but their importance is deduced from the nature of growth and structural responses to physical forces.

In tomato, symptoms of waterlogging injury include low rates of stem extension, transpiration and growth in shoot weight together with stimulations of epinastic curvature and adventitious rooting. They arise primarily from a deficiency of molecular oxygen in the rooting medium. Treating the foliage with benzyladenine (BA) and gibberellic acid (GA) can mitigate some of the effects of anaerobiosis and promote the growth of plants under anaerobic and also aerobic conditions.

Extension growth by the roots of barley seedlings is reduced by mechanical impedance and poor soil aeration. In compacted soil both stresses are likely to be experienced by the plant root system. Using an artificial system designed to permit the study of mechanical impedance in the absence of a deficiency of oxygen or other stresses, very low pressures down to 0.2 bar have been found to depress elongation. Under these conditions and application to the roots of 3, 5-diiodo-4-hydroxybenzoic acid (DIHB) stimulates extension growth to a small extent; a similar proportional increase can also be obtained when DIHB is applied to roots which are not subjected to mechanical constraint.

When the mechanisms controlling the actions of growth regulators are more fully understood, treatments may be devised which will be of practical relevance for the relief of stress conditions in the soil.

INTRODUCTION

The fundamental concept that many of the growth responses of plants to their environment are mediated by hormones may be traced back to Darwin's famous experiments with gravity and light which were published in 1880(1). In recent years it has become evident that growth is regulated by at least five groups of hormones, namely the auxins, gibberellins, cytokinins, ethylene and inhibitors such as abscisic acid. Although much research has been specifically concerned only with shoot tissue, available evidence also implicates hormones in the control of growth in roots and its co-ordination with shoot development.

In this paper we consider evidence for the involvement of hormones in the responses of plants to the waterlogging of the soil or to mechanical impedance, and

consider the possibility of alleviating the symptoms by applying growth regulators. These stress conditions are somewhat dissimilar but are nevertheless considered together since in the field both can occur in association with inadequate aeration of the root system.

WATERLOGGED SOIL CONDITIONS

(a) Effect on the soil and on plant growth. Saturation of the soil with water will impede gaseous exchange between the soil and the aerial environment since the coefficient of diffusion for most gases is several thousand times less in solution than in air. If, as a result, the demands for oxygen by respiring roots and soil organisms exceed the restricted supply from the atmosphere, an anaerobic rooting medium will develop. Many of the symptoms of waterlogging injury can be reproduced by growing plants in anaerobic solution cultures and it is therefore evident that a deficiency in the supply of oxygen to roots is a major cause of damage to waterlogged plants. A loss of nutrient ions from the soil, the production of phytotoxic substances and the accumulation of gaseous products of respiration may also have a deleterious influence(2).

Symptoms arising from this much changed soil environment include poor root growth or root death, low rates of shoot growth, leaf senescence and abscission, accelerated adventitious or nodal root production, epinastic curvature, abortion of young flowers and premature termination of growth by the shoot apex. The effects on any one plant are, however, subject to considerable variation. The duration of waterlogging and other environmental conditions such as temperature, and content of soil organic matter can influence the extent of injury. The age of the plant is also an important determinant of sensitivity to anaerobiosis. For example peas have been shown to be more tolerant as young vegetative plants than at the time of flowering(3). Marked differences in susceptibility also exist between species. Plants such as the pea or tobacco are severely injured and can be killed by several days of waterlogging while others such as winter wheat respond in ways which appear conducive to survival under these conditions. The physiological basis for these differences are not understood, but the involvement of plant hormones cannot be excluded.

(b) Effect on the content of hormones in plants. The possibility that waterlogging the rooting medium has implications for the hormonal relationships of plants was considered first by Went(4) who provided indirect evidence for the synthesis by roots of a hormone like factor 'caulocaline', which is required for stem growth and produced in reduced amounts when roots are submerged in non-aerated water. At about the same time Chibnall(5) concluded that "... some influence of the root system, possibly hormonal, is responsible for the regulation of protein synthesis (senescence) in leaves". It is only within the last 15 years that evidence has accumulated to support this concept, now widely held, that for normal growth and longevity, the aerial parts of plants are largely dependant upon hormones (mainly cytokinins and gibberellins) produced by healthy roots (6,7,8,9,10,11). It is therefore to be expected that when roots experience anaerobic conditions, normal metabolism is disrupted and their effectiveness as a source of hormones for the shoots is diminished. The concentration of substances with gibberellin(12,13) or cytokinin(14) like activity in xylem-exudates from decapitated plants has been found to fall within the first 24 h of waterlogging; this implies that the total flux of such hormones into the shoots of intact plants is also much reduced. However detailed analyses of the gibberellin and cytokinin content of shoot tissues using modern physical methods of assay have not been reported. One attempt using bioassay techniques yielded somewhat complex result(15).

In contrast to the gibberellins and cytokinins, the remaining groups of hormones have been reported to increase following waterlogging. Auxin(16), abscisic acid

(17,18), and ethylene(19,20,21) have all been found in higher concentrations, albeit sometimes transiently, in the shoot systems of various species. Some of the ethylene may be derived from the soil(22,23),from extra production in shoot tissues (24) or from the physical trapping effect of water surrounding plant parts(25,26). The degree to which hormonal changes bring about the effects of waterlogging is still a matter for speculation. Their influence is largely inferred from the similarity between certain responses to short periods of waterlogging and the generally cited properties of the substances concerned or from the results of applying growth substances to plants. The high concentrations of the growth substances used(12,27,28) are often difficult to relate to the comparatively small changes which develop endogenously.

(c) Effects of applying growth substances to plants. The possibility that applying gibberellic acid and/or a cytokinin to the shoots of waterlogged plants may compensate for deficiencies in endogenous hormones of this type and also oppose the actions of others present in greater amounts has been investigated primarily in the tomato plant.

There is little doubt that when benzyladenine (BA) a synthetic cytokinin, and gibberellic acid (GA) are applied, some remission of injury from waterlogging can be achieved(27,28,29), although the effects have been less marked for some responses than for others. In our experiments these hormones affected not only waterlogged plants but also plants growing in well drained soil. Broadly similar results have also been obtained in aerobic and anaerobic solution cultures. Benzyladenine and GA (10 mg l⁻¹) were given as a foliar spray to plants at about the time of flowering on the day before anaerobic soil conditions were induced and again on each of the following three days. Stem extension was promoted throughout the 7 days of the experiment and was sufficient to overcome the waterlogging stress. However, in absolute terms elongation by the stem was promoted by BA and GA more in plants growing in aerobic rather than anaerobic conditions (Table 1). In some experiments more stem growth occurred in anaerobically treated plants which received the hormones than in aerobically grown plants not treated with the hormone spray.

Growth in shoot fresh weight can be reduced by up to 45 per cent of non-waterlogged controls by 7 days of waterlogging but treatment with BA and GA increased the growth of waterlogged plants to within 10 per cent of controls (Table 1). Waterlogging also halved the growth in shoot dry weight. Although BA and GA offset this to some extent, growth in dry weight by waterlogged plants was still about 33 per cent below that of non-waterlogged controls. As was found for shoot extension, BA and GA were more effective in stimulating the growth of non-waterlogged plants than waterlogged plants (Table 1).

Waterlogging reduced the rate of transpiration (Table 1), a result compatible with the observation that the stomata of tomatoes are induced to close by waterlogging(24). The increase in transpiration brought about by BA and GA (Table 1) is in accord with the conclusion of Livne and Vaadia(30) that these hormones can promote stomatal opening; this may explain why waterlogged plants sprayed with BA and GA tended to wilt (Table 1). The maintenance of open stomata may also be implicated in the increased growth in dry weight by hormone treated plants, since by this means the fixation of carbon-dioxide by photosynthesis could be enhanced(31).

In experiments using solution cultures, anaerobic conditions were found to reduce growth in root dry weight by 64 per cent over 6 days, while applying BA and GA inhibited root growth even further (Table 1). In contrast, anaerobiosis stimulated adventitious rooting at the base of the stem, an effect which was less evident when BA and GA were also given (Table 1).

Several authors have reported that BA and/or GA can reduce the epinastic curvature of leaves which develops when the roots of tomato (or sunflower) plants are

TABLE 1

Effects of benzyladenine (BA) and gibberellic acid (GA) each at a concentration of 10 mg l⁻¹ on tomato plants at the 8-10 leaf stage which were grown for 7 days in waterlogged and non-waterlogged soil

	Non-waterlogged		Waterlogged	
	Control	BA & GA	Control	BA & GA
Shoot growth over 7 days				
Shoot extension (cm)	5.8 ± 0.2	8.6 ± 0.4	3.1 ± 0.2	5.5 ± 0.3
Shoot fresh weight (g)	27.2 ± 1.7	43.7 ± 1.3	12.4 ± 1.6	24.4 ± 2.2
Shoot dry weight (g)	4.1 ± 0.2	5.0 ± 0.2	2.1 ± 0.1	2.8 ± 0.2
Transpiration ⁽¹⁾ (ml plant ⁻¹ day ⁻¹)	144.1 ± 4.5	180.8 ± 7.5	103.9 ± 7.8	137.0 ± 11.2
Number of wilted plants (day 6)	0	0	0	4
Number of adventitious roots	0	0	14.4 ± 1.6	8.8 ± 1.9
Root growth over 6 days ⁽²⁾				
Root dry weight x 10 (g)	2.33 ± 0.35	0.90 ± 0.26	0.84 ± 0.28	0.61 ± 0.25
Petiole epinasty (degrees)	12.4 ± 3.4	14.6 ± 4.8	64.9 ± 4.4	26.6 ± 3.5

The means of 11 replicates are shown together with their standard errors.

(1) Measured between day 2 and day 4 in a separate experiment.

(2) From plants grown in solution culture.

subject to anaerobic conditions(32,27,28). The effect is shown in Table 1. Epinastic growth is intimately connected with an increase in the ethylene content of leaf tissue. This ethylene may give rise to the epinastic growth since the application of low amounts of the gas (< 0.07 ppm) also induces this response while inhibitors of ethylene action retard epinasty(33). When BA and GA were applied, epinasty was inhibited (Table 1) but the high rate of ethylene production by waterlogged plants was not reduced. Epinastic curvature is therefore not inhibited by these hormones through a regulation of ethylene biosynthesis. An alternative explanation is therefore required. Observations that BA and GA can reduce the epinasty response to applied ethylene suggest that these hormones may reduce the sensitivity of petiole tissue of waterlogged plants to the gas (Jackson & Campbell, unpublished).

MECHANICAL IMPEDANCE

(a) Occurrence in the field. Wiersum(34) has shown that roots are unable to decrease in diameter in order to penetrate small pores. Thus under field conditions unless there are sufficient pores or fissures which roots can enter freely, the soil must, necessarily be deformed. The reaction of the soil exerts a pressure on the root and may modify its form and structure. Since the diameter of roots is normally in the range 0.1 to 2 mm and pores smaller than this do not drain under gravity, the conditions which give rise to mechanical stress on roots are also likely to be associated with anaerobiosis. However unlike anaerobiosis, mechanical stress does not modify the growth of the whole plant providing the supply of water and nutrients is adequate.

(b) Effect on root extension. Although roots can develop a maximum pressure of 10 to 15 bars(35) the minimum pressure which restricts extension is of greater significance to the plant since it can limit the ability of roots to obtain adequate water and mineral nutrients. This subject is best investigated in artificial systems where precisely measured mechanical stresses can be applied while other factors affecting growth remain favourable. Using such techniques it is now well established that if, when expanding pores, roots are subject to pressures greater than a small fraction of a bar, their rate of elongation is considerably reduced(36,37,38). In barley, for example, a pressure of 0.2 bar reduces root extension by 50%(38). Although species differ in their sensitivity, external pressures of 0.5 bar or less considerably reduce root extension in all plants which have been studied(38).

(c) Evidence for the involvement of hormones. The mechanisms by which the extension of roots is so greatly affected by small pressures is little understood but its complexity is evident.

When apices of main root axes are subject to mechanical stress, branching patterns are modified. Laterals emerge closer to the apex than in controls and they elongate at a faster rate(38). The position in which laterals develop can also be modified when roots are caused to bend; lateral initials typically develop on the convex side(39,40). The pattern of lateral root production is believed to be under hormonal control(41). Other evidence for the likely involvement of growth substances in the response to mechanical stress comes from studies on the time course of effects caused by changes in external pressure. When roots are grown against an externally applied pressure which is subsequently removed, their rate of extension does not return to that of unimpeded controls until 2 or 3 days later(42) -i.e. after cells formed since the pressure was removed have reached the stage of rapid expansion. Such a 'lag' is readily compatible with the postulate that the response is initiated within the apical meristematic tissues.

The sensitivity of the root apex to mechanical stress and the particular importance of the root cap have also been demonstrated(43). The rate of extension is considerably reduced when roots of maize collide with small glass spheres (ballotini)

TABLE 2

Effect of 10^{-6} M 3,5-diiodo-4-hydroxybenzoic acid (DIHB) during the first 6 days after germination on elongation by the roots of barley seedlings grown in ballotini (pore size 70 μ m) against 0 or 0.2 bar of applied pressure

Experiment No	Applied pressure (bar)	Root Length (cm)		
		1 -DIHB	2 +DIHB	Ratio:2/1
1	0	(a) 11.3	12.2	1.08*
	0.2	(b) 3.7	4.2	1.14*
Ratio ^b /a		0.32*	0.34*	1.06
2	0	(a) 9.1	11.8	1.27*
	0.2	(b) 3.4	3.7	1.11
Ratio ^b /a		0.37*	0.31*	0.87

* Ratios significantly different from 1.00, $p < 0.05$.

which can be displaced readily. The slower rate of growth continues for some 15 minutes before returning to the original rate. Indeed, after the initial inhibition the extension of many roots may be enhanced for a period of between 15 to 30 minutes before returning to the control rate. In contrast, roots which have their caps removed by microsurgery show no significant changes in root elongation as a result of contact with the ballotini. The root cap is known to be a source of inhibitors such as abscisic acid(44), furthermore, transient increases in another hormone (ethylene) have been observed when roots of broad bean grow against an obstruction (45).

Although several aspects of the response clearly indicate effects on hormonal mechanisms, a number of papers have ignored this aspect and have sought to explain the effect of mechanical stress entirely in terms of osmoregulation in expanding cells(37,46,47,48). A necessary consequence of an osmoregulatory mechanism is that the final cell volume should be smaller in impeded roots but available evidence is to the contrary. In maize an external pressure which restricted root extension by 80 per cent resulted in the length of cortical cells being reduced by 67 per cent but cell volume was unaltered(49). In the cortex of barley roots, a pressure of 0.2 bar reduced cell length while the mean-diameter increased and the outer layers cell volume was enhanced(50). Such gross changes in the orientation of cell enlargement are most likely to be regulated by hormones and Osborne (1976)(51) has shown that auxin (indol-3yl-acetic acid) and ethylene will induce similar changes.

The evidence indicates that responses to mechanical impedance cannot be explained in terms of simple osmoregulation. Further studies are therefore needed to elucidate the complex mechanisms involved.

(d) Effects of applying 3, 5-diiodo-4-hydroxybenzoic acid (DIHB)

As indicated above, roots are most likely to experience mechanical stress in compacted soil with few large pores where aeration is also liable to be impaired(52). Chemical treatments which aim to reduce the effects of compaction may do so by counteracting those due to mechanical stress or poor aeration or both. In addition, since mechanical stress reduces the relative growth rate of roots, compounds which enhance the absolute growth rate could help overcome the effects of a thin layer of resistant soil. For example, Wilkins et al,(53) have shown that DIHB can have a small but significant effect on root extension of Pisum sativum when soil is compacted. Work has now been carried out with barley in artificial systems to identify the principal effects of the compound. Plants were grown in cells containing beds of ballotini forming pores 70 μ m in diameter. The side walls of the cells were made of flexible polyester membrane so that a known pressure (0.2 bar) could be applied to the roots when the cells were sealed into a pressure tank(38). A constant flow of aerated nutrient solution was maintained through the ballotini so that mechanical stress was separated from anaerobiosis. Results (Table 2) show that although DIHB did not overcome mechanical impedance - the rate of root extension against an external pressure of 0.2 bar was not changed relative to controls in the presence of the compound - the absolute rate of growth in the presence of DIHB was increased under these conditions. In addition, other work has suggested that DIHB can be more effective in overcoming the adverse effects of poor aeration(54) and the presence of inhibiting amounts of light or ethylene(55).

CONCLUSIONS

Most symptoms of waterlogging injury may be ascribed directly or indirectly to the anaerobic nature of the saturated soil environment. This in turn can give rise to an imbalance of endogenous hormones which is believed to mediate several responses of the shoot system to short term flooding. The extent to which hormones can be implicated in explaining differences in tolerance of waterlogging between species or between plants in their vegetative or reproductive phases has not been resolved.

The considerable changes in hormonal content which can take place at the time of flowering(56) suggest that their involvement is not unlikely. However, not all the effects of waterlogging are linked necessarily with hormones and their importance may well diminish when the stress is imposed for long periods.

Little is known of hormonal changes in roots which are subjected to mechanical impedance. A high degree of sensitivity to low pressures, the rapidity of the response and the morphological characteristics of impeded roots such as the pattern of branching and the reorientation of cell growth strongly implicate the action of endogenous growth substances.

Some relief from the effects of anaerobiosis or from mechanical stress has been achieved using gibberellic acid, benzyladenine or DIHB although their effects cannot be explained simply in terms of rectifying an imbalance of endogenous hormones. When more is known of the mechanisms of hormone action, in its broadest sense, it may then be possible to devise techniques of practical relevance for the relief of stress arising from unfavourable soil conditions. Treatment with growth regulators may be of greatest benefit during short periods of stress, for example, when imposed by a horizontal band of compacted soil or by transient waterlogging.

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