ETHYLENE PRODUCTION, EPINASTY, AND GROWTH OF TOMATO PLANTS AS INFLUENCED BY ROOT AERATION

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ABSTRACT

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By

Kent Jay Bradford

Three sources have been suggested for the increase in ethylene content of plants following waterlogging: the soil, the root, and the shoot. A system was designed which allowed simultaneous measurement of root and shoot ethylene synthesis of tomato (*Lycopersicon esculentum* Mill.) plants with varying atmospheres in the root zone. Ethylene production by soil microorganisms was eliminated by using Turface, an inert cultural medium. Anaerobiosis of the root zone was maintained by a flowing stream of nitrogen gas to facilitate ethylene diffusion from the root. Under these conditions, ethylene production by tomato shoots increased fivefold within two days, while that by roots remained constant or declined. Growth responses normally induced by flooding, such as epinasty, adventitious root formation, reduced growth, and chlorosis, were also observed. These results indicate that the primary effect of flooding is deprivation of oxygen to the root, and this causes elevated ethylene synthesis to occur in the shoot. High light intensity during growth or pretreatment with ethylene can render the plants relatively insensitive to anaerobic root atmospheres.

A review of the pertinent literature revealed that the developmental changes associated with flooding injury can be attributed to imbalances in hormonal control mechanisms. It is suggested that ethylene may serve a coordinating role in mediating plant responses to root stress. A paradigm is proposed under which a variety of experimental observations may be subsumed.

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Kent Jay Bradford

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INTRODUCTION

Typical symptoms of flooding injury include leaf epinasty (93, 94, 110, 157, 209), chlorosis of lower leaves (26, 93, 94, 102, 110), adventitious root formation (92, 110, 157), hypocotyl swelling (93, 102, 157), leaf abscission (48, 92), wilting (26, 93, 110) and reduction in stem growth (92, 93, 94, 110, 176). Although some species may not exhibit all of these symptoms, susceptible plants manifest a definite syndrome of responses following saturation of the soil. In addition, environmental conditions such as temperature, nutrient status, or previous stresses can modify the expression of the flooding syndrome (27, 61, 80, 137, 190). Such a variety of growth responses from a single perturbation of the root environment suggests that flooding alters a fundamental control mechanism within the plant (probably hormonal), which in turn has a series of consequences (110, 158). This hypothesis would explain why a characteristic sequence of events follows flooding of the root system. It would also explain modification of the response by environmental factors, since these are known to alter plant hormone levels (121).

Turkova (209) was perhaps the first to suggest the ethylene might be involved in flooding injury. He observed that "when a ground becomes swampy by rainfall, the outward appearance of tomatoes growing on it is strikingly similar to the usual habitus of plants that were under the influence of ethylene". Zimmerman and coworkers at the Boyce Thompson Institute for Plant Research had shown that epinasty (40), adventitious rooting accompanied by stem hypertrophy (229), and leaf senescence (231) are characteristic responses of plants exposed to low concentrations of ethylene. Subsequent investigations have also linked ethylene to reduction in growth (54, 64), leaf abscission (17, 91), and wilting (190). Thus, all of the primary symptoms of flooding injury can be induced by ethylene treatment. The fact that high auxin concentrations can elicit similar responses led Kramer (110) and Phillips (157, 158) to suggest that accumulation of auxin in shoots of waterlogged plants might be responsible for the flooding syndrome. However, in light of the ability of auxin to stimulate ethylene production (140, 232), responses to elevated levels of auxin are likely responses to ethylene (25). The ability of ethylene application to mimic the effects of flooding, coupled with the recognition of ethylene as an endogenous plant growth regulator, suggests that elevated ethylene production may be a basic response to flooding.

This hypothesis has been tested by several workers who measured the ethylene content of flooded plants in relation to symptom development. Kawase (101) found elevated ethylene levels in intact chrysanthemum, radish, sunflower and tomato plants after 24 hours of flooding. Highest ethylene concentrations were found in submerged tissues, but nonsubmerged shoots also increased in ethylene content. In a subsequent study with sunflower plants, Kawase (102) discovered correlations between the ethylene concentration in stems of flooded plants and the development of epinasty, chlorosis, and stem hypertrophy. He also showed that treatment of the root system with ethephon (2-chloroethylphosphonic acid), which breaks down to release ethylene (225), caused reduction in height, loss of chlorophyll, epinasty, and stem hypertrophy which were virtually identical to the flooding treatment. El-Beltagy and Hall (48) found a similar situation in *Vicia faba*, where the ethylene concentration in stems and leaves of flooded plants increased within 24 hours and remained higher than that of control plants for up to 10 days. They also observed epinasty, abscission, and reduction in growth in waterlogged plants. Jackson and Campbell (85-89) have shown that waterlogging or lowered oxygen in the root zone causes increased ethylene levels in shoots within 12 hours. The ethylene concentration peaks at 48 hours and then declines. Epinastic growth of petioles is most rapid in the period from 24 to 48 hours, when ethylene content is increasing rapidly. These studies strongly suggest that an increase in ethylene levels in the plant is a key event in eliciting the flooding syndrome.

Support for this hypothesis also comes from work on the influence of ethylene on individual growth responses. Palmer (150, 152) has shown that the epinastic response is specific for ethylene, possibly resulting from a differential sensitivity to ethylene between the adaxial and abaxial surfaces of the petiole. The upper side of the petiole elongates in response to ethylene, while the lower side is inhibited. When ethylene is no longer present, the lower side elongates more rapidly than the upper, allowing recovery of the normal orientation. Osborne (149) has suggested that epinasty is due to a Type 2 response (ethylene-induced elongation) by the upper side, while recovery involves Type 1 and Type 3 responses (auxin-stimulated growth) by the lower side. This specificity of the epinastic response makes it a good indicator of the internal ethylene status of flooded plants. Jackson and Campbell (89) have reported that 0.04 to 0.07 ul/1 ethylene in the aerial environment of tomato plants is sufficient to promote epinasty. Reduced elongation and increased radial expansion of the hypocotyl are also well-documented plant responses to ethylene (3, 4, 65, 102, 234). Induction of lateral roots, which can occur under waterlogged conditions (21, 57), also requires ethylene in tomato (233, 234). Although formation of adventitious roots in tomato apparently is not dependent upon ethylene (233, 234), the gas does potentiate their development (89, 229). The observed increase in endogenous ethylene during flooding and the similarity between responses of plants to ethylene and the symptoms of flooding injury support the hypothesis that elevation of ethylene content in flooded plants is responsible for what has been termed the flooding syndrome. The question now is: how does flooding the root zone cause an increase in ethylene within the plant?

Blockage of Gas Diffusion

Kawase (101-103) has suggested that water surrounding the roots prevents diffusion of ethylene from submerged portions of the plant due to the low solubility of ethylene in water. (Ethylene is slightly more soluble than oxygen in water.) The ethylene normally produced in the tissue builds up, and then gradually moves up the stem, causing elevated levels of ethylene in the stem and leaves. This conclusion is based on the observation of a decreasing gradient of ethylene from the lower, submerged portion of the plant to the upper, aerial portion. In addition, symptoms of injury are usually more severe in the lower leaves and stem. Recently, Zeroni, *et al.*, (228) found that while diffusion of ethylene from aerial portions of *Vicia faba* is extremely rapid, ethylene can be channelled to any part of the plant if escape of the gas is blocked by a diffusion barrier. According to this hypothesis, the rise in ethylene during flooding is purely passive; the plant essentially gasses itself with ethylene from within.

Microbial Ethylene Production

The discovery of ethylene in anaerobic soil by Smith and Russell (193) in 1969 stimulated work on the significance of this discovery for plant growth. Smith and coworkers (44, 188, 191) found a clear relationship between high moisture content of field soils and both elevated ethylene and reduced oxygen concentrations in soil atmospheres. Up to 20 ul/1 ethylene was detected in some soils, enough to inhibit the growth of cereal roots by 60 to 80 per cent (192). Other workers have also measured significant levels of ethylene in anaerobic soils (179, 186, 187), and it has been suggested that ethylene of microbial origin plays a regulatory role in soil biology (184-186). The possibility that microorganisms in the soil surrounding plant roots could be the source of excess ethylene in flooded plants has been thoroughly investigated by Jackson and Campbell (85-89). They have shown (87) that when ethylene surrounding the roots of tomato plants exceeds 2 ul/1, epinasty and elevated ethylene levels are observed in petioles. The use of ^{14}C-labelled ethylene established that the gas can move rapidly from roots to shoots. In plants flooded in soil, the rise in ethylene in the shoots was paralleled by a similar rise (at approximately tenfold higher concentration) in the soil water (85). Thus, movement of ethylene from the soil, into the root, and subsequently into the shoot, could be a factor in flooding injury. In this hypothesis also, the plant plays an essentially passive role, being influenced by soil ethylene as it moves through the plant.

Oxygen Deprivation of the Root

In their most recent publications, Jackson and Campbell (88, 89) have proposed a third hypothesis. In experiments with tomato plants grown in nutrient solution, they discovered that low oxygen (less than 3 per cent) in the root zone was sufficient to cause increases in ethylene in the shoots and epinasty of the petioles. Roots were required for this effect, for removing the roots and subjecting the basal portion of the shoot to anaerobic conditions did not promote epinasty. Since ethylene was not released from the nutrient solution alone, these experiments indicate that oxygen stress to the roots can in some way increase the ethylene concentration in the shoot. Anaerobiosis of the roots has long been suspected as being the primary cause of injury to waterlogged plants. Flooding rapidly (within 24 hours) decreases the oxygen concentration surrounding roots, since diffusion of oxygen through water is approximately 10,000 times slower than through air (69), and the oxygen remaining in the soil is rapidly consumed by root and microbial respiration (89). The extensive experiments of Cannon (27) and Clements (34) in the 1920's demonstrated the deleterious effects of deficient oxygen on root growth. Kramer (110) and Jackson (94) also attributed the primary cause of injury to oxygen deficiency and subsequent effects on water absorption, phloem transport, and hormone metabolism. Numerous experiments in both soil and nutrient solutions have established the benefits of root aeration on plant growth (49, 50, 51, 60, 213, 227; for reviews, see 12, 68). The experiments of Jackson and Campbell indicate that the effect of low oxygen may be to alter the ethylene physiology of the plant. They have hypothesized (89) that a transmissible factor which is produced in anaerobic roots can cause increased ethylene production in the shoot. This hypothesis requires an active rather than passive role of the plant in responding to the stress, since a specific factor (or factors) must be made by the root and transported to the shoot. It also allows for the possibility that the flooding syndrome may have adaptive significance, rather than simply being a pathological response to excessive ethylene accumulation.

The three hypotheses outlined above can be distinguished according to the proposed source of ethylene in flooded plants. Kawase (103) has concluded that the submerged portions of the plant produce ethylene, which then accumulates due to blockage of diffusion by water. Jackson and Campbell (89) propose that soil ethylene of microbial origin can move into the plant and up the stem, or that root anaerobiosis in some way induces ethylene production in the shoot. Therefore, the soil, the root, and the shoot have all been suggested as the site of ethylene synthesis. Of course, it is apparent that all three processes may operate simultaneously under field conditions.

Thesis Objectives

The purpose of the present study was to distinguish between the three extant hypotheses. The experiments of Kawase could be confounded by soil ethylene, while Jackson and Campbell did not measure ethylene production by roots in their low oxygen treatment. It was desirable to grow plants either in soil or inert media, rather than in nutrient solution, in order to facilitate diffusion of gases from the root. In addition, there is some indication that roots grown in nutrient solution may be adapted physiologically or anatomically to lower oxygen levels (21, 110, 135). Low-oxygen stress could be effected by flowing nitrogen gas through the root zone without altering the diffusion properties of the root surface. If plants under these conditions developed epinasty, then the hypothesis of Kawase would be untenable. By growing the plants in inert media and measuring the ethylene production by roots and shoots simultaneously, one could determine the source of the ethylene. This approach would isolate the minimum necessary condition in the root zone which would cause epinasty. That is, if low oxygen in the absence of a blockage of diffusion or a contribution from microbial ethylene still produces epinasty in the shoot, it is apparent that the source of the ethylene is within the plant, probably in the shoot. Simultaneous measurement of root and shoot ethylene production should settle the latter point. If the experiments suggest that oxygen stress to the root affects shoot physiology, further experiments could be designed to determine the nature of this root-shoot communication.

MATERIALS AND METHODS

Plant material. Dwarf tomato plants (_Lycopersicon esculentum_ Mill. cv. Tiny Tim) were used in most of the experiments and, where noted, cv. Chico III, a nondwarf variety, was used. Dwarf plants were chosen since the entire shoot could be enclosed for ethylene determinations. In addition, more mature plants could be conveniently employed, and the slower growth rate lowered the variability resulting from differential growth of treatment and control plants.

Growing conditions. Plants were grown in a greenhouse at 18 C minimum with natural lighting between October 1976 and March 1977. Plants for preliminary experiments were grown in a silty loam soil, pH 6.7, organic matter 4.97 per cent, or in Perlite. For the main experiments, plants were grown in Turface (BASF Wyandotte Corporation, Wyandotte, MI 48192), an inert clay medium. Plants were watered with half-strength Hoagland's solution 1 (79) plus 132 mg/l ammonium sulfate, as recommended by Shive and Robbins (180). The plants were moved into a controlled environment room at least three days before beginning an experiment. Illumination was from incandescent and fluorescent lights at an intensity of 1000-1300 ft-c (4000-5000 uW/cm) in a 16-hour photoperiod. Temperature was maintained at 25 ± 2 C. Six-week-old plants were generally used, when flower buds were just visible on the dwarf plants.

Preliminary experiments. For preliminary experiments, plants were grown in 1-pint (453 ml) canning jars. Three holes were drilled in the metal lid and fitted with rubber serum stoppers, one for the plant stem and one each for entrance and exit gas. Seeds were pregerminated and planted through the center hole in the lid. Just before the experiment, the opening surrounding the stem was sealed with lanolin or with RTV-11 silicone rubber with a nonphytotoxic catalyst. The roots were therefore isolated from the atmosphere, while the shoots were not enclosed. Air or nitrogen gas at a rate of 3-4 ml/min was introduced through a stainless steel tube to the bottom of the root zone and exited through an outlet in the lid. The outlet was closed with a serum cap punctured with a syringe needle to limit back diffusion of gases and to facilitate sampling. Ethylene analyses were made on the effluent gas or by sealing the root chamber for one hour and sampling the accumulated ethylene. The jars were initially brought to field capacity and subsequently maintained by adding nutrient solution back to the original weight. The jars were covered with aluminum foil to exclude light.

Main experiments. Following the results of the preliminary experiments, a more convenient experimental apparatus was designed (Figure 1). Plants were grown in 2.5 cm diameter by 19 cm long glass tubes capped at one end by a one-hole rubber stopper, and at the other with a serum cap from which the septum had been removed. Turface was used as the growing medium to facilitate gas exchange and to allow easy removal of the roots for examination and measurement. A glass T-joint inserted into the lower stopper provided for irrigation and entrance of the flow gas. A parawax (vaseline and paraffin, 8:1) seal isolated the root zone from the shoot chamber. Flow gas from the root zone exited through an 18-gauge syringe needle into a 1.6 mm diameter Tygon tube and was vented through the stopper covering the shoot chamber. A serum cap covered the outlet and the gas passed through a 25-gauge syringe needle to prevent back diffusion. The shoot chamber was of pyrex and was fitted with a sampling port. The chamber was sealed at the top and bottom with rubber stoppers. Flow gas to the shoot entered through the lower stopper by way of an 18-gauge syringe needle and exited through a second needle in the upper stopper. The shoot chamber was of sufficient size to enclose a six-week-old dwarf tomato plant without restraint. An apparatus was constructed to hold 30 plants and to provide irrigation through tubes connected to each plant. The roots were maintained in darkness and were irrigated with nutrient solution daily.

The root zone was ventilated at a rate of 1.5 ml/min employing capillary flow meters. Since the free air space of the filled tube was approximately 30 ml, this rate supplied approximately three volumes per hour. Assuming complete mixing, this would be sufficient to remove 95 per cent of the gas originally in the root zone after one hour (129).

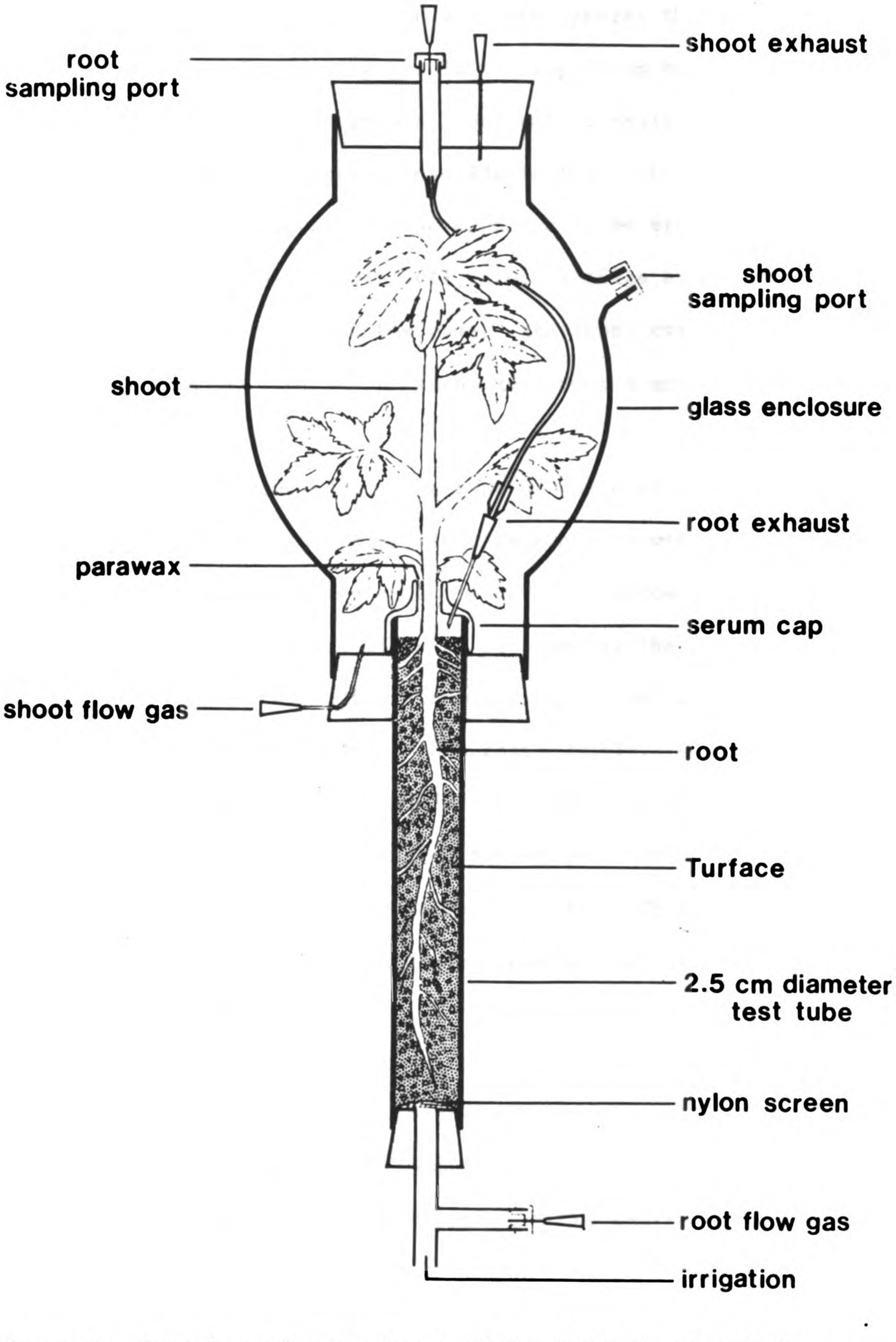

Figure 1. Experimental apparatus used for the main experiments.

Romell (173) has calculated from rates of carbon dioxide production and levels of carbon dioxide found in soil atmospheres that normal soil aeration completely exhanges the gas in the top 20 cm of soil once every hour. Thus, the flow rate chosen represents a realistic rate of supply of oxygen and removal of carbon dioxide in the root zone. Diffusion of ethylene from the root can also be expected to be similar to that under natural conditions in a porous soil, since surface hydration (the major block to diffusion) would be the same. Ethylene, carbon dioxide, and oxygen were sampled from the effluent gas under semi-equilibrium conditions.

The shoot was ventilated at a rate of 45-60 ml/min, sufficient to provide one chamber volume approximately every 10 min. This high flow rate was used to prevent depletion of carbon dioxide in the shoot chamber. Ethylene sampling was effected by stopping the flow gas and sealing the chamber for two hours before taking a sample. Otherwise, the shoot and root chambers were flushed continuously. Control roots and all shoots were flushed with humidified compressed air which had been filtered through Purafil (potassium permanganate on an alumina base) to remove ethylene. Treatment roots received 99.9 PCP cylinder nitrogen which was free of ethylene. Root flow gas was also filtered with Ascarite to remove carbon dioxide.

This system maintained oxygen levels of less than 0.5 per cent in the root zone of nitrogen-treated plants. It also allowed for the nondestructive, simultaneous measurement of ethylene production by both the roots and shoot of a single plant over the course of several days.

Ethylene, carbon dioxide, and oxygen determinations. Three ml gas samples were taken with gas-tight syringes and injected into a gas chromatograph which used nitrogen at 60 C as the carrier gas and was equipped with a 2 mm by 1 m column of activated alumina and a flame ionization detector. Concentrations of ethylene above 1 nl/1 were easily detected. Carbon dioxide and oxygen were determined in 3 ml samples injected into a gas chromatograph with a thermal conductivity detector. Helium at 70 C was used as the carrier gas. Carbon dioxide, oxygen, and nitrogen were separated on a 6 mm by 0.6 m silica gel and a 6 mm by 3 m molecular seive column (20). Ethylene, carbon dioxide, and oxygen concentrations were determined by comparison of peak heights with those of standard mixtures.

Epinasty measurements. Epinasty was measured as an increase in the angle between the adaxial surface of a petiole and the stem. A transparent protractor was used to determine the petiole angles. In one case ($AgNO_3$ experiment), epinasty was measured as the vertical distance moved by a marked spot on the petiole 1.5 cm from the stem.

Chlorophyll determinations. Total chlorophyll was determined in 1 cm leaf discs by the method of Arnon (7), using 80 per cent acetone extracts and measuring the absorbance at 652 nm.

Silver nitrate application. Plants were treated with solutions of 0.1 per cent Tween 20 plus or minus 500 mg/1 $AgNO_3$. The entire shoot was dipped into the appropriate solution for approximately 10 seconds. Treatment of plants with various gases was begun the following day.

Ethylene pretreatments. In some experiments, plants were pretreated with ethylene before altering root aeration. Plants were gassed with 8.6 ul/1 ethylene in air with continuous flow for one day, and returned to air for the following day. Control plants were treated similarly with ethylene-free air. This procedure was then repeated for three consecutive cycles. This allowed recovery from the severe epinasty induced by ethylene treatment and prevented abscission (1, 229). Following the ethylene treatments, a week to ten days elapsed before beginning the experiments.

Statistical analysis. Statistical analyses of data were by the analysis of variance procedure. The significance of mean differences was tested by Duncan's multiple range test.

RESULTS

Preliminary Experiments

The first experiment was designed to measure ethylene production by the soil plus roots or the soil alone and petiole epinasty. Eight Chico III tomato plants and four jars of soil without plants were used in each treatment. The treatments were air or nitrogen flow gas through the root system. Observations were also made on adventitious root development and chlorosis.

Epinasty of the third leaf (first true leaf = 1) began within the first day of treatment with nitrogen (Figure 2A; treatments statistically significant at $p \leq 0.01$). Epinasty continued to develop rapidly during the second day, and subsequently increased at a much slower rate for the remainder of the experiment. Ethylene production data (Figure 2B) is shown for the nitrogen treatment only, as no ethylene significantly above the blank (empty jar) was detected in the aerobic treatment. Ethylene evolved from the soil plus roots increased in parallel with the development of epinasty, then declined to a lower constant level. Soil alone produced a low level of ethylene which gradually increased to a peak on the third day, then declined. By the third day, nitrogen-treated plants had

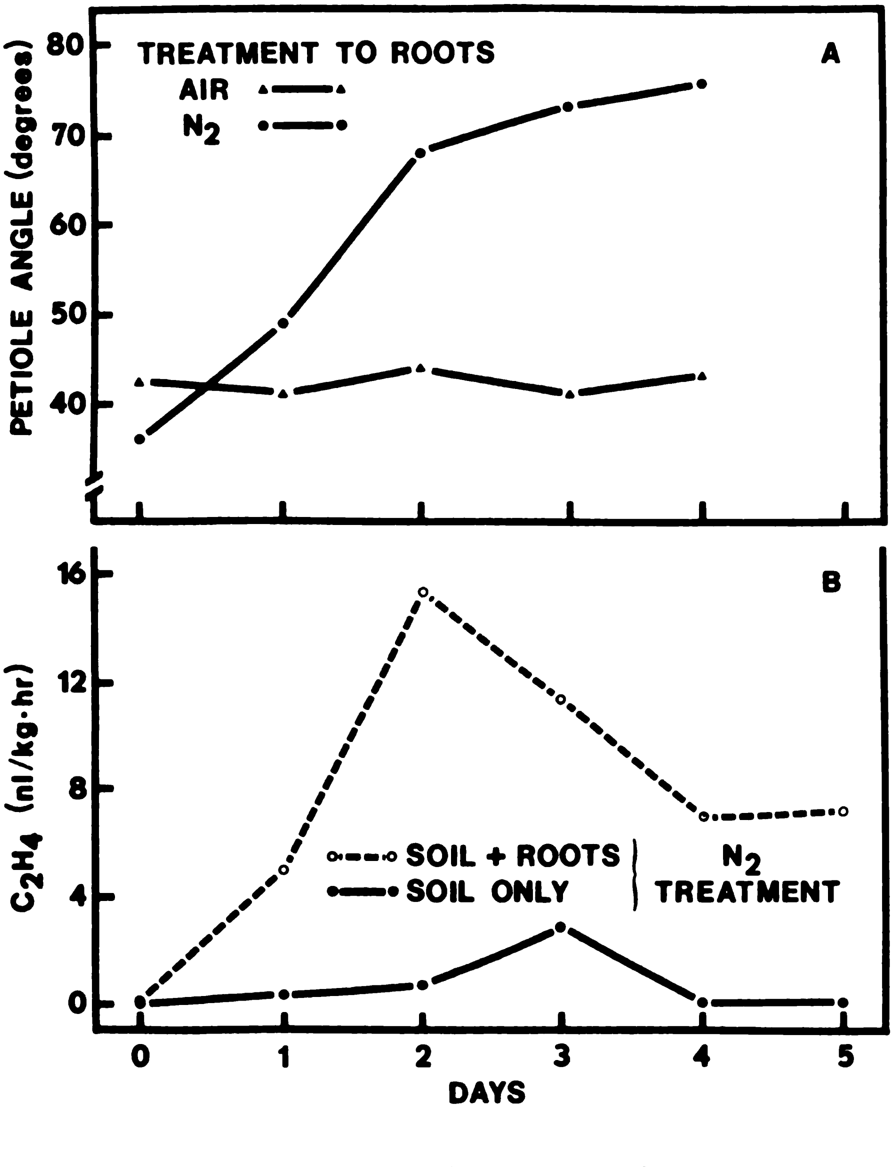

Figure 2. (A) Epinasty of tomato plants as influenced by oxygen supply to the root. (B) Ethylene production by soil plus roots or by soil alone in the nitrogen treatment.

begun to develop adventitious roots on the stem and by the fourth day had an average of 6.2 ± 1.3 roots per plant. Three control plants developed one adventitious root each, and the remaining five had none. Severe chlorosis was evident in the lower leaves of nitrogen-treated plants, but this effect was not quantitated.

This experiment was repeated using Tiny Tim tomatoes with Perlite as an inert growing media to ascertain the contribution of ethylene from the soil and from the root. In addition, the shoots of half the plants in each treatment were dipped in a solution of $AgNO_3$ to determine which responses of the flooding syndrome were directly attributable to ethylene. Silver (I) ion has been reported to be a selective inhibitor of ethylene action (14, 15).

Again, as shown in Figure 3A, epinasty (average of leaves 3, 4, and 5) began within 12 hours and was pronounced after 24 hours of nitrogen treatment to the roots. Epinasty was entirely prevented by $AgNO_3$ pretreatment. As silver treatment had no effect on root ethylene production, the data have been combined in Figure 3B. Ethylene production by the root system (corrected for blanks containing Perlite only) declined significantly ($p \leq 0.05$) under anaerobic conditions for 12 hours, then began to rise. Thus, ethylene production by the root was lowest at a time when epinasty was rapidly developing. The later rise may have been a senescence phenomenon, as much of the root appeared to be dead by the fourth day. Callus growth had appeared on the stems of the nitrogen-treated plants by the sixth day, but adventitious roots had not yet emerged. This response was also prevented by silver ion. Abscission of lower leaves was also observed, but this was confounded by a slight toxicity of the silver treatment.

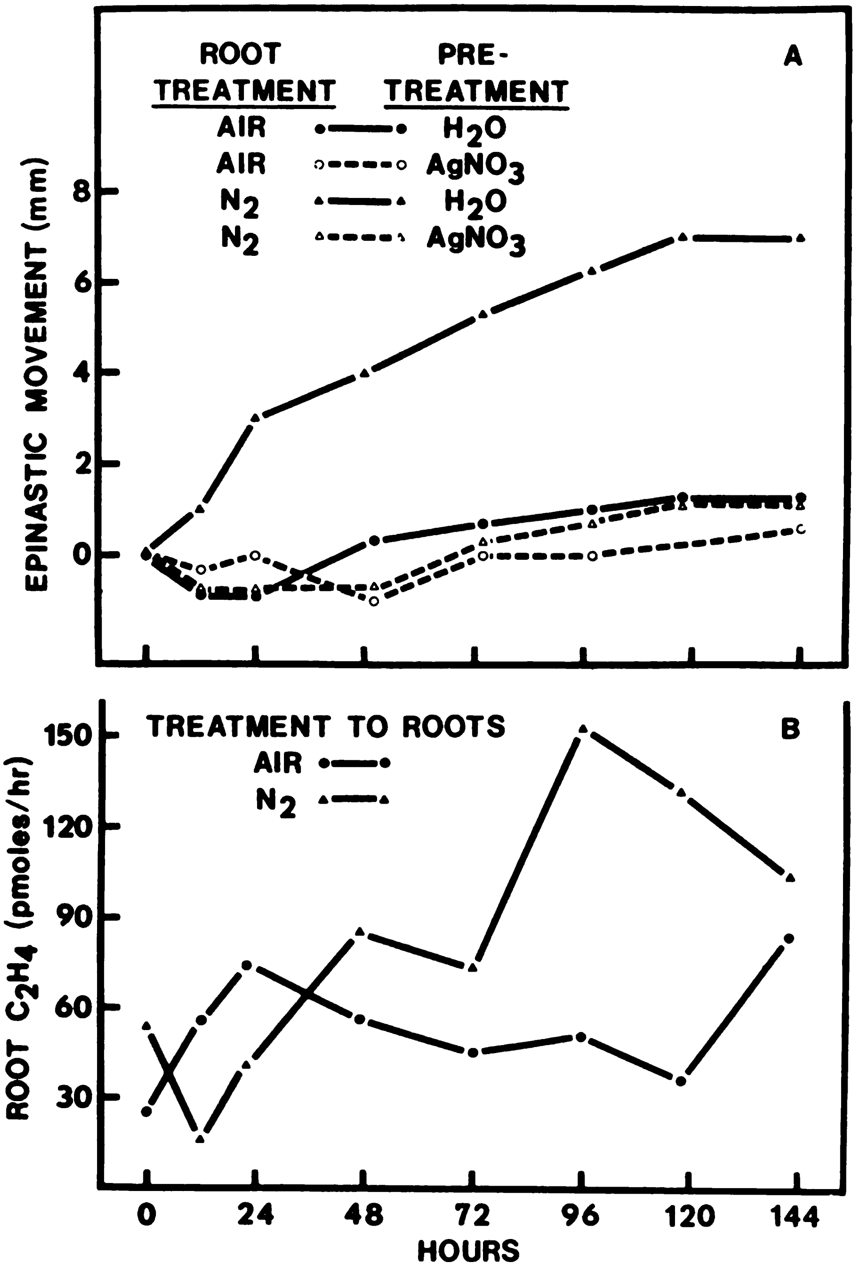

Figure 3. (A) Epinasty of tomato plants as influenced by aeration of the root and $AgNO_3$ treatment of the shoot. (B) Ethylene production by roots under different aeration regimes.

Table 1 shows some effects of the treatments on chlorophyll, fresh weight, and surface area of the sixth leaf from these plants. Nitrogen treatment reduced ($p \leq 0.01$) chlorophyll levels on both a per dm^2 and a per g fresh weight basis, as well as reducing ($p \leq 0.01$) the fresh weight and surface area. Silver caused an increase in chlorophyll retention ($p \leq 0.05$) when expressed on an area basis, but was not significant on a fresh weight basis. This is apparently due to differential effects of silver on fresh weight and leaf expansion, as shown in the table.

From the preliminary experiments it was apparent that oxygen deprivation of the root caused epinasty, adventitious root development, chlorosis, and reduced growth of tomato shoots. Neither flood water nor soil ethylene are required for the response. Silver ion completely prevented epinasty and adventitious root development and partly ameliorated chlorosis and the reduction in growth, implicating ethylene as a factor in these responses.

Main Experiments

Based on the results of the preliminary experiments, it was decided to simultaneously measure ethylene production by both the roots and the shoot under differing aeration. Turface, which had been screened to remove particles less than 2 mm^2, was used as a root medium as it allowed easy removal of the roots for observation and measurement of growth. As a short equilibrating period was required to achieve anaerobic conditions, time "zero" in all graphs is designated as the time when the oxygen concentration in the root effluent gas became less than 1 per cent. In all cases, the data have been corrected for ethylene or carbon dioxide detected in identical systems containing Turface but no plant. Data are expressed on a per plant basis, as weights could be taken only at

Table 1. Effects of Anaerobiosis of the Root Zone on Chlorophyll Content, Fresh Weight, and Surface Area of the Sixth Leaf of Tomato Plants.

Parameter After 7 Days of Treatment	Main Effects^x	Treatment to Roots			
		Air		Nitrogen	
		Pretreatment			
		H_2O	$AgNO_3$	H_2O	$AgNO_3$
Chlorophyll, mg/dm^2	**	3.62 ab^z	4.40 a	1.85 c	2.53 bc
	± N_2**	4.01 a		2.19 b	
	± $AgNO_3$*		3.47 a	2.73 b	
Chlorophyll, mg/g Fr. wt.	*	1.32 ab	1.41 a	0.77 c	1.00 bc
	± N_2**	1.36 a		0.88 b	
Fresh Wt., g	**	0.51 ab	0.56 a	0.35 c	0.40 bc
	± N_2**	0.53 a		0.37 b	
Surface Area, dm^2	†	0.19	0.18	0.14	0.16
	± N_2*	0.18 a		0.15 b	

x = Statistical significance of mean differences. Variance partitioned into main effects when significant.
† = Mean difference not statistically significant.
* = Means significantly different at $p \leq 0.05$.
** = Means significantly different at $p \leq 0.01$.
z = Means followed by the same letter are not significantly different at the indicated confidence level.

the completion of the experiment. However, the usual effect of nitrogen treatment was to reduce fresh weight, which would tend to amplify observed differences between treatments.

The first experiment using this system was simply to determine the effect of root anaerobiosis on ethylene production by roots and shoots and on various growth parameters. Six-week-old Tiny Tim tomato plants were used, with twelve plants per treatment. The upper stoppers of the shoot chambers were left ajar except during ethylene measurements to reduce condensation on the inner walls of the chambers. Ethylene synthesis by shoots of nitrogen-treated plants increased ($p \leq 0.01$) within 18 hours to five times the control level and remained five- to ninefold greater for the remainder of the experiment (Figure 4). Control shoots maintained a fairly constant low level of ethylene evolution. Anaerobic roots sustained a low rate of ethylene production similar to control roots for two days, then showed a slight decline on the third and fourth days. Epinasty was not measured daily, as removal and replacement of the shoot chambers would have been too damaging to the plants. However, epinasty was not obvious until the second day, subsequent to the rise in ethylene levels.

Root respiration, as measured by carbon dioxide production, was greatly decreased in anaerobic roots and continued to decline for the remainder of the experiment (Figure 5). Control root respiration, on the other hand, increased at a fairly constant rate as growth continued.

Epinasty, shoot and root fresh weight, root dry weight, chlorophyll content, and total leaf area were all significantly affected ($p \leq 0.001$) by root oxygen stress (Table 2). Root growth was particularly sensitive, both fresh and dry weight being approximately half of the control values. Shoot dry weight, however, was not affected.

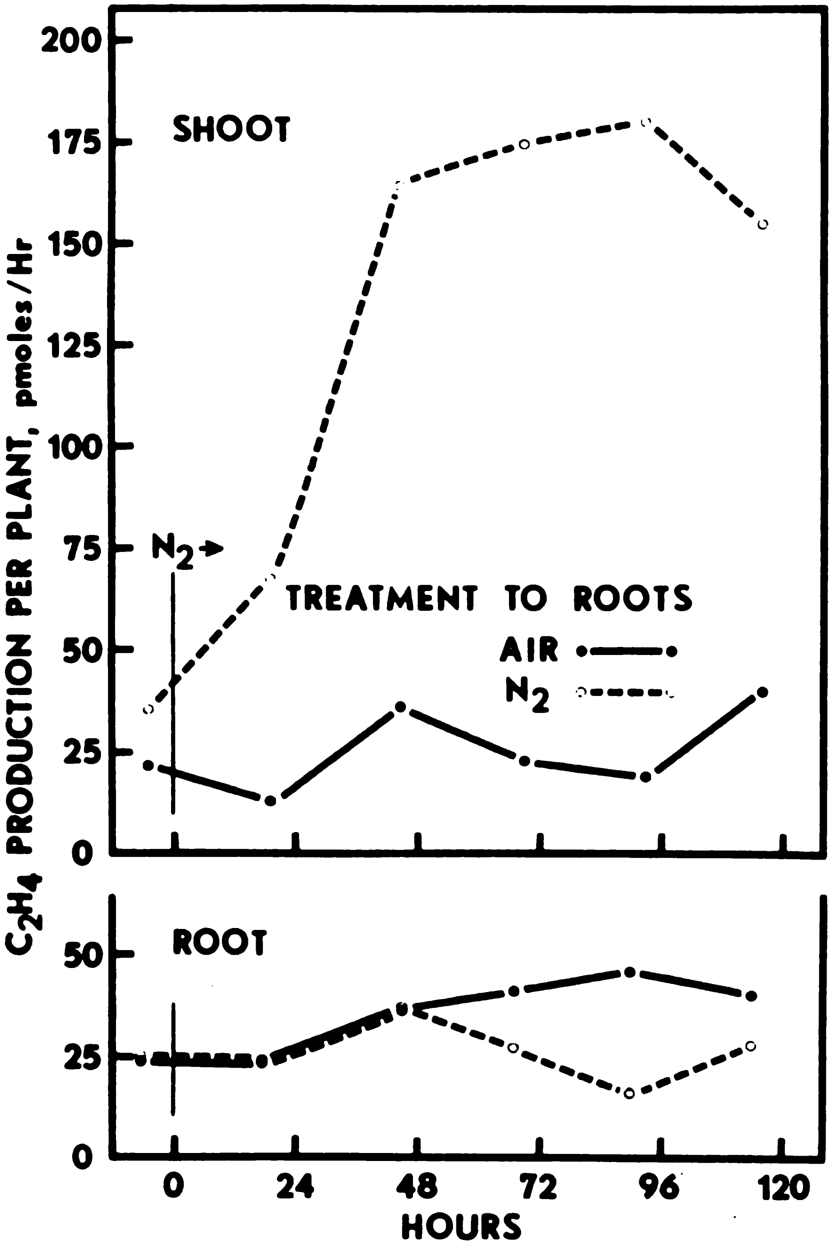

Figure 4. Shoot and root ethylene production as influenced by aeration of the root.

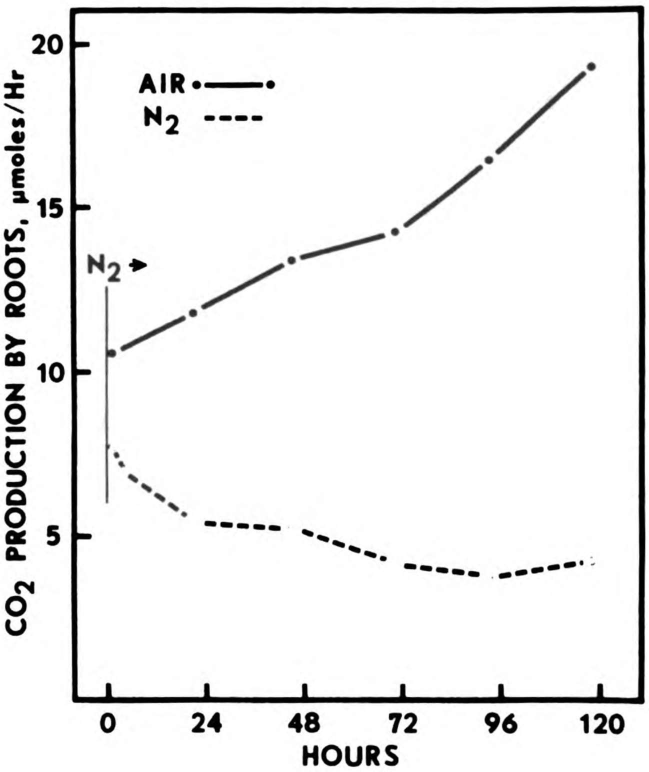

Figure 5. Root carbon dioxide production as influenced by aeration. Time "zero" is taken to be the time when the oxygen level in the nitrogen effluent gas became less than 1 per cent.

Several other observations deserve mention. The root tips from the anaerobic treatment were blackened and few root hairs were present. Upon harvesting the shoots, the stumps of control plants continued to exude under root pressure, while anaerobic roots had no root pressure. Although wilting did not occur under the conditions of the experiment, nitrogen-treated plants quickly wilted after removal of the shoot chambers. Stem thickening was apparent in nitrogen-treated plants and callus growth had begun along the stem, although no adventitious roots had emerged at the termination of the experiment.

The experiment was repeated using Chico III seedlings. Due to size constraints of the apparatus, four-week-old plants in which the third

Table 2. Effects of Anaerobiosis of the Root Zone on Various Growth Parameters of Tiny Tim Tomato Plants.

Observation after five days of treatment		Treatment to Roots	
		Air	Nitrogen
Petiole angle, degrees	***	50.0	80.8
Shoot Fresh Wt., g	***	3.28	2.54
Root Fresh Wt., g	***	2.29	1.04
Shoot Dry Wt., g	†	0.286	0.293
Root Dry Wt., g	***	0.121	0.066
Chlorophyll, mg/g Fresh Wt.	***	1.62	1.35
Leaf area, dm^2	***	0.80	0.64

† = Mean differences not statistically significant.
*** = Means significantly different at $p \leq 0.001$.

true leaf had just emerged were used. Again, ethylene synthesis in nitrogen-treated plants increased ($p \leq 0.01$) within 20 hours to about twice the control level, and increased further after 43 hours (Figure 6). Unlike the previous experiment, control ethylene production also increased on the second and third days of the experiment, such that the treatment differences were no longer significant on a per plant basis. However, if the data points at 72 hours are expressed on a per gram fresh weight basis, the treated shoots produced an average of 204 pmoles/g fresh weight/hr, while the control shoots produced 128 pmoles/g fresh weight/hr. This difference was significant at the 5 per cent level. The very rapid growth of the control plants and the negligible growth of the treated

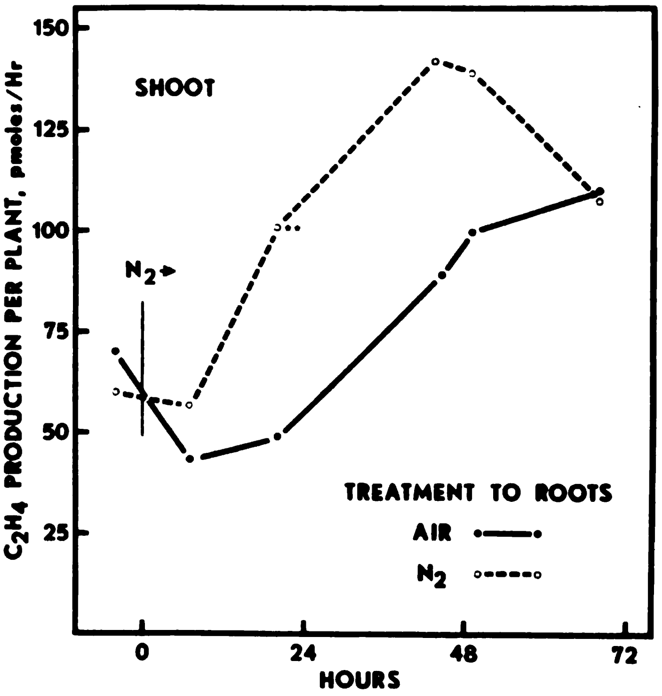

Figure 6. Ethylene production by shoots of Chico III tomato seedlings as influenced by aeration of the root. Data are expressed on a per plant basis. (** = Treatments significantly different at $p \leq 0.01$)

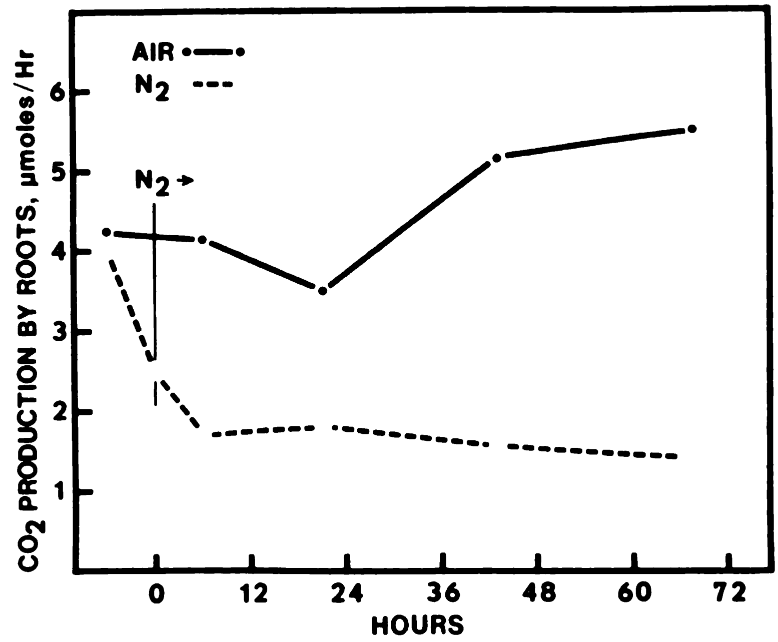

Figure 7. Carbon dioxide production by roots of Chico III tomato seedlings as influenced by aeration.

plants accounts for the discrepancy (see also Table 3). Root ethylene production was below detection in both control and treated plants, probably due to the small size of the root systems (approximately one-fourth the size of those in the previous experiment). Root respiration showed a similar pattern (Figure 7). It should be noted that this experiment lasted only 72 hours due to the rapid growth of the control plants and the equally rapid deterioration of the treated plants.

The effects of root anaerobiosis on various growth parameters of Chico III tomato seedlings is shown in Table 3. Depression of shoot fresh and dry weights, height, and chlorophyll retention is evident. Observations on root appearance and root pressure were also similar to those in the previous experiment.

Table 3. Effects of Anaerobiosis of the Root Zone on Various Growth Parameters of Chico III Tomato Plants.

Observation after three days of treatment		Treatment to Roots	
		Air	Nitrogen
Petiole angle, degrees	***	47.9	62.9
Shoot Fresh Wt., g	***	0.844	0.577
Root Fresh Wt., g	***	0.556	0.284
Shoot Dry Wt., mg	+	66.5	57.2
Root Dry Wt., mg	***	27.4	18.8
Height, cm	*	4.6	3.8
Chlorophyll, mg/g Fresh Wt.	**	1.82	1.02

+ = Means significantly different at $p = 0.1$.
* = Means significantly different at $p = 0.05$.
** = Means significantly different at $p = 0.01$.
*** = Means significantly different at $p = 0.001$.

These results indicate that oxygen deprivation of the root is sufficient to cause increased ethylene synthesis in the shoot. A variety of other symptoms of flooding injury, as documented in Tables 2 and 3, are also exhibited in response to root anaerobiosis. Therefore, although water jacketing or soil ethylene may contribute to flooding damage in the field, the capability exists within tomato plants to respond to root oxygen stress in the absence of these factors.

If the plant does have the inherent capability to respond to root oxygen stress by increasing ethylene production, how is this response mediated? Is ethylene synthesis "deregulated" due to a disturbance of normal metabolism, or is there a "factor" which specifically stimulates ethylene synthesis? Went (218) and Jackson (92) had discovered that the presence of adventitious roots on the stems of tomato plants before the original root system was flooded could largely prevent injury. Furthermore, the presence of a stressed root system was necessary for symptom expression (89, 110). The healthy root system apparently performs a function, which can be replaced by adventitious roots, in maintaining shoot integrity. A stressed root in the absence of adventitious roots seemingly promotes shoot injury. In light of the previous experiments, the hypothesis could be advanced that the presence of adventitious roots at the time of oxygen stress to the original root in some way prevents the production of ethylene by the shoot and the accompanying responses. This would further implicate the root in the control of ethylene physiology of the shoot.

An experiment was designed to test this hypothesis; adventitious roots would be induced on plants before subjecting the original root to oxygen stress. All plants were pretreated with ethylene to induce root formation. Half of the plants were given nitrogen gas to the roots, the other half air. After three days (sufficient time for a response to appear) half the plants in each aeration treatment had the adventitious roots removed to determine their influence on ethylene production by the shoot. In addition, one set of plants was included which had been gassed with ethylene, but which had not been induced to form visible adventitious roots. It was considered that these plants would serve as an additional control for the possible effects of ethylene treatment other than adventitious root formation. Due to space limitations, only three plants were included in each treatment.

For the initial 72 hours, there was no significant difference in ethylene production between the three treatments (Figure 8). Excision of the adventitious roots caused a slight increase in ethylene production

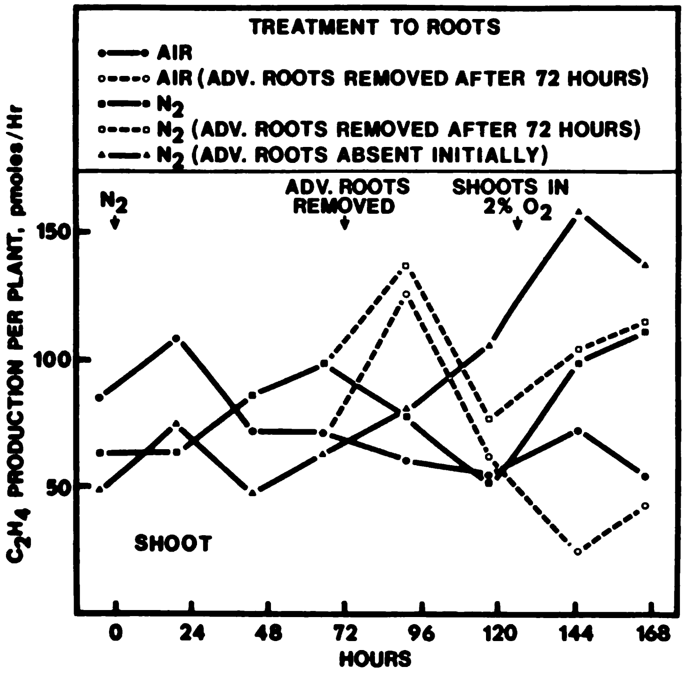

Figure 8. Ethylene production by Tiny Tim tomato shoots as influenced by adventitious roots and aeration of the original root system.

on the following day, which then returned to the control level. This apparent "wound" ethylene synthesis was not influenced by root aeration. Since gassed plants not exhibiting adventitious roots also seemed unaffected by root anaerobiosis, the possibility existed that ethylene treatment could have caused morphological adaptations which would increase internal diffusion of oxygen to the root (3, 28, 102, 110). Lowering the oxygen concentration surrounding the shoot would limit the diffusion of oxygen through the stem to the roots. When the flow gas through the shoot chambers was reduced to 2 per cent oxygen, ethylene production by those plants with anaerobic roots began to rise, being significantly ($p \leq 0.05$) higher than the aerobic plants after 40 hours (Figure 8). (Shoot ethylene production was determined during a two hour interval following a one hour recovery period in 21 per cent oxygen.) Thus, internal diffusion of oxygen must be considered as a factor in this experiment.

Growth parameters for this experiment are listed in Table 4. Significant epinasty was not observed in any treatment. Shoot fresh weight was decreased ($p \leq 0.05$) by nitrogen treatment, but chlorophyll was lost only from those plants lacking adventitious roots and receiving nitrogen to the original roots. Plants with adventitious roots present and an anaerobic primary root system did not exhibit loss of chlorophyll. Observation of the nitrogen-treated roots revealed no blackened tips and a generally healthy condition, compared to roots from previous experiments. Root pressure was also evident at harvest in both aerobic and anaerobic roots.

Although the above observations tend to indicate that the original roots, while being in an external environment of less than 0.5 per cent

Table 4. Effects of Anaerobiosis of the Root Zone on Growth Parameters of Tomato Plants Pretreated with Ethylene.

Observation after seven days of treatment		Treatment to Roots				
		Air		Nitrogen		
		Adventitious Roots				
		present	removed after 72 hrs	present	removed after 72 hrs	"absent"
Epinasty, degrees	†	3.3	0.0	3.3	3.3	10.0
Shoot Fresh Wt., g	*	4.97 a^z	5.09 a	4.00 b	4.13 b	4.07 b
Root Fresh Wt., g	†	2.22	2.15	1.63	1.67	1.74
Shoot Dry Wt., g	†	0.39	0.38	0.39	0.41	0.40
Root Dry Wt., g	†	0.12	0.12	0.09	0.10	0.10
Chlorophyll, mg/g Fr. Wt.	**	1.29 a	1.26 a	1.23 a	0.95 b	0.82 b
Shoot Growth, 3 to 7 days, cm	*	1.5 ab	2.0 a	1.0 b	0.9 b	0.8 b

† = Mean differences not statistically significant.
* = Means significantly different at $p \leq 0.05$.
** = Means significantly different at $p \leq 0.01$.
^{z} = Means followed by the same letter are not significantly different at the indicated confidence level.

oxygen, were able to survive relatively unharmed, other evidence suggests that the plants did respond to the stress. Figure 9 is a histogram of adventitious root growth in each treatment. Initially, 3 to 4 adventitious roots were present on each plant (except the "roots absent initially" treatment). Nitrogen treatment of the original root caused this number to increase to 9 roots per plant, while control plants developed only 1 additional root. When the adventitious roots were removed from control plants, essentially no new roots appeared. The nitrogen-treated plants, however, responded by producing an average of 11 new roots per plant. After three days of nitrogen treatment to the roots, no adventitious roots had developed on plants initially lacking them. By the seventh day, after the reduction of shoot oxygen levels, approximately 12 roots per plant were present. Similar plants kept in the greenhouse did not develop adventitious roots.

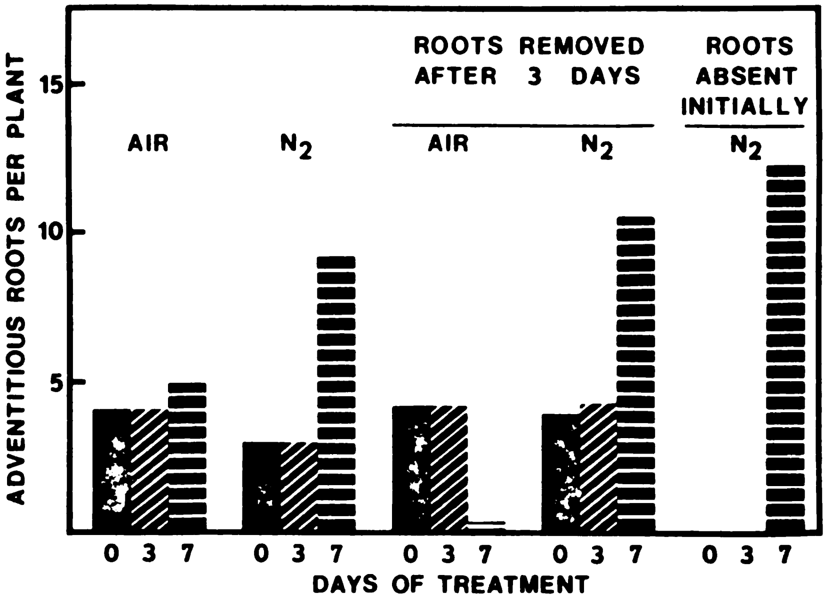

Figure 9. Adventitious root formation as influenced by pretreatment and root aeration.

It should be noted for future reference that the relative humidity in the shoot chambers during this experiment was maintained at virtually 100 per cent by keeping the upper stoppers in place. Root respiration (data not shown) indicated that growth was very slow and was not as drastically reduced by nitrogen treatment as in previous experiments.

Since it appeared that ethylene pretreatment had an ameliorating effect on subsequent root oxygen stress, it was decided to compare ethylene-pretreated with non-pretreated plants. Half the plants were treated with ethylene as before, while half were given an identical cycle using ethylene-free air. Half of each group received nitrogen ventilation of the root zone, while the other half received air. The effect of humidity was also investigated by lowering the relative humidity in the shoot chambers after seventy-two hours by leaving the upper stoppers ajar. Since humidified air was constantly flowing through the chambers and some condensation was still observed, it was estimated that the relative humidity was still quite high.

Shoot ethylene production was not significantly affected by any treatment (note expanded scale on ordinate), although plants with anaerobic roots tended to be higher after the humidity was reduced (Figure 10). Root ethylene production was very low, and while aerobic roots tended to increase in production after reducing the humidity, anaerobic roots did not. At high relative humidity, root respiration was slowly increasing in both aerobic and anaerobic roots, although the absolute rate was much lower in anaerobic ones (Figure 11). After the relative humidity was reduced, aerobic root respiration increased in rate, while that of anaerobic roots declined. On the sixth day of treatment, aerobic roots respired at a rate of 8.0 umoles CO_2/ g fresh wt./ hr, and anaerobic roots at a rate of 2.4 umoles/ g fresh wt./ hr.

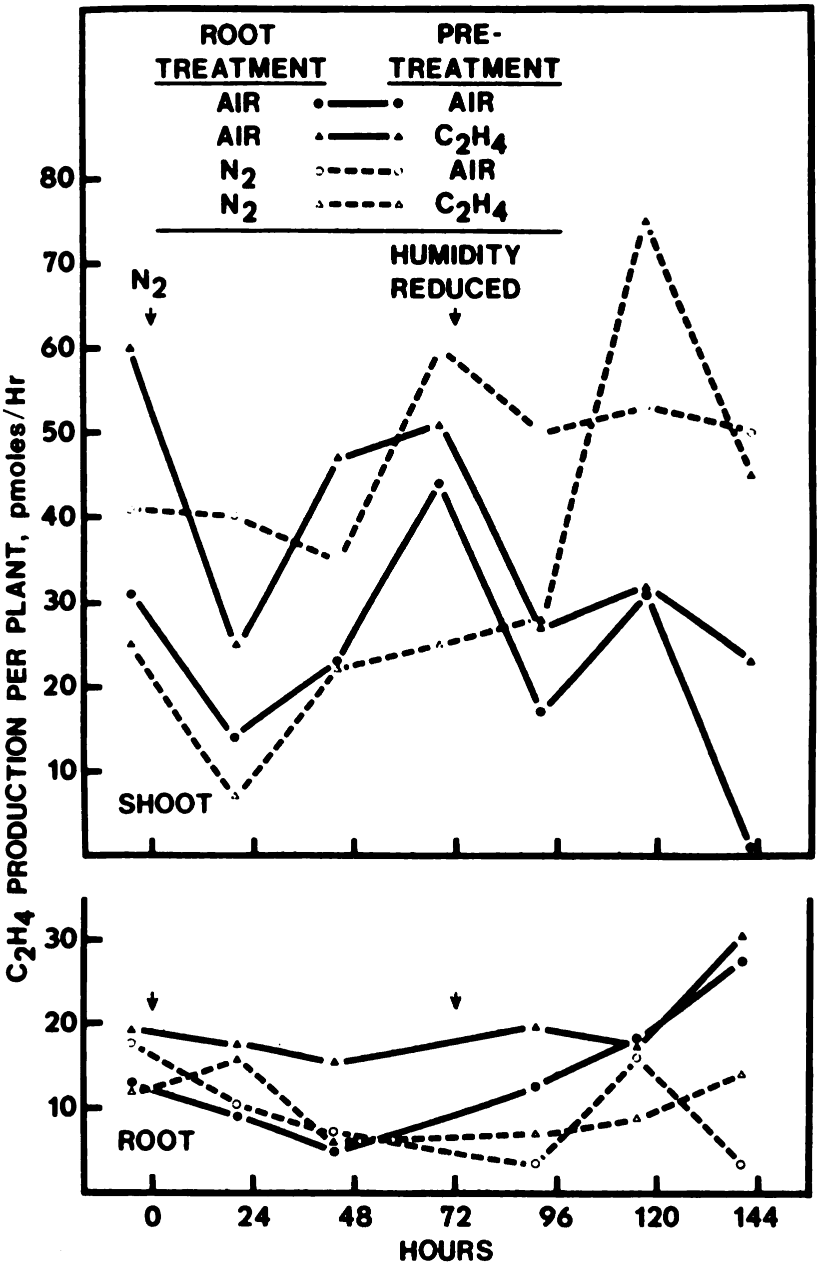

Figure 10. Ethylene production by tomato plants as influenced by pretreatment and root aeration.

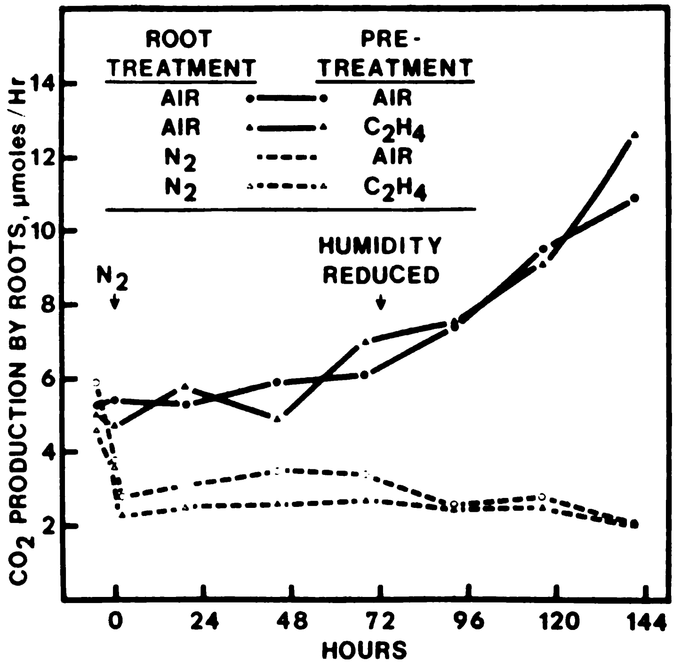

Figure 11. Root respiration (carbon dioxide production) as influenced by pretreatment and root aeration.

Plants with no ethylene pretreatment and anaerobic roots developed 15 degrees of epinastic curvature, while similar plants with aerobic roots showed only about 7 degrees of curvature (Table 5). The nitrogen treatment was significant ($p \leq 0.05$) regardless of pretreatment. Ethylene pretreatment, however, significantly ($p \leq 0.01$) prevented the development of epinasty regardless of root conditions. The amount of curvature seen here is approximately half that observed in an earlier experiment (Table 2). Shoot fresh weight, root fresh weight, and root dry weight were all reduced ($p \leq 0.001$) by nitrogen treatment, but were not affected by ethylene pretreatment. Shoot dry weight was not changed by any treatment. Nitrogen treatment reduced ($p \leq 0.001$) leaf chlorophyll content by about 30 per cent, and ethylene pretreatment also reduced ($p \leq 0.05$) chlorophyll content in the absence of aeration effects. Shoot growth

Table 5. Effects of Anaerobiosis of the Root Zone and Pretreatment on Growth Parameters of Tomato Plants.

Observation after six days of treatment	Main Effects[x]	Treatment to Roots			
		Air		Nitrogen	
		Pretreatment			
		Air	Ethylene	Air	Ethylene
Epinasty, degrees	*	6.7 b[z]	-1.7 c	15.0 a	2.5 bc
	± C_2H_4**		0.4 b	10.8 a	
	± N_2*	2.5 a		8.8 b	
Shoot Fresh Wt., g	***	2.76 a	2.86 a	2.03 b	1.87 b
Root Fresh Wt., g	***	1.49 a	1.45 a	0.92 b	0.83 b
Shoot Dry Wt., g	†	0.23	0.22	0.25	0.21
Root dry Wt., mg	***	81.8 a	73.1 a	49.6 b	46.0 b
Chlorophyll, mg/g Fr. Wt.	*	1.47 a	1.32 ab	1.09 bc	0.85 c
	± C_2H_4*		1.08 b	1.28 a	
	± N_2***	1.40 a		0.97 b	
Shoot Growth, cm	**	2.75 ab	3.45 a	1.32 c	1.68 bc
	± N_2***	3.10 a		1.50 b	
New Adventitious Roots	*	0.0 a	0.2 a	5.7 b	3.0 c
	± N_2**	0.1 a		4.4 b	

x = Statistical significance of mean differences. Variance partitioned into main effects when significant.

† = Mean differences not statistically significant.

* = Means significantly different at $p \leq 0.05$.

** = Means significantly different at $p \leq 0.01$.

*** = Means significantly different at $p \leq 0.001$.

z = Means followed by the same letter are not significantly different at the indicated confidence level.

was halved by root anaerobiosis. Ethylene pretreatment alone did not induce adventitious roots in these plants, and in nitrogen-treated plants, ethylene pretreatment actually reduced ($p \leq 0.05$) the number of new adventitious roots developed. As in the previous experiment, nitrogen-treated roots still had positive root pressure at harvest and did not appear to be dead, even though they were smaller than control roots.

In summary, the previous history of a plant can modify the response of the plant to an imposed stress. The main experiments described occurred between January and March, 1977. Plants grown in the low light and short days of winter are more sensitive to oxygen deprivation than those grown under more favorable conditions. The ability of the latter plants to maintain root integrity in an environment of less than 0.5 per cent oxygen is the key observation in the last reported experiments; virtually all of the other phenomena can be explained on this basis.

DISCUSSION

The three hypotheses outlined in the introduction will each be reexamined in light of the present results. A review of possible consequences of root oxygen deprivation will then be undertaken in an attempt to formulate a tentative model of plant responses to the stress. It is hoped that such a model might serve to pinpoint areas of ignorance for further research. A paradigm will be proposed under which many experimental observations may be seen as perturbations of a fundamental control mechanism. Finally, a brief intimation as to the possible adaptive significance of these plant responses will be given.

Examination of Hypotheses

Attention will first be directed to Kawase's proposal that blockage of gas diffusion from the root by flood water leads to increased internal ethylene levels. Kawase (102) found that upon flooding, ethylene concentration increased first in the submerged roots, then in the stem of sunflower plants. In addition, earlier results (101) showed that ethylene concentrations were higher in submerged portions than in adjacent aerial portions of the plant. Finally, blocking diffusion from stem cuttings by submersion or by covering with plastic film increased the ethylene concentration within the tissue (103). On this basis, Kawase (103) concluded, "Conclusively, the first and major cause for ethylene increase in flooded plants is the blockade of ethylene escape out of the flooded portion, inducing high ethylene concentration in the flooded portion. Ethylene gas, thus accumulated, moves upward through the stem causing flooding injury above water level". This hypothesis receives some support from the recent work of Zeroni, _et al_. (228), who found that while diffusion of ethylene from roots is normally extremely facile, blockage of diffusion could increase retention of ethylene supplied to the root tip.

The question asked in the present work is not "Does blockage of diffusion increase internal ethylene concentration?", but rather, "Is such blockage the 'first and major' cause of flooding injury?". The results of the first four experiments conducted clearly indicate that virtually all of the symptoms of flooding injury can be caused by oxygen deprivation of the root, without a blockage of diffusion. Root ethylene production, as shown in Figures 3 and 4, declines or remains constant during the period of rapid development of epinasty. The fact that root ethylene production continues in a low-oxygen environment is an indication that internal diffusion of oxygen is occurring, at least to the more basal portions of the root, since oxygen is required for ethylene synthesis (226). Kawase (103) also mentioned the requirement for internal diffusion of oxygen in order to maintain root ethylene production. His data (102) indicate, however, that while root ethylene concentration increased within twenty-four hours of flooding, stem concentration did not increase above control levels until the fourth day. Kawase (102) suggests that ethylene is concentrated in the submerged portions of the plant, then "gradually" starts to move up the stem. However, it is apparent that if an internal route for the diffusion of oxygen exists to sustain root ethylene synthesis, the same route would be available for ethylene escape. The ethylene should therefore move quickly to the aerial portions of the plant and be dissipated. In the absence of such a concentrating mechanism, it is doubtful that the root could produce sufficient ethylene to cause the observed increases, especially at a time when the original root system is rapidly deteriorating (i.e., after four to ten days of flooding (102)).

Experiments with cuttings submerged or wrapped in plastic film (103) were of short duration (up to twelve hours) and seem to have little significance for the response of an intact plant to flooding. The submerged root of an intact plant can carry on gaseous exchange with the atmosphere through the shoot, while a submerged cutting is completely isolated. Furthermore, only shoot cuttings were used for submersion, while during actual flooding, the root is the stressed organ.

The conclusion from the present work must be that while blockage of diffusion from the root may contribute to elevated internal ethylene concentrations, it is not required for such elevation and cannot be termed the "first and major" cause of flooding injury.

An alternative explanation for many of Kawase's results is that the ethylene observed in submerged roots came from the soil. All of his experiments with intact plants were flooded in soil. Jackson and Campbell (85) have shown that flooded soils can rapidly accumulate several ul/l ethylene in the soil water. While recognizing this possibility, Kawase did not measure soil ethylene concentrations and stated that "flood water....should also work against ethylene entry to the plants" (103). However, ethylene present in the soil water at concentrations greater than that in the plant would tend to move into the inner air space of the root. The rate limiting step would be diffusion of ethylene through water to the air/water interface in the root, where it would rapidly pass into the gas phase. The enormous difference in diffusion rate (four orders of magnitude greater in air) would cause rapid movement of ethylene up the stem and out the plant and maintain a gradient of ethylene <u>into</u> the plant (see also 228). This process could account for the initial rise in root ethylene concentration and the much lower rise in shoot ethylene (101). A role for soil ethylene is also supported by Kawase's (102) finding that ethephon applied to the soil could cause virtually identical symptoms as flooding. The rise in shoot ethylene begins only on the fourth day of flooding and coincides with the development of epinasty (102). Both Kawase (102) and Burrows and Carr (26) have reported that sunflowers can be flooded for up to three days without injury, but more than three days causes damage to the root. Thus, increased shoot ethylene levels occur only upon injury to the root. This would confirm the findings of Jackson and Campbell (86, 89) and the present work (compare Figures 4 and 5 with Figures 8 and 10) that elevated shoot ethylene levels are a response to an injured root.

Although soil ethylene may have been a factor in Kawase's experiments and in flooding in the field, is it a primary cause of flooding injury? The first experiment (Figure 2) indicated a general correlation between ethylene production by the root-soil system and development of epinasty. Similar results have been observed in plants flooded in soil by Jackson and Campbell (85, 89). Further experiments in nutrient solution (85, 88, 89) or with inert media in the present work have shown, however, that contribution of ethylene from the root environment is not necessary for the response. The present experiments are especially clear on this point, since root and shoot ethylene production were measured simultaneously. Shoot ethylene evolution increased in the absence of a concentration gradient from the root (Figure 4). Consequently, the flooding syndrome can be elicited without external ethylene or blockage of diffusion simply by subjecting the root to anaerobic conditions.

Jackson and Campbell (89) have proposed that oxygen stress in the root causes the synthesis of a factor which moves from the root to the shoot and stimulates ethylene synthesis. Before concluding that such a factor is required, the possible mechanisms of plant response to the stress should be examined to determine whether known physiological, hormonal, or morphological changes can account for the observations. Does the plant possess the ability to adapt to the stress without invoking specific ethylene-promoting factors? If so, what might these adaptations be? Is increased ethylene synthesis the initial response to the stress, or is it the result of other changes in the stressed plant? How does ethylene, in turn, influence these other factors? Is the flooding syndrome a pathological condition, or a programmed attempt by the plant to survive in an unfavorable situation? These questions will certainly not be answered here, but by reviewing the options open to the plant, it is hoped that the directions in which to look for the answers may become clearer. Although the root and shoot responses will be discussed separately, it should be borne in mind that all these processes are occurring simultaneously in the intact plant.

Root Responses to Oxygen Deprivation

Physiological

Water permeability. As shown in Table 6, roots respond in a variety of ways to insufficient areation. One of the first effects of oxygen stress in many plants is a decrease in water uptake. Livingston and Free (123) reported that the most sensitive species, such as *Coleus blumei*, suffered an almost complete cessation of water uptake within 12 to 24 hours after soil oxygen was removed. Kramer (109) found that root permeability was reduced 10 to 50 per cent by short periods of oxygen deficiency or excess carbon dioxide to the roots, carbon dioxide being more effective than low oxygen. In the present experiments, carbon dioxide was not allowed to build up due to the continuous flow technique. Tobacco is especially susceptible to wilting after aeration is reduced (113). Tomato plants, on the other hand, generally do not wilt after flooding, or if wilting does occur, it develops much later than other responses such as epinasty. The use of chambers enclosing the shoots in the present work maintained a high relative humidity and precluded wilting. Reduction in root permeability could be more important under high temperature and bright light conditions, where injury is more severe (113). Kramer (110) has concluded that flooding injury is "not

Table 6. Root Responses to Oxygen Deprivation

Physiological	Hormonal	Morphological
Reduced CO_2 production (Figures 5, 7, 11)	Ethylene synthesis remains constant or decreases (Figures 3, 4, 10)	Large cortical air spaces and less suberization (21, 111, 135)
Inhibition of TCA cycle; accumulation of ethanol, malate, pyruvate, succinate, alanine, proline, and γ-aminobutyric acid (18, 45, 55, 56, 72)	Ethylene content increases if diffusion is blocked (101, 102, 228)	Proliferation, reduced length, and increased diameter of roots (21)
Induction of alcohol dehydrogenase and pyruvate decarboxylase (37, 38, 72, 221)	IAA content increases; IAA oxidase inhibited (158, 211, 224)	Shorter, thicker, more branched, and fewer root hairs on roots (111)
Malate accumulation in helophytes; succinate and ethanol accumulation in non-helophytes (39)	GA content and export to shoot through xylem decreases (168-170)	More lateral roots per unit length (57)
Permeability to water reduced (12, 109, 110, 112, 113, 123)	Cytokinin content and export to shoot through xylem decreases (26, 170)	
Ion uptake inhibited; transport to the shoot not affected (80)	ABA content increases (?) (137)	
Degradation of polyphenols and export to the shoot (32)		
Death of root tips (26, present work)		

simply the result of interference with absorption following injury to the root system." However, reduced root permeability and the resulting turgor changes could be a means of root-shoot communication. Even slight water stress is known to alter many hormone relations (124). While water stress alone is probably not the cause of flooding injury, it could serve as a mechanism to initiate adaptive changes within the plant.

Metabolism. The tricarboxylic acid cycle is inhibited by anaerobiosis (56), and various fermentation products accumulate, such as ethanol, malate, pyruvate, and succinate (18, 55, 56). The amino acids alanine, proline, and γ-aminobutyric acid also increase in anaerobic roots and in the xylem sap (45, 56). Methionine, which has been suggested as the ethylene-promoting factor (89), does not increase in the sap, according to the data of Dubinina (45).

It has been proposed that flooding injury is due to accumulation of toxic quantities of ethanol (37, 55). Erickson and coworkers (18, 55) have shown that anaerobic roots produce considerable quantities of ethanol, much of which moves into the stem with the transpiration stream. Crawford (37) has found that only those plants subject to flooding injury (nonhelophytes) accumulate ethanol during flooding. Plants not subject to injury (helophytes) do not produce ethanol under anaerobic conditions. Furthermore, alcohol and malic dehydrogenase activities are induced by flooding (specifically by acetaldehyde) in nonhelophytes, but not in helophytes (38, 39). Under anaerobic conditions, nonhelophytes primarily accumulate ethanol and succinate, which can be toxic. Helophytes, on the other hand, accumulate malate under similar conditions, which is not toxic and can be remetabolized upon return of oxygen to the system. Rice roots, which are adapted to flooded conditions, increase their carbon dioxide production as oxygen levels fall, unlike most mesophytes (201). Rice roots also accumulate ethanol, but are more tolerant to it than are corn or wheat roots (72, 201).

The possibility exists that roots may be able to physiologically adapt to low oxygen conditions by changes in metabolism. Livingston and Free (123) noted that roots which develop after the original roots have died, due to oxygen starvation, are able to survive at oxygen levels which were insufficient for the original roots. Cannon and Free (29) observed that when corn roots are given an anaerobic treatment, growth stops for several days, then resumes at a slower rate. "This suggests," they conclude, "that some kind of physiological readjustment of the roots to anaerobic conditions occurs when the soil oxygen is removed and is necessary to continued anaerobic existence of the roots." The response in corn differs from that in sunflower "in that the physiological changes are not accompanied by perceptible morphological modifications." Kramer (110, 111) and others have remarked on the ability of adventitious roots to grow well in the same flood water which killed the original root. Such physiological adaptation may be partly responsible for the relative immunity of ethylene-pretreated plants to anaerobic conditions (Figures 8 and 10). Ethylene is known to evoke changes in respiratory pathways in plants (195), and this may increase the resistance of the plants to subsequent oxygen stress.

Phenols. Catlin, et al. (32), have reported that during flooding, phenols decrease in the roots of walnut seedlings. They postulate that export of phenolic compounds to the shoot could be responsible for certain inhibitory effects. Released phenols might also influence auxin metabolism (see below).

Hormonal

Ethylene. The present work is apparently the first to measure ethylene production by intact roots under varying aeration. As shown in Figures 3, 4, and 10, root ethylene production remains constant or decreases under anaerobic conditions. Ethylene production by control roots was also relatively low and remained fairly constant as growth continued (Figures 4 and 5). This suggests that ethylene is synthesized in the growing region of the root, as the volume of these regions would remain relatively constant as total root volume increases. However, the fact that root ethylene production continued after visible injury to the root tips indicates that other regions of the root may also synthesize the gas. In soil (Figure 1), it is likely that rhizosphere microorganisms contribute to ethylene production (185). Roots in inert media are not sterile, and microorganisms may synthesize ethylene under anaerobic conditions (186). However, the low rates of production and the absence of an increase under anaerobiosis tend to discount microorganisms as a significant source of ethylene in these experiments, at least as a cause of the rapid, initial symptoms. The later rise in ethylene seen in Figure 3 could be due to microbial decomposition of dead tissues, as also mentioned by Kawase (102).

Auxin. Indole-3-acetic acid (IAA) levels in flooded sunflower roots are maintained at higher levels than in control roots (158), perhaps by an inhibition of IAA oxidase in anaerobic tissues (104, 211, 224). Phenols released in waterlogged roots could also reduce IAA oxidase acitvity (120, 163). Auxin transport in roots is primarily acropetal, and it requires energy (2, 10). Thus, auxin could accumulate at the point where oxygen and energy become limiting.

Cytokinins. Cytokinin synthesis occurs primarily in the root apices (30, 105, 181, 216). Flooding reduces the cytokinin levels in xylem exudate of sunflowers in parallel with the decline in metabolic activity of the root apices (26). Other stresses, such as water deficit (83) or salinity (82), also result in decreased translocation of cytokinins from the root.

Gibberellins. Gibberellins (GA) have also been detected in xylem sap, and there is evidence that they, too, may be synthesized in the root (30, 31, 161). Gibberellin export to the shoot is greatly reduced in flooded plants (168, 169). The possible consequences of reduced GA and cytokinin synthesis in the root will be discussed in the sections on morphological adaptation.

Abscisic acid. There is little evidence for abscisic acid (ABA) synthesis in roots. Tal, *et al.* (200), found indirect evidence from grafting experiments that a factor which closes stomates and reduces transpiration originated in the root. Abscisic acid application to the root increased exudation from detopped plants (199). Abscisic acid also increases the movement of water through roots under a hydrostatic pressure gradient (63). Abscisic acid synthesized in the shoot and transported to the root in response to root stress may influence plant water status (137, see discussion of ABA in shoots).

Morphological

Cortical air spaces. Several morphological changes take place when roots develop under deficient aeration. Bryant (21) found that the cortex of barley roots grown in unaerated solution culture developed large air passages and increased in diameter relative to aerated roots. McPherson (135) observed a similar situation in corn roots and showed that cortical air spaces could be eliminated by maintaining optimum oxygen levels around the roots. Geisler (57) subjected pea roots to varying aeration and observed greater lateral root development per length of root in unaerated culture, although elongation was retarded. Cortical air spaces may allow internal diffusion of oxygen adequate for maintenance of cell viability and initiation of lateral roots, but not enough to support elongation. Studies on oxygen movement through plants (9, 70, 76) indicate that the internal supply of oxygen is adequate to sustain root respiration to a depth of approximately 10 cm, but beyond this an external source is required. Treatment of roots with ethylene or IAA results in swelling of the cortex and reduction in elongation (33). Thus, higher auxin and ethylene concentrations in flooded roots may cause cortical swelling which would increase intercellular air space and facilitate the passage of oxygen to the root. In addition, pretreatment with ethylene could induce swelling and ameliorate the effects of a subsequent oxygen deficiency, which may explain the insensitivity of such plants so treated in the present study. Ethylene-pretreated roots may be similar to roots produced in water, which show a considerable tolerance to deficient aeration, possibly resulting from "their modified structure rather than from increased tolerance of anaerobic respiration" (111). Through internal modifications, the ethylene-pretreated roots may have been able to maintain their synthetic and degradative functions without elongation. This view is supported by the change in ethylene production following lowering of the shoot oxygen level (Figure 8) and by the observation that the hypocotyl and basal root region were swollen following ethylene pretreatment.

Lateral branching. The increase in lateral branching of poorly aerated roots could have several explanations. Auxin can stimulate lateral branching of roots (230), and this stimulation can be overcome by cytokinins (197, 204). The generally slower growth and even death of the root tips may also cause lateral root formation analogous to the promotion of lateral shoots by removal of the apical bud (71, 202, 203). It has been suggested that lateral root initiation is controlled by a balance of IAA and cytokinin (66). IAA entering the root from the shoot has been found to accumulate in lateral root primordia (10, 141). Flooding prevents the destruction of IAA and stops the synthesis of cytokinins, thus shifting the balance in favor of IAA and promoting lateral root growth. In addition, greater oxygen availability nearer the root-shoot junction probably also promotes new root formation in that region (62).

Shoot Responses to Oxygen Deprivation of the Root

Physiological

Growth. The most commonly reported effect of flooding on plants is a reduction in shoot growth (see Table 7 for references). Tables 2, 3, 4, and 5 all document the effects of root anaerobiosis on growth. It is interesting that in each case, shoot fresh weight was reduced, while shoot dry weight was relatively unaffected by root oxygen stress. Apparently assimilation could continue in the absence of expansion. Growth responses such as epinasty, hypocotyl swelling, and adventitious root formation may increase dry matter without contributing equally to expansion and water uptake. The data of Selman and Sandanam (176) also show a greater reduction in fresh weight than in dry weight of unaerated tomato shoots, while root fresh and dry weight both decrease to a similar

Table 7. Shoot Responses to Oxygen Deprivation of the Root

Physiological	Hormonal	Morphological
Loss of chlorophyll (26, 106, 93, Tables 1-5)	Ethylene synthesis and content increases (48, 85-89, 101-103, Figures 4, 6)	Epinasty (48, 85-89, 102, 157, 158, 209, Tables 1, 2, 3, 5)
Reduction in growth (48, 86, 88, 92, 93, 94, 168, 176, 218, Tables 1-5)	IAA content increases (110, 157, 158)	Adventitious roots (12, 29, 92, 110, Table 5, Figure 9)
Accumulation of carbohydrates in lower stem (43, 110)	GA content decreases (157, 168-170)	Stem hypertrophy (102, 110)
Increase in ethanol content (18)	Cytokinin content decreases (26, 170)	Radial enlargement of cortical cells and development of numerous air spaces (102, 157)
Increase in polyphenol oxidase and peroxidase activity (58)	ABA content increases (137, 223)	Reduction in leaf expansion (176, Table 2)

extent. The discrepancy could also be due to the effect of flooding on leaf expansion (Table 2), as Selman and Sandanam (176) found that inhibition of leaf expansion in flooded plants may be due to a deficiency of GA.

The possibility that reduction in growth could be due to toxic products produced in anaerobic roots was examined by Jackson (93). Using grafting and split-root techniques, he concluded that "it is unlikely that a significant amount of reduced shoot growth or chlorosis of lower leaves is due to a toxin produced in the flooded roots or in the root environment." Accumulation of ethanol, for example, may have secondary effects on growth, but is probably not the major cause of growth inhibition. A similar conclusion had been reached by Went (218), who found that although reduced water and salt uptake could be experimentally excluded, plants with unaerated roots still suffered reduced shoot growth. He also showed that if only a small fraction of the root was in an aerobic environment, shoot growth could be restored. The conclusion of both Went and Jackson was that some factor normally produced by the aerobic root is no longer transported to the shoots of flooded plants.

Chlorosis. Chlorosis of lower leaves is also a common response to flooding (Table 7). Tables 1-5 show that chlorophyll is lost from the leaves of plants with anaerobic root systems. Plants with an anaerobic root system but possessing adventitious roots had chlorophyll levels equivalent to that of control plants (Table 4). This is further support for the hypothesis that adventitious roots can fulfill a function that is lost in anaerobic roots (92, 218).

Substrate accumulation. Kramer (110) has suggested that accumulation of carbohydrates at the base of the stem could be involved in swelling and adventitious root formation. Phloem transport is blocked by lack of oxygen (43). However, substrate accumulation may be the result of simultaneous hormonal changes in the same tissues (143, 177, 178).

Hormonal

Ethylene. It is now well established that plants such as tomato and sunflower respond quickly to root oxygen stress by increased production of ethylene (Table 7). As shown in Figure 4, a highly significant increase in ethylene production occurred within 18 hours following modification of the root environment. This increase precedes any visible symptoms of damage, satisfying the condition stated by Jackson (94): "If ethylene is the causal agent inducing epinasty in tomato leaves following flooding of the roots, then it must be produced upon very slight injury to the root systems, ...[which is] insufficient to cause any of the other injury symptoms in the shoots." A unique feature of the present work is the ability to measure ethylene production from intact shoots in real time. Virtually all of the previous investigations have used excised tissues taken from plants at different times and vacuum extracted according to the method of Beyer and Morgan (16). The present method allows the same plant to be followed throughout the response period.

A question may arise as to whether collection and measurement of ethylene in the air space surrounding a plant accurately reflects internal concentrations, especially since closure of stomates has been reported following flooding (48). Furthermore, resistance to gaseous diffusion in response to stress has been found to cause an underestimation of internal ethylene levels (122). Burg (22) has considered this problem and has concluded that (a) measurement of ethylene collected by enclosing a plant tissue invariably underestimates the internal concentration, and

(b) a rate of ethylene production of 3 to 5 nl/g-hr corresponds to an internal concentration of "a few ppm". A specific example given by Burg (22) correlates a rate of 3.4 nl/g-hr with an internal concentration of 1 ul/1. Using data in Figure 4 and Table 2, it can be calculated that nitrogen-treated plants produced ethylene at a rate of approximately 0.6 nl/g-hr after 18 hours and 1.5 nl/g-hr after 43 hours. The control plants maintained a rate of about 0.3 nl/g-hr. The internal concentrations corresponding to these rates of production, according to Burg (22), would be 0.09 ul/1 in the control, 0.18 ul/1 at 18 hours, and 0.44 ul/1 at 43 hours. Jackson and Campbell (89) reported that epinasty in tomato occurred in an atmosphere of 0.04 to 0.07 ul/1 ethylene. Exogenous ethylene would be additive with the endogenous ethylene (22), which was reported to be about 0.3 ul/1 (89). Thus, only a slight increase in internal ethylene levels is sufficient to cause epinasty. At maximum epinasty, Jackson and Campbell (88) found about 1 ul/1 ethylene in leaf tissues, as compared with 0.44 ul/1 from the present calculation. Considering (a) the expected underestimation by the collection technique, (b) the possible reduced diffusion due to stomatal closure, (c) the range of uncertainty in the assumptions for the calculations, and (d) the fact that the present calculations included the entire shoot, all of which may not be active in ethylene production, it appears that the present technique measured realistic values for the rates of ethylene production and correlated fairly well with previous reports. In addition, the relatively small increase in ethylene levels necessary to cause epinasty could explain the observed epinasty (Table 5) with only a slight increase in the rate of ethylene production (Figure 10).

Auxin. Auxin levels in flooded plants have been much discussed, but only two studies seem to have measured IAA directly. Phillips (158) found that shoot IAA levels in sunflowers increased after 10 days of flooding. After only one day, there was no difference between flooded and control plants. Hartung and Witt (75), on the other hand, found decreased IAA activity in sunflower plants waterlogged for one to four weeks as compared to control plants at optimum soil moisture content. Plants flooded for 2 1/2 days were slightly lower in auxin content than control plants. Regardless of the discrepancy upon long-term flooding, it appears that in the time interval at issue here, i.e., within 48 hours, auxin levels do not change appreciably. Instead of elevated IAA levels being responsible for the rise in ethylene synthesis (140), it seems more likely that the elevated ethylene levels are influencing IAA physiology (23, 139). Morgan, et al. (139) reported that a 15-hour exposure of tomato and sunflower plants to ethylene caused an increase in both uptake and transport of auxin through stem segments. This is in contrast to the usually reported effect of ethylene, which is to inhibit the capacity of the auxin transport system (23, 71, 139). Burg and Burg (23) explained certain symptoms which appear to be auxin deficiencies on the basis of reduced transport. Adventitious rooting, stem swelling, and epinasty, however, are symptoms of excess auxin (232). Ethylene may promote auxin transport in flooded tomatoes, thereby causing the continued high levels of ethylene production (Figure 4) and secondary effects such as adventitious roots (see below).

Cytokinins. Following the discoveries that cytokinins are synthesized in roots (105, 216) and that kinetin can delay loss of chlorophyll in detached leaves (172), Burrows and Carr (26) and Carr and Reid (30) hypothesized that reduction in the supply of cytokinins from the root could be a cause of chlorosis during flooding. They found that the cytokinin concentration in xylem sap of flooded sunflower plants declined for up to 72 hours in parallel with the decline in metabolic activity of the roots. After 96 hours, there was a drastic reduction in both the volume of exudate and the concentration of cytokinins, and examination of the roots revealed that the apices were blackened and apparently dead. Chlorosis also became severe on the fourth day of flooding. Application of benzyladenine (BA) to waterlogged plants prevented loss of chlorophyll, epinasty, and adventitious rooting in flooded plants (166, 170, 176).

Kawase (102) reported that shoot ethylene levels increased slightly for the first four days of flooding, then rose at a much faster rate. Thus, ethylene production increased markedly coincident with the dramatic decline in cytokinin supply from the root (26). Lau, et al. (115-118) have reported a synergistic enhancement of IAA-stimulated ethylene production by cytokinins in mung bean. The correlation between reduced cytokinin supply and increased ethylene production seen in flooded intact plants is therefore unexpected. According to the Lau mechanism, cytokinin prevents conjugation of IAA, which maintains higher free IAA levels in the tissue and stimulates ethylene production. However, the use of high concentrations of both IAA (10^{-5} to 10^{-4} M) and kinetin (10^{-4} M) can be criticized as being nonphysiological (see also 88). On the basis of the data available for flooded plants, it appears that a reduction in cytokinin supply to the shoot may be involved in elevated ethylene production. Unfortunately, ethylene production was not measured in experiments where exogenous cytokinin was applied (88, 166, 170, 176), so whether the effect of cytokinin was to influence synthesis of, or sensitivity to ethylene, cannot be answered.

That sensitivity to ethylene can be altered by kinetin was shown by Mayak and Kofranek (133) and Mayak and Dilley (131). In the presence of sucrose, kinetin increased the longevity of carnation flowers following an exposure to ethylene. Eisinger (47) has recently obtained evidence that kinetin can influence both sensitivity to, and synthesis of ethylene in carnation flowers. He found that kinetin-treated flowers were less responsive to applied ethylene. In addition, ethylene production was reduced by moderate kinetin concentrations, but was accelerated by supra-optimal concentrations. If the cytokinin supply from the roots of unflooded plants is optimal, then a reduction in cytokinin could lead to increased ethylene production, as in carnation. Application of cytokinin could either reduce sensitivity to ethylene or decrease synthesis, or both. At higher cytokinin concentrations, synergistic enhancement of ethylene synthesis could come into play.

Indirect evidence for a connection between cytokinin levels and ethylene production comes from recent measurements of these two hormones in *Verticillium*-infected tomato plants. The vascular wilt diseases produce symptoms in infected plants strikingly similar to those resulting from ethylene treatment, such as loss of chlorophyll, abscission, and epinasty (78). Pegg and Cronshaw (156) measured increased ethylene production in susceptible, but not in resistant, isolines of tomato following infection with *V. albo-atrum*. The rise in ethylene coincided with the onset of symptom expression (between 6 and 9 days following inoculation). Virtually identical results were obtained with *Fusarium*-infected tomato plants by Gentile and Matta (58). Patrick, *et al.* (154)

conducted a similar study, but measured the concentration of cytokinins in the xylem sap of tomato plants infected with _V. dahliae_. Total cytokinins, both per plant and per ml of exudate, fell by about 50 per cent between the sixth and twelfth days following inoculation, the period of rapid symptom development. Collectively, these results correlate increased ethylene production with decreased cytokinin levels in tomato shoots. The timing of sampling periods is not precise enough in these experiments to determine which event is more precocious. Furthermore, Pegg (155) found that exposure to 5 ul/l ethylene for 48 hours following inoculation with _V. albo-atrum_ almost completely prevented injury from the pathogen. This was attributed to an increase in the number of xylem vessels and an inhibition of vessel colonization by the fungal hyphae. As part of the reduction in cytokinin levels in infected plants was due to decreased exudation (154), the increase in unplugged xylem vessels induced by ethylene treatment may have maintained cytokinin levels in the shoots and prevented injury. The stimulation of lateral roots by ethylene (229) could also provide more sites of cytokinin synthesis in treated plants. While this hypothesis is based on relatively scanty evidence, it could partially explain the relative immunity of ethylene-pretreated plants to restriction of root oxygen availability (Figures 8 and 10), as exogenous cytokinin prevents many symptoms of flooding injury (166).

Gibberellins. Flooding has also been found to alter GA synthesis in the root (Table 7). Phillips (157) deduced from experiments involving combinations of girdling and flooding that in addition to auxin, a factor from the root was also involved in the response. He found that application of GA could partially prevent flooding injury. Phillips and

Jones (161) and Carr and Reid (30) obtained evidence of GA synthesis in roots, and Reid, _et al_. (168-170) measured decreased levels of GA in roots, shoots, and xylem sap of flooded plants. Exogenous GA has been found to partially overcome some of the flooding symptoms, particularly reduction in growth (88, 168, 170, 176). Reid and Railton (170) have suggested that cytokinins may control the synthesis of GA, as BA application increased GA levels in both flooded and unflooded tomatoes. Conversely, ethylene has been found to reduce the endogenous GA content of cucumber plants (174). Thus, GA levels may be the result not only of reduced synthesis in the root, but also of changes in other hormones. Since GA is also synthesized in shoots (98), flooding effects on GA levels may be secondary to those on ethylene or cytokinins (107).

Abscisic acid. Waterlogging of dwarf bean plants caused a fivefold increase in ABA activity in the leaves after five days (223). In wheat leaves, Wright (222) found that only a slight decrease in water potential (from -7 to -8 bars) could double ethylene production, and a further doubling occurred between -8 and -9 bars, a stress insufficient to cause wilting. ABA levels followed a similar curve, but the increases occurred at water potentials about one bar more negative than for ethylene. This result opens the possibility that ethylene may be responsible for the rise in ABA, but no direct evidence is available of a causal connection (222). An effect of ABA on root oxygen stress was discovered by Mizrahi, _et al_. (137). They observed that tobacco plants previously exposed to salination of the nutrient solution did not suffer injury upon cessation of aeration, while control plants quickly wilted. Salinated plants contained higher ABA concentrations, and addition of ABA to the nutrient solution could replace the effect of salination. The ability of ABA to close stomates (97) and increase root permeability (63, 199) is probably responsible for the maintenance of turgor in unaerated plants. Salination has been reported to increase ethylene production by citrus leaves (167). Ethylene application can increase ABA levels in rose petals and leaves (132) and in cucumber plants (174), while ABA-induced ethylene synthesis is seen in carnation (130). The close and apparently reciprocal relationship between ABA and ethylene strongly suggests that these two hormones interact in the response of plants to root stress. Elucidation of the cause and effect sequence in this relationship would be quite interesting and revealing.

Morphological

Epinasty. Perhaps the most obvious symptom of the flooding syndrome is petiole epinasty. Turkova (209) commented on this response to deficient aeration and noted the similarity of flooded plants to plants treated with ethylene. Workers in the early 1930's established that ethylene at extremely low concentrations could promote epinasty in a variety of plants (e.g., 40). The endogenous regulation of petiole epinasty, however, is still a source of controversy. Since epinasty is the result of more rapid elongation in the upper (adaxial) side of the petiole than in the lower (abaxial) side, Lyon (127) proposed that asymetric distribution of auxin could be responsible. He envisioned a continuous upward movement of auxin in petioles which was counterbalanced by gravitational direction of auxin downwards. If the gravitational component was removed by rotation on a clinostat, auxin would accumulate in the upper side of the petiole and cause epinasty. His early work (127) tended to support this hypothesis, as more IAA was found in the upper than in the lower side of epinastic petioles, and triiodobenzoic acid (TIBA, an auxin transport inhibitor) could block the epinasty-producing effect of applied IAA. Lyon concluded (127), "Auxin transport is an essential factor in epinasty."

Following the discovery that auxin can stimulate ethylene synthesis (e.g., 140), Stewart and Freebairn (196) attempted to separate the effects of ethylene and auxin on epinasty. By giving tomato seedlings a short exposure to 40 C, ethylene synthesis could be prevented. Subsequent exposure to exogenous ethylene caused epinasty, while IAA application did not. Thus, auxin-induced epinasty appears to be mediated by an intervening step requiring ethylene synthesis. Lyon (128) examined whether ethylene might inhibit the gravitational component in auxin transport, leading to accumulation of auxin in the upper petiole. Exposure to ethylene for 24 hours was found to increase by 30 to 40 per cent the amount of IAA transported to the upper side of tomato and pepper petioles. Kang and Burg (99) reported similar results and hypothesized that ethylene actually reverses the direction of geostimulated lateral auxin transport. This reversal leads to a redistribution of auxin and consequently causes epinasty.

This hypothesis has been challenged recently by Palmer (150, 152). In sunflower, he showed that epinasty was independent of lamina factors (the presumed source of auxin) and that ethylene would promote epinasty in the absence of an auxin supply (150). He has criticized the earlier work on auxin distribution on several counts (152): (a) While epinasty is a rapid response to ethylene, commencing within 1 to 2 hours and completed within 15 to 20 hours (40), Lyon did not measure IAA distribution until after 24 hours of exposure to ethylene (128), and Kang and Burg used an 18-hour exposure (99). The major epinastic growth was completed before the measurements were made, and auxin redistribution may have been a secondary effect. (b) Radioactivity from ^{14}C-IAA was not shown to be associated exclusively with IAA at the time of measurement. (c) In several of Lyon's experiments (128), ethylene treatment increased the amount of IAA transported to both the upper and lower sides of the petiole as compared to control plants.

When IAA transport in Coleus petioles was measured during the period of rapid epinastic growth, no redistribution of IAA was found in the petioles (152). Furthermore, DPX1840, which inhibits both lateral and polar auxin transport (13), did not prevent the initial epinastic response (152). After 6 hours, the rate of growth decreased, indicating that IAA may be a cofactor for continued growth. Leather, et al. (119) have also found that the final degree of curvature is greater with both IAA and ethylene treatment than with ethylene alone. In contrast to tissues which elongate in response to auxin, where ethylene reduces auxin transport, Musgrave and Walters (145) have reported that tissues which elongate in response to ethylene have increased auxin transport capacity. Palmer (152) has interpreted his work as being contradictory to this finding concerning auxin transport, since no difference in IAA content was seen between tissues responding to ethylene (adaxial) and those not responding to ethylene (abaxial). However, the data of Musgrave and Walters (145) indicates that while transport of IAA through ethylene-treated tissues was increased, IAA levels present in the tissue were not changed. Since the upper petiole has increased in volume relative to the lower, yet the concentrations of IAA are equal in the two halves, it follows that more IAA must have been transported through the upper half. Thus, the evidence is not contradictory, but rather mutually supportive.

The cells of the upper side of the petiole elongate in response to ethylene, while those of the lower side are inhibited by ethylene and stimulated by auxin (150). Osborne (149) has interpreted this response in terms of a target cell concept, the upper petiole cells being Type 2 cells (elongate in response to ethylene), and the lower ones Type 1 or 3 cells (elongate in response to auxin). If the upper petiole cells are indeed Type 2 cells, several associated phenomena can be explained. Type 2 cells in abscission zones enlarge in response to ethylene only when the supply of auxin from the leaf or fruit is reduced (91, 149). If auxin transport through the Type 2 cells of the petiole is increased by exposure to ethylene (145), this may explain why such exposure usually does not cause leaf abscission in tomato, as it does in species where ethylene inhibits auxin transport (17). Turkova (cited by Abeles, 1) reported that exposure to ethylene increased the amount of auxin in the base of tomato petioles. It is also interesting that two species which show a dramatic response to flooding (tomatoes and sunflower) are also the only two of the species tested which increased auxin transport following exposure to ethylene (139). In flooded plants, increased ethylene production may be an initial response which alters auxin distribution and results in the secondary effects of flooding, such as adventitious rooting.

If this analysis of hormonal control of epinasty is correct, the question becomes, "How does flooding the root cause ethylene production in the shoot?" Both changes in turgor and reduction in root-synthesized hormones have been discussed previously as possible means of root-shoot communication. These suggestions were based in part on the ability of applied ABA, GA, and cytokinins to prevent or reduce flooding injury.

Jackson and Campbell (88) have criticized these hypotheses and have argued for a specific ethylene promoting signal, which is formed in anaerobic roots and is transported to the shoot. First of all, spraying with BA plus GA prevented epinasty in plants gassed with ethylene, so the effect is probably on ethylene action rather than synthesis (88). The authors also state that since only 0.04 to 0.07 ul/l ethylene is required to cause epinasty in an intact plant, which presumably has an adequate supply of root hormones, the presence of normal levels of cytokinins and GA do not prevent petiole epinasty. However, since the internal concentration of ethylene in tomato leaves was reported to be 0.3 ul/l (89), it appears than sensitivity to ethylene is attenuated in the intact plant. The data of Table 4 of the present work can also be interpreted in a way to support the hypothesis of Jackson and Campbell. Only those plants lacking adventitious roots suffered loss of chlorophyll under anaerobic root conditions. If a reduced cytokinin supply is responsible for the loss of chlorophyll and also for the increase in ethylene production, one would have expected to see a much greater increase in ethylene production by those plants lacking adventitious roots (Figure 8). That there was not a large increase indicates that other factors besides cytokinin play a role in regulation of ethylene production. However, the slight rise in ethylene production seen after lowering the shoot oxygen level (Figure 8) was roughly proportional to the loss in chlorophyll, which suggests that cytokinins may play a part in regulation of ethylene synthesis.

The hormonal situation in cuttings is undoubtedly different from that in the intact plant. For example, auxin can diffuse from the cutting or be inactivated by enzymes at the cut surface (219). It has also been reported that tomato petioles produce ethylene at an accelerated rate following excision (90, 91) and that this wound ethylene synthesis does not promote abscission (91). In sunflower, excised petioles did not become epinastic (151), although it is probable that they also exhibit wound ethylene production. If epinasty or abscission were simply matters of increasing ethylene levels by a given amount, then wound ethylene should also promote the response. One cannot, therefore, ignore the influence of differing sensitivities to hormones under different physiological conditions. The work of Jackson and Campbell with cuttings (88), while suggesting that an ethylene-promoting factor may be involved, cannot be considered as conclusive evidence of the noninvolvement of root hormones in the flooding syndrome.

It is not surprising that all of the observed facts do not fit into a single, well-defined hypothesis. The complexity of the situation is shown in Figure 12, where many of the interactions which have been discussed are presented in graphical form. Ethylene has been placed at the focal point, as it is the hormone directly responsible for epinasty. Other hormones could assume that position in the etiology of other responses to flooding. The arrows indicate interactions which have been observed in the present work or reported in the literature. No temporal order is implied by the arrangement of factors, as all are occurring simultaneously in the intact plant. Exogenous events affecting epinasty are shown in boxes, while endogenous events are in circles. It is clear even from this simplified model that adequate mechanisms exist that may used by the plant in responding to the stress. The point to be emphasized by Figure 12 is the interdependency of hormonal action. Application or synthesis of a hormone simultaneously influences all the mechanisms

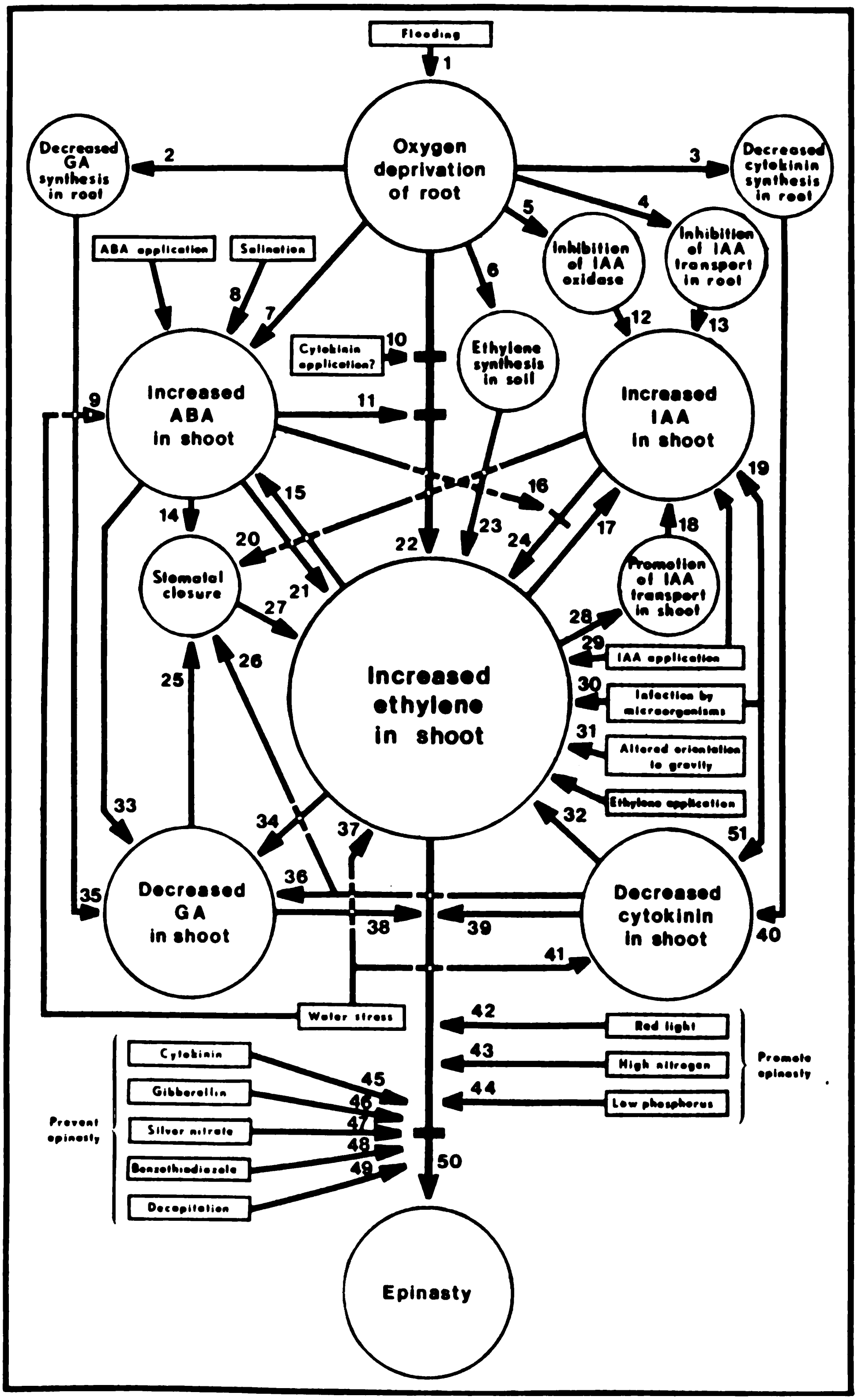

Figure 12. Possible hormonal interactions involved in the epinastic response. The numbers refer to literature citations on the following page.

References for Figure 12

1. 44, 164, 179, 188
2. 30, 168-170
3. 26, 30, 170
4. 10, 158
5. 32, 120, 130, 163, 211, 224
6. 44, 85-89, 185-193
7. 223
8. 137
9. 114, 137, 222, 223
10. 47
11. 132, 137
12. 158
13. 10, 141, 158
14. 198, 214
15. 11, 132, 137

16 59, 108

17. 139
18. 139, 145
19. 58
20. 198, 199
21. 130
22. 48, 85-89, 101-103, Figures 4 and 6
23. 87, 102
24. 22, 100, 140
25. 124, 198-200
26. 124, 198-200, 210
27. 48, 122
28. 139, 145
29. 22, 100, 140
30. 58, 78, 122, 156, 217
31. 40, 119, 151
32. 47
33. 215
34. 174
35. 10, 158
36. 170
37. 48, 58, 134, 222
38. 88, 157, 166, 176
39. 88, 166, 176
40. 26, 30, 170
41. 84, 210
42. 136
43. 153
44. 46
45. 86, 88, 157, 166, 176
46. 88, 150, 157, 176
47. 14, 15, Figure 3
48. 89
49. 157, 158
50. 40, 152
51. 154

of which it is a part. Developmental or growth events, such as epinasty, are the resultants of the various hormonal, environmental, genetic, and nutritional vectors.

Adventitious roots. This confluence of hormonal action is also evident in the control of adventitious root initiation and growth. Went (218) showed that continued shoot growth was dependent upon factors supplied by an aerated root system. If even a small fraction of the total root was in an aerobic environment, shoot growth could be maintained and chlorosis prevented. In experiments with tomatoes, Jackson (92) found that continuous removal of adventitious roots from flooded plants prevented recovery from the injury. The presence of adventitious roots on the stem before flooding the original root system offered partial protection from epinasty and reduced shoot growth. The work of Jackson and Went suggests that either the aerobic roots produce a factor essential to continued shoot growth, or that aerobic roots can prevent injury from toxic factor(s) produced in anaerobic roots.

To determine whether adventitious roots influence ethylene synthesis in plants with an anaerobic root system, the experiment shown in Figure 8 was performed on plants in which adventitious roots had been induced by ethylene pretreatment. Presence or absence of adventitious roots did not significantly affect ethylene production of these plants (Figure 8). However, as shown in Table 4, none of these plants suffered severe injury due to the anaerobic treatment. Ethylene pretreatment may have increased the resistance of the plants to the stress. Figure 9, however, indicates that the growth of new adventitious roots following ethylene pretreatment was influenced by the aeration status of the original root. Only plants with anaerobic root systems produced adventitious roots in excess of those present at the start of the experiment. Removal of adventitious roots stimulated the formation of new adventitious roots only in plants made anaerobic. Gill (62) has reported a similar situation in a woody species (Alnus glutinosa (L.) Gaern.). Alternatively, data in Table 5 could indicate that the presence of adventitious roots inhibits the formation of additional adventitious roots. Ethylene pretreatment in this case stimulated the formation of only a few adventitious roots (average of 0.2 per plant). When the original roots were subsequently subjected to an anaerobic atmosphere, ethylene pretreatment significantly reduced the number of adventitious roots which developed. Primordia induced following ethylene exposure may have prevented differentiation of new primordia during root anaerobiosis. The existence of correlative control of root formation is therefore suggested.

The hormonal control of root initiation and growth may be even more complex than that of epinasty. In flooded plants, adventitious root formation occurs in a milieu of hormonal changes like that shown in Figure 12. A specific hormone responsible for adventitious root formation is not as obvious as in the case of epinasty, although auxin has long been known to be a minimal requirement (c.f., e.g. 219). Ethylene also stimulates adventitious roots on a variety of plants (229), although the concentrations and lengths of exposure required are much greater than for most ethylene-mediated responses (1). As in epinasty, adventitious rooting seems to be associated with auxin-ethylene interactions.

The work of Zobel with an ethylene-requiring tomato mutant, diageotropica (dgt), has been most instructive on this point. The primary lesion in this single gene mutant apparently is an inability to produce ethylene in response to auxin (233). The morphological results of this defect are characterized by "diageotropic growth of both shoots and roots, thin stems without large secondary xylem vessels but with abnormally thick phloem fibers, dark green hyponastic leaf segments, primary and adventitious roots without lateral branching, and an open hypocotyl hook" (234). The alteration of gravitational responses, leaf orientation, and hook opening are consistent with earlier hypotheses concerning auxin-ethylene interactions in the regulation of those events (25, 99). In the present connection, it is most interesting that while dgt is a "prolific adventitious rooting plant," lateral root formation on existing roots requires ethylene (234). Thus, adventitious root initiation on stem tissue is a separate process from lateral root initiation on root tissue. While the increase in lateral roots seen in flooded plants may be due to ethylene (57, 234), adventitious rooting apparently is not controlled by the gas.

How, then, is adventitious rooting controlled, and why is the trait generally expressed only in response to injury to the main root? Due to the economic and practical importance of rooting to the plant propagation industry, virtually all of the work on adventitious roots has been done with cuttings. As was discussed earlier, stem cuttings present a somewhat different situation from the intact plant, but much pertinent information can be obtained from these studies. The major findings can be generalized as follows: (a) auxin is generally required for root initiation (35, 74, 183, 212, 219); (b) at a given auxin concentration, low rates of cytokinins increase rooting, while high levels inhibit rooting (52, 162, 183 for adventitious roots; 197, 204 for lateral root initiation); (c) GA generally has little influence on rooting, although it can be inhibitory even in the presence of auxin (8, 35, 95, 205);

(d) nutritional factors can modify the root-promoting effect of auxin (126, 138, 162, 171); (e) light intensity may either promote or inhibit the rooting response through effects of both the nutritional and hormonal factors (74, 77, 125, 132, 147, 162; see later discussion of environmental factors). It has been suggested that lateral root initiation is controlled by the ratio of auxin to cytokinin, high ratios promoting and low ones inhibiting lateral roots (66, 204). A similar mechanism may be involved in adventitious root formation in flooded plants. The hormonal changes upon flooding (Figure 12) are likely to shift the auxin:cytokinin balance in favor of auxin, promoting adventitious and lateral roots. As application of BA prevented adventitious roots (but not stem hypertrophy) in flooded plants (166), shifting the balance in favor of cytokinins may prevent adventitious root formation. In an intact, unstressed plant, the normal levels of auxin and cytokinin present may prevent adventitious root initiation. Injury to the original root can rapidly reduce cytokinin synthesis and export (83), favoring adventitious root development. This double control of adventitious rooting by shoot-synthesized auxin and root-synthesized cytokinin could be responsible for the rapid development of adventitious roots following flooding (102, Figure 9, Table 5).

The role of ethylene in adventitious rooting remains unclear. In the present work, the ability of ethylene to induce adventitious roots was inconsistent, apparently depending upon the growing conditions of the plants before treatment (Figure 9 and Table 5). In mung bean, Mullins (144) found that ethylene (1 ul/1) inhibited the initiation of adventitious root primordia and he suggested that the balance between IAA (promotion) and ethylene (inhibition) regulates root primordia initiation, if other factors are not limiting. Apelbaum and Burg (5) demonstrated that ethylene prevents cell division and deoxyribonucleic acid synthesis in the apical hook region of pea seedlings. The direct effect may be to inhibit DNA polymerase activity (6). Cell division is required for root primordia initiation in plants lacking preformed root initials (19, 144). Inhibition of cell division in the apical meristem of pea roots by both ethylene and auxin (2,4-D) was also observed (5). Auxin, however, stimulated divisions in the basal root region which ultimately formed lateral roots, while ethylene did not stimulate lateral roots (5). This situation in pea is different from that in tomato, where work with the mutant dgt has shown that ethylene, but not auxin, will promote lateral root formation (233). This is another instance of a different ethylene-auxin relationship in tomato as compared to pea (c.f. 139). The ability of an inhibitor of ethylene action ($AgNO_3$) to prevent adventitious rooting in the present study further suggests that in tomato, ethylene may be involved in the endogenous regulation of root formation. In addition, the high rates of auxin often used commercially to promote rooting undoubtedly stimulate ethylene production. Further work is needed on the role of ethylene in adventitious rooting and its possible influence on the auxin:cytokinin balance.

Phenolic compounds may also be involved in the induction of adventitious roots. Catlin, *et al*. (32) found degradation of phenols in flooded walnut roots and proposed that their export to the shoot could be a factor in flooding injury. Ortho-dihydroxyphenols, such as catechol, have been reported to act synergistically with IAA to promote adventitious rooting in cuttings, possibly by inhibiting IAA oxidase activity (77). Chlorogenic acid has a similar effect on auxin catabolism and increases root auxin content (163). Furthermore, cells with high polyphenol oxidase activity were found to be sites of root primordia initiation (77). Hess (77) has proposed a model for adventitious root initiation involving a mobile phenol, auxin, polyphenol oxidase, carbohydrates, and nitrogenous substances. Cytokinins, at least, and probably ethylene, must be added to this model in order to approach the in vivo situation. It is perhaps relevant that ethylene treatment of tomato plants was found to induce polyphenol oxidase activity and raise the endogenous level of phenols (58). Thus, the effect of ethylene on adventitious rooting may involve both hormonal and enzymic interactions.

Once induced, how do adventitious roots affect plant growth? When adventitious roots were induced to form on an otherwise normal plant, Jackson (92) reported that the plants were "pale green, slender, and not as vigorous as those in the other [control] group." The only effect on growth in the present study was an insignificant increase in shoot growth following the removal of the adventitious roots (Table 4). Schramm (175) found that adventitious roots on flooded tomato plants had a higher rate of oxygen consumption than any other roots on a per gram fresh weight basis. In addition, carbon dioxide production of adventitious roots under anaerobiosis was higher than that of other roots under similar conditions. This may indicate that adventitious roots exhibit a Pasteur effect, switching to fermentation when oxygen becomes limiting. Anatomical considerations also support this view, as in meristems the respiratory quotient (RQ) has been found to be greater than unity (148). The high rate of metabolism and close packing of the cells apparently limits oxygen diffusion to the area, resulting in a high RQ. Low surface-to-volume ratios (i.e., thicker roots) also increase the RQ (148). Schramm (175) observed larger steles and 3 to 4 more layers of cells in the cortex of adventitious roots than in the original roots. Adventitious roots have high respiratory activity and a low surface-to-volume ratio (175), suggesting that they may be under some oxygen limitation even in an aerobic environment. Their ability to tolerate and even grow rapidly under such conditions is significant, considering their role in restoring growth to flooded plants (92). However, adventitious roots cannot function in completely anaerobic conditions, as evidenced by injury to plants with both original and adventitious roots under water (92). It is possible that substrate competition between the rapidly respiring adventitious roots, the original roots, and the growing shoot could be responsible for the decreased vigor observed in adventitiously rooted, but unflooded plants (92).

The synthetic and degradative activities of roots have been discussed earlier. Adventitious roots could therefore be expected to synthesize mainly cytokinins and GA, and to oxidize shoot auxin. If the hypothesis presented previously is correct, these activities would tend to correct the high auxin to cytokinin ratio induced by flooding, and prevent initiation of new roots. It would also correct the hormonal imbalance in the shoot (Figure 12) leading to resumption of shoot growth and recovery from epinasty.

Stem hypertrophy. Swelling of the lower stem has often been reported as a response to flooded root conditions (110). In sunflower, Kawase (102) found that flooding caused the stem cortex cells to enlarge radially with the formation of numerous large intercellular spaces. This effect was highly correlated with internal ethylene concentrations, and ethephon treatment produced a virtually identical result. Goeschl, et al. (65) determined that endogenous ethylene was responsible for the swelling of pea epicotyls in response to physical stress. Ethylene has been shown to cause altered orientation of cell wall microfibrils, which results in a cessation of elongation and isodiametric growth of cortical cells (3, 4). It would appear that elevated ethylene levels in flooded plants is directly responsible for stem hypertrophy. This view is also supported by the smaller stem diameter of the ethylene-requiring mutant *dgt* as compared to its isogenic control line (234). That ethylene directly influences stem hypertrophy is supported by the observation that BA application to flooded tomato plants will prevent epinasty and adventitious rooting, but not stem thickening (166).

Ethylene may also play a role in secondary vascular development. The tomato mutant *dgt* does not develop secondary xylem vessels, although the primary vascular system appears normal (234). From this, one can conclude that ethylene is required for normal secondary vascularization in tomato. When Pegg (155) gassed tomato plants with ethylene, increased numbers of xylem vessels were observed, primarily in the region where secondary thickening was commencing. He attributed the greater resistance of ethylene-treated plants to *Verticillium* infection to this increase in vascularization, as was discussed earlier.

The net result of exposure of stem tissue to ethylene would appear to be an increased capacity for transport of both air and water through the root-shoot transition zone. Since increased ethylene production is a rapid response of shoot tissue to flooding, these morphological changes may be adaptations to restricted root oxygen availability. Studies have shown that movement of oxygen through the shoot to the root can occur in mesophytes (9, 70, 76, 96). The proportion of the root oxygen demand satisfied in this way is dependent upon the ambient oxygen concentration surrounding the root (70, 194). Shoot oxygen uptake of bean seedlings more than doubled when the roots were in an anaerobic atmosphere (194). Studies on oxygen movement through plants (9, 70, 76) indicate that the depth to which the internal oxygen supply is adequate for root growth depends on both the rate of respiration of the roots and the rate of diffusion from the root. Kramer (110) has questioned whether increased cortical air spaces would, in fact, increase oxygen diffusion to the root. He has also noted that by the time the intercellular spaces have developed, the critical period of oxygen starvation has passed and injury has already occurred. The studies cited above, however, have established that internal gas movement occurs primarily through the intercellular spaces and that this source of oxygen is sufficient to sustain root growth to a limited depth. Although to this author's knowledge the hypothesis has not been tested, it would seem reasonable to predict that ethylene treatment would facilitate internal movement of gases due to increased intercellular spaces. This increased diffusion would only become apparent upon lowering the oxygen content in the root atmosphere, as only then would a concentration gradient be established between the root and the shoot (70, 194).

These observations are pertinent in understanding the results shown in Figures 4, 6, 8, and 10. In plants not pretreated with ethylene (Figures 4 and 6), the adaptive response is not rapid enough to prevent injury to the root, as evidenced by blackened apices and an absence of root pressure. Root injury is probably involved in the increased ethylene production exhibited by these plants, as Gentile and Matta (58) found that injuring the roots of tomato plants by immersion in a copper sulfate solution caused a four- to fivefold increase in ethylene synthesis by the shoots. In ethylene-pretreated plants (Figures 8 and 10), anaerobic root atmospheres were much less damaging, as apices were still alive and root pressure was evident. This suggests that ethylene-induced internal modifications allowed for greater oxygen diffusion to the roots and prevented injury. This hypothesis is supported the fact that when the shoot atmosphere was changed to 2 per cent oxygen, shoot ethylene production immediately began to rise in plants with anaerobic roots (Figure 8).

Environmental Factors

Temperature

In extensive experiments concerning the effects of deficient oxygen on root growth, Cannon (27) found that in general, the higher the temperature, the greater the injury. This can be related to the rise in respiration with temperature, such that any given oxygen concentration will become less adequate with increasing temperature. Kramer and Jackson (113) noted that the initial response (wilting) in tobacco plants was more rapid at 20 C than at 34 C, but subsequent injury was much more severe at the higher temperature. The roots at 20 C had only limited decay, but permeability to water was very low. In a soil situation, the increase in microbial oxygen consumption with temperature must also be considered. In general, increasing temperature accelerates injury due to an elevated demand and reduced supply of oxygen.

Light

The involvement of light in many developmental processes in plants suggests that it may also be involved in responses to flooding. For example, high light intensity, especially red light, promoted epinasty in Salvia occidentalis (136). The effect of light on flooding injury has not been directly tested, but one response to flooding, adventitious rooting, is related to light. Van Overbeek, *et al.* (212) found that in addition to auxin, leaves were also required for rooting of *Hibiscus* cuttings. Sugars and nitrogenous substances could replace the leaves in promoting rooting. Subsequent work in a variety of plants has demonstrated that carbohydrates, in addition to auxin, are required for root initiation (73, 74, 77, 125, 138, 162). Light intensity can influence rooting of cuttings through its effects on the carbohydrate content of the stock plant (74, 77). Rooting of cuttings generally increases with increasing light intensity to a certain point, beyond which an inhibitory effect is seen (74, 77, 125, 126). Auxin can promote or inhibit rooting, depending on the carbohydrate concentration or nutritional status of the plant (146). Thus, the balance between carbohydrate and auxin may be more important than the absolute amount present.

Nutritional status can also influence sensitivity to other hormones. Mayak and Dilley (131) found that the longevity of fall- or spring-grown carnations was increased by kinetin, but that winter-grown flowers did not respond. The extension of longevity by kinetin was related to the incident radiation during the period prior to anthesis. Addition of sucrose to the kinetin media restored the senescence-delaying effect of the hormone in winter-grown flowers. Sucrose supplementation also lowered the sensitivity of the flowers to ethylene and ABA.

In addition to nutritional effects, light also influences hormone levels directly. Light is known to decrease auxin content and transport in plant tissues (53, 104, 147), which may account for the inhibition of rooting by high light intensity (74). Tucker (206, 207) measured IAA, ABA, GA, and cytokinin levels in tomato plants grown under winter (low light intensity) or summer (high light intensity) conditions. In general, he found decreased IAA and ABA and increased GA and cytokinin concentrations in summer-grown plants as compared to winter-grown plants. Tucker (208) also showed that far-red illumination, which inhibits lateral bud growth in tomato, increased the auxin and ABA levels in the plants. The nutritional and hormonal changes initiated by light can be expected to influence plant responses to flooding injury, as many of the symptoms can be related to hormonal imbalances (e.g., Figure 12).

The inconsistent ability of ethylene to promote adventitious rooting in the present work (Figure 9 and Table 5) indicates that the gas probably acts in conjunction with other hormonal and/or nutritional factors. The plants used in the first experiment (Figure 9) were grown during the low light conditions of February, and they formed adventitious roots following ethylene treatment. The plants used in the second experiment (Table 5) were grown in March under greater light intensity, and ethylene treatment stimulated few roots. With reference to the work of Tucker (206, 207), plants grown under low light conditions would likely have a higher auxin:cytokinin ratio than plants grown under higher light conditions. If the effect of ethylene is to shift this balance in favor of auxin, and thereby promote adventitious root formation, less of a perturbation would be required in the plants with a higher ratio initially. Since relatively high levels of ethylene are required to induce adventitious roots (1), such an indirect mechanism may be operative. In addition, the higher carbohydrate status of the plants grown in March would increase the concentration of auxin required for primordia initiation (146). Thus, by creating a condition of supraoptimal carbohydrate and suboptimal auxin, high light intensity may prevent adventitious root formation in response to ethylene. This block apparently cannot be overcome by ethylene alone, but an anaerobic root atmosphere can cause adventitious rooting even when ethylene levels are not significantly increased (Figure 10 and Table 5). The fact that epinasty also occurred in plants with anaerobic roots without a large increase in ethylene production once again points out that sensitivity to a particular hormone may be as important as absolute concentrations. Various workers (e.g., 77, 144) have noted the interdependency of the factors (nutritional, hormonal, and environmental) involved in adventitious root formation, all of which must be adequate to permit the process to proceed.

Relative Humidity

The transpirational demand also influences the response of plants to deficient root aeration. Injury due to flooding is more severe on sunny than on cloudy days (49). Poor aeration can decrease root permeability (109), which would accentuate the water deficit in the plant. Figure 11 also indicates that the vapor pressure gradient in the shoot can influence root respiration; at high relative humidity in the shoot chamber, root respiration increased slowly in both aerobic and anaerobic roots. When the relative humidity was lowered, aerobic root respiration immediately increased, while that of anaerobic roots decreased. Much less injury due to poor aeration was seen in the root systems of plants which had a low transpirational demand. This lessened claim upon root absorption, coupled with the internal modifications occasioned by ethylene pretreatment, probably combined to prevent severe injury to the roots. The interaction between oxygen supply, relative humidity, and root respiration apparently is related to the severity of injury under conditions where transpirational demand is high (49, 109, 112).

Microorganisms

Microorganisms in the root environment may also play a role in flooding injury. The ability of soil microbes to synthesize ethylene and its accumulation under anaerobic conditions has already been discussed. Kramer (110) found that flooding in soil was more deleterious than submersion of the roots in water. The more rapid lowering of the oxygen level due to microbial respiration was suggested as the cause. Toxic compounds might also be formed by microorganisms and cause injury to the plant (110), but Jackson's grafting experiments suggest that this is not a major factor (93). In the present work, the ability of low oxygen to mimic virtually all of the major symptoms of flooding injury in inert media indicates that microorganisms are an accessory, not a primary cause of injury. Nevertheless, the situation of the plant growing in soil is much more complex than the model system employed here, and degradation of the original root by microorganisms during periods of anaerobiosis may limit recovery upon return of oxygen to the root (113).

A Paradigm for Plant Responses to Flooding

Following this discussion of hormonal mediation of plant responses to flooding, it is perhaps pertinent to recall the introduction by Went and Thimann to their classic book, Phytohormones (219): "The field of plant hormones is perhaps now at the stage of its most rapid development. The number of facts is becoming so large, and their distribution so scattered, that there is danger of losing sight of the general trend." In the forty years since that statement was made, the accumulation of facts and their distribution has obviously accelerated. The danger of losing sight of the "general trend" is therefore even greater today. On the other hand, the opportunity for significant insight is enhanced by the proliferation of observations. What general trend or paradigm for the plant's response to flooding can be drawn from this large body of facts? This author would like to suggest that the concept of correlative control of organ formation in both shoots and roots could be used to provide a conceptual framework for understanding the varied responses of plants to root stresses.

Correlative control is linked to the polarity of the plant, defined as "a tendency to produce regenerates of different nature in apical and basal parts, the apical ends tending to give rise to shoots, the basal ends to roots" (219). The phenomenon known as apical dominance (the inhibition of lateral bud growth by the growing apex) is the most well known example of correlative control. While a great deal is known about this process in shoots, very little work has been done on apical dominance in roots (c.f. 159, 160). Reports of the inhibition of lateral roots by the growing root tip have occasionally appeared, suggesting the existence of correlative control of lateral root initiation (81, 203, 230). Recent work with cereal roots (42, 67) indicates that when the primary root is injured or impeded by mechanical stress, compensatory growth of lateral roots occurs. Ethylene also stimulates the development of lateral roots in cereals, possibly by inhibiting growth of the main axis (41), or by causing a redistribution of metabolites between the parts of the root or between the root and shoot (36).

The possibility that the auxin:cytokinin balance regulates adventitious and lateral root formation has been discussed earlier. Auxins generally promote root formation, and while low levels of cytokinin are apparently required for initiation of meristematic activity, high levels are generally inhibitory (52, 66, 204). The inverse situation obtains in the shoot, where auxins inhibit and cytokinins promote lateral shoot growth (220). Transport studies have shown that IAA moving from the shoot into the root accumulates at adventitious and lateral root primordia (10, 141). Cytokinins applied to the root tip move through the root and into the shoot (142, 165). Although increased accumulation of cytokinin was not observed in lateral buds released from dominance (165), the reduction in auxin due to decapitation would lower the auxin:cytokinin ratio without any change in cytokinin levels. The precise regulation of apical dominance is still not clear, and ethylene, GA, and ABA may also be involved in both the shoot and root (24, 160, 234). The point to be made here (which has certainly been emphasized before, c.f. 107, 182) is that localization of the sites of hormone synthesis in certain organs and the subsequent transport to other tissues offers the plant a mechanism for precise correlation of root and shoot growth.

From this point of view, the major symptoms of the flooding syndrome, epinasty, reduced shoot growth, and adventitious rooting, can be seen as perturbations of the normal correlative control mechanisms of the plant. Epinasty may be considered as an apical dominance phenomenon (153, 160). Adventitious rooting has been discussed in the present work as being under correlative control. Ethylene is involved in both of these processes, possibly by influencing the distribution of the correlative signals. The rapid synthesis of ethylene in the shoot following root stress may retard shoot growth until the stress is relieved, or until a new root system is formed to balance the existing shoot. Flooding or oxygen deprivation of the root has the effect of shifting the root-shoot junction farther up the stem to a region of more favorable aeration, where a new equilibrium between root and shoot is established. This is a compensatory mechanism within the whole plant similar to that found within root systems, where injury to one part causes increased growth in another part (42, 67). In this light, the "fundamental control mechanism" referred to in the introduction is not a single hormone, but rather the complex interactions which together maintain the polarity of organ formation within the plant. Ethylene produced in response to stress can perhaps be viewed as an "early warning" which, through direct and indirect effects on morphological, physiological, and hormonal events, shifts this polar balance to a new equilibrium position. This "coordinating" function of ethylene may be compared to the hypothesis that the gas serves a similar role in the control of senscence of various plant parts (1, 106).

Gill (62), in discussing adventitious root formation in woody plants, has remarked, "It is interesting and, from the point of view of natural selection, slightly perplexing that many of the herbaceous species shown to generate or to already possess these primordia have no apparent ecological connection with flooding. Much of the research on flooding response has been conducted using crop plants such as tomato (_Lycopersicon esculentum_) and sunflower (_Helianthus annuus_) which are normally flooded only by mishap, and even then only for short periods." Although these plants are grown agriculturally now, they of course evolved under natural conditions where flooding may have been a frequent occurrence, as on flood plains. Whereas trees are generally more tolerant of flooded conditions and can replace in subsequent years any loss of growth, annual plants have only one season in which to achieve maturity and produce seed. There would obviously be great selective advantage in a mechanism which provided for rapid replacement of an injured root system and restored shoot growth. Plants which show the most rapid response to flooding are least injured, while plants with slower responses may die before recovery can begin (110). If a plant evolved under fluctuating soil water conditions, selective pressures would have favored those which could rapidly readjust their root-shoot relationships to meet the changing environment. The present analysis suggests that the basic ability to respond to root stress already exists in plants in the form of correlative controls. The existence in herbaceous species of mechanisms for survival under flooded conditions is therefore not surprising, but rather another example of the remarkable ability of plants to adapt to their environment.

SUMMARY

A series of experiments was performed which distinguished between extant hypotheses concerning the source(s) of elevated ethylene levels found in waterlogged plants. Anaerobiosis of the root zone, without soil ethylene accumulation or blockage of gas diffusion from the root, was sufficient to cause increased ethylene synthesis by the shoots of tomato plants. Epinasty, adventitious roots, chlorosis, and reduced growth were also observed in plants with anaerobic roots. The effect was most pronounced in plants grown under low light intensity. Ethylene pretreatment or high light intensity during growth apparently attenuate the response to root anaerobiosis.

A review of the pertinent literature suggests that perturbation of hormonal control mechanisms is the probable cause of the symptoms of flooding injury. It is proposed that many of the responses can be viewed as alterations of the correlative control process in both roots and shoots. Ethylene may serve a coordinating role in shifting the root-shoot balance to a new equilibrium position.

LITERATURE CITED

LITERATURE CITED

1. Abeles FB 1973 Ethylene in Plant Biology. Academic Press, New York

2. Altman A, R Mozes 1977 Comparative basipetal transport of 6-benzylaminopurine-8-^{14}C, gibberellin A3-^{3}H, IAA-2-^{14}C, and sucrose-^{14}C in the root of intact *Citrus amantium* seedlings. Physiol Plant 39: 233-235

3. Apelbaum A, SP Burg 1971 Altered cell microfibrillar orientation in ethylene-treated *Pisum sativum* stems. Plant Physiol 48: 648-652

4. Apelbaum A, SP Burg 1972 Effects of ethylene and 2,4-Dichlorophenoxyacetic acid on cellular expansion in *Pisum sativum*. Plant Physiol 50: 125-131

5. Apelbaum A, SP Burg 1972 Effect of ethylene on cell division and deoxyribonucleic acid synthesis in *Pisum sativum*. Plant Physiol 50: 117-124

6. Apelbaum A, E Sfakiotakis, DR Dilley 1974 Reduction in extractable deoxyribonucleic acid polymerase activity in *Pisum sativum* seedlings by ethylene. Plant Physiol 54: 125-128

7. Arnon DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1-15

8. Bachelard EP, BB Stowe 1963 Rooting of cuttings of *Acer rubrum* L. and *Eucalyptus camaldulensis* Dehn. Aust J Biol Sci 16: 751

9. Barber DA, M Ebert, NTS Evans 1962 The movement of ^{15}O through barley and rice plants. J Exp Bot 13: 397-403

10. Batra MW, KL Edwards, TK Scott 1975 Auxin transport in roots: its characteristics and relationship to growth. *In* JG Torrey, DT Clarkson, eds, The Development and Function of Roots, Academic Press, New York pp 299-325

11. Ben-Yehoshua S, B Aloni 1974 Effect of water stress on ethylene production by detached leaves of Valencia orange (*Citrus sinensis* Osbeck). Plant Physiol 53: 863-865

12. Bergman HF 1959 Oxygen deficiency as a cause of disease in plants. Bot Rev 25: 417-485

13. Beyer EM Jr 1972 Auxin transport: a new synthetic inhibitor. Plant Physiol 50: 322-327

14. Beyer EM Jr 1976 Silver ion: a potent antiethylene agent in cucumber and tomato. HortScience 11: 195-196

15. Beyer EM Jr 1976 A potent inhibitor of ethylene action in plants. Plant Physiol 58: 268-271

16. Beyer EM Jr, PW Morgan 1970 A method for determining the concentration of ethylene in the gas phase of vegetative plant tissues. Plant Physiol 46: 352-354

17. Beyer EM Jr, PW Morgan 1971 Abscission: the role of ethylene modification of auxin transport. Plant Physiol 48: 208-212

18. Bolton EF, AE Erickson 1970 Ethanol concentration in tomato plants during soil flooding. Agron J 62: 220-224

19. Borthwick HA, KC Hammer, MW Parker 1937 Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. Bot Gaz 98: 491-519

20. Brenner N, E Cieplinski 1958 Gas chromatographic analysis of mixtures containing oxygen, nitrogen, and carbon dioxide. Ann NY Acad Sci 72: 705-713

21. Bryant AE 1934 Comparison of anatomical and histological differences between roots of barley grown in aerated and non-aerated culture solutions. Plant Physiol 9: 389-391

22. Burg SP 1968 Ethylene, plant senescence, and abscission. Plant Physiol 43: 1503-1511

23. Burg SP, EA Burg 1967 Inhibition of polar auxin transport by ethylene. Plant Physiol 42: 1224-1228

24. Burg SP, EA Burg 1968 Ethylene formation in pea seedlings: its relation to the inhibition of bud growth caused by indoleacetic acid. Plant Physiol 43: 1069-1074

25. Burg SP, EA Burg 1968 Auxin stimulated ethylene formation: its relationship to auxin inhibited growth, root geotropism, and other plant processes. In F Wightman, G Setterfield, eds, Biochemistry and Physiology of Plant Growth Substances, Runge Press Ltd, Ottowa, Canada pp 1275-1294

26. Burrows WJ, DJ Carr 1969 Effects of flooding the root system of sunflower plants on the cytokinin content in the xylem sap. Physiol Plant 22: 1105-1112

27. Cannon WA 1925 The physiological features of roots, with special reference to the relation of roots to aeration of the soil. Carnegie Inst Wash Publ 368

28. Cannon WA 1932 Absorption of oxygen by roots when the shoot is in darkness or in light. Plant Physiol 7: 673-684

29. Cannon WA, EE Free 1920 Root adaptation to deficient soil aeration. Carnegie Inst Wash Year Book 19: 62-63

30. Carr DJ, DM Reid 1968 The physiological singificance of the synthesis of hormones in roots and of their export to the shoot system. *In* F Wightman, G Setterfield, eds, Biochemistry and Physiology of Plant Growth Substances, Runge Press Ltd, Ottowa, Canada pp 1169-1185

31. Carr DJ, DM Reid, KGM Skene 1965 The supply of gibberellins from the root to the shoot. Planta 63: 382-92

32. Catlin PB, GC Martin, EA Olsson 1977 Differential sensitivity of *Juglans hindsii*, *J regia*, Paradox hybrid, and *Pterocarya stenoptera* to waterlogging. J Amer Soc Hort Sci 102: 101-104

33. Chadwick AV, SP Burg 1967 An explanation of the inhibition of root growth caused by indole-3-acetic acid. Plant Physiol 42: 415-420

34. Clements FE 1921 Aeration and air content: the role of oxygen in root activity. Carnegie Inst Wash Publ 315

35. Coleman WK, RI Greyson 1977 Analysis of root formation in leaf discs of *Lycopersicon esculentum* Mill. cultured *in vitro*. Ann Bot 41: 307-320

36. Cornforth IS, RJ Stevens 1973 Ethylene and the germination and early growth of barley. Plant and Soil 38: 581-587

37. Crawford RMM 1967 Alcohol dehydrogenase activity in relation to flooding tolerance in roots. J Exp Bot 18: 458-464

38. Crawford RMM, M McManmon 1968 Inductive responses of alcohol and malic dehydrogenases in relation to flooding tolerance on roots. J Exp Bot 19: 435-441

39. Crawford RMM, PO Tyler 1969 Organic acid metobolism in relation to flooding tolerance in roots. J Ecol 57: 235-244

40. Crocker W, PW Zimmerman, AE Hitchcock 1932 Ethylene-induced epinasty of leaves and the relation of gravity to it. Contrib Boyce Thompson Inst Plant Res 4: 177-218

41. Crossett RN, DJ Campbell 1975 The effects of ethylene in the root environment upon the development of barley. Plant and Soil 42: 453-464

42. Crossett RN, DJ Campbell, HE Stewart 1975 Compensatory growth in cereal root systems. Plant and Soil 42: 673-683

43. Curtis OF 1929 Studies on solute translocation in plants. Experiments indicating that translocation is dependent on the action of living cells. Amer J Bot 16: 154-168

44. Dowdell RJ, KA Smith, R Crees, SWF Restall 1972 Field studies of ethylene in the soil atmosphere. Equipment and preliminary results. Soil Biol Biochem 4: 325-331

45. Dubinina IM 1961 Metabolism of roots under various levels of aeration. Fiziol Rast (Soviet Plant Physiol) 8: 314-322

46. Eckerson SH 1931 Influence of phosphorous deficiency on metabolism of the tomato (Lycopersicon esculentum). Contrib Boyce Thompson Inst Plant Res 3: 197

47. Eisinger W 1977 Role of cytokinins in carnation flower senescence. Plant Physiol 59: 707-709

48. El-Beltagy AS, MA Hall 1974 Effect of water stress upon endogenous ethylene levels in Vicia faba. New Phytol 73: 47-60

49. Erickson AE 1965 Short term oxygen deficiencies and plant responses. Proc Conf Drainage for Efficient Crop Production, Amer Soc Agr Eng, St Joseph, Mi pp 11-12

50. Erickson AE, DM VanDoren 1960 The relation of plant growth and yield to soil oxygen availability. Proc 7th Int Cong Soil Sci 3: 428-434

51. Erickson, LC 1946 Growth of tomato roots as influenced by oxygen in the nutrient solution. Amer J Bot 33: 551-561

52. Eriksen EN 1974 Root formation in pea cuttings. III. The influence of cytokinin at different developmental stages. Physiol Plant 30: 163-167

53. Fletcher RA, S Zalik 1964 Effect of light quality on growth and free indoleacetic acid content in Phaseolus vulgaris. Plant Physiol 39: 328-331

54. Fuchs Y, M Lieberman 1968 Effects of kinetin, IAA, and gibberellin on ethylene production, and their interactions in growth of seedlings. Plant Physiol 43: 2029-2036

55. Fulton JM, AE Erickson 1964 Relation between soil aeration and ethyl alcohol accumulation in xylem exudate of tomatoes. Proc Soil Sci Soc Am 28: 610-614

56. Fulton JM, AE Erickson, NE Tolbert 1964 Distribution of C^{14} among metabolites of flooded and aerobically grown tomato plants. Agron J 56: 527-529

57. Geisler G 1965 The morphogenetic effect of oxygen on roots. Plant Physiol 40: 85-88

58. Gentile IA, A Matta 1975 Production of and some effects of ethylene in relation to Fusarium wilt of tomato. Physiol Plant Path 5: 27-37

59. Gertman E, Y Fuchs 1972 Effect of abscisic acid and its interactions with other plant hormones on ethylene production in two plant systems. Plant Physiol 50: 194-195

60. Gilbert SG, JW Shive 1942 The significance of oxygen in nutrient substrates for plants I. The oxygen requirement. Soil Sci 53: 143-152

61. Gilbert SG, JW Shive 1945 The importance of oxygen in the nutrient substrate for plants. Relation of the nitrate ion to respiration. Soil Sci 59: 453-460

62. Gill CJ 1975 The ecological significance of adventitious rooting as a response to flooding in woody species, with special reference to Alnus glutinosa (L.) Gaertn. Flora 164: 85-97

63. Glinka Z 1977 Effects of abscisic acid and of hydrostatic pressure gradient on water movement through excised sunflower roots. Plant Physiol 59: 933-935

64. Goeschl JD, HK Pratt 1968 Regulatory roles of ethylene in the etiolated growth habit of Pisum sativum. In F Wightman, G Setterfield, eds, Biochemistry and Physiology of Plant Growth Substances, Runge Press Ltd, Ottowa, Canada pp 1229-1242

65. Goeschl JD, L Rappaport, HK Pratt 1966 Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. Plant Physiol 41: 877-885

66. Goldacre PL 1959 Potentiation of lateral root induction by root initials in isolated flax roots. Aust J Biol Sci 12: 388-394

67. Goss MJ 1977 Effects of mechanical impedance on root growth in barley (Hordeum vulgare L.) I. Effects on elongation and branching of seminal root axes. J Exp Bot 28: 96-111

68. Grable AR 1966 Soil aeration and plant growth. Advances in Agron 18: 57-106

69. Greenwood DJ, D Goodman 1967 Direct measurement of the distribution of oxygen in soil aggregates and in columns of fine soil crumbs. J Soil Sci 18: 182-196

70. Greenwood DJ, D Goodman 1971 Studies on the supply of oxygen to the roots of mustard seedlings. New Phytol 70: 85-96

71. Hackett C 1971 Relations between the dimensions of the barley root system: effects of mutilating the axes. Aust J Biol Sci 24: 1057-1064

72. Hageman RH, D Flesher 1960 The effect of an anaerobic environment on the activity of alcohol dehydrogenase and other enzymes of corn seedlings. Arch Biochem Biophys 87: 203-209

73. Hansen J 1976 Adventitious root formation induced by gibberellic acid and regulated by the irradiance to the stock plants. Physiol Plant 36: 77-81

74. Hansen J, EN Eriksen 1974 Root formation of pea cuttings in relation to the irradiance of the stock plants. Physiol Plant 32: 170-173

75. Hartung W, S Witt 1968 Über den Einfluss der Bodenfeuchtigkeit auf den Wuchsstoffgehalt von *Anastatica hierochuntica* und *Helianthus annuus*. Flors Abt B 157: 603-614

76. Healy MT, W Armstrong 1972 The effectiveness of internal oxygen transport in a mesophyte (*Pisum sativum* L.). Planta 103: 302-309

77. Hess CE 1969 Internal and external factors regulating root initiation. *In* WJ Whittington, ed, Root Growth, Plenum Press, New York pp 42-53

78. Hislop EC, GV Hoad, SA Archer 1973 The involvement of ethylene in plant diseases. *In* RJW Byrde, CV Cutting, eds, Proc 3rd Long Ashton Symp pp 87-117

79. Hoagland DR, DI Arnon 1950 The water-culture method for growing plants without soil. Calif Agr Exp Sta Circ 347: 1-32

80. Hopkins HT, AW Spect, SB Hendricks 1950 Growth and nutrient accumulation as conrolled by oxygen supply to plant roots. Plant Physiol 25: 193-209

81. Horsley SB, B Wilson 1971 The development of the woody portion of the root system of *Betula papyrifera*. Amer J Bot 58: 141-147

82. Itai C, A Richmond, Y Vaadia 1968 The role of root cytokinins during water and salinity stress. Israel J Bot 17: 187-195

83. Itai C, Y Vaadia 1965 Kinetin-like activity in root exudate of water-stressed sunflower plants. Physiol Plant 18: 941-944

84. Itai C, Y Vaadia 1971 Cytokinin activity in water-stressed shoots. Plant Physiol 47: 87-90

85. Jackson MB, DJ Campbell 1974 Ethylene and the epinastic response of tomato plants to waterlogging. Agr Res Council Letcomb Lab Ann Report 1973 pp 23-26

86. Jackson MB, DJ Campbell 1975 Hormones and the responses of plants to waterlogged soil. Agr Res Council Letcomb Lab Ann Report 1974 pp 45-48

87. Jackson MB, DJ Campbell 1975 Movement of ethylene from roots to shoots, a factor in the responses of tomato plants to waterlogged soil conditions. New Phytol 74: 397-406

88. Jackson MB, DJ Campbell 1975 Ethylene and waterlogging effects in tomato. Ann Appl Biol 81: 102-105

89. Jackson MB, DJ Campbell 1976 Waterlogging and petiole epinasty in tomato: the role of ethylene and low oxygen. New Phytol 76: 21-29

90. Jackson MB, DJ Campbell 1976 Production of ethylene by excised segments of plant tissue prior to the effect of wounding. Planta 129: 273-274

91. Jackson MB, DJ Osborne 1970 Ethylene, the natural regulator of leaf abscission. Nature 225: 1019 -1022

92. Jackson WT 1955 The role of adventitious roots in recovery of shoots following flooding of the original root system. Amer J Bot 42: 816-819

93. Jackson WT 1956 Flooding injury studied by approach-graft and split root system techniques. Amer J Bot 43: 496-502

94. Jackson WT 1956 The relative importance of factors causing injuries to shoots of flooded tomato plants. Amer J Bot 43: 637-639

95. Jansen H 1967 Die Wirkung von Gibberellinsäure und Indolylessigsäure auf die Wurzelbildung von Tomatenstecklingen. Planta 74: 371-378

96. Jensen CR, LH Stolzy, J Letey 1967 Tracer studies of oxygen diffusion through roots of barley, corn, and rice. Soil Science 103: 23-29

97. Jones RJ, TA Mansfield 1970 Suppression of stomatal opening in leaves treated with abscisic acid. J Exp Bot 21: 714-719

98. Jones RL, IDJ Phillips 1966 Organs of gibberellin synthesis in light-grown sunflower plants. Plant Physiol 41: 1381-1386

99. Kang BG, SP Burg 1974 Ethylene action on lateral auxin transport in tropic responses, leaf epinasty, and horizontal nutation. In Plant Growth Substances 1973, Hirokawa Publishing Co, Inc, Tokyo pp 1090-1094

100. Kang BG, W Newcomb, SP Burg 1971 Mechanism of auxin-induced ethylene production. Plant Physiol 47: 504-509

101. Kawase M 1972 Effect of flooding on ethylene concentration in horticultural plants. J Amer Soc Hort Sci 97: 584-588

102. Kawase M 1974 Role of Ethylene in induction of flooding damage in sunflower. Physiol Plant 31: 29-38

103. Kawase M 1976 Ethylene accumulation in flooded plants. Physiol Plant 36: 236-241

104. Kefford NP 1962 Auxin-gibberellin interaction in rice coleoptile elongation. Plant Physiol 37: 380-386

105. Kende H 1965 Kinetinlike factors in the root exudate of sunflowers. Proc Nat Acad Sci (USA) 53: 1302-1307

106. Kende H, A Hanson 1976 Relationship between ethylene evolution and senescence in morning-glory flower tissue. Plant Physiol 57: 523-527

107. Kende H, D Sitton 1967 The physiological significance of kinetin- and gibberellin-like root hormones. Ann NY Acad Sci 144: 235-243

108. Kondo K, A Watanabe, H Imaseki 1975 Relationships in actions of indoleacetic acid, benzyladenine and abscisic acid in ethylene production. Plant Cell Physiol 16: 1001-1007

109. Kramer PJ 1940 Causes of decreased absorption of water by plants in poorly aerated media. Amer J Bot 27: 216-220

110. Kramer PJ 1951 Causes of injury to plants resulting from flooding of the soil. Plant Physiol 26: 722-736

111. Kramer PJ 1965 Effect of deficient aeration on the roots of plants. Proc Conf on Drainage for Efficient Crop Production, Amer Soc Agr Eng, St Joseph Mi pp 13-14

112. Kramer PJ 1969 Plant and Soil Water Relationships: A Modern Synthesis, McGraw-Hill Book Company, New York 482 p

113. Kramer PJ, WT Jackson 1954 Causes of injury to flooded tobacco plants. Plant Physiol 29: 241-245

114. Kriedemann PE, BR Loveys 1974 Hormonal mediation of plant responses to environmental stress. In RL Bieleski, AR Ferguson, MM Cresswell, eds, Mechanisms of Regulation of Plant Growth, Bulletin 12, The Royal Society of New Zealand, Wellington pp 461-465

115. Lau OL, WW John, SF Yang 1977 Effect of different cytokinins on ethylene production by mung bean hypocotyls in the presence of indole-3-acetic acid or calcium ion. Physiol Plant 39: 1-3

116. Lau OL, SF Yang 1973 Mechanism of a synergistic effect of kinetin on auxin-induced ethylene production: suppression of auxin conjugation. Plant Physiol 51: 1011-1014

117. Lau OL, SF Yang 1974 Synergistic effect of calcium and kinetin on ethylene production by the mungbean hypocotyl. Planta 118: 1-6

118. Lau OL, SF Yang, KH Yung 1974 Suppression of IAA conjugation by kinetin in relation to a synergistic effect of kinetin on IAA-induced ethylene production. *In* Plant Growth Substances 1973, Hirokawa Publishing Co, Inc, Tokyo pp 364-373

119. Leather GR, LE Forrence, FB Abeles 1972 Increased ethylene production during clinostat experiments may cause leaf epinasty. Plant Physiol 49: 183-186

120. Lee TT 1977 Role of phenolic inhibitors in peroxidase-mediated degradation of indole-3-acetic acid. Plant Physiol 59: 372-375

121. Leopold AC, PE Kriedemann 1975 Plant Growth and Development, 2nd Edition, McGraw-Hill Book Co, New York 545 p

122. Levy D, S Marco 1976 Involvement of ethylene in epinasty of CMV-infected cucumber cotyledons which exhibit increased resistance to gaseous diffusion. Physiol Plant Path 9: 121-126

123. Livingston BE, EE Free 1917 The effect of deficient soil oxygen on the roots of higher plants. Johns Hopkins Univ Circ NS3: 182-185

124. Livne A, Y Vaadia 1972 Water deficits and hormone relations. *In* TT Kozlowski, ed, Water Deficits and Plant Growth vol 3, Academic Press, New York pp 255-276

125. Lovell PH, A Cobb, KG Moore 1971 The control of root initiation and development in detached cotyledons of *Sinapis alba* L. and *Raphanus sativus* L. Ann Bot 35: 501-509

126. Lovell PH, A Illsley, KG Moore 1972 The effects of light intensity and sucrose on root formation, photosynthetic ability, and senescence in detached cotyledons of *Sinapis alba* L. and *Raphanus sativus* L. Ann Bot 36: 123-134

127. Lyon CJ 1963 Auxin transport in leaf epinasty. Plant Physiol 38: 567-574

128. Lyon CJ 1970 Ethylene inhibition of auxin transport by gravity in leaves. Plant Physiol 45: 644-646

129. Marynick DS, MC Marynick 1975 A mathematical treatment of rate data obtained in biological flow systems under nonsteady state conditions. Plant Physiol 56: 680-683

130. Mayak S, DR Dilley 1976 Rgulation of senescence in carnation (*Dianthus caryophyllus*). Effect of abscisic acid and carbon dioxide on ethylene production. Plant Physiol 58: 663-665

131. Mayak S, DR Dilley 1976 Effect of sucrose on response of cut carnation to kinetin, ethylene, and abscisic acid. J Amer Soc Hort Sci 101: 583-585

132. Mayak S, AH Halevy 1972 Interrelationships of ethylene and abscisic acid in the control of rose petal senescence. Plant Physiol 50: 341-346

133. Mayak S, AM Kofranek 1976 Altering the sensitivity of carnation flowers (*Dianthus caryophyllus* L.) to ethylene. J Amer Soc Hort Sci 101: 503-506

134. McMichael BL, WR Jordan, RD Powell 1972 An effect of water stress on ethylene production by intact cotton petioles. Plant Physiol 49: 658-660

135. McPherson DC 1939 Cortical air spaces in the roots of *Zea mays*. New Phytol 38: 190-202

136. Meijer G 1957 The influence of light quality on the flowering response of *Salvia occidentalis*. Acta Bot Neerl 6: 395

137. Mizrahi Y, A Blumenfeld, AE Richmond 1972 The role of abscisic acid and salination in the adaptive response of plants to reduced root aeration. Plant Cell Physiol 13: 15-21

138. Moe R 1977 Effect of light, temperature and CO_2 on the growth of *Campanula isophylla* stock plants and on the subsequent growth and development of their cuttings. Scientia Horticulturae 6: 129-141

139. Morgan PW, E Beyer Jr, HW Gausman 1968 Ethylene effects on auxin physiology. *In* F Wightman, G Setterfield, eds, Biochemistry and Physiology of Plant Growth Substances, Runge Press Ltd, Ottowa, Canada pp 1255-1273

140. Morgan PW, WC Hall 1964 Accelerated release of ethylene by cotton following application of indoyl-3-acetic acid. Nature 201: 99

141. Morris DA, RE Briant, PG Thompson 1969 The transport and metabolism of ^{14}C-labelled indoleacetic acid in intact pea seedlings. Planta 89: 178-197

142. Mozes R, A Altman 1977 Characteristics of root-to-shoot transport of cytokinin 6-benzylaminopurine in intact seedlings of *Citrus aurantium*. Physiol Plant 39: 225-232

143. Mullins MG 1970 Hormone-directed transport of assimilates in decapitated internodes of *Phaseolus vulgaris* L. Ann Bot 34: 897-909

144. Mullins MG 1970 Auxin and ethylene in adventitious root formation in *Phaseolus aureus* (Roxb.). *In* DJ Carr, ed, Plant Growth Substances 1970, Springer-Verlag, New York pp 526-533

145. Musgrave A, J Walters 1973 Ethylene-stimulated growth and auxin transport in *Ranunculus sceleratus* petioles. New Phytologist 72: 783-789

146. Nanda KK, MK Jain, S Malhotra 1971 Effect of glucose and auxins in rooting etiolated stem segments of *Populus nigra*. Physiol Plant 24: 387-391

147. Naqvi SM, SA Gordon 1967 Auxin transport in *Zea mays* coleoptiles. II. Influence of light on the transport of indole-acetic acid-2-^{14}C. Plant Physiol 42: 138-143

148. Obroucheva NV 1975 Physiology of growing roots. *In* JG Torrey, DT Clarkson, eds, The Development and Function of Roots, Academic Press, New York pp 279-298

149. Osborne DJ 1977 Ethylene and target cells in the growth of plants. Science Progress 64: 51-63

150. Palmer JH 1972 Roles of ethylene and indol-3yl-acetic acid in petiole epinasty in *Helianthus annuus* and the modifying influence of gibberellic acid. J Exp Bot 23: 733-743

151. Palmer JH 1973 Ethylene as a cause of transient petiole epinasty in *Helianthus annuus* during clinostat experiments. Physiol Plant 28: 188-193

152. Palmer JH 1976 Failure of ethylene to change the distribution of indoleacetic acid in the petiole of *Coleus blumei* x *frederici* during epinasty. Plant Physiol 58: 513-515

153. Palmer JH, IDJ Phillips 1963 The effect of the terminal bud, indoleacetic acid, and nitrogen supply on the growth and orientation of the petiole of *Helianthus annuus*. Physiol Plant 16: 572-584

154. Patrick TW, R Hall, RA Fletcher 1977 Cytokinin levels in healthy and *Verticillium*-infected tomato plants. Can J Bot 55: 377-382

155. Pegg GF 1976 Response of ethylene-treated tomato plants to infection by *Verticillium albo-atrum*. Physiol Plant Path 9: 215-226

156. Pegg GF, DK Cronshaw 1976 Ethylene production in tomato plants infected with *Verticillium albo-atrum*. Physiol Plant Path 8: 279-295

157. Phillips IDJ 1964 Root-shoot hormone relations. I. The importance of an aerated root system in the regulation of growth hormone levels in the shoot of *Helianthus annuus*. Ann Bot 28: 17-35

158. Phillips IDJ 1964 Root-shoot hormone relations. II. Changes in endogenous auxin concentration produced by flooding of the root system in Helianthus annuus. Ann Bot 28: 37-45

159. Phillips IDJ 1969 Apical dominance. *In* MB Wilkins, ed, Physiology of Plant Growth and Development, McGraw-Hill, London, pp 165-202

160. Phillips IDJ 1975 Apical dominance. Ann Rev Plt Physiol 26: 341-367

161. Phillips IDJ, RL Jones 1964 Gibberellin-like activity in bleeding sap of root systems of *Helianthus annuus* detected by a new dwarf pea epicotyl assay and other methods. Planta 63: 269-278

162. Pierik RLM, HHM Steegmans 1975 Analysis of adventitious root formation in isolated stem explants of *Rhododendron*. Scientia Horticulturae 3: 1-20

163. Pilet PE 1964 Effect of chlorogenic acid on the auxin catabolism and the auxin content of root tissue. Phytochem 3: 617-621

164. Ponnamperuma FN 1972 The chemistry of submerged soils. Advances in Agron 24: 29-96

165. Procházka S, H Schraudolf, J Sonka 1977 Transport of ^{14}C-kinetin in intact and decapitated pea seedlings (*Pisum sativum* L.). Z. Pflanzenphysiol 81: 308-313

166. Railton ID, DM Reid 1973 Effects of benzyladenine on the growth of waterlogged tomato plants. Planta 111: 261-266

167. Rasmussen GK, JR Furr, WC Cooper 1969 Ethylene production by citrus leaves from trees grown in artificially salinized plots. J Amer Soc Hort Sci 94: 640-641

168. Reid DM, A Crozier 1971 Effects of waterlogging on the gibberellin content and growth of tomato plants. J Exp Bot 22: 39-48

169. Reid DM, A Crozier, BMR Harvey 1969 The effects of flooding on the export of gibberellins from the root to the shoot. Planta 89: 376-379

170. Reid DM, ID Railton 1974 Effect of flooding on the growth of tomato plants: involvement of cytokinins and gibberellins. *In* RL Bieliski, AR Ferguson, MM Cresswell, eds, Bulletin 12, the Royal Society of New Zealand, Wellington, pp 789-792

171. Reid MA 1924 Relation of kind of food reserves to regeneration in tomato plants. *Bot Gaz* 77: 103-110

172. Richmond A, A Lang 1957 Effects of kinetin on protein content and survival of detached *Xanthium* leaves. Science 125: 650-651

173. Romell LG 1923 Soil Aeration. Int Inst Agr Bull (Int Rev of the Science and Practice of Agr) 1: 281-297

174. Rudich J, AH Halevy, N Kedar 1972 The level of phytohormones in monoecious and gynoecious cucumbers as affected by photoperiod and ethephon. Plant Physiol 50: 585-590

175. Schramm RJ 1960 Anatomical and physiological development of roots in relation to aeration of the substrate. Diss Abstr 21: 2089

176. Selman IW, S Sandanam 1972 Growth responses of tomato plants in non-aerated water culture to foliar sprays of gibberellic acid and benzyladenine. Ann Bot 36: 837-848

177. Seth AK, PF Wareing 1964 Interaction between auxins, gibberellins and kinins in hormone-directed transport. Life Sci 3: 1483-1486

178. Seth A, PF Wareing 1967 Hormone-directed transport of metabolites and its possible role in plant senescence. J Exp Bot 18: 65-77

179. Sheard RW, AJ Leyshon 1976 Short-term flooding of soil: its effect on the composition of gas and water phases of soil and on phosphorous uptake of corn. Can J Soil Sci 56: 9-20

180. Shive JW, WR Robbins 1937 Methods of growing plants in solution and sand cultures. NJ Agr Exp Sta Bull 636

181. Short KC, JG Torrey 1972 Cytokinins in seedling roots of pea. Plant Physiol 49: 155-160

182. Skene KG 1975 Cytokinin production by roots as a factor in the control of plant growth. In JG Torrey, DT Clarkson, eds, The Development and Function of Roots, Academic Press, New York pp 365-396

183. Skoog F, CO Miller 1957 Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp Soc Exptl Biol 11: 118-131

184. Smith AM 1973 Ethylene as a cause of fungistasis. Nature 246: 311-313

185. Smith AM 1976 Ethylene in soil biology. Ann Rev Phytopath 14: 53-73

186. Smith AM 1976 Ethylene production by bacteria in reduced microsites in soil and some implications to agriculture. Soil Biol Biochem 8: 293-298

187. Smith AM, RJ Cook 1974 Implications of ethylene production by bacteria for biological balance of soil. Nature 252: 703-705

188. Smith KA, RJ Dowdell 1974 Field studies of the soil ethylene. I. Relationships between ethylene, oxygen, soil moisture content, and temperature. J Soil Sci 25: 217-230

189. Smith KA, RJ Dowdell, KC Hall, R Crees 1974 Effect of cultivation on the content of oxygen and ethylene in soil. Agr Res Council Letcombe Lab Ann Report 1973, pp 35-36

190. Smith KA, MB Jackson 1974 Ethylene, waterlogging and plant growth Agr Res Council Letcombe Lab Ann Report 1973, pp 60-75

191. Smith KA, SWF Restall 1971 The occurrence of ethylene in anaerobic soil. J Soil Sci 22: 430-443

192. Smith KA, PD Robertson 1971 Effect of ethylene on root extension in cereals. Nature 234:148-149

193. Smith KA, RS Russel 1969 Occurrence of ethylene, and its significance, in anaerobic soil. Nature 222: 769-771

194. Soldatenkov SV, HT Chao 1962 The role of bean and corn leaves in respiration of oxygen deprived roots. Fiziol Rast (Soviet Plant Physiol) 8: 307-313

195. Solomos T, GC Laties 1975 The mechanism of ethylene and cyanide action in triggering the rise in respiration in potato tubers. Plant Physiol 55: 73-78

196. Stewart ER, HT Freebairn 1969 Ethylene, seed germination, and epinasty. Plant Physiol 44: 955-958

197. Street HE 1966 The physiology of root growth. Ann Rev Plant Physiol 17: 315-344

198. Tal M, D Imber 1970 Abnormal stomatal behavior and hormonal imbalance in <u>flacca</u>, a wilty mutant of tomato. II. Auxin and abscisic acid-like activity. Plant Physiol 46: 373-376

199. Tal M, D Imber 1971 Abnormal stomatal behavior and hormonal imbalance in <u>flacca</u>, a wilty mutant of tomato. III. Hormonal effects on the water status in the plant. Plant Physiol 47: 849-850

200. Tal M, D Imber, C Itai 1970 Abnormal stomatal behavior and hormonal imbalance in <u>flacca</u>, a wilty mutant of tomato. I. Root effect and kinetin-like activity. Plant Physiol 46: 367-372

201. Taylor DL 1942 Influence of oxygen tension on respiration, fermentation, and growth in wheat and rice. Amer J Bot 29: 721-738

202. Thimann KV 1936 Auxins and the growth of roots. Amer J Bot 23: 561-569

203. Torrey JG 1950 The induction of lateral roots by indoleacetic acid and root decapitation. Amer J Bot 37: 257-265

204. Torrey JG 1962 Auxin and purine interactions in lateral root initiation in isolated pea root segments. Physiol Plant 15: 177-85

205. Torrey JG 1976 Root hormones and plant growth. Ann Rev Plant Physiol 27: 435-484

206. Tucker DJ 1976 Endogenous growth regulators in relation to side shoot development in the tomato. New Phytol 77: 561-568

207. Tucker DJ 1977 Apical dominance in the "rogue" tomato. Ann Bot 41: 181-190

208. Tucker DJ 1977 The effects of far-red light on lateral bud outgrowth in decapitated tomato plants and the associated changes in the levels of auxin and abscisic acid. Plant Sci Letters 8: 339-344

209. Turkova NS 1944 Growth reactions in plants under excessive watering. Compt Rend (Doklady) Acad Sci USSR 42: 87-90

210. Vaadia Y, C Itai 1969 Interrelationships of growth with reference to the distribution of growth substances. In WJ Whittington, ed, Root Growth, Plenum Press, New York pp 65-79

211. Valdovinos JG, LC Ernest, EW Henry 1967 Effects of ethylene and gibberellic acid on auxin synthesis in plant tissue. Plant Physiol 42: 1803-1806

212. Van Overbeek J, SA Gordon, LE Gregory 1946 An analysis of the function of the leaf in thè process of root formation in cuttings. Amer J Bot 33: 100-107

213. Vlamis J, AR Davis 1944 Effects of oxygen tension on certain physiological responses of rice, barley, and tomato. Plant Physiol 19: 33-50

214. Walton DC, E Galson, MA Harrison 1977 The relationship between stomatal resistance and abscisic acid levels in leaves of water-stressed bean plants. Planta 133: 145-148

215. Wareing PF, J Good, J Manuel 1968 Some possible physiological roles of abscisic acid. In F Wightman, G Setterfield, eds, Biochemistry and Physiology of Plant Growth Substances, Runge Press Ltd, Ottowa, Canada pp 1561-1579

216. Weiss C, Y Vaadia 1965 Kinetin-like activity in root apices of sunflower plants. Life Sci 4: 1323-1326

217. Wellman FL 1941 Epinasty of tomato, one of the earliest symptoms of Fusarium wilt. Phytopath 31: 281-283

218. Went FW 1943 Effect of the root system on tomato stem growth. Plant Physiol 18: 51-65

219. Went FW, KV Thimann 1937 Phytohormones, The MacMillan Publishing Co, New York 294 p

220. Wickson M, KV Thimann 1958 The antagonism of auxin and kinetin in apical dominance. Physiol Plant 11: 62-74

221. Wignarajah K, H Greenway 1976 Effect of anaerobiosis on activities of alcohol dehydrogenase and pyruvate decarboxylase in roots of Zea mays. New Phytol 77: 575-584

222. Wright STC 1976 The relationship between leaf water potential (Ψ_{leaf}) and the levels of ABA and ethylene in excised wheat leaves. In PE Pilet, ed, 9th Int Conf on Plant Growth Substances, Lausanne pp 436-438

223. Wright STC, RWP Hiron 1970 The accumulation of abscisic acid in plants during wilting and other stress conditions. In DJ Carr, ed, Plant Growth Substances 1970, Springer-Verlag, New York pp 291-298

224. Yamada N 1954 Auxin relationships of the rice coleoptile. Plant Physiol 29: 92

225. Yang SF 1969 Ethylene evolution from 2-chloroethylphosphonic acid. Plant Physiol 44: 1203-1204

226. Yang SF 1974 The biochemistry of ethylene: biogenesis and metabolism. In VC Runeckles, E Sondheimer, DC Walton, eds, Recent Advances in Phytochemistry vol 7, Academic Press, New York pp 131-164

227. Yu PT, LH Stolzy, J Letey 1969 Survival of plants under prolonged flooded conditions. Agron J 61: 844-847

228. Zeroni M, PH Jerie, MA Hall 1977 Studies on the movement and distribution of ethylene in *Vicia faba* L. Planta 134: 119-125

229. Zimmerman PW, AE Hitchcock 1933 Initiation and stimulation of adventitious roots caused by unsaturated hydrocarbon gases. Contrib Boyce Thompson Inst Plant Res 5: 351-369

230. Zimmerman PW, AE Hitchcock 1935 Responses of roots to "root-forming" substances. Contrib Boyce Thompson Inst Plant Res 7: 439-445

231. Zimmerman PW, AE Hitchcock, W Crocker 1931 The effect of ethylene and illuminating gas on roses. Contrib Boyce Thompson Inst Plant Res 3: 459-481

232. Zimmerman PW, F Wilcoxon 1935 Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib Boyce Thompson Inst Plant Res 7: 209-230

233. Zobel RW 1973 Some physiological characteristics of the ethylene-requiring tomato mutant *diageotropica*. Plant Physiol 52: 385-389

234. Zobel RW 1974 Control of morphogenesis in the ethylene-requiring tomato mutant, *diageotropica*. Can J Bot 52: 735-741