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Effects of Root Anaerobiosis on Ethylene Production, Epinasty, and Growth of Tomato Plants[1]

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ABSTRACT

Experiments were performed to determine the source(s) of ethylene-causing epinasty in flooded tomato plants (*Lycopersicon esculentum* Mill.). Simultaneous measurements were made of ethylene synthesized by the roots and shoots of tomato plants exposed to either aerobic or anaerobic atmospheres in the root zone. When the root zone was made anaerobic by a flowing stream of N_2 gas, petiole epinasty and accelerated ethylene synthesis by the shoots were observed. In soil-grown plants, ethylene synthesis by the root-soil complex increased under anaerobic conditions; but when grown in inert media under the same conditions, ethylene synthesis by roots remained constant or declined during the period of rapid epinastic growth by the petioles. Other characteristic symptoms of flooding, *e.g.* reduced growth and chlorosis, were also observed in plants with anaerobic roots. Pretreatment of plants with $AgNO_3$, an inhibitor of ethylene action, completely prevented epinasty, demonstrating that ethylene is the agent responsible for waterlogging symptoms. These results indicate that deprivation of O_2 to the roots is the primary effect of soil flooding, and that this is sufficient to cause increased ethylene synthesis in the shoot. The basis of the observed root-shoot communication is unknown, but root-synthesized hormones or specific ethylene-promoting factors may be involved.

Waterlogging of higher plants can cause petiole epinasty, chlorosis, stem hypertrophy, reduced growth, and adventitious rooting (16). Epinastic growth of tomato petioles commenced within 12 hr following flooding of the soil (12). Exposure to ethylene will also cause petiole epinasty in a variety of plants (5). Recent investigations have revealed that flooding-induced epinasty is due to elevated ethylene concentrations in the shoots (9, 12, 14). Several hypotheses have been proposed suggesting that this excess ethylene may be produced in the soil, the root, or the shoot (11, 12, 15).

Kawase (13) found that submersion increased the ethylene content in a variety of plant tissues. Ethylene content of flooded intact sunflower plants increased first in the submerged portion, then in the aerial parts, and was correlated with the development of epinasty, adventitious rooting, and stem hypertrophy (14). Kawase (15) suggested that blockage of ethylene diffusion from the submerged roots allows the accumulation of ethylene which then moves up the plant, causing visible symptoms of flooding injury. Zeroni *et al.* (27) reported that ethylene may be diverted throughout a plant if escape is blocked by a diffusion barrier.

Following the discovery (26) of relatively high concentrations (>20 $\mu l\ l^{-1}$) of ethylene in anaerobic soils, Jackson and Campbell (8) suggested that microbially synthesized ethylene may enter the plant and cause epinasty. They later showed that concentrations of ethylene in the root zone in excess of 2 $\mu l\ l^{-1}$ were sufficient to cause epinasty (11) and proposed that ethylene diffusing from the soil into the plant under waterlogged conditions may be responsible for the rise in shoot ethylene content and symptom expression.

Using tomato plants grown in nutrient solution, Jackson and Campbell (9, 12) found that low O_2 (less than 3%, v/v) in the root zone caused epinasty and elevated ethylene levels in the shoots. Since no ethylene was evolved from the nutrient solution alone, this suggested that root O_2 deficiency can stimulate shoot ethylene production.

The first two hypotheses for the origin of the ethylene increase (*i.e.* root and soil) are passive models which require a concentration gradient between the root and the shoot to allow diffusion of ethylene up the stem. The plant is essentially gassed from within by root-synthesized ethylene, or from without by microbially synthesized ethylene. The third hypothesis requires some form of root-shoot interaction whereby a root stress stimulates ethylene synthesis in the shoot.

This study tested these hypotheses by using N_2 ventilation of the root zone, rather than flood water, to impose an O_2 deficiency upon the root without blocking gas diffusion. Microbial contribution of ethylene was prevented by using inert growing media. A special apparatus maintained the desired atmosphere in the root zone and allowed simultaneous measurement of root and shoot ethylene synthesis.

MATERIALS AND METHODS

Plant Material. Dwarf tomato plants (*Lycopersicon esculentum* Mill. cv. Tiny Tim) were used for some experiments (Figs. 3, 4, and 5) and a nondwarf cultivar, Chico III, for others (Figs. 2 and 6).

Growing Conditions. Plants were grown in a greenhouse at 18 C minimum with natural lighting between October 1976 and February 1977. Plants were grown in a silty loam soil (pH 6.7), organic matter 5% (w/w), in perlite, or in Turface (BASF Wyandotte Corp.) an inert clay medium. Plants were watered with 0.5 Hoagland solution (7) and were acclimated in the controlled environment room at least 3 days prior to an experiment. A 16-hr photoperiod was used with incandescent and fluorescent lights at an intensity of 1,000 to 1,300, ft-c (4–5 mw cm^{-2}). Temperature was maintained at 25 ± 2 C. Six-week-old plants were generally used, when flower buds were just visible on the dwarf plants.

Data for Figures 2 and 3 were obtained from plants (eight/treatment) grown in 453-ml canning jars with metal lids fitted with rubber serum stoppers and tubing for introducing gases. The stem of a young plant was sealed to the lid with lanolin or with RTV-11 silicone rubber (General Electric) with a nonphytotoxic catalyst (Härter T1, Wacker Chemie Gmbh, Münich). Thus, the roots were isolated from the atmosphere, while the shoots were not enclosed. Ethylene-free air or N_2 gas at a rate of 3 to 4 ml min^{-1} was introduced to the bottom of the root zone through a stainless steel tube and exited through an outlet in the

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lid. Ethylene analyses were made on the effluent gas or by sealing the root chamber for 1 hr and sampling the accumulated ethylene. The rooting media 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.

For other experiments, a special apparatus was constructed to permit simultaneous measurement of ethylene synthesis by the roots and shoot (Fig. 1). The shoot chamber accommodated a 6-week-old dwarf tomato plant without restraint. Twelve plants were used/treatment. The test unit held 30 chambers and provided irrigation through tubes connected to each chamber. 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^{-1} employing capillary flow meters. Since the free air space of the filled tube was approximately 30 ml, this rate supplied about 3 volumes/hr. Assuming complete mixing, this would remove 95% of the gas originally in the root zone after 1 hr (17). Romell (22) calculated from rates of CO_2 production and levels of CO_2 found in soil atmospheres that normal aeration completely exchanges the gas in the top 20 cm of soil once every hr. Thus, the selected flow rate represents a realistic rate of supply of O_2 and removal of CO_2 in the root zone. Ethylene, CO_2, and O_2 were sampled from the effluent gas under steady-state conditions.

The shoot chamber was ventilated at a rate of 45 to 60 ml min^{-1}, sufficient to provide one chamber volume approximately every 10 min. For ethylene sampling the flow gas was stopped and the chamber sealed for 2 hr. Otherwise, the shoot and root chambers were flushed continuously with humidified, ethylene-free air. Treated roots received 99.9% N_2 which was also free of ethylene. Flow gas to the roots was filtered with Ascarite to remove CO_2. This system maintained O_2 levels of less than 0.5% in the root zone of N_2-treated plants and allowed simultaneous measurement of ethylene production by both the roots and shoot of a single plant over the course of several days.

Ethylene, CO_2, and O_2 Determination. Ethylene was determined in 3-ml gas samples taken with gas-tight syringes and injected into a gas chromatograph which used N_2 as the carrier gas and was equipped with a column (2 mm × 1 m) of activated alumina at 60 C and a flame ionization detector. Concentrations of ethylene above 1 nl l^{-1} were detectable. CO_2 and O_2 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. CO_2, O_2, and N_2 were separated on silica gel (6 mm × 0.6 m) and molecular sieve (6 mm × 3 m) columns mounted in parallel (3).

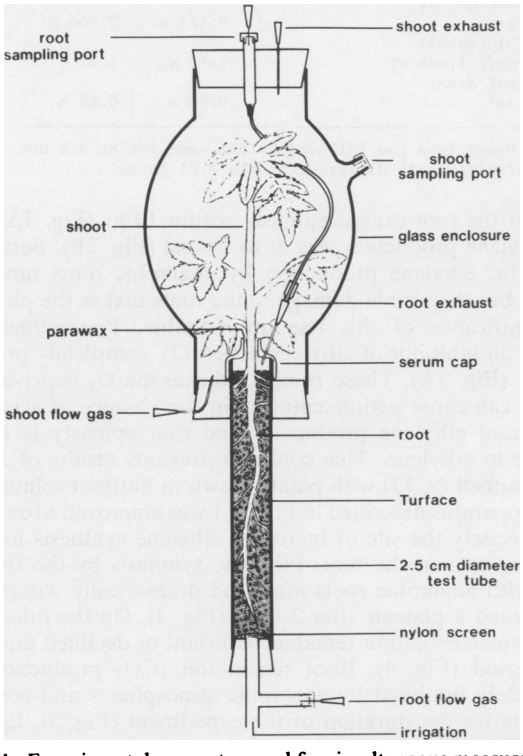

FIG. 1. Experimental apparatus used for simultaneous measurement of ethylene production by roots and shoots.

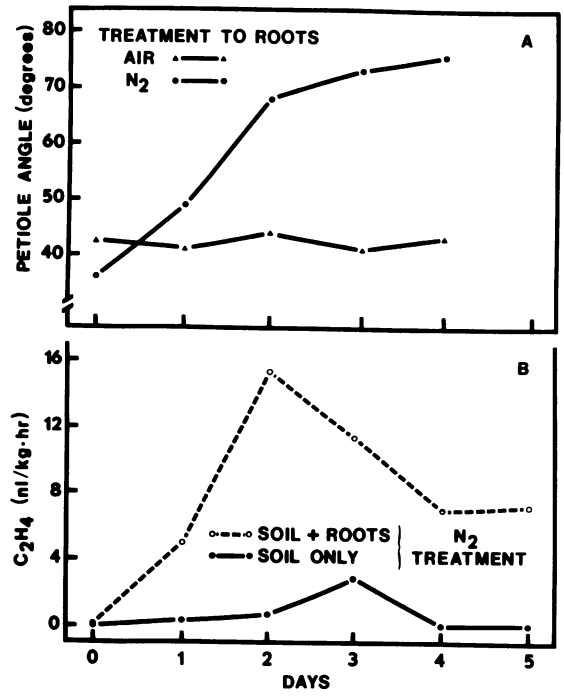

FIG. 2. A: Epinasty of Chico III tomato plants as influenced by O_2 availability to the roots; B: ethylene production by soil and roots or by soil alone under anaerobic conditions.

Epinasty Measurements. Epinasty was measured (with a transparent protractor) as an increase in the angle between the adaxial surface of a petiole and the stem. In one case (Fig. 3), epinasty was measured as the distance moved by a marked spot on the petiole 1.5 cm from the stem.

Chl Determination. Total Chl was determined in 1-cm leaf discs from corresponding leaves on each plant by the method of Arnon (1), using 80% acetone extracts and measuring the *A* at 652 nm.

$AgNO_3$ Application. Plants were treated with solutions of 0.1% Tween 20 ± 500 mg l^{-1} $AgNO_3$. The entire shoot was dipped into the appropriate solution for approximately 10 sec. Treatment of plants with various gases was begun the following day.

RESULTS

Epinastic growth commenced within 1 day and continued for 3 days after O_2 was excluded from the root zone of soil-grown plants (Fig. 2A). Ethylene production by the root-soil complex also increased for the first 2 days and then declined (Fig. 2B). Ethylene evolution from the anaerobic soil was low and showed a slight rise only on the 3rd day (Fig. 2B). No ethylene was detected in the effluent gas of the aerobic treatment. The difference between the "soil + roots" and the "soil only" curves cannot be attributed entirely to root ethylene production due to the rhizosphere effect on soil microorganisms and the ability of such microorganisms to produce ethylene anaerobically (23, 25, 26). Although ethylene evolution from the root zone appears to parallel the development of epinasty, the actual concentration of ethylene in the soil atmosphere never exceeded 0.8 μl l^{-1}. Since Jackson and Campbell

(11) reported that an ethylene concentration of 2 $\mu l\ l^{-1}$ in the root zone is required for significant movement of the gas from roots to shoots, it is unlikely that soil or root ethylene synthesis is the direct cause of epinasty.

The hypothesis that ethylene produced by the root or the rhizosphere is the underlying cause of petiole epinasty was tested by growing plants in Perlite, an inert growing medium. O_2 depri-

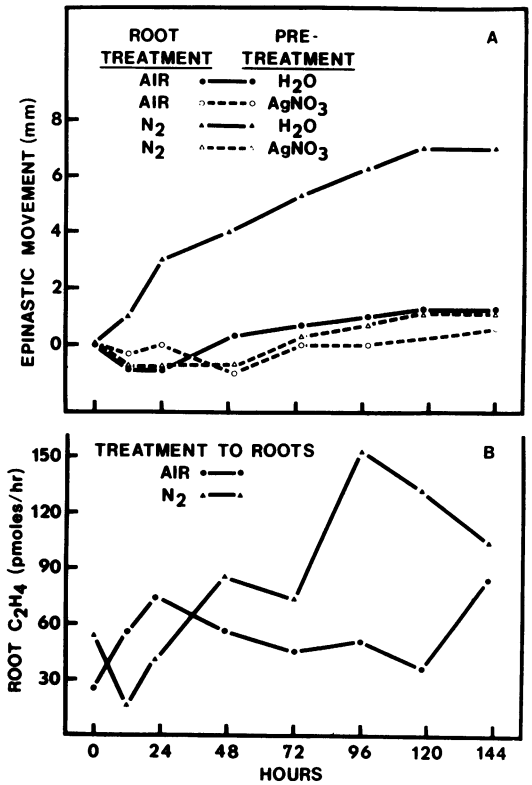

FIG. 3. A: Epinasty of Tiny Tim tomato plants as influenced by aeration of the roots and $AgNO_3$ treatment of the shoot; B: ethylene production by roots under different aeration regimes.

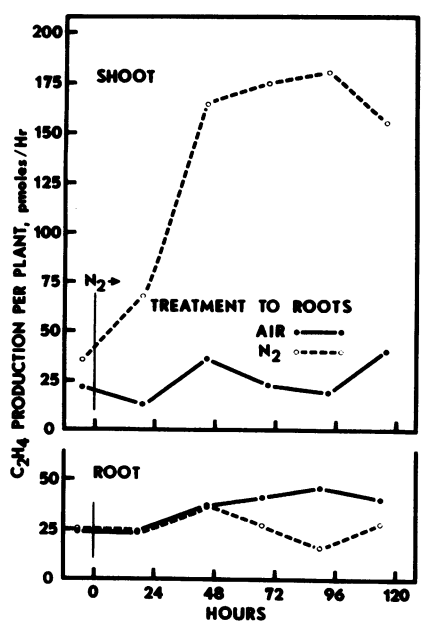

FIG. 4. Shoot and root ethylene production by Tiny Tim tomato plants as influenced by aeration of the roots.

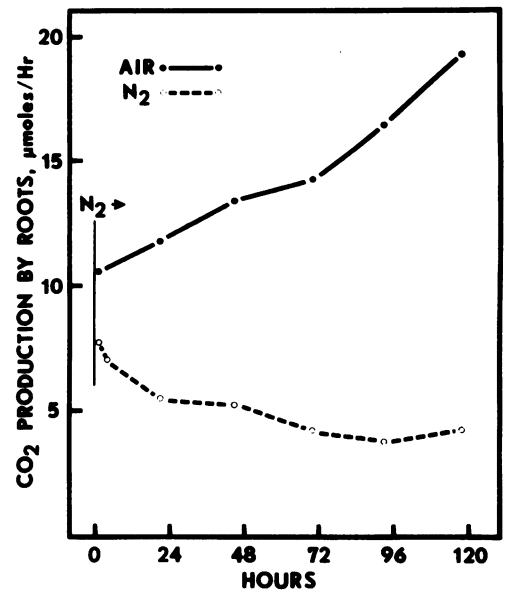

FIG. 5. CO_2 production by roots of Tiny Tim tomato plants as influenced by aeration. Time "zero" is when the O_2 level in the N_2 effluent gas became less than 1%.

Table I

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	N_2
Petiole angle, degrees	50.0 a[z]	80.0 b
Shoot fresh wt, g	3.28 a	2.54 b
Shoot dry wt, g	0.286 a	0.293 a
Root fresh wt, g	2.29 a	1.04 b
Root dry wt, g	0.121 a	0.066 b
Chlorophyll mg/g fresh wt	1.62 a	1.35 b
Leaf area, dm^2	0.80 a	0.64 b

[z] Means in a row followed by the same letter are not significantly different at the 0.01 level

vation of the root caused epinasty within 10 hr (Fig. 3A), while root ethylene production was at its lowest (Fig. 3B). Between 72 and 96 hr, ethylene production by anaerobic roots rapidly increased, but the visible damage to the roots makes the physiological significance of this rise questionable. Pretreatment with $AgNO_3$, an inhibitor of ethylene action (2), completely prevented epinasty (Fig. 3A). These results indicate the O_2 deprivation of the root can cause petiole epinasty in the absence of accelerated soil or root ethylene production and that epinasty is a direct response to ethylene. This confirms previous results of Jackson and Campbell (9, 12) with plants grown in nutrient solution.

The apparatus described in Figure 1 was employed to determine more precisely the site of increased ethylene synthesis following O_2 deprivation of the root. Ethylene synthesis by the shoots of plants with anaerobic roots increased dramatically within 1 day and reached a plateau after 2 days (Fig. 4). On the other hand, root ethylene evolution remained constant or declined during the same period (Fig. 4). Root respiration (CO_2 production) was immediately inhibited by anaerobic atmospheres and continued to decline for the duration of the experiment (Fig. 5). Epinasty, loss of Chl, and reduction in growth were evident in plants with anaerobic roots (Table I).

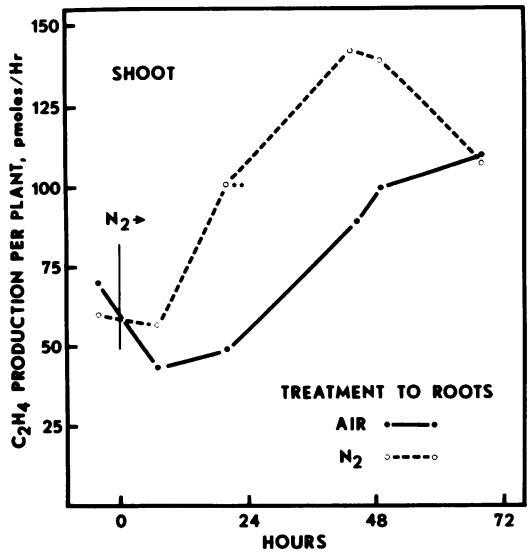

FIG. 6. Ethylene production by shoots of Chico III tomato seedlings as influenced by aeration of the root. (* *): treatments significantly different at $P \leq 0.01$.

This experiment was repeated with nondwarf tomato plants (Fig. 6). Shoot ethylene production/plant increased significantly within 24 hr of depriving the root of O_2, and continued to rise on the 2nd day before declining on the 3rd. Unlike the previous experiment, ethylene production by control plants also increased during this period. However, if the rates of production at 72 hr are expressed on a per g fresh wt basis, the shoots of plants with anaerobic roots produced significantly more ethylene (204 pmol g fresh wt^{-1} hr^{-1}) than control shoots (128 pmol g fresh wt^{-1} hr^{-1}). This discrepancy can be accounted for by the very rapid growth of the control plants and the negligible growth of the treated plants. Shoot and root fresh wt were drastically reduced by anerobic root ventilation, and other symptoms of flooding injury, such as epinasty and chlorosis, were also apparent with data similar to those in Table I.

DISCUSSION

These results (Figs. 4 and 6) indicate that depriving the root of O_2 is sufficient to cause accelerated ethylene synthesis by tomato shoots. Since the increase in shoot ethylene content occurs in the absence of a concentration gradient from the root to the shoot (Fig. 4), passive diffusional models of soil or root ethylene involvement in shoot responses to flooding are inadequate (11, 15). Diffusion of ethylene from the root or soil to the shoot may occur under flooded conditions in the field, but it is not necessary for the response. Thus, the primary cause of flooding-induced ethylene is deprivation of O_2 to the root, not ethylene production by microorganisms or blockage of ethylene escape from the root.

Jackson and Campbell (12) came to a similar conclusion and found that an injured root system was required for increased ethylene synthesis by the shoot. We have unpublished results which suggest that factors which may allow survival of the root system under anaerobic conditions (*i.e.* ethylene pretreatment or environmental conditions during growth) also prevent elevated shoot ethylene synthesis. Kawase's data (14) show that the ethylene content of sunflower shoots first began to rise after 4 days of flooding, which is sufficient time to cause permanent injury to the roots (4). Furthermore, Gentile and Matta (6) found that injuring the roots of tomato plants by immersion in a copper sulfate solution caused a 4- to 5-fold increase in ethylene synthesis by the shoots. Therefore, accelerated ethylene synthesis by the shoot seems to be an initial, rapid response to root injury.

Several researchers (4, 20, 21) have measured decreased cytokinin and gibberellin concentrations in xylem exudate from flooded roots. However, application of cytokinins, gibberellins, or mixture of the two hormones can only partially relieve flooding injury (10, 18, 19, 24). Reduced supply to the shoot of root-synthesized hormones may be involved in flooding injury, but is probably not the only contributing factor. Jackson and Campbell (12) have proposed that a specific signal is transmitted from injured roots to the shoot which stimulates ethylene synthesis. While our experiments did not address the question of the mechanism of root-shoot communication, they clearly demonstrate that such communication exists and that the plant responds to root O_2 stress by increasing the rate of ethylene synthesis by the shoot. The present results are consistent with both a hormonal imbalance theory and/or a specific factor theory of promoting ethylene synthesis, but they clearly rule out theories requiring passive diffusion of ethylene from the root zone to the shoot. The exact mechanism by which root injury promotes shoot ethylene synthesis awaits further experimentation.

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