

# Involvement of Plant Growth Substances in the Alteration of Leaf Gas Exchange of Flooded Tomato Plants<sup>1</sup>

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## ABSTRACT

Ethylene, abscisic acid, and cytokinins were tested for their ability to either induce or prevent the changes which occur in gas exchange characteristics of tomato (*Lycopersicon esculentum* Mill. cv. Rheinlands Ruhm) leaves during short-term soil flooding. Ethylene, which increases in the shoots of flooded plants, had no effect on stomatal conductance or photosynthetic capacity of drained plants. Abscisic acid, which also accumulates in the shoots of flooded plants, could reproduce the stomatal behavior of flooded plants when sprayed on the leaves of drained plants. However, photosynthetic capacity of drained plants was unaffected by abscisic acid sprays. Cytokinin export from the roots to the shoots declines in flooded plants. Spray applications of benzyladenine increased stomatal conductance in both flooded and drained plants. In addition, the decline in photosynthetic capacity during flooding was largely prevented by supplementary cytokinin applications. The possible involvement of these growth substances in modifying leaf gas exchange during flooding is discussed.

Both stomatal behavior and photosynthetic capacity of tomato plants were rapidly altered following soil flooding (4). Stomatal conductance decreased, and the sensitivity of the stomata to changes in CO<sub>2</sub> concentration increased. Assimilation rates were reduced due to (a) the lower stomatal conductance and (b) a fall in photosynthetic capacity of the mesophyll. Evidence has been presented which suggests that the stressed roots influence leaf gas exchange by a mechanism not involving decreases in leaf water potential (4, 7). Flooding promotes the synthesis of the ethylene precursor, ACC<sup>3</sup>, in the roots and its transport in the transpiration stream to the shoot, where it is converted into ethylene (10). Despite earlier reports to the contrary (24), recent studies have described both stomatal (25) and nonstomatal (16) inhibition of photosynthesis by ethylene. It is therefore possible that ethylene is involved in altering leaf gas exchange of flooded tomato plants. ABA also accumulates in the leaves of flooded plants (17, 29), and ABA applications can cause stomatal closure and increase stomatal sensitivity to CO<sub>2</sub> (8, 13, 28). On the other hand, the export of cytokinins from the roots of flooded plants is reduced by soil flooding (11). As cytokinins are known to delay senescence

and maintain photosynthetic capacity (1), a decline in cytokinin supply to the shoots during flooding might also influence assimilation rates. These plant growth substances were therefore tested for their ability to either induce (ethylene and ABA) or prevent (cytokinin) the characteristic changes which occur in stomatal behavior and carbon assimilation of flooded tomato plants.

## MATERIALS AND METHODS

Culture of the tomato plants (*Lycopersicon esculentum* Mill. cv. Rheinlands Ruhm), gas exchange techniques, and measurement protocol have been described previously (4).

For ethylene treatment, plants were enclosed overnight (16 h) in a 0.1 m<sup>3</sup> plexiglass box and exposed to 4 μl l<sup>-1</sup> ethylene in air at a flow rate of 5 l min<sup>-1</sup>. Leaf gas exchange was measured before and after this treatment with a portable system, the cuvette of which could be placed inside the plexiglass box (8). Ethylene (5 μl l<sup>-1</sup>) was present in both the ambient and the measuring gas streams during the second measurement. ABA was dissolved in a small quantity of ethanol and diluted to the desired concentration with water. BA, BA-riboside, or kinetin were dissolved in 1 N KOH and neutralized with HCl after dilution to give 100 μM solutions. Hormone sprays contained 0.01% Triton X-100, and control plants received identical sprays without the active substance. Gas exchange measurements were made on day 0, and the plants were sprayed at 12:00, 15:00, and 18:00 on day 1, and again at 6:00 on day 2. Gas exchange was then remeasured on day 2. This protocol was adopted to match the time course of stress in plants flooded at 8:00 on day 1. Root hypoxia would begin as O<sub>2</sub> became depleted from the soil water, which would take several hours to reach stressful levels (19). Thus, the earliest that the shoots might experience the effects of root stress would be noon of day 1. By the morning of day 2, changes in stomatal behavior and photosynthesis were evident (4, 7). If the growth substances are involved in these changes, they would have to be effective within the same time scale.

## RESULTS AND DISCUSSION

Increased ethylene synthesis in the shoots of flooded plants has been well documented as a cause of petiole epinasty in tomato (9). However, ethylene has generally been thought to have no effect on stomata and photosynthesis (24). Recently, Pallas and Kays (25) reported that a 2.5-h exposure to ethylene (1 μl l<sup>-1</sup>) inhibited photosynthesis by 30% in peanut (*Arachis hypogaea* L.) leaves, apparently due to stomatal closure. The net photosynthetic rate of potato (*Solanum tuberosum* L.) leaves was also reduced by 18% after a 3-h exposure to 1 μl l<sup>-1</sup> ethylene (16). Erkan and Bangerth (14) found that ethephon sprays increased stomatal resistance and decreased carbon uptake in tomato and pepper (*Capsicum annuum* L.) leaves. It was therefore logical to test whether ethylene might be responsible for the

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<sup>3</sup> Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; *A*, assimilation rate; *C*<sub>i</sub>, intercellular CO<sub>2</sub> partial pressure; *g*<sub>s</sub>, leaf epidermal conductance to water vapor; RuBP, ribulose biphosphate.

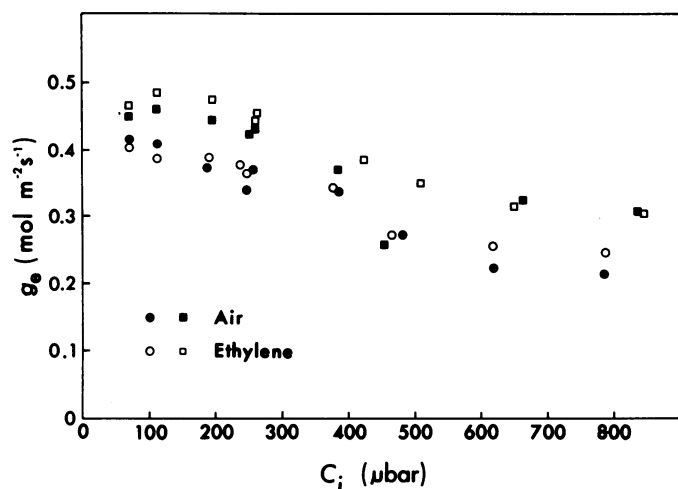


FIG. 1. Epidermal conductance to water vapor ( $g_e$ ) as a function of intercellular CO<sub>2</sub> partial pressure ( $C_i$ ) before (●, ■) and after (○, □) overnight exposure to 5  $\mu\text{l l}^{-1}$  ethylene. Ethylene was present in both the ambient and the measuring gas streams during the second measurement. Data for two replicate plants (●, ○, ■, □) are shown.

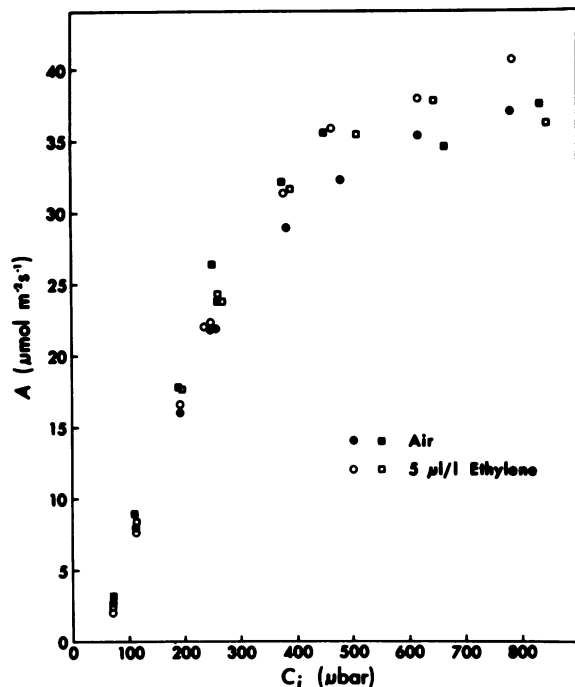


FIG. 2. Assimilation rate ( $A$ ) as a function of  $C_i$  before and after overnight exposure to 5  $\mu\text{l l}^{-1}$  ethylene. Same plants as in Figure 1.

stomatal closure and reduced photosynthetic capacity of flooded tomato plants. Previous experiments involving feeding ACC to excised shoots and monitoring  $g_e$  with a diffusion porometer had detected no effect of ethylene on the first day of feeding (7). On the second day,  $g_e$  fell due to the effects of root excision. In the present experiments, intact plants were exposed to 5  $\mu\text{l l}^{-1}$  ethylene overnight. The plants exhibited extreme epinasty, but there was no effect on  $g_e$  and no change in stomatal sensitivity to CO<sub>2</sub> (Fig. 1). Assimilation rates as a function of  $C_i$  were also insensitive to ethylene (Fig. 2). The ability of ethylene to influence  $g_e$  and  $A$  is apparently species-specific, as shown by Pallas and Kays (25). In tomato, the compound is ineffective in altering the gas exchange characteristics of drained plants. It is unlikely, therefore, that the high levels of ethylene in the shoots of flooded plants are responsible for the reduction in  $g_e$  and  $A$ .

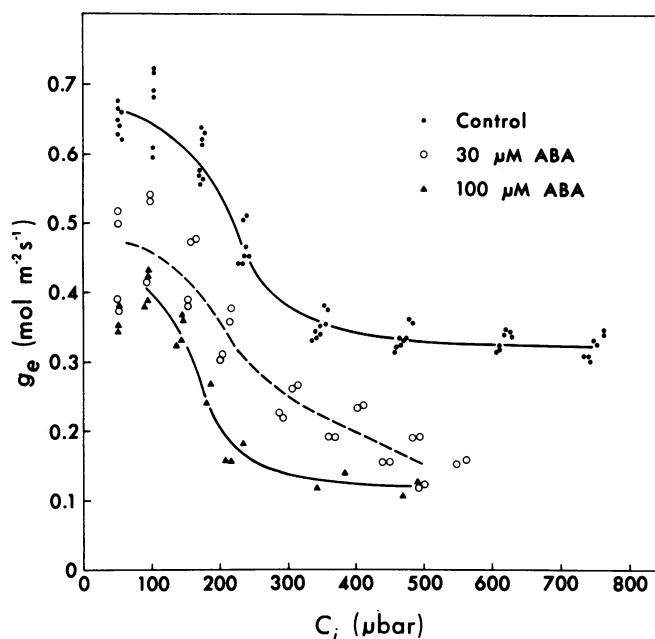


FIG. 3. Influence of ABA sprays on  $g_e$  of unflooded tomato plants. Conductance was measured on day 0 (control), and the plants were sprayed three times in the afternoon of day 1 and again on the morning of day 2. Measurements were then repeated. Data for two replicates at each ABA concentration are shown. Data for 10  $\mu\text{M}$  ABA are omitted as they did not differ significantly from the control.

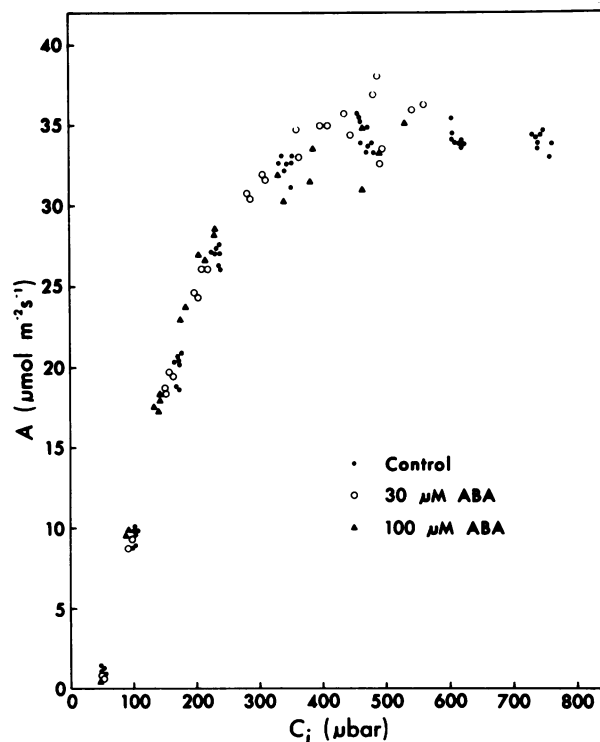


FIG. 4.  $A$  as a function of  $C_i$  and ABA treatment. Same plants as in Figure 3.

A plant growth substance known to influence stomatal behavior is ABA (28). The ABA content of the leaves of tomato seedlings increased almost 8-fold after 4 d of soil flooding, even though wilting did not occur (17). When ABA was sprayed on the leaves of drained plants,  $g_e$  was reduced at all  $C_i$  (Fig. 3). At a concentration of 100  $\mu\text{M}$ , ABA sprays reproduced the pattern

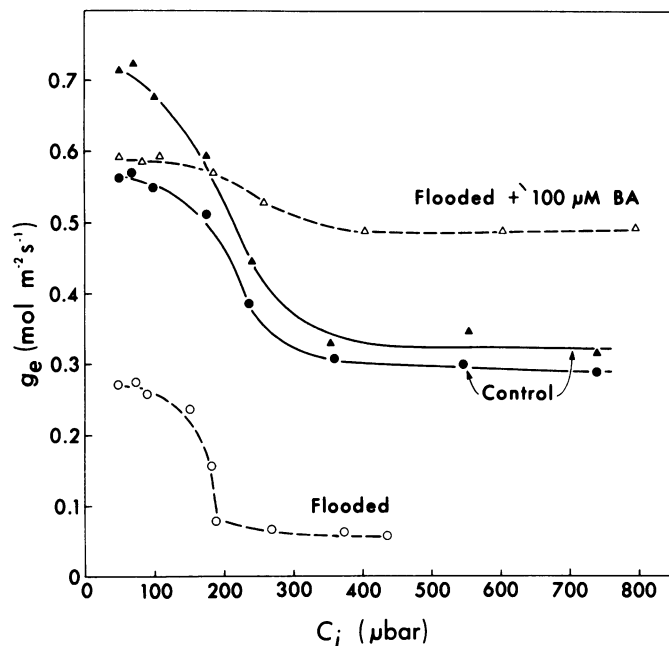


FIG. 5. Influence of BA sprays on  $g_e$  of flooded tomato plants. Plants were measured on day 0 (control) and flooded on day 1. One plant received three sprays of  $100 \mu\text{M}$  BA that afternoon and once again the next morning before being remeasured.

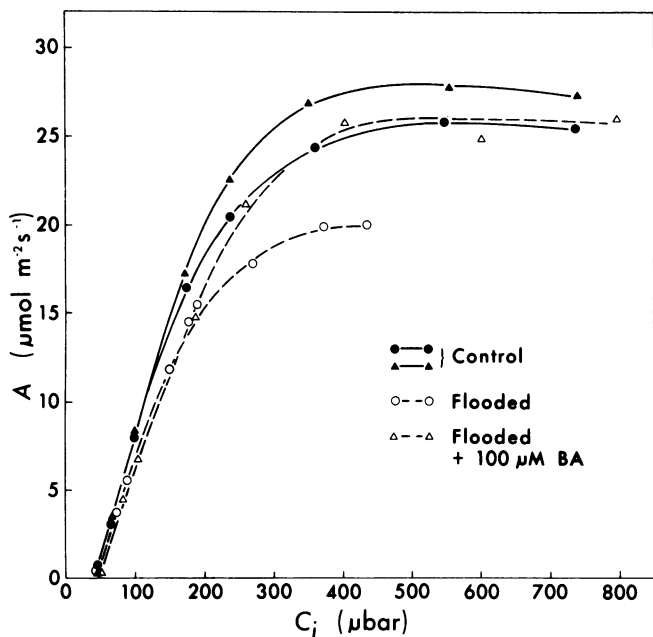


FIG. 6. Influence of BA sprays on  $A$  of flooded tomato plants. Same plants as in Figure 5.

of stomatal closure observed in flooded plants (*cf.* Figs. 3 and 5; see also Refs. 4, 5). Despite the marked effect on  $g_e$ , ABA had no effect on the relationship between  $A$  and  $C_i$  (Fig. 4), in agreement with previous short-term (13) and long-term (8) experiments. Raschke, on the other hand, has reported that ABA caused varying degrees of nonstomatal inhibition of photosynthesis in 13 different species (28). As in the case of ethylene, the effects of ABA on assimilative capacity appear to be species-specific. ABA synthesis and degradation is affected by leaf turgor pressure (26), but stomatal closure during flooding can occur without changes in leaf turgor (7). Measurements of elevated ABA levels in waterlogged plants have not included simultaneous

estimates of turgor pressure (17, 29), so the stimulus for ABA accumulation remains uncertain. Further investigations of ABA metabolism in flooded plants with careful attention to time course, water relations, and transport are needed to establish whether endogenous ABA is indeed responsible for causing stomatal closure.

Cytokinin activity in xylem exudate of sunflower plants declined following flooding (11). To test whether a deficiency of cytokinins might be responsible for the fall in  $g_e$  and  $A$ , plants were flooded in the morning, then sprayed with  $100 \mu\text{M}$  BA, BA-riboside, or kinetin during that afternoon and again the next morning. Gas exchange measurements were made before and after this treatment. Plants sprayed with BA or BA-riboside did not have lower conductances as usually occurs following flooding; instead,  $g_e$  was actually greater than in the control (Fig. 5). Conductance of drained plants was also increased by BA, with a  $\text{CO}_2$  response curve similar to that for flooded plants (5). Inasmuch as the measurement protocol went from low to high  $\text{CO}_2$ , the major effect of BA was to reduce stomatal closure once opening had occurred in low  $\text{CO}_2$ . The initial  $g_e$  under standard conditions was often not markedly affected by BA relative to that in control plants. In a single experiment with kinetin, no change in stomatal behavior occurred. Stimulation of transpiration and stomatal opening by cytokinins is well-known in grasses, but the compounds are often ineffective on dicotyledonous species (21, 23). In the present case, the major effect of BA was to inhibit stomatal closure, rather than to promote opening, and previous results with kinetin (2, 3, 22) and with BA plus GA (18) can be interpreted similarly. Since ABA seems to be a primary signal for stomatal closure, particularly in response to water stress (8), one can envisage an antagonism between ABA and cytokinins in regulating stomatal aperture, as has often been suggested (see Ref. 6). The interaction between ABA and cytokinins can also be shifted toward stomatal closure by nitrogen deficiency (27), which may also occur in flooded plants (12).

The cytokinin sprays largely prevented the fall in  $A$  which usually follows flooding (Fig. 6). In three experiments with BA, one with BA-riboside, and two with kinetin, the average inhibition of  $A$  at  $C_i = 400 \mu\text{bar}$  was 18% in flooded plants without supplementary cytokinins, but only 6% with cytokinin treatment. In the same experiment,  $A$  increased over the 2 d by 6% in unflooded plants and by 9% in unflooded plants sprayed with cytokinin. (Main effects of flooding and cytokinin treatment were significant at  $P < 0.001$  and  $P < 0.025$ , respectively. The interaction was not significant.) Although cytokinin supplementation did not completely ameliorate the effect of flooding on  $A$ , it did considerably offset the inhibition, particularly at high  $\text{CO}_2$  levels. Since the photosynthetic rate at saturating  $\text{CO}_2$  is limited by RuBP regeneration (30), supplementary cytokinins prevented the apparent decline in RuBP regeneration capacity which occurred during flooding (4). At least partial alleviation of some flooding stress symptoms by cytokinin or cytokinin plus gibberellin sprays has been reported previously (9, 12, 18). Furthermore, cytokinins can promote the synthesis of photosynthetic enzymes and electron transport components in expanding or greening leaves (15). The ability of applied cytokinins to delay senescence, accompanied by maintenance of photosynthetic rates (1), is well known, and a decrease in the capacity for noncyclic electron transport is an early indication of senescence (20). These and other data (4) suggest that the capacity for RuBP regeneration may be particularly sensitive to stresses, and that cytokinins may be involved in the endogenous regulation of photosynthetic capacity.

In conclusion, ethylene is apparently not responsible for the partial stomatal closure and decline in photosynthetic capacity in flooded tomato plants. It may have indirect effects on transpiration and assimilation by promoting epinasty and altering

light interception. ABA treatment did result in stomatal behavior identical to that in flooded plants. If endogenous ABA is responsible for the flooding effect, the question of the mechanism regulating ABA accumulation or redistribution remains, as the plants did not experience a leaf water deficit. Supplementary cytokinins prevented stomatal closure and considerably ameliorated the effect of flooding on assimilative capacity. The results provide additional evidence for the contrasting influences of ABA and cytokinins on stomatal behavior, and suggest that cytokinins may also be involved in the maintenance of photosynthetic capacity.

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