Effects of Soil Flooding on Leaf Gas Exchange of Tomato Plants[1]

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ABSTRACT

Carbon dioxide and water vapor exchange of tomato (*Lycopersicon esculentum* Mill. cv Rheinlands Ruhm) leaves were measured before and after 24 h of soil flooding to characterize both stomatal and nonstomatal responses to the stress. Leaf epidermal conductance to water vapor decreased by 47% after flooding, accompanied by an increase in the sensitivity of stomata to changes in CO_2 concentration. Assimilation rates under ambient conditions fell by 27%, and the inhibition could not be overcome by elevated CO_2 partial pressures. Stomatal conductance limited the assimilation rate to approximately the same degree both before and after flooding. The reduction in photosynthetic capacity was not due to a decrease in apparent quantum yield or to an increase in photorespiration. The results were analyzed according to a recent model of photosynthesis, and possible mechanisms underlying the flooding effect are discussed.

In some species, notably tobacco, soil flooding is closely followed by leaf wilting (18). Recent studies on a variety of herbaceous and woody species, however, suggest that the more common response to flooding is partial stomatal closure and the maintenance of high leaf ψ[3] (4, 17, 25, 29). In flooded tomato, stomatal closure acts to prevent leaf water deficits rather than being a response to low leaf ψ (4, 17). It has been suggested (4) that the stressed roots are involved in altering stomatal behavior in flooded plants. The postulated root effect could act directly on the guard cells to cause stomatal closure without affecting photosynthetic capacity, in which case C_i would be reduced. On the other hand, root stress could affect stomatal conductance indirectly by diminishing photosynthetic capacity, if the observed correlation between stomatal conductance and photosynthetic capacity (31) is due to capacity effects on conductance. Intercellular CO_2 concentrations could then remain constant or even increase under ambient conditions. Photosynthetic rates do decline during flooding or root anaerobiosis (24, 30), as would be expected following a reduction in g_e. However, Moldau (21)

found that nonstomatal conductance to CO_2 uptake was also reduced by flooding (but not by drought) in *Phaseolus vulgaris*. Moldau's experiments were all performed at low ambient CO_2 concentrations, making it uncertain whether the photosynthetic capacity at saturating CO_2 levels was similarly affected.

In the present study, leaf gas exchange of tomato plants was measured to determine the effects of soil flooding on stomatal conductance and on leaf photosynthetic capacity. The responses of stomatal conductance and assimilation rate to varied CO_2 partial pressures were assessed before and after 24 h soil flooding. Photosynthetic responses to quantum flux and O_2 partial pressure were also determined. The results indicate that stomatal behavior and photosynthetic capacity are independently altered by root stress. Analysis of the data according to a recent model of photosynthesis (10, 11) suggests several mechanisms which could account for the observations.

MATERIALS AND METHODS

Seeds of tomato (*Lycopersicon esculentum* Mill. cv Rheinlands Ruhm) were germinated in vermiculite and transplanted to 12-cm pots of potting soil after 14 d. The pots were watered daily with a complete nutrient solution and twice daily with tap water. Conditions in the unshaded greenhouse were: 30°C maximum, 15°C minimum; 50 to 80% RH (uncontrolled); quantum flux (PAR) 1800 to 2000 μE m^{-2} s^{-1} at midday. Plants were flooded in the early morning by placing the pots inside larger pots lined with plastic and filling with tap water to the cotyledonary node.

Gas exchange parameters were measured using an open system with a clamp-on chamber which enclosed 2.4 cm^2 leaf area. CO_2 and water vapor exchange were measured separately for upper and lower surfaces by CO_2- and H_2O-sensing IR gas analyzers (Beckman model 865 and ADC model 225, respectively). Water content of the ingoing air was regulated by a condenser at 16°C, and CO_2 concentration was controlled by a mass-flow controller (Tylan) and measured by a third IR gas analyzer (Hartmann and Braun URAS 2). Light from a xenon arc lamp passed through 5 cm of water and a glass IR filter before reaching the leaf. Intensity was varied by interposing metal screens or neutral density filters between the light source and the leaf. Leaf temperature was controlled by the water-jacketed cuvette, and was measured by a thermocouple appressed for a length of 1.5 cm to the abaxial leaf surface. The air flow rate was 0.7 l min^{-1} and boundary layer conductances measured with wet filter paper were 0.98 and 1.33 mol m^{-2} s^{-1} for the upper and lower surfaces, respectively. Gas exchange parameters were calculated according to von Caemmerer and Farquhar (28), and represent the total for both sides of the leaf for the projected leaf area. The conductance values include the stomatal plus cuticular components and are termed the epidermal conductance (g_e). Conductances in the dark were <0.05 mol m^{-2} s^{-1}, which would be a maximum estimate of the cuticular conductance.

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[3] Abbreviations: A, assimilation rate; A_{max}, maximum assimilation rate at saturating CO_2; C_a, ambient CO_2 partial pressure; C_i, intercellular CO_2 partial pressure; E, transpiration rate; g_e, leaf epidermal conductance to water vapor; I, incident quantum flux (PAR); ψ, water potential; RuBP, ribulose bisphosphate; RuBPC/O, ribulose bisphosphate carboxylase/oxygenase; Γ, CO_2 compensation point.

Measurements were made on the fourth or fifth leaves of 5-week-old plants. The penultimate leaflet was placed into the gas exchange cuvette and allowed to attain steady rates of E and A under standard conditions (1800 μE m^{-2} s^{-1}, 25.8°C, 16 mbar leaf to air vapor pressure difference, 340 μbar C_a). This usually required 1 h for control plants, and up to 2.5 h for flooded plants due to damped stomatal oscillations following flooding. After this initial measurement, other parameters were held constant as C_a was varied from 50 to 950 μbar. Thirty min was allowed after each change in C_a, which was sufficient time for the establishment of a new steady state. For low O_2 measurements, the air stream was diluted 10:1 with N_2 to give 2% O_2 prior to the injection of CO_2. Gas exchange parameters were measured on

day 0, flooding occurred on the morning of the next day (day 1), and gas exchange was remeasured on day 2. Previous measurements of g_e (4) and preliminary tests here showed no changes in gas exchange characteristics or leaf water relations during day 1. The results therefore represent the changes occurring in leaf gas exchange after one full day of flooding. The experiments were confined to this short time scale to identify the initial responses to root flooding.

RESULTS

Leaf epidermal conductance was reduced at all C_i after 24 h of flooding (Fig. 1). Stomata failed to open as widely at low C_i after flooding, and closed to very low g_e as C_i increased above 200 μbar. The open loop gains for the feedback loop involving g_e and C_i were calculated according to Farquhar et al. (13). Flooding increased this gain at all levels of C_a, particularly at and above atmospheric (Table I), indicating that C_i is less affected by changes in C_a following flooding. Under standard conditions ($C_a = 340$ μbar), g_e was reduced an average of 47% by flooding and A fell by 27%, resulting in an average drop in C_i from 235 to 204 μbar (Table II). This is reflected in the 20% decrease in the transpiration ratio (E/A) following flooding (Table II).

Assimilation was reduced by flooding at all C_i, but the degree of inhibition was greater at high C_i than at low C_i (Fig. 2). The initial slope of the A versus C_i curve (for C_i <100 bar) decreased by 16%, while A_{max} was inhibited by 25% (Table II). The reduction in A caused by flooding was similar when measured at either atmospheric or saturating CO_2 levels (Table II), indicating that the stomatal limitation of photosynthesis was approximately the same both before and after flooding. The rates of RuBP regeneration, calculated according to the model of Farquhar and Caemmerer (10), should increase at low C_i's, then remain fairly constant as C_i rises further. Instead, RuBP regeneration rates rose and then declined sharply as C_i increased (Fig. 2, inset). Possible reasons for this deviation from the model will be discussed below. However, it should be noted that in both control and flooded plants, stomata were acting under ambient conditions to maintain C_i in the region where the RuBP regeneration rates stopped increasing and began to decline (Fig. 2, inset).

Dark respiration was 32% less following flooding, but the compensation point was unchanged (Table II).

The dependence of A upon incident quantum flux was altered by 24 h flooding (Fig. 3). The apparent quantum yield at low light intensity ($I < 200$ μE m^{-2} s^{-1}) was not significantly affected by the stress, being 0.050 mol CO_2/mol incident quanta in the control, and 0.049 mol CO_2/mol quanta after flooding. Assimilation rates of the flooded plants were reduced, however, at $I >$ 300 μE m^{-2} s^{-1}. The light compensation point decreased from 38 to 16 μE m^{-2} s^{-1} following flooding, apparently due to the decline in dark respiration.

Inhibition of A by short-term flooding was maintained even when measured in 2% O_2 (Fig. 4). In control plants, low O_2 stimulated A at $C_i < 600$ μbar, but inhibited A at $C_i > 600$ μbar. Promotion of A by O_2 at high C_i has been observed in a variety of species, although the underlying mechanism remains uncer-
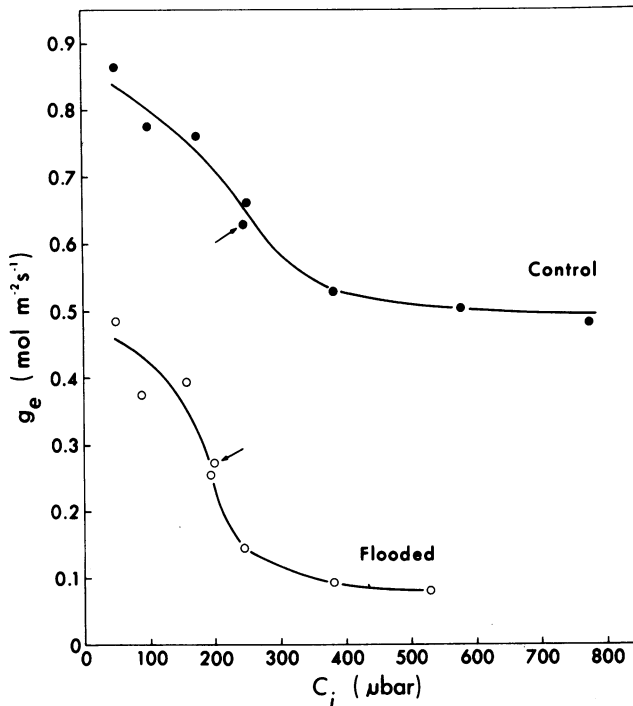

FIG. 1. Leaf epidermal conductance (g_e) as a function of C_i before and after 24 h soil flooding. The arrows indicate initial points obtained at $C_a = 340$ μbar.

Table I. *Gains of the Feedback Loop Involving Stomatal Conductance and CO_2 before and after 24 Hours Soil Flooding*

Differences between control and flooded treatments are highly significant (P < 0.01) at all levels of C_a ($n = 10$).

	Loop Gains (Dimensionless) at Following C_a (μ bar)		
	175	340	500
Control	−0.034 ± 0.008[a]	−0.28 ± 0.05	−0.28 ± 0.07
Flooded	−0.12 ± 0.03	−1.49 ± 0.31	−2.56 ± 0.63

[a] Mean ± SE.

Table II. *Gas Exchange Parameters of Tomato Leaves before and after 24 Hours Soil Flooding*

A, g_e, C_i, and E were measured under standard conditions and $C_a = 340$ μbar. dA/dC_i is the slope of linear regressions of A on C_i for $C_i < 100$ μbar, and the compensation point (Γ) is the intercept on the C_i axis. A_{max} is the maximum assimilation rate observed, and R_d is the dark respiration rate. Means for measurements on 12 replicate plants are shown. All treatment comparisons are highly significant (P < 0.01) except Γ.

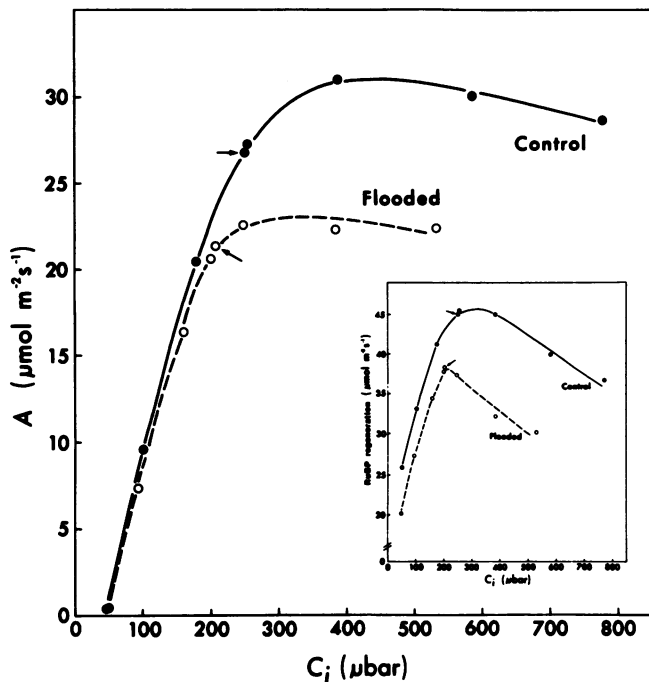|	A	g_e	C_i	E/A	dA/dC_i	A_{max}	R_d	Γ
	$\mu mol\ m^{-2}\ s^{-1}$	$mol\ m^{-2}\ s^{-1}$	μbar	$mol\ mol^{-1}$	$mol\ m^{-2}\ s^{-1}$	$\mu mol\ m^{-2}\ s^{-1}$		μbar
Control	27.9	0.537	234	249	0.177	34.1	2.6	43
Flooded	20.5	0.285	204	198	0.149	25.5	1.7	44
Percent of control	73	53	87	80	84	75	68	102

FIG. 2. Assimilation rate (A) as a function of C_i before and after 24 h soil flooding. The arrows indicate initial points obtained at $C_a = 340$ μbar. Inset, RuBP regeneration rates calculated from the A versus C_i curves. Same plant as in Figure 1.
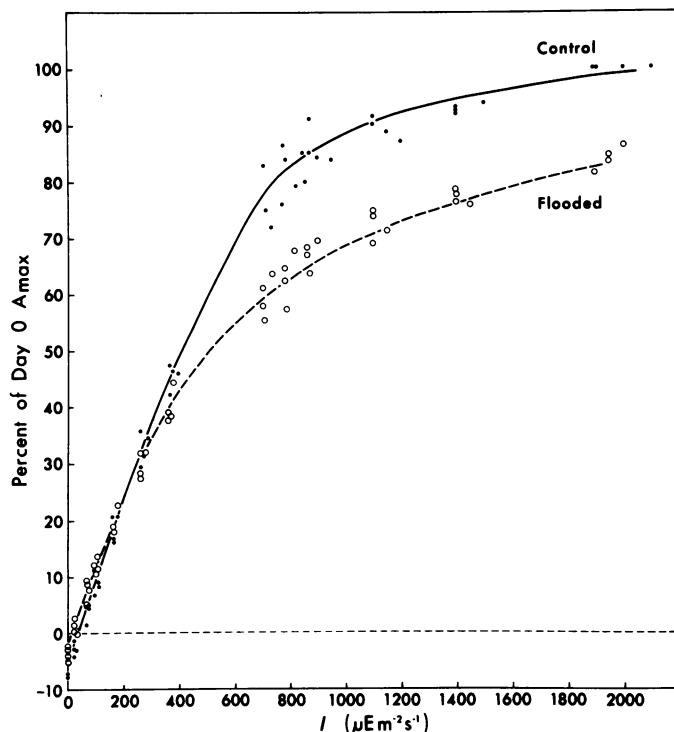

FIG. 3. Assimilation as a function of incident quantum flux before and after 24 h soil flooding. Data are plotted as a percent of the A_{max} on day 0 for four replicate plants. The average A_{max} on day 0 was 35.2 ± 1.6 μmol m^{-2} s^{-2}. C_a was maintained at 950 μbar as I was varied.

tain (21, 27, 32). Following flooding, the stimulation of A by 2% O_2 at $C_i < 200$ μbar was reduced, and the crossover from promotion to inhibition occurred at $C_i = 300$ μbar (Fig. 4). These results make it unlikely that the inhibition of A by flooding is due to an increase in photorespiration.
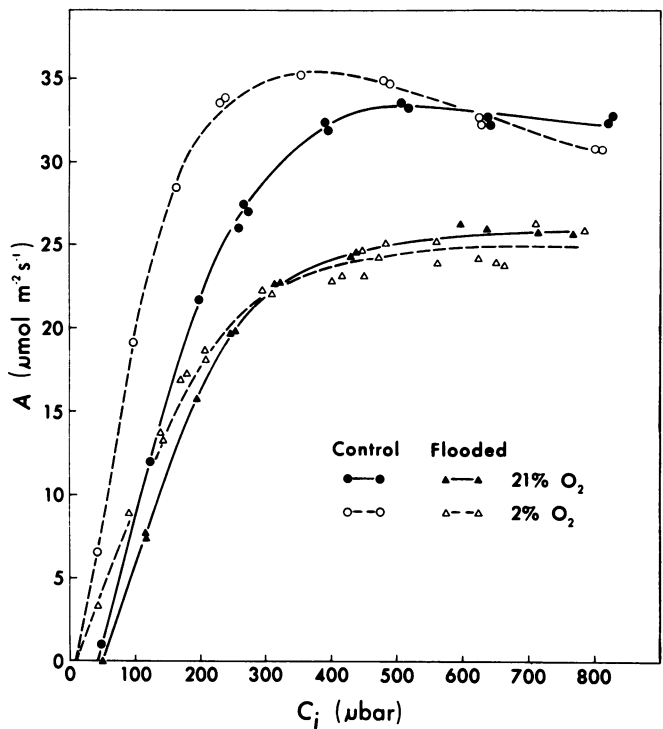

FIG. 4. Effect of 21% and 2% O_2 on A before and after 24 h soil flooding. A was measured as C_a was increased in 21% O_2, then remeasured in 2% O_2 as C_a was decreased.

DISCUSSION

Short-term flooding had marked effects on both stomatal behavior and photosynthetic capacity. In agreement with previous measurements using diffusion porometry (4), g_e was reduced under ambient conditions by an average of 47% (Table II). In addition, the sensitivity of the stomata/C_i system to changes in CO_2 was sharply increased, as indicated by the higher values for the CO_2 loop gain after flooding (Table I). The transpiration rates of flooded plants may be sufficiently reduced that leaf ψ is actually greater than in the controls (17, 25, 29). This suggests a 'feedforward' of stomatal response to the stress, analogous to the feedforward responses of stomata to humidity (9). That is, a feedback mechanism operating to regulate stomatal aperture by sensing a decline in leaf ψ cannot account for stomatal behavior which results in an increase in leaf ψ. Other experiments with root pruning (1, 4, 5, 7, 33), stem girdling (4), and soil water depletion (2) also implicate the roots in influencing stomatal conductance independently of changes in leaf ψ.

Wong et al. (31) found that g_e was linearly correlated with A under a variety of environmental conditions, but this linear correlation was not maintained during flooding. In flooded plants, g_e was reduced to a greater extent than was A, resulting in a fall in C_i and a decrease in the transpiration ratio (Table II). In one experiment, A of a flooded plant at saturating C_i declined from 93% to 75% of the day 0 value between 11:30 and 17:30 on day 2. Conductance at the same C_i, however, was only 31% of the control initially, and did not decline further during the day. On another occasion, A_{max} was only slightly affected by the stress, but g_e was reduced nonetheless. Thus, while in the majority of cases the changes in stomatal behavior and assimilative capacity were coincident, there seemed to be no necessary linkage between them.

Models of leaf gas exchange based on the properties of RuBPC/ O have identified the initial slope region of the A versus C_i curve (at low C_i) with the activity of RuBPC/O, and the high C_i region of the curve with RuBP regeneration capacity (10, 11, 28). Thus,

at low C_i, A would be limited by the amount and activity of RuBPC/O, while at high C_i, A would be limited by the rate at which RuBP could be synthesized. Under ambient CO_2 conditions, stomata of both flooded and control plants maintained C_i in the region of transition between the two types of limitation (Fig. 2). Von Caemmerer and Farquhar (28) have noted that optimal water use often occurs when C_i is maintained in this transition region. Similar stomatal behavior has also been observed in other species in response to a variety of environmental parameters (13).

According to the model (10, 28), the greater inhibition of A at high C_i than at low C_i (Fig. 2; Table II) would suggest that flooding primarily affects RuBP regeneraztion, which includes photosynthetic electron transport, NADPH and ATP synthesis, and the reductive pentose phosphate cycle. The apparent quantum yield was unaffected by flooding (Fig. 3), and there was no change in Chl in these short-term experiments (data not shown), indicating that the lesion was not in the light harvesting components of the chloroplast. If the photosynthetic rate was limited solely by the maximum rate of RuBP regeneration, A should continue to rise slightly with increasing C_i as carboxylations are substituted for oxygenations, and the calculated RuBP regeneration rate should be essentially independent of C_i (10). However, in both flooded and control plants, A either remained constant or actually declined at high C_i's (Figs. 2 and 4), resulting in a sharp drop in calculated RuBP regeneration rates (Fig. 2, inset). This indicates that some additional factor(s) became limiting as C_i increased. The inability of low O_2 pressure to increase A in flooded plants (except at low C_i) (Fig. 4) also indicates that RuBP availability, per se, was not the sole factor limiting photosynthesis. In control plants, low O_2 actually reduced A at the highest C_i (Fig. 4), as has been reported in other species (20, 27, 32). Woo and Wong (32) have recently discussed several possible mechanisms which could account for these observations, including changes in the activity of RuBPC/O.

In the case of flooded plants, several lines of evidence suggest that the availability of Pi for RuBP regeneration may be limiting. Harris et al. (14) were able to induce responses to CO_2 and O_2 like those shown in Figures 2 and 4 in spinach leaf discs by feeding mannose to sequester Pi. Limitation of RuBP regeneration by the availability of Pi would make A independent of CO_2 and O_2 at high C_i (10), as was observed in flooded plants (Figs. 2 and 4). Reduction in sink activity of the flooded roots might cause accumulation of sucrose in the leaves, which could result in a build-up of triose phosphates and depletion of the cytoplasmic Pi pool (15). A consequence of this would be diversion of photosynthate into starch (22), which has recently been observed in the leaves of flooded sunflower plants (R. L. Wample and R. W. Davis, personal communication). However, root anaerobiosis did not significantly alter the incorporation of ^{14}C-photoassimilates into starch in the leaves of wheat seedlings (30). Further studies of the levels of photosynthetic metabolites in flooded plants will therefore be required to substantiate this scheme.

In addition to the possibility of metabolic sequestration of Pi, poor root aeration also severely reduces uptake of both P and N (16, 26). A lowering of the C_i at which O_2 neither promotes nor inhibits A, as observed here following flooding (Fig. 4), was induced by nitrogen deficiency in cotton (32). It remains to be determined whether changes in nutrient content are sufficiently rapid to account for the present results. It should be noted that root excision caused a nonstomatal inhibition of A in bean leaves which could not be mimicked by nutrient starvation (6). The possible involvement of plant growth substances in the etiology of the flooding syndrome is considered in a separate report (3).

Partial stomatal closure and an increased sensitivity to CO_2 appear to be characteristic responses of stomata to environmental stress. In addition to the present results for flooded plants (Fig.

1; Table I), similar data have been reported for plants subjected to drought or chilling temperatures (8, 19, 23). Such a change in stomatal behavior may be symptomatic of an altered water use strategy. Prior to stress, A would be slightly favored at the expense of a higher transpiration ratio (Table II). Following stress, E would be minimized within the constraint of maintaining A near the maximum rate allowed by the reduced photosynthetic capacity (Fig. 2). The changes occurring in mesophyll cells and in guard cells, while generally synchronous, appeared to be largely independent, and g_e was reduced to a greater extent than was A. The net effect was a fall in C_i and in the transpiration ratio (Table II), which reflects a more conservative water use strategy after flooding.

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