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A Negative Hydraulic Message from Oxygen-Deficient Roots of Tomato Plants?[1]

Influence of Soil Flooding on Leaf Water Potential, Leaf Expansion, and Synchrony between Stomatal Conductance and Root Hydraulic Conductivity

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Four to 10 h of soil flooding delayed and suppressed the normal daily increase in root hydraulic conductance (L_p) in tomato (*Lycopersicon esculentum* Mill. cv Ailsa Craig) plants. The resulting short-term loss of synchrony between L_p and stomatal conductance decreased leaf water potential (ψ_L) relative to well-drained plants within 2 h. A decrease in ψ_L persisted for 8 h and was mirrored by decreased leaf thickness measured using linear displacement transducers. After 10 h of flooding, further closing of stomata and re-convergence of L_p in flooded and well-drained roots returned ψ_L to control values. In the second photoperiod, L_p in flooded plants exceeded that in well-drained plants in association with much increased L_p and decreased stomatal conductance. Pneumatic balancing pressure applied to roots of intact flooded plants to prevent temporary loss of ψ_L in the 1st d did not modify the patterns of stomatal closure or leaf expansion. Thus, the magnitude of the early negative hydraulic message was neither sufficient nor necessary to promote stomatal closure and inhibit leaf growth in flooded tomato plants. Chemical messages are presumed to be responsible for these early responses to soil flooding.

An important goal in studies of plants stressed at the roots is to establish the relative contributions of hydraulic and chemical messages in mediating adaptive responses of the shoot. In flooded plants, reductions in g_s and leaf elongation can occur in the absence of any marked perturbation in leaf water status (Pereira and Kozlowski, 1977; Jackson et al., 1978; Bradford and Hsiao, 1982; Zhang and Davies, 1986, 1987; Jackson and Hall, 1987), supporting the view that chemical messages predominate. However, it may be premature to disregard completely the involvement of a hydraulic message, since frequent measurements of ψ_L at high resolution are rare. Previous studies have provided some evidence that, soon after inundation of the soil or sparging nutrient solution with nitrogen, a transient decrease in ψ_L may take place within a few hours of the start of the treatment (Jackson et al., 1978; Jackson and Hall, 1987; Everard and Drew, 1989; Schildwacht, 1989), especially if flooding commences at the end of the photoperiod (Bradford and Hsiao, 1982). Such a loss of shoot hydration could conceivably trigger stomatal closure and slow leaf expansion. This early and temporary leaf water deficit has been ascribed to a reduction in L_p following flooding (Kramer, 1969; Hiron and Wright, 1973; Schildwacht, 1989), apparently arising from an O_2 deficit (Mees and Weatherley, 1957) and/or an accumulation of CO_2 around the roots (Kramer, 1940; Smit and Stachowiak, 1988). However, there are contrasting reports of the existence and duration of the reduced hydraulic conductivity of flooded roots (Kramer 1940; Mees and Weatherley, 1957; Jackson et al., 1978; Bradford and Hsiao, 1982; Everard and Drew, 1987; Reece and Riha, 1991), arising, in part, from the different methods used to calculate L_p. Many of these problems have their origin in the influence of hidden osmotic driving forces affecting J_Vs. One method of overcoming this difficulty is to pressurize detopped root systems sufficiently to induce a flow of xylem sap that is as fast as transpiration and dilutes sap solutes to osmotically less effective concentrations. This approach generates linear increases in J_V with increases in applied pressure above a certain value. The slopes of these lines give osmotically less dependent, and thus more reliable, measures of L_p (Dalton et al., 1975; Fiscus, 1975; Passioura, 1984, 1988; Jackson et al., 1996). We adopt this approach in the present paper in which we examine the possible involvement of a negative hydraulic message from roots of tomato (*Lycopersicon esculentum*) plants in the initiation of leaf responses to soil flooding for up to 36 h.

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Abbreviations: ANOVA, analysis of variance; ΔP, applied pneumatic pressure; g_s, stomatal conductance; J_V, sap flow rate; L_p, root hydraulic conductivity; ψ_L, leaf water potential.

MATERIALS AND METHODS

Plant Material

Tomato (*Lycopersicon esculentum* Mill. cv Ailsa Craig) plants were grown in peat-based Levington compost in a controlled environment room providing a 16-h photoperiod (8 AM to 12 AM) with a PAR of 400 μmol m^{-2} s^{-1}, 25/20°C light/dark temperature, and 50% RH as described previously (Else et al., 1994). All pots were irrigated automatically via capillary matting, and side shoots were removed from plants regularly. Plants at the eight-leaf stage were flooded at 9 AM for 36 h by placing plant pots into larger plastic pots filled with 1.2×10^{-3} m^3 of tap water at 25°C; water was maintained 10 mm above soil level.

g_s, Whole-Plant Transpiration, and Leaf Elongation Rates

Abaxial conductances of leaf 4 (counting from the base) of flooded and well-drained plants were determined at 2-h intervals during the first and second photoperiods using a Delta-T Mk3 diffusion porometer (Delta-T Devices, Cambridge, UK). Whole-plant transpiration was measured gravimetrically and corrected for evaporation from the soil surface during a similar time course. Leaf elongation was determined every 4 h during the two photoperiods by measuring with calipers (Camlab, Cambridge, UK) the length of the terminal leaflet of the youngest expanding leaf (leaf 8).

ψ_L

Water potentials of leaf 4 were measured at 2-h intervals with a pressure chamber. Immediately after excision, the leaf was sealed into the chamber and pressurized at a rate of 0.02 MPa s^{-1}. Erroneous pressure chamber values caused by bubbling at lower pressures were avoided by blotting the cut surface before applying pressure. No adjustments to ψ_L were made for xylem sap solute potential. Continuous, indirect estimates of leaf water status, and thus of ψ_L, were made by monitoring changes in leaf thickness every 30 s throughout 24 h of flooding using displacement transducers capable of detecting changes of <1 μm (Malone, 1992). Briefly, eight ST 200 transducers (Sangamo, Bognor Regis, UK) were mounted on a metal frame. The core of each transducer was connected by a counterbalanced lever to a plastic foot resting on the center of a leaf. Voltage outputs from the transducers were demodulated (CAH-16 convertor, Sangamo) and logged on a personal computer via a multichannel analog-digital convertor card (MITC-1; 3D Digital, Interface House, London) using custom software. To control effects of thermal expansion, blank transducers were always included. These were mounted on the same frame and logged in exactly the same way, but they contained no leaves.

L_P

Estimates of L_P were made every 2 h during the first photoperiod and every 4 h during the second photoperiod. Plants were detopped with a razor blade and well-drained or flooded root systems were immediately placed inside pressure chambers designed to collect sap from detached root systems (Else et al., 1994). A series of pressures were applied (0.1–0.4 MPa) using compressed air for well-drained plants and O_2-free nitrogen for flooded plants. Preliminary experiments indicated that the response of sap flow to applied pressure was linear for both flooded and well-drained roots over the pressures chosen. Furthermore, the range of J_Vs generated encompassed the rates of whole-plant transpiration in this study. The initial 200 mm^3 of sap, known to be contaminated with a wound-induced release of solutes (Else et al., 1994), was discarded. Sap was collected in Eppendorf tubes for 5 min at each pressure and weighed.

Collection of Soil Water

Soil water from flooded pots was withdrawn through a 70-mm length of silicon rubber tubing (1.5 mm i.d.) attached at one end to a ceramic pot buried in the soil and at the other to a three-way tap (Nipro Medical, Tokyo, Japan) connected to a 2×10^3 mm^3 hypodermic syringe. Pots containing plants in well-drained soil were squeezed to enable soil water samples to be taken. After collection, samples were frozen in liquid nitrogen before being stored at −20°C.

CO2 and O2 Dissolved in Soil Water

CO_2 partial pressures in 2×10^{-6} m^3 samples of flood water were measured by head-space analysis using a Pye Unicam (Cambridge, UK) 104 gas chromatograph fitted with a katherometer detector and two stainless steel analytical columns (3 m × 3 mm i.d.). One column was packed with Porapak (Waters) (60/80 mesh), the other was packed with molecular sieve 5A (Phase Separations Ltd, Clwyd, UK) (60/80 mesh), and both were maintained at 90°C, with helium as the carrier gas (Hall and Dowdell, 1981). The system was calibrated by injecting 1×10^{-6} m^3 samples of gas from the gas space above water samples equilibrated with 0, 1, 5, or 10 kPa CO_2 (British Oxygen Co., London, UK). O_2 partial pressures in 10-mm^3 samples of flood water were determined with a Pye-Unicam 104 gas chromatograph fitted with a ^{63}Ni electron capture detector, a stainless steel precolumn (50 × 4 mm i.d.) containing magnesium perchlorate to remove water, and an analytical column (1.5 m × 2 mm i.d.) maintained at 110°C and packed with Carbosieve B (Supelco, Bellefonte, PA) (60/80 mesh). O_2-free nitrogen was the carrier gas (Hall, 1978).

Testing Physiological Significance of Decreased ψ_Ls

Plants were flooded and their root systems immediately sealed inside pressure chambers situated in the controlled environment room. The chambers were designed with split-tops that allowed pressure sealing around the hypocotyl region to enable sap to be collected from the shoot system. Well-drained plants were sealed inside similar chambers. The terminal leaflet (approximately 3×10^3 mm^2) was removed from leaf 6 of both flooded and well-drained plants, and a 40-mm length of silicon rubber tubing (1.5 mm i.d.) was carefully placed over the petiole stump. Pressure was applied slowly to each root system until xylem sap from the cut petiole barely exuded into the tubing. At this point, the pressure applied to roots corresponded to the hydrostatic component of the shoot xylem water potential (see Passioura, 1984, for a full discussion). A mark on the silicone tubing was made at the meniscus of the sap 5 mm distal to the cut end of the petiole. As flooding progressed, pressure to each root system was adjusted continuously to maintain the xylem sap meniscus at the original point. Throughout the photoperiod, measurements of g_s (terminal leaflet, leaf 4) and leaf length (terminal leaflet, leaf 8) were made on both flooded and well-drained plants. Plants in the pressure chambers were depressurized and removed from the chambers after 12 h. g_s and leaf length were measured on the same leaflets during the next photoperiod, to check the influence of eliminating negative xylem water potentials during the first photoperiod on stomatal behavior and leaf growth the following day.

Statistical Analyses

Treatment effects on g_s were compared by ANOVA after $\log_e$ transformation. Linear regression lines were fitted to leaf length and L_p data with respect to time and pressure, respectively, and mean slopes of flooded and well-drained plants were then tested for differences by ANOVA. All other data were analyzed by ANOVA.

RESULTS

Leaf Responses to Soil Flooding

g_ss of well-drained plants showed a marked daily variation, increasing rapidly at the beginning of each photoperiod and reaching a maximum between midday and early afternoon before declining gradually during the evening. Whole-plant transpiration fluctuated in tandem with g_s (Fig. 1, A and B). However, during the first 2 h of flooding, stomata opened less than those of well-drained plants (Fig. 1A). Thereafter, g_s decreased steadily throughout the remainder of the photoperiod to 33 to 50% of the value for well-drained values. At the beginning of the second photoperiod, stomata of flooded plants failed to open fully, with g_s being maintained at a smaller, more constant level than in controls until the end of the experiment (Fig. 1A). Again, transpiration rates mirrored changes in g_s (Fig. 1B).

A divergence in leaf elongation between flooded and well-drained plants was observed after 4 h. The youngest expanding leaves of well-drained plants grew steadily throughout the experiment (Fig. 1C).

ψ_L

ψ_Ls measured with a pressure chamber decreased by approximately −0.1 MPa after only 2 h of flooding (Fig. 2A). Thereafter, ψ_L recovered slowly until values approximated those of well-drained plants after 8 to 10 h. During the second photoperiod, leaves of flooded plants had significantly higher ψ_L values than their well-drained counterparts (Fig. 2A). ψ_Ls of well-drained plants remained at approximately −0.5 MPa throughout both photoperiods.

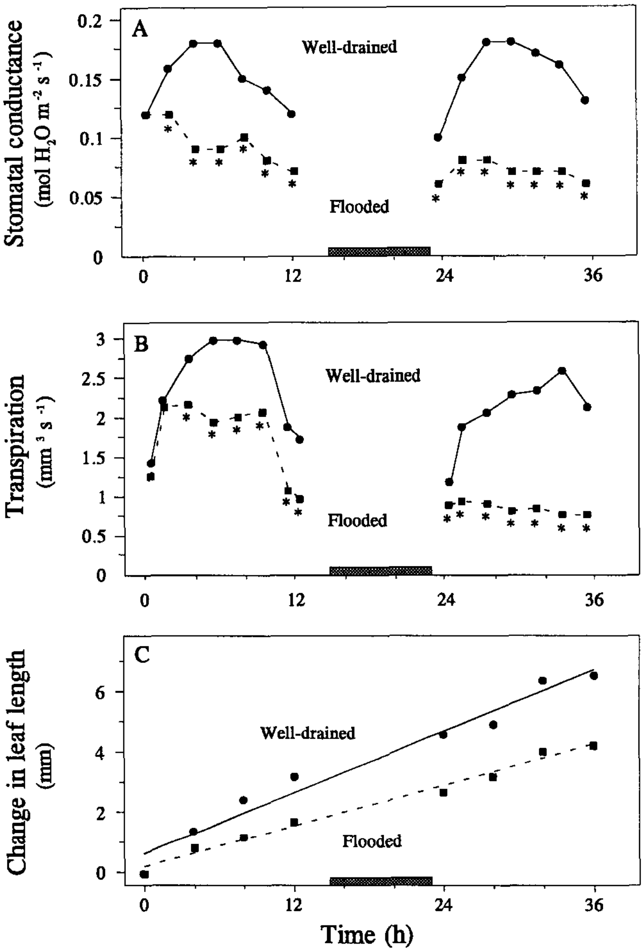

Figure 1. Effect of flooding during 36 h on abaxial g_s of the fourth-oldest leaf (LSD 0.216, $P < 0.05$, $n = 8$) (A), whole-plant transpiration rates (LSD 0.198, $P < 0.05$, $n = 8$) (B), and change in length of the eighth-oldest leaf of tomato plants (C). Asterisks (*) indicate significant differences between means. Leaf extension rates were significantly different as estimated from the mean slopes of linear regression lines fitted to flooded (b [slope of the regression line] = 0.113) and well-drained (b = 0.175) length data.

In a separate experiment, leaf thickness, used as a marker for leaf water status, was measured continuously for 24 h (Fig. 2B). Leaf thickness in flooded plants decreased steadily after a lag period of 90 min, reaching a minimum 3 to 4 h after flooding that was well below that of control leaves. Thereafter, leaf thickness increased slowly during the remainder of the photoperiod, approaching well-drained values after approximately 10 h of flooding. Thus, the pattern of change in leaf thickness caused by flooding was similar to that for ψ_L. There was no detectable daily variation in leaf thickness in well-drained plants, although a steady downward drift in leaf thickness was evident that was not linked to ψ_L. During the dark period, leaf thickness of both flooded and well-drained plants increased considerably (Fig. 2B), with thickness of flooded leaves exceeding that of well-drained leaves. Increased thickness of flooded

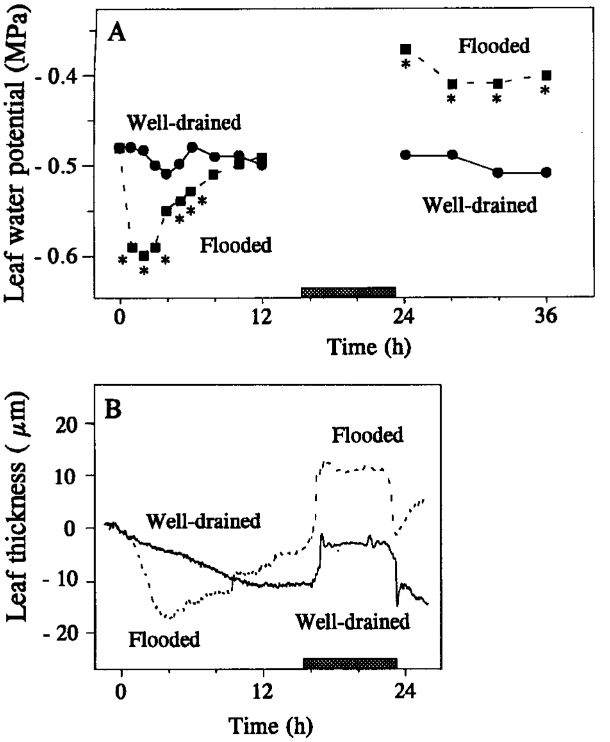

Figure 2. A, Effect of soil flooding on ψ_L of leaf 4 of tomato plants measured at intervals throughout 36 h. No adjustments were made for xylem sap solute potential. Asterisks (*) indicate significant differences between means (LSD = 0.0213, $P < 0.05$, $n = 8$). B, Effect of 24 h of flooding on leaf thickness (marker for ψ_L) measured with displacement transducers. Data represent the mean leaf thicknesses from three flooded and three well-drained plants. The experiment was repeated three times with similar results. The flooding treatment started 1 h into the photoperiod.

leaves, compared to that of leaves of well-drained plants, was also apparent during the subsequent photoperiod, corresponding to the less negative ψ_L recorded at this time.

L_p

L_p was estimated for flooded and well-drained root systems every 2 to 4 h during the two photoperiods using slopes of the linear relationships between J_V and ΔP (e.g. Figure 3A). During each photoperiod, well-drained roots showed a daily peak in conductivity that corresponded temporally to the daily peak in transpiration and g_s (Fig. 3B). Flooding suppressed this diurnal rhythm in L_p, although conductivity values were not reduced below their initial early morning value. Thus, during the first 2 to 6 h of flooding, L_p was held lower than in well-drained roots. Throughout the remainder of the first photoperiod, hydraulic conductivities were similar in both treatments (Fig. 3B). By the end of the second photoperiod, the character of the flooding effect had changed fundamentally, giving rise to hydraulic conductivities that exceeded those of well-drained controls.

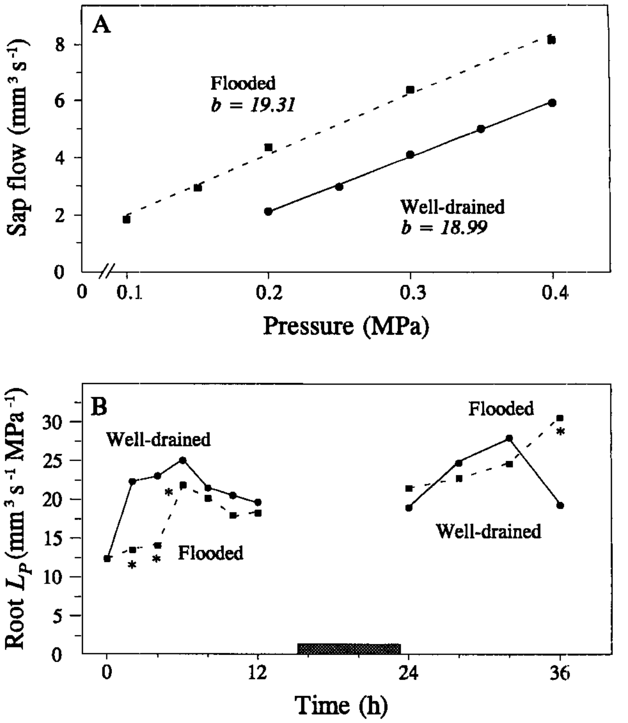

Figure 3. A, Effect of pneumatic pressure applied to the roots on J_V through detopped root systems of well-drained tomato plants and plants flooded for 24 h. B, Effect of flooding on the L_p of detopped tomato root systems during 36 h. L_p was estimated from the slopes of linear relationships between J_V and applied pressure (ΔP). The flow rates generated encompassed the range that occurs in intact plants as transpiration (Fig. 1B). Each point represents a mean of five replicates. ANOVA of the slopes used to generate L_p indicated a SE of difference of 2.486 (75 degrees of freedom). Asterisks (*) represent a significant difference between means ($P < 0.05$).

CO_2 and O_2 Dissolved in Soil Flood Water

CO_2 in soil water increased from near atmospheric equilibrium partial pressure of approximately 0.035 to 8.9 kPa after only 1 h of flooding and continued to increase for the next 24 h, reaching a maximum of 12.4 kPa. Conversely, O_2 partial pressure declined from 20.8 to 7.9 kPa within 1 h and to 6 kPa after 6 h and was at the lower limits of reliable quantification (1 kPa) by 24 h (Table I).

Table I. *Equilibrium partial pressures of dissolved O_2 and CO_2 in flood water taken from soil at various times after the start of flooding*

Partial pressures of CO_2 and O_2 surrounding well-drained roots were assumed to be atmospheric. Means are of five replicates ± SES.

Time	Partial Pressures of Gases in Flood Water	
	CO_2	O_2
h	*kPa*	
1	8.9 ± 0.29	7.9 ± 0.65
6	10.4 ± 0.31	6.1 ± 0.43
24	12.4 ± 0.10	<1.0

Assessing the Physiological Significance of the Decrease in ψ_L

Application of pressure to roots of intact flooded plants allowed the hydrostatic component of xylem sap water potential, and thus ψ_L, to be maintained at constant levels throughout the first 12 h of flooding. Any decrease in ψ_L caused by the temporary reduction in L_p of flooded plants should have been prevented by this treatment. As flooding progressed, the magnitude of the balancing pressure necessary to maintain the hydrostatic pressure of xylem sap close to zero mirrored the transient changes in ψ_L and leaf thickness seen in unpressurized plants (cf. Fig. 4C with Fig. 2). This pattern confirmed the existence of a smaller ψ_L in whole plants during the first few hours of flooding. However, the application of a balancing pressure did not alleviate flooding-induced stomatal closure during the first photoperiod (Fig. 4A). Similarly, the decrease in growth in leaf length caused by flooding was not overcome by applying balancing pressure to the roots (Fig. 4B). Furthermore, the application of balancing pressure during the first photoperiod did not ameliorate the effect of flooding on stomatal behavior and leaf growth during the second (unpressurized) photoperiod. Stomatal behavior of well-drained plants was unaffected by the application of balancing pressure (Fig. 4A) except at 4 and 6 h of the first photoperiod, when g_s of pressurized plants was greater than in unpressurized plants. Rates of leaf extension were similar in well-drained plants irrespective of pressure treatment (Fig. 4B).

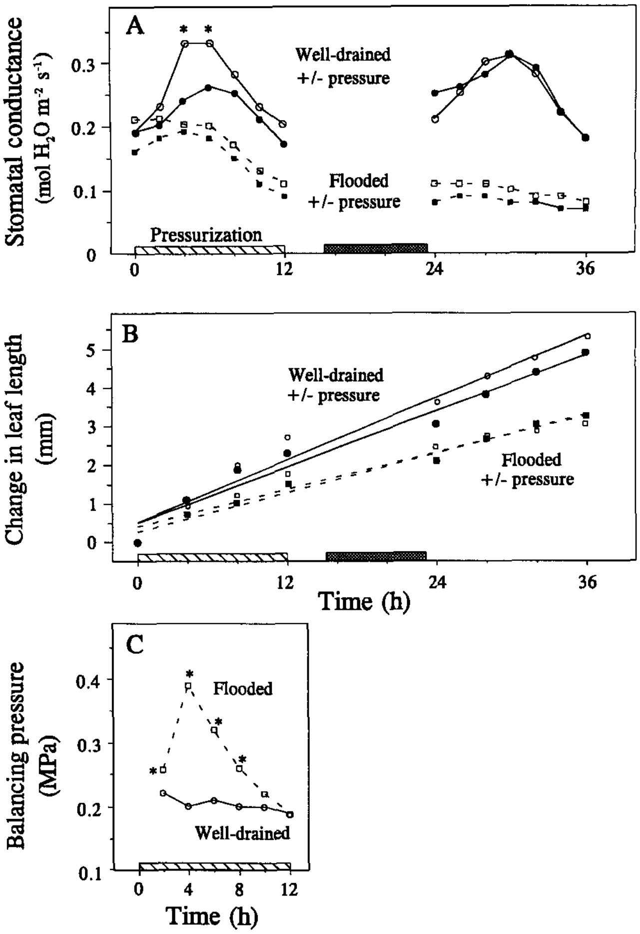

Figure 4. Effects of applying balancing pressures to roots of intact flooded and well-drained tomato plants for 12 h during the first photoperiod on abaxial g_s in the fourth-oldest leaf (A), change in length of the eighth-oldest leaf (B), and the balancing pressure applied to roots required to maintain xylem sap water potentials close to zero throughout the first photoperiod (C). ●, Well-drained, not pressurized; ○, well-drained, pressurized; ■, flooded, not pressurized; □, flooded, pressurized. After 12 h, all plants were removed from the chambers. Asterisks (*) indicate a statistically significant difference ($P < 0.05$). At no time during the 36 h did application of balancing pressures during the first photoperiod decrease the effect of flooding on g_s (LSD of $\log_e$ transformed data = 0.173, $P < 0.05$). There were no marked differences in g_s between well-drained plants given balancing pressure and those aspirated normally except between 4 and 6 h, when g_s of pressurized well-drained plants was statistically greater than in their unpressurized counterparts. Leaf extension rate in flooded plants (23.33 nm s^{-1}) was significantly slower than in well-drained plants (35.28 nm s^{-1}) (SE of difference of $\log_e$ transformed data was 3.806) and was not altered by the application of balancing pressures. Balancing pressures applied to flooded plants were significantly greater than those applied to well-drained plants during the first 8 h of inundation (LSD = 0.04, $P < 0.05$, $n = 8$).

DISCUSSION

We have examined changes in water relationships of tomato plants during the first 36 h of flooding, concentrating on events during the first photoperiod. The questions we addressed were: (a) Does soil flooding depress ψ_Ls and what is the duration and intensity of the effect? (b) Does soil flooding change L_p? (c) What are the consequences of changes in L_p for ψ_L? (d) Is decreased ψ_L responsible for stomatal closure and slowing of leaf expansion during the 1st d of flooding? (e) Does decreased ψ_L of flooded plants during the first photoperiod influence g_s and leaf expansion in the 2nd d of flooding? Answers to these questions determine the extent to which nonhydraulic chemical messages generated by flooded roots need to be invoked to explain the initiation of stomatal closure and slower leaf expansion that occur in flooded plants.

Does Soil Flooding Depress ψ_Ls?

Frequent measurements of ψ_L with the pressure chamber revealed a transient decrease in flooded plants during the first 16-h photoperiod, with a maximum depression of −0.12 MPa after 2 h. A full recovery of ψ_L was achieved by the end of the first photoperiod. During the subsequent photoperiod, ψ_L was maintained approximately −0.1 MPa above that of well-drained plants. This pattern confirms earlier studies under glasshouse or growth-room conditions with flooded tomato (Jackson et al., 1978) and pea plants (*Pisum sativum*) (Jackson and Hall, 1987). However, it contrasts with reports of unchanged ψ_L during flooding (Pereira and Kozlowski, 1977; Zhang and Davies, 1986; Smit and Stachowiak, 1988; Smit et al., 1988), or of marked and sustained decreases in ψ_L (Kramer and Jackson, 1954; Hiron and Wright, 1973), or of the absence of less negative

ψ_Ls following the initial decrease (Schildwacht, 1989). These discrepancies may be explained, in part, by the transient nature of the decline in ψ_L, which would be overlooked if measurements of leaf water status were made at longer intervals (Schildwacht, 1989), by the varying magnitude of the water vapor deficit between the leaf and the atmosphere in the different experiments (Neuman and Smit, 1991), and by the time flooding starts within the photoperiod (Bradford and Hsiao, 1982).

To help resolve some of these contradictions, we sought an alternative, in situ method to monitor changes in leaf water status that (a) was frequent enough to detect short-term changes, (b) would obviate perturbations associated with the leaf excision necessary for pressure bomb measurements, and (c) would avoid the inevitable variation among destructively sampled plants. Leaf thickness can vary with ψ_L and can be measured continuously and nondestructively with high resolution (Malone, 1992). Our measurements of leaf thickness provided additional evidence that ψ_L decreased markedly within 90 min of flooding and then increased steadily to approach, and at later times, exceed that of well-drained plants. This crossover in ψ_L was presumably a consequence of increasingly extensive stomatal closure (see below) and a tendency for L_p to return to and then exceed control levels.

Does Soil Flooding Change L_p?

The effect of flooding during the first 8 h was not to decrease L_p but to dampen the amplitude of the rise and fall seen in well-drained plants as the photoperiod progressed (Parsons and Kramer, 1974). In the second photoperiod, increases in L_p of flooded plants again lagged behind the steep daily increase in well-drained plants. However, by the end of the second photoperiod, the hydraulic conductivity of flooded roots increased sharply to exceed that of well-drained counterparts, indicating a sudden loss of the resistance mechanism. This was probably a result of cell death. Previous claims that flooding quickly decreases hydraulic conductivity to values well below those at the start of flooding may be incorrect. This is because the earlier work used a simple Ohm's law calculation ($L_p = 1/[J_V/\Delta P]$) (Kramer, 1940; Jackson et al., 1978; Bradford and Hsiao, 1982) to compute L_p that is vulnerable to hidden influences of osmotic driving forces or used unrealistically slow J_Vs. If this approach were to be used with our pressure/flow data (Fig. 3A), estimates of L_p would give very different values from those obtained using the slope of the relationship between J_V and ΔP. When we used slopes to calculate L_p, the effect of flooding was seen as a dampening, during the first few hours, of the normal daily increase in L_p. This is in line with the conclusions of Everard and Drew (1987, 1989), although their results were affected by using very slow flow rates that were subject to poorly quantified influences of osmotic driving forces and reflection coefficients.

Increased CO_2 and shortage of O_2 at the roots (Table I) are known to interfere with water uptake by roots (Kramer, 1940; Glinka and Reinhold, 1962; Smit and Stachowiak, 1988). We assume changes in these gases in the flood water (Table I) interfere with the normal increase and decrease of L_p during the 1st d of flooding and induce a temporary depression of conductivity across membranes (Zhang and Tyerman, 1991). Such effects may be mediated by conformational changes in the water channel proteins (aquaporins). These membrane proteins are thought to regulate the passage of water (Chrispeels and Maurel, 1994) symplastically from external to internal apoplastic pools. As flooding injury progresses into the 2nd d, the measured increase in L_p to values that exceed those of well-drained plants is a likely outcome of membrane degeneration. This can be expected as the supply of metabolic energy and unsaturated fatty acid components (Vartapetian et al., 1978) of the lipid bilayers declines because of O_2 deprivation.

Are Changes in L_p Responsible for Early Decreases in ψ_Ls?

Divergence in L_p between well-drained and flooded plants during the first photoperiod paralleled a reduction of ψ_L and leaf thickness in flooded plants. This temporal coincidence suggests a causal relationship. Although some stomatal closure and reduction in transpiration had already started in flooded plants, it was insufficient, at this time, to offset an otherwise inevitable depressing effect of a smaller L_p on ψ_L. During the second photoperiod, stomatal closure in flooded plants was more complete than on the 1st d. The resulting large decrease in transpiration rate, coupled with the recovery of L_p, can account for the less negative ψ_L of flooded plants in the second photoperiod when compared to well-drained plants.

Does the Early Decrease in ψ_L Initiate Stomatal Closure and Slower Leaf Expansion in Flooded Plants?

Flooding the soil partially closed stomata and slowed leaf expansion after 4 h, the close temporal association with lower ψ_L suggesting a causal connection. If verified, this could be interpreted in terms of an effective negative hydraulic message from O_2-deficient roots generated by the suppression of the rhythm in L_p during the early hours of flooding. To test this hypothesis, leaf water status of flooded intact plants was artificially maintained at a constant level by applying a balancing pressure to the roots according to the principles established by Passioura (1984). We assume that these apply even if there is a degree of hydraulic isolation between stomatal guard cells and the rest of the leaf. Applying balancing pressure did not alter patterns of stomatal closure and leaf growth in flooded plants. Thus, although a temporary loss of leaf hydration and ψ_L was unambiguously established during the first few hours of flooding, it was not sufficiently intense or prolonged to influence markedly g_s or leaf growth. Consequently, there must have been some other cause, presumably chemical messages generated directly or indirectly by O_2-deficient roots and sensed by stomatal guard cells and expanding leaf cells. We do not doubt that upon the imposition of a much larger evaporative demand, ψ_L could have been depressed sufficiently to induce stomatal closure and slow leaf growth markedly.

Does the Decrease in ψ_L of Flooded Plants during the First Photoperiod Influence Stomatal Conductivity and Leaf Expansion in the Second Photoperiod?

There remains a possibility that decreased leaf expansion and stomatal closure during the 2nd d of flooding were consequences of the brief decrease of ψ_L on the 1st d. To test this carryover hypothesis, roots of intact plants were pressurized throughout the first photoperiod to nullify the effect of the smaller L_p on ψ_L. However, this had no influence on stomatal behavior or leaf growth during the following day. The lack of effect of early leaf water deficits on later events substantiates the conclusions of Jackson et al. (1978) and Bradford and Hsiao (1982).

Synchrony of Daily Changes in Stomatal Closure and L_p

In well-drained plants, g_ss and transpiration rates peaked about half way through each photoperiod in association with changes in L_p, which increased and then decreased coincidentally during the same time period. This synchrony among L_p, transpiration, and g_s presumably explains the relative consistency of ψ_L throughout each photoperiod. This conclusion is supported by the grafting experiments of Sanders and Markhart (1992), which indicate that L_p can influence ψ_L, at least in drought-treated plants. Without a close coordination between L_p and g_s, ψ_L would necessarily adjust to any mismatch. How the synchrony between stomatal aperture and L_p is achieved is not known. The possibility that L_p increases as water flux increases in response to stomatal opening (the rotameter flow gauge analogy of Tinklin and Weatherley, 1966) seems unlikely, since we found a linear rather than an upwardly curving relationship between driving force and the resultant J_V that the Tinklin and Weatherley analogy would predict (Fig. 3A). This applies even when additional osmotic driving forces generated by soil and xylem sap solutes are taken into account (Jackson et al., 1996). There is evidence that g_ss are linked to L_p by chemical signals transported in the transpiration stream (Meinzer et al., 1991). Whatever the mechanism that coordinates vapor and liquid phase conductivities, it is sufficiently disrupted by a few hours flooding to cause a temporary decrease in ψ_L.

SUMMARY

During the first few hours of soil flooding, the normal daily increase in L_p of tomato plants was dampened. This interfered with synchronization of L_p with g_ss and transpiration rates. The resulting mismatch temporarily decreased ψ_L in flooded plants. However, this was not sufficiently severe or prolonged to initiate stomatal closure or slow leaf expansion. Thus, chemical rather than hydraulic messages generated directly or indirectly by O_2-deficient roots are probably involved. ABA, ethylene, and the ethylene precursor ACC are likely candidates, since they increase in shoots as a result of flooding (Jackson, 1993; Else et al., 1995). Other, as-yet-uncharacterized solutes of transpiration fluid that are active in slowing leaf expansion or closing stomata may also need to be considered (Munns and King, 1988; Trejo and Davies, 1991; Netting et al., 1992; Munns, 1992), as will changes in levels of substances such as nitrate and protons (Gollan et al., 1992) that can affect the activity or partitioning of ABA between apoplast and symplast within the leaf.

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