

# A Negative Hydraulic Message from Oxygen-Deficient Roots of Tomato Plants?<sup>1</sup>

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

Mark A. Else, William J. Davies, Michael Malone, and Michael B. Jackson\*

IACR-Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol BS18 9AF, United Kingdom (M.A.E., M.B.J.); Division of Biological Sciences, University of Lancaster, Lancaster LA1 4YQ, United Kingdom (M.A.E., W.J.D.); and Horticulture Research International Wellesbourne, Warwick CV35 9EF, United Kingdom (M.M.)

---

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 ( $\psi_L$ ) relative to well-drained plants within 2 h. A decrease in  $\psi_L$  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  $\psi_L$  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  $\psi_L$  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  $\psi_L$  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  $\psi_L$  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  $J_v$ s. 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.

---

<sup>1</sup> This work was supported by the Biotechnology and Biological Sciences Research Council (UK) under its Linked Research Group Scheme.

\* Corresponding author; e-mail mike.jackson@bbsrc.ac.uk; fax 441-1275-394-281.

Abbreviations: ANOVA, analysis of variance;  $\Delta P$ , applied pneumatic pressure;  $g_s$ , stomatal conductance;  $J_v$ , sap flow rate;  $L_p$ , root hydraulic conductivity;  $\psi_L$ , 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  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ , 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  $1.2 \times 10^{-3} \text{ m}^3$  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).

### $\psi_L$

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  $0.02 \text{ MPa s}^{-1}$ . Erroneous pressure chamber values caused by bubbling at lower pressures were avoided by blotting the cut surface before applying pressure. No adjustments to  $\psi_L$  were made for xylem sap solute potential. Continuous, indirect estimates of leaf water status, and thus of  $\psi_L$ , were made by monitoring changes in leaf thickness every 30 s throughout 24 h of flooding using displacement transducers capable of detecting changes of  $<1 \mu\text{m}$  (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  $\text{O}_2$ -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  $200 \text{ mm}^3$  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  $2 \times 10^3 \text{ mm}^3$  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  $-20^\circ\text{C}$ .

### $\text{CO}_2$ and $\text{O}_2$ Dissolved in Soil Water

$\text{CO}_2$  partial pressures in  $2 \times 10^{-6} \text{ m}^3$  samples of flood water were measured by head-space analysis using a Pye Unicam (Cambridge, UK) 104 gas chromatograph fitted with a katharometer detector and two stainless steel analytical columns ( $3 \text{ m} \times 3 \text{ mm}$  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  $90^\circ\text{C}$ , with helium as the carrier gas (Hall and Dowdell, 1981). The system was calibrated by injecting  $1 \times 10^{-6} \text{ m}^3$  samples of gas from the gas space above water samples equilibrated with 0, 1, 5, or 10 kPa  $\text{CO}_2$  (British Oxygen Co., London, UK).  $\text{O}_2$  partial pressures in  $10\text{-mm}^3$  samples of flood water were determined with a Pye-Unicam 104 gas chromatograph fitted with a  $^{63}\text{Ni}$  electron capture detector, a stainless steel precolumn ( $50 \times 4 \text{ mm}$  i.d.) containing magnesium perchlorate to remove water, and an analytical column ( $1.5 \text{ m} \times 2 \text{ mm}$  i.d.) maintained at  $110^\circ\text{C}$  and packed with Carbosieve B (Supelco, Bellefonte, PA) (60/80 mesh).  $\text{O}_2$ -free nitrogen was the carrier gas (Hall, 1978).

### Testing Physiological Significance of Decreased $\psi_L$ s

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  $3 \times 10^3 \text{ mm}^2$ ) was removed from leaf 6 of both flooded and well-drained plants, and a 40-mm length of silicon rubber tub-

ing (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

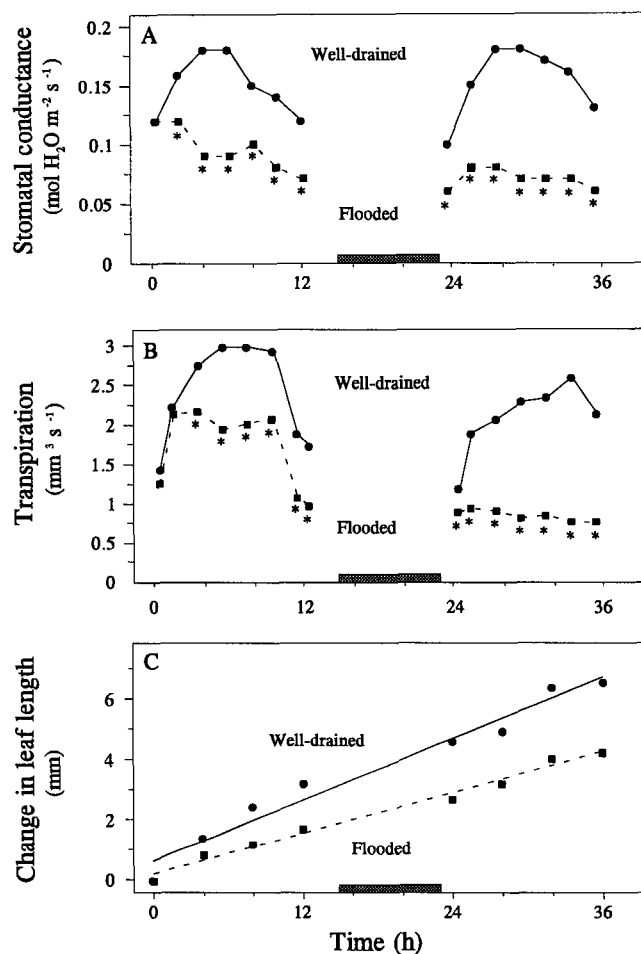
### Leaf Responses to Soil Flooding

$g_s$ s 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).

### $\psi_L$

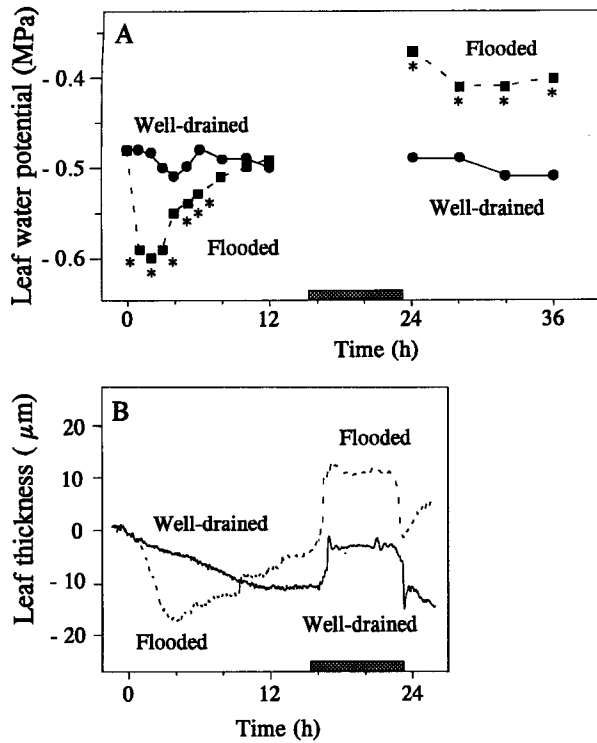
$\psi_L$ s measured with a pressure chamber decreased by approximately  $-0.1$  MPa after only 2 h of flooding (Fig. 2A). Thereafter,  $\psi_L$  recovered slowly until values approximated those of well-drained plants after 8 to 10 h. During the second photoperiod, leaves of flooded plants had significantly higher  $\psi_L$  values than their well-drained coun-



**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.

terparts (Fig. 2A).  $\psi_L$ s of well-drained plants remained at approximately  $-0.5$  MPa throughout both photoperiods.

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  $\psi_L$ . 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  $\psi_L$ . 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

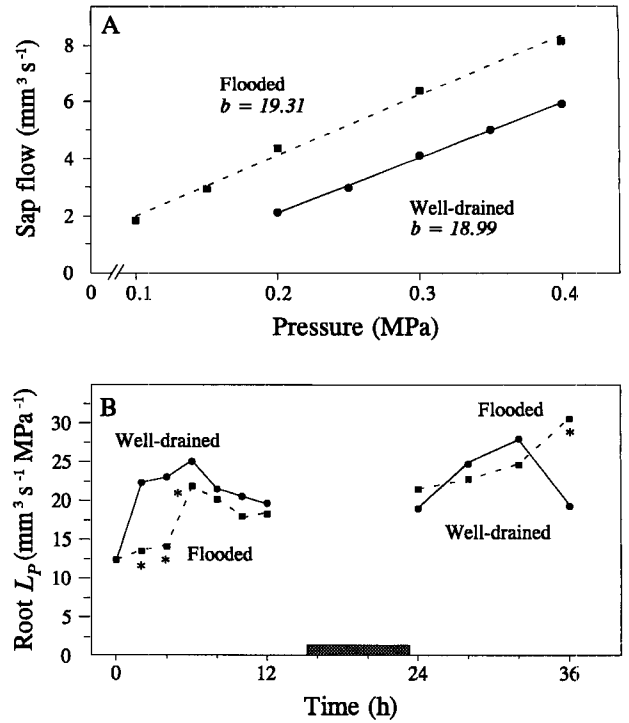


**Figure 2.** A, Effect of soil flooding on  $\psi_L$  of leaf 4 of tomato plants measured at intervals throughout 36 h. No adjustments were made for xylem sap solute potential. Asterisks (\*) indicate significant differences between means (LSD = 0.0213,  $P < 0.05$ ,  $n = 8$ ). B, Effect of 24 h of flooding on leaf thickness (marker for  $\psi_L$ ) 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  $\psi_L$  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  $\Delta P$  (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 ( $\Delta P$ ). 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<sub>2</sub> and O<sub>2</sub> Dissolved in Soil Flood Water**

CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> and CO<sub>2</sub> in flood water taken from soil at various times after the start of flooding

Partial pressures of CO<sub>2</sub> and O<sub>2</sub> surrounding well-drained roots were assumed to be atmospheric. Means are of five replicates  $\pm$  sts.

Time	Partial Pressures of Gases in Flood Water	
	CO <sub>2</sub>	O <sub>2</sub>
<i>h</i>	<i>kPa</i>	
1	8.9 $\pm$ 0.29	7.9 $\pm$ 0.65
6	10.4 $\pm$ 0.31	6.1 $\pm$ 0.43
24	12.4 $\pm$ 0.10	<1.0

### Assessing the Physiological Significance of the Decrease in $\psi_L$

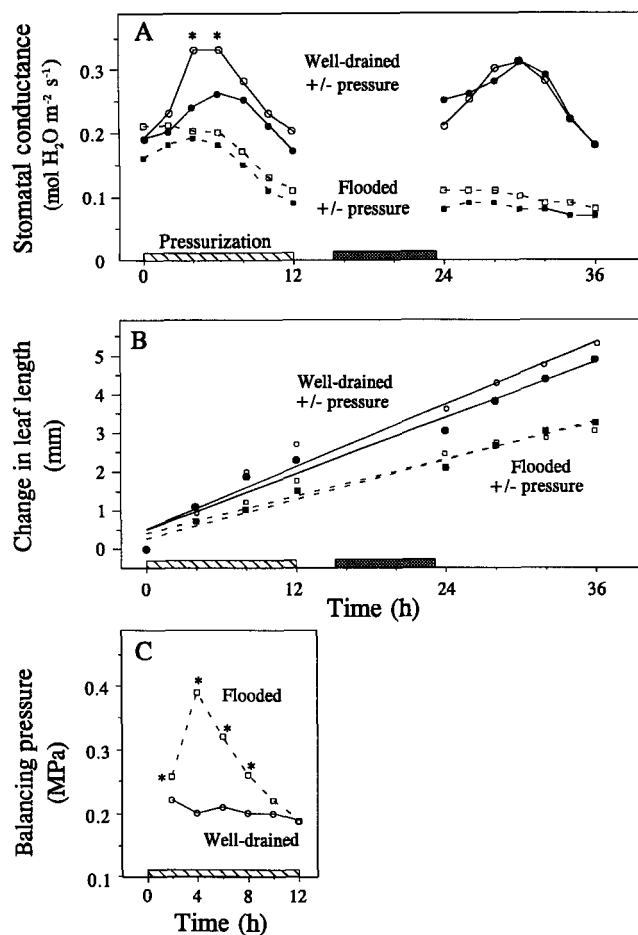
Application of pressure to roots of intact flooded plants allowed the hydrostatic component of xylem sap water potential, and thus  $\psi_L$ , to be maintained at constant levels throughout the first 12 h of flooding. Any decrease in  $\psi_L$  caused by the temporary reduction in  $L_p$  of flooded 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  $\psi_L$  and leaf thickness seen in unpressurized plants (cf. Fig. 4C with Fig. 2). This pattern confirmed the existence of a smaller  $\psi_L$  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).

### 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  $\psi_L$ s 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  $\psi_L$ ? (d) Is decreased  $\psi_L$  responsible for stomatal closure and slowing of leaf expansion during the 1st d of flooding? (e) Does decreased  $\psi_L$  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 $\psi_L$ s?

Frequent measurements of  $\psi_L$  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  $\psi_L$  was achieved by the end of the first photoperiod. During the subsequent photoperiod,  $\psi_L$  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,



**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 ( $23.33 \text{ nm s}^{-1}$ ) was significantly slower than in well-drained plants ( $35.28 \text{ nm s}^{-1}$ ) (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,  $P < 0.05$ ,  $n = 8$ ).

it contrasts with reports of unchanged  $\psi_L$  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  $\psi_L$  (Kramer and Jackson, 1954; Hiron and Wright, 1973), or of the absence of less negative

$\psi_L$ s following the initial decrease (Schildwacht, 1989). These discrepancies may be explained, in part, by the transient nature of the decline in  $\psi_L$ , 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  $\psi_L$  and can be measured continuously and non-destructively with high resolution (Malone, 1992). Our measurements of leaf thickness provided additional evidence that  $\psi_L$  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  $\psi_L$  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  $J_v$ s. 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  $\Delta P$ . 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  $\text{CO}_2$  and shortage of  $\text{O}_2$  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  $\text{O}_2$  deprivation.

#### Are Changes in $L_p$ Responsible for Early Decreases in $\psi_L$ ?

Divergence in  $L_p$  between well-drained and flooded plants during the first photoperiod paralleled a reduction of  $\psi_L$  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  $\psi_L$ . 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  $\psi_L$  of flooded plants in the second photoperiod when compared to well-drained plants.

#### Does the Early Decrease in $\psi_L$ 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  $\psi_L$  suggesting a causal connection. If verified, this could be interpreted in terms of an effective negative hydraulic message from  $\text{O}_2$ -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  $\psi_L$  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  $\text{O}_2$ -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,  $\psi_L$  could have been depressed sufficiently to induce stomatal closure and slow leaf growth markedly.

### Does the Decrease in $\psi_L$ 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  $\psi_L$  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  $\psi_L$ . 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,  $g_s$ s 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  $\psi_L$  throughout each photoperiod. This conclusion is supported by the grafting experiments of Sanders and Markhart (1992), which indicate that  $L_p$  can influence  $\psi_L$  at least in drought-treated plants. Without a close coordination between  $L_p$  and  $g_s$ ,  $\psi_L$  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  $g_s$ s 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  $\psi_L$ .

### 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  $g_s$ s and transpiration rates. The resulting mismatch temporarily decreased  $\psi_L$  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.

### ACKNOWLEDGMENTS

We thank Mr. R.G. Parkinson for plant propagation, Mr. R.S. Cole and Mr. D.J. Mills for construction of split-top pressure chambers, and Ms. G.M. Arnold for help with statistical analyses.

Received March 13, 1995; accepted July 20, 1995.

Copyright Clearance Center: 0032-0889/95/109/1017/08.

### LITERATURE CITED

- Bradford KJ, Hsiao TC (1982) Stomatal behaviour and water relations of waterlogged tomato plants. *Plant Physiol* **70**: 1508–1513
- Chrispeels MJ, Maurel C (1994) Aquaporins. The molecular basis of facilitated water movement through living plant cells. *Plant Physiol* **105**: 9–13
- Dalton FN, Raats PAC, Gardner WR (1975) Simultaneous uptake of water and solutes by plant roots. *Agron J* **67**: 334–339
- Else MA, Davies WJ, Whitford PN, Hall KC, Jackson MB (1994) Concentrations of abscisic acid and other solutes in xylem sap from root systems of tomato and castor-oil plants are distorted by wounding and variable sap flow rates. *J Exp Bot* **45**: 317–323
- Else MA, Hall KC, Arnold GM, Davies WJ, Jackson MB (1995) Export of ABA, ACC phosphate and nitrate from roots to shoots of flooded tomato plants. Accounting for effects of xylem sap flow rate on concentration and delivery. *Plant Physiol* **107**: 377–384
- Everard JD, Drew MC (1987) Mechanisms of inhibition of water movement in anaerobically treated roots of *Zea mays* L. *J Exp Bot* **38**: 1154–1165
- Everard JD, Drew MC (1989) Water relations of sunflower (*Helianthus annuus*) shoots during exposure of the root system to oxygen deficiency. *J Exp Bot* **40**: 1255–1264
- Fiscus EL (1975) The interaction between osmotic- and pressure-induced water flow in plant roots. *Plant Physiol* **55**: 917–922
- Glinka Z, Reinhold L (1962) Rapid changes in permeability of cell membranes to water brought about by carbon dioxide and water. *Plant Physiol* **37**: 481–486
- Gollan T, Schurr U, Schultze E-D (1992) Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. I. The concentration of cations, anions, amino acids in, and pH of, the xylem sap. *Plant Cell Environ* **15**: 551–560
- Hall KC (1978) A gas chromatographic method for the determination of oxygen dissolved in water using an electron capture detector. *J Chromatogr Sci* **16**: 311–313
- Hall KC, Dowdell RJ (1981) An isothermal gas chromatographic method for the simultaneous estimation of oxygen, nitrous oxide, and carbon dioxide content of gases in the soil. *J Chromatogr Sci* **19**: 107–111
- Hiron RWP, Wright STC (1973) The role of endogenous abscisic acid in the response of plants to stress. *J Exp Bot* **4**: 769–781
- Jackson MB (1993) Are plant hormones involved in root to shoot communication? *Adv Bot Res* **19**: 103–187
- Jackson MB, Gales K, Campbell DJ (1978) Effect of waterlogged soil conditions on the production of ethylene and on water relationships. *J Exp Bot* **29**: 183–193
- Jackson MB, Hall KC (1987) Early stomatal closure in waterlogged pea plants is mediated by abscisic acid in the absence of foliar water deficits. *Plant Cell Environ* **10**: 121–130
- Jackson MB, Davies WJ, Else MA (1996) Pressure-flow relationships, xylem solutes and hydraulic conductivity in roots of flooded tomato plants. *Ann Bot* (in press)

- Kramer PJ** (1940) Causes of decreased absorption of water by plants in poorly aerated media. *Am J Bot* **27**: 216–220
- Kramer PJ**, ed (1969) *Plant and Soil Water Relationships: A Modern Synthesis*. McGraw Hill, London
- Kramer PJ, Jackson WT** (1954) Causes of injury to flooded tobacco plants. *Plant Physiol* **29**: 241–245
- Malone M** (1992) Kinetics of wound-induced hydraulic signals and variation potentials in wheat seedlings. *Planta* **187**: 505–510
- Mees GC, Weatherley PE** (1957) The mechanism of water absorption by roots. II. The role of hydrostatic pressure gradients across the cortex. *Proc R Soc Lond B Biol Sci* **14**: 381–391
- Meinzer FC, Grantz DA, Smit B** (1991) Root signals mediate coordination of stomatal and hydrostatic conductivity in growing sugarcane. *Aust J Plant Physiol* **18**: 329–338
- Munns R** (1992) Leaf elongation assay detects an unknown growth inhibitor in xylem sap from wheat and barley. *Aust J Plant Physiol* **19**: 127–135
- Munns R, King R** (1988) Abscisic acid is not the only stomatal inhibitor in the transpiration stream of wheat plants. *Plant Physiol* **88**: 703–708
- Netting AG, Willows RD, Milborrow BV** (1992) The isolation and identification of the prosthetic group released from a bound form of abscisic acid. *Plant Growth Regul* **11**: 327–334
- Neuman DS, Smit BA** (1991) The influence of leaf water status and ABA on leaf growth and stomata of *Phaseolus* seedlings with hypoxic roots. *J Exp Bot* **42**: 1499–1506
- Parsons LR, Kramer PJ** (1974) Diurnal cycling in root resistance to water movement. *Physiol Plant* **30**: 19–23
- Passioura JB** (1984) Hydraulic resistance of plants. I. Constant or variable? *Aust J Plant Physiol* **11**: 333–339
- Passioura JB** (1988) Water transport in and to roots. *Annu Rev Plant Physiol Plant Mol Biol* **39**: 245–265
- Pereira JS, Kozłowski TT** (1977) Variations among angiosperms in response to flooding. *Physiol Plant* **42**: 181–192
- Reece CF, Riha SJ** (1991) Role of root systems of eastern larch and white spruce in response to flooding. *Plant Cell Environ* **14**: 29–34
- Sanders PL, Markhart AH III** (1992) Interspecific grafts demonstrate root system control of leaf water status in water-stressed *Phaseolus*. *J Exp Bot* **43**: 1563–1567
- Schildwacht PM** (1989) Is a decreased water potential after withholding oxygen from the roots the cause of the decline of leaf-elongation rates in *Zea mays* L. and *Phaseolus vulgaris* L.? *Planta* **177**: 178–184
- Smit B, Stachowiak M** (1988) Effects of hypoxia and elevated carbon dioxide concentration on water flux through *Populus* roots. *Tree Physiol* **4**: 153–165
- Smit B, Stachowiak M, Van Volkenburgh E** (1988) Cellular processes limiting leaf growth in plants under hypoxic root stress. *J Exp Bot* **40**: 89–94
- Tinklin R, Weatherley PE** (1966) On the relationship between transpiration rate and leaf water potential. *New Phytol* **65**: 509–517
- Trejo CL, Davies WJ** (1991) Drought-induced closure of *Phaseolus vulgaris* L. stomata precedes leaf water deficit and any increase in xylem sap ABA. *J Exp Bot* **42**: 1507–1515
- Vartapetian BB, Mazliak P, Lance C** (1978) Lipid biosynthesis in rice coleoptiles grown in the presence or in the absence of oxygen. *Plant Sci Lett* **13**: 321–328
- Zhang J, Davies WJ** (1986) Chemical and hydraulic influences on the stomata of flooded plants. *J Exp Bot* **37**: 1479–1491
- Zhang J, Davies WJ** (1987) ABA in roots and leaves of flooded pea plants. *J Exp Bot* **38**: 1649–659
- Zhang WH, Tyerman SD** (1991) Effect of low O<sub>2</sub> concentration and azide on hydraulic conductivity and osmotic volume of the cortical cells of wheat roots. *Aust J Plant Physiol* **18**: 603–613