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Export of Abscisic Acid, 1-Aminocyclopropane-1-Carboxylic Acid, Phosphate, and Nitrate from Roots to Shoots of Flooded Tomato Plants[1]

Accounting for Effects of Xylem Sap Flow Rate on Concentration and Delivery

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We determined whether root stress alters the output of physiologically active messages passing from roots to shoots in the transpiration stream. Concentrations were not good measures of output. This was because changes in volume flow of xylem sap caused either by sampling procedures or by effects of root stress on rates of whole-plant transpiration modified concentrations simply by dilution. Thus, delivery rate (concentration × sap flow rate) was preferred to concentration as a measure of solute output from roots. To demonstrate these points, 1-aminocyclopropane-1-carboxylic acid (ACC), abscisic acid, phosphate, nitrate, and pH were measured in xylem sap of flooded and well-drained tomato (*Lycopersicon esculentum* Mill., cv Ailsa Craig) plants expressed at various rates from pressurized detopped roots. Concentrations decreased as sap flow rates were increased. However, dilution of solutes was often less than proportional to flow, especially in flooded plants. Thus, sap flowing through detopped roots at whole-plant transpiration rates was used to estimate solute delivery rates in intact plants. On this basis, delivery of ACC from roots to shoots was 3.1-fold greater in plants flooded for 24 h than in well-drained plants, and delivery of phosphate was 2.3-fold greater. Delivery rates of abscisic acid and nitrate in flooded plants were only 11 and 7%, respectively, of those in well-drained plants.

When roots are stressed, the amounts of hormone, hormone precursor, or other solutes entering the shoots via the transpiration stream can alter. These changes may constitute physiologically active messages that modify shoot physiology and development (Kende, 1964; Itai and Vaadia, 1965; Jackson and Campbell, 1975; Bradford and Yang, 1980; Davies et al., 1994). These modifications include stomatal closure, slower leaf expansion, and petiole epinasty, among a range of effects of root stresses on target tissues some distance from the signaling root system. Tests of root signaling must include good estimates of amounts of physiologically active solutes entering shoots from root systems at the time developmental responses first take place. With relatively few exceptions (von Wagner and Michael, 1971; Beever and Woolhouse, 1973; Bradford and Yang, 1980; Loveys, 1984; Neumann and Stein, 1984; Munns, 1990; Meinzer et al., 1991; Schurr et al., 1992), solute concentrations in xylem sap have commonly been used to estimate levels of export from roots. Often, the sap analyzed was collected exuding from detopped roots under osmotic forces (Heindle et al., 1982), or displaced from root systems, or shoots, by arbitrary levels of applied suction, pressure, or centrifugal force (Neales et al., 1989; Neuman et al., 1990; Noodén et al., 1990). However, this interpretation of concentration may mislead for at least two reasons (Jackson, 1993). First, concentration values are inherently unstable because, being solvent:solute ratios, they could change with volume flow by dilution independently of solute flux. In many published experiments, flow rates of analyzed sap are slower than whole-plant transpiration, leading to overestimates of concentrations in xylem sap of intact, transpiring plants. Second, even if concentrations are correctly estimated, faster or slower rates of whole-plant transpiration can change the concentration, through dilution effects, even when the amounts of solute actually carried in xylem sap remain unchanged (Jackson, 1991, 1993; Else et al., 1993, 1994).

In the present paper we address these problems. We describe relationships among xylem sap flow rates, concentrations, and delivery rates (concentration × sap flow rate) of ACC, ABA, phosphate, and nitrate in xylem sap and compare flooded and well-drained plants. We show that delivery rates give more realistic estimates of solute entry into the shoot from the root system than do concentrations. Because flow and delivery were not always independent, we estimated deliveries in intact plants using sap flowing at rates of whole-plant transpiration. By these means, we demonstrate that delivery of ACC and phosphate from roots into shoots is increased by 24 h of soil flooding and is augmented by direct entry of these solutes from soil water. Delivery of ABA and nitrate from roots to shoots is shown to be decreased by soil flooding.

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MATERIALS AND METHODS

Plant Material

Seedlings of tomato (*Lycopersicon esculentum* Mill., cv Ailsa Craig) were transplanted into 95-mm-diameter plastic pots filled with general-purpose peat-based compost (Fisons' Levington M3, Ipswich, UK) containing a slow-release fertilizer (Osmocote, 2 kg m^{-3}, Grace Sierra [UK Ltd.], Nottingham, UK) and grown in a Fisons' 1550 controlled environment room. Day/night temperatures were 25 and 20°C, and the photoperiod was 16 h at 400 μmol m^{-2} s^{-1} PPFD with an RH of 50%. All pots were irrigated automatically, and side shoots of the plants were removed regularly. Plants at the seven- to eight-leaf stage were flooded for 24 h by placing the plant pots into larger plastic pots (1.2×10^{-3} m^3) filled with tap water. Flooding times were staggered to ensure that each plant was flooded for 24 h. Water in the flooded pots was maintained 10 mm above soil level. Whole-plant transpiration was determined by loss of weight during 1 to 2 h after evaporative losses from pots without plants were subtracted.

Collection of Xylem Sap and Soil Water

Flooded or well-drained plants were detopped just below the cotyledonary node with a sharp razor blade, and a 20-mm length of rubber sleeving was attached immediately to the stump. The assembly was placed in a pressure chamber designed specifically to collect sap expressed from the cut stump while minimizing damage caused by constriction of the hypocotyl stump (Else et al., 1994). Compressed air or oxygen-free nitrogen were used to pressurize (0.02–0.4 MPa) root systems of well-drained or flooded plants, respectively, thus generating a range of sap flows that included whole-plant transpiration rates. Sap for solute analysis was collected for 10 min at each pressure in Eppendorf tubes or in glass scintillation vials with screw lids. The initial 200 mm^3 of sap from each root system was discarded to avoid contamination that was restricted to this first sample (Else et al., 1994). ACC and ABA concentrations were unchanged in sap flowing at a constant rate from detopped roots for 25 min to 10 h after shoot removal (Else et al., 1994, and our unpublished data). Thus, in our short-term experiments, excising the shoot, and thus removing a potential input from the phloem, did not change hormone output from roots. Immediately after collection, sap samples were weighed and frozen in liquid nitrogen before storing at −20°C. Osmotically driven sap flowing from detopped plants was collected using a 20-mm-long rubber sleeve placed over the hypocotyl and capped by a small rubber stopper threaded onto silicon rubber tubing (1 mm i.d.) that channeled the sap into Eppendorf tubes.

Samples of soil water were withdrawn through ceramic pots (40 × 10 mm) buried in the center of pots of soil prior to planting. These were joined to a 70-mm length of silicon rubber tubing (3 mm i.d.) ending in a Nipro three-way tap (Sansho Medical Industries Ltd., Tokyo, Japan) connected to a 5×10^{-6} m^3 hypodermic syringe. Pots containing plants growing in well-drained soil were immersed for 5 min to enable soil water samples to be taken. After collection, samples were frozen in liquid nitrogen before storage at −20°C.

Assessing Integrity of Flooded Roots Using an Apoplastic Dye

Light Green dye (Light Green SF Yellowish; Aldrich Chemical Co. Ltd., Gillingham, Dorset, UK) moves through healthy plant tissue apoplastically but not symplastically (Epel and Bandurski, 1990). Tomato plants were flooded with dye solution (252.3 mmol m^{-3}) for 24 h, after which time concentration in the soil water had declined to 82.4 mmol m^{-3}. Xylem sap was collected from detopped roots pressurized from 0.2 to 0.4 MPa, as described above. Well-drained plants were immersed in dye solution for 15 min prior to sap collection. Because the dye is unstable when exposed to light and air, samples of xylem sap or soil water were assayed immediately using a Unicam (Pye Unicam, Cambridge, UK) SP1800 UV spectrophotometer at 630 nm.

Solute Analysis

ACC in xylem sap was measured using GC and a nitrogen/phosphorous detector after derivatization to *N*-benzoyl *n*-propyl ACC and sample purification by HPLC. The method and its confirmation by GC-MS were described by Hall et al. (1993). [^{14}C]ACC (CEA, Gif-sur-Yvette, France) was used as an internal standard and *N*-benzoyl *n*-propyl ACC was the UV-absorbing marker for HPLC. *N*-benzoyl isobutyl ACC was synthesized and used as the internal GC-nitrogen/phosphorous detector standard. ACC in soil water was measured by GC-MS.

ABA in xylem sap and soil water was assayed by GC-MS using an OV 1701 capillary column (30 m × 0.22 mm i.d.). Before GC-MS, 5 ng of [$^2H_3$]ABA was added to samples of sap or soil water before they were passed through a Sep-Pak C_{18} cartridge. The dried eluate was taken up in 50 mm^3 of methanol, methylated with ethereal diazomethane, and dried prior to taking it up in 10 mm^3 of ethyl acetate. Ions at *m/z* 190 and 162 (methyl abscisate) and *m/z* 193 and 165 ([*methyl*-$^2H_3$]abscisate) were monitored under conditions described by Whitford and Croker (1991).

Phosphate and nitrate concentrations were determined by anion-exchange chromatography using an IonPac AS4 analytical column (250 × 4 mm i.d.) with an AG4-SC guard column and a Dionex Conductivity Detector-3 (Dionex UK, Ltd., Camberley, Surrey, UK). High background conductivity was lowered prior to chromatography by an Anion Self-Regenerating Suppressor-1 (Dionex [UK] Ltd). A standard solution of anions was chromatographed to establish retention times. Anions were eluted with a buffer containing 2.24 mol m^{-3} sodium hydrogen carbonate and 1.8 mol m^{-3} sodium carbonate (BDH Chemicals, Ltd., Poole, UK) at a flow rate of 2×10^{-6} m^3 min^{-1}.

Acidity of xylem sap was measured in 15-mm^3 samples with a Camlab pH Boy meter (Camlab, Ltd., Cambridge, UK).

Statistical Analyses

Data for xylem sap were analyzed from eight or nine root systems. Effects of increasing pressure on sap flow rates were compared between treatments using analysis of variance after $\log_e$ transformation. Linear or "linear plus exponential" curves were fitted to back-transformed means for sap flow data with respect to pressure for flooded or well-drained treatments, respectively, using approximate logarithmic weighting. For solute analyses, curves of the form $y = ax^b$ were fitted to data from each root; both x and y data were $\log_e$ transformed to increase variance homogeneity and to enable the parameters of the curves to be estimated by linear regression of $\log_e(y)$ on $\log_e(x)$. Well-drained and flooded treatments were then compared by analysis of variance of individual values of the fitted parameters a and b from each root. Back-transformed means were plotted together with "mean" fitted curves given by the means of individual parameters obtained from each root system. Whole-plant transpiration rates were inserted into individual regression equations to estimate concentrations or delivery rates in intact plants. Experimentally derived mean values of b for flooded and well-drained plants were compared against the value of -1 for a theoretical dilution curve, using t tests.

RESULTS

Transpiration and Osmotic Sap Flows

Flooding for 24 h reduced transpiration rates by 70% and slowed osmotically driven sap flow from detopped roots by 58% (Table I). On the other hand, removing the shoot reduced the flow of xylem sap to 8.8% of the transpiration rate in well-drained plants and to 12.5% of the transpiration rate in flooded plants. We investigated the extent to which such differences in flow rate influence xylem sap solute concentrations and deliveries.

Sap Flow from Pressurized Roots

Each root system was subjected to an incremental series of pressures. The resulting range of sap flows included the transpiration rates of flooded and control plants (Fig. 1). Sap flow from well-drained roots was significantly slower than from flooded roots at all applied pressures greater than 0.02 MPa. The shape of response curves of well-drained plants comprised an initial exponential portion followed by a linear portion at higher pressures. In plants flooded for 24 h, the early, curved portion was absent but the slope of the linear portion was similar to that for well-drained plants (Fig. 1). The linear character of this line over the entire range of flows through flooded roots demonstrates that an abnormal pathway for water entry does not set in as flow rates come to exceed those of whole-plant transpiration. In both flooded and well-drained plants, sap increased in alkalinity with flow rate (Fig. 1).

Table I. *Effect of 24 h of soil flooding on whole-plant transpiration and rates of osmotically driven sap flow from detopped tomato root systems*

Means of eight replicate plants (transpiration) or five replicate plants (sap flow), with SE values given.

Treatment	Whole-Plant Transpiration Rate	Rate of Osmotically Driven Sap Flow
	$mm^3\ s^{-1}$	$mm^3\ s^{-1}$
Well-drained	3.51 ± 0.17	0.31 ± 0.02
Flooded	1.04 ± 0.07	0.13 ± 0.01

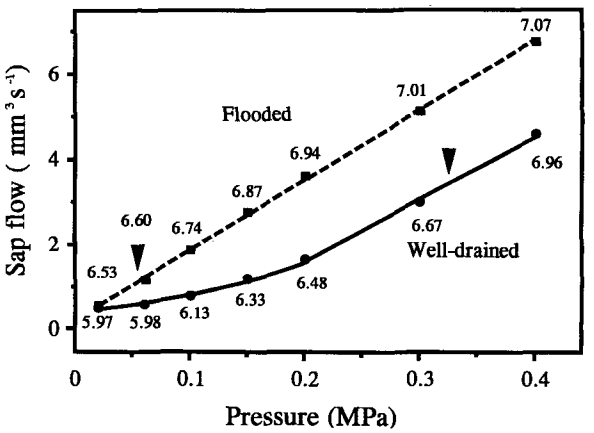

Figure 1. Effect of pressure (0.02–0.4 MPa) on rates of sap flow from roots of well-drained tomato plants and of plants flooded for 24 h. Arrowheads indicate rates of transpiration for comparable intact plants. The pH at the various flows is also given. Well-drained and flooded root systems were pressurized with compressed air and oxygen-free nitrogen, respectively. The initial 200 mm^3 of sap was discarded. Sap was collected for 10 min at each pressure. Linear and "linear plus exponential" relationships were fitted to flooded and well-drained plants, respectively. Mean points are back-transformed from $\log_e$ (Well-drained, $n = 8$; Flooded, $n = 9$).

Effects of Sap Flow Rate on Solute Concentration

ACC concentrations in sap from roots flooded for 24 h were greater than those from well-drained roots over the range of sap flows tested and declined from both flooded and well-drained roots as sap flow rates increased (Fig. 2A). However, dilution was less than that expected on a flow rate basis in all plants. For individual regression curves of the form $y = ax^b$, calculated mean values of the slope b were significantly different from the theoretical value of -1 (well-drained $b = -0.46 \pm 0.173$; flooded $b = -0.72 \pm 0.074$). Transpiration rates of comparable well-drained and flooded whole plants are shown by arrowheads (Fig. 2A). Concentrations at these flow rates are considered to be good estimates of those present in the transpiration stream of whole plants. These concentrations, obtained arithmetically by inserting the transpiration rates into the appropriate regression equations for each plant, showed that 24-h flooding increased ACC concentrations in the transpiration stream 10-fold (Table II).

Phosphate concentrations in xylem sap were increased by flooding at all flow rates (Fig. 2B). Phosphate became more dilute as sap flow was increased. In well-drained plants this dilution was approximately in proportion to flow rate ($b = -0.89 \pm 0.024$), but in flooded plants, dilu-

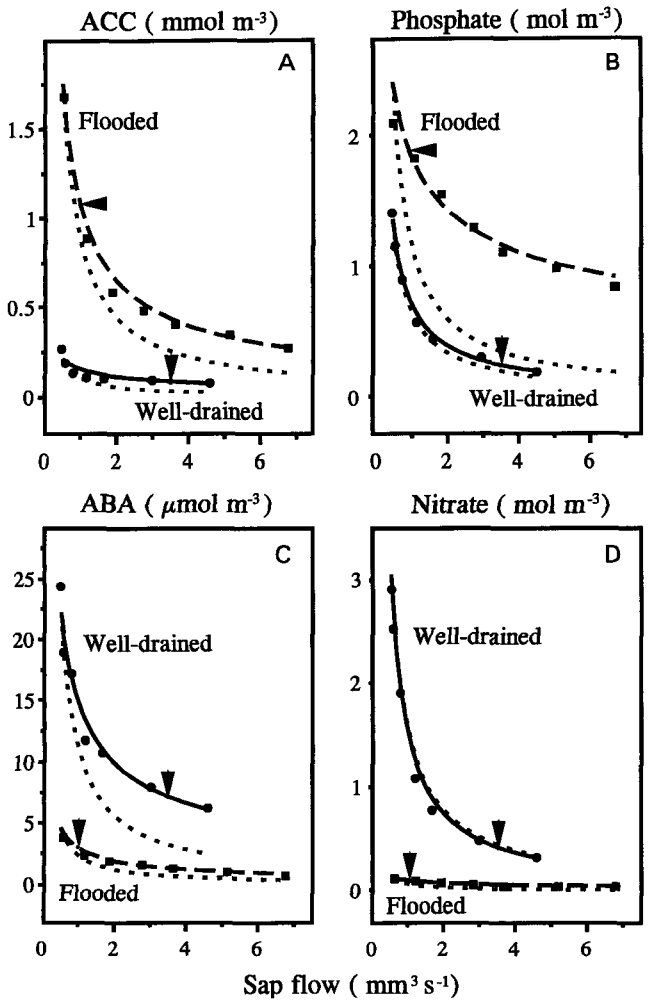

Figure 2. Effects of sap flow rate on concentrations of ACC (A), phosphate (B), ABA (C), and nitrate (D) in xylem sap expressed from roots of well-drained and flooded tomato plants. Back-transformed means are plotted along with curves of the form $y = ax^b$ with "mean" parameters (Well-drained, $n = 8$; Flooded, $n = 9$). Theoretical curves representing dilution of xylem sap solutes in strict proportion to sap flow rates are shown as dotted lines, starting from the slowest sap flow rate. Arrowheads show transpiration rates of comparable intact plants.

tion was not proportional to flow ($b = -0.37 \pm 0.038$). Arrowheads show transpiration rates of comparable intact plants (Fig. 2B). These rates were used to calculate the likely phosphate concentrations in the transpiration stream of intact plants. Flooding for 24 h increased phosphate levels 8-fold in the transpiration stream (Fig. 2B; Table II).

In contrast to ACC and phosphate, concentrations of ABA and nitrate were smaller in sap from the roots of flooded plants at all flow rates compared to well-drained plants (Fig. 2, C and D). ABA was diluted as flow increased, but dilution was less than proportional in both well-drained ($b = -0.58 \pm 0.02$) and flooded plants ($b = -0.65 \pm 0.040$). In well-drained plants, nitrate was diluted in proportion to sap flow ($b = -1.03 \pm 0.030$), but in flooded plants dilution was less than proportional ($b = -0.39 \pm 0.056$) (Fig. 2D). The estimated concentration of ABA in the transpiration stream was decreased by flooding to 41% of control levels, and nitrate decreased to 25% of the concentration in well-drained plants (Table II).

Effects of Sap Flow on Delivery

ACC delivery increased with sap flow, with the effect being more marked in flooded plants (Fig. 3A). This interaction between flow and delivery was the inevitable outcome of dilution being less than proportional as flow rates were increased. To avoid distortions caused by this interaction, estimates of ACC delivery rates in intact plants were made using concentrations in sap flowing at the rate of whole-plant transpiration (arrowheads in Fig. 3A). Table II shows these deliveries to be increased 3-fold by flooding. Unlike that of ACC, delivery of phosphate was little changed by increasing sap flow through well-drained roots; only in flooded plants did delivery increase with flow (Fig. 3B). Deliveries in sap flowing at the rates of whole-plant transpiration are given in Table II. Receipt of phosphate by the shoots of intact plants was increased 2.3-fold by 24-h flooding.

Delivery of ABA increased with sap flow rate in both flooded and well-drained plants (Fig. 3C). Delivery of nitrate was proportional to flow in well-drained plants but not in flooded plants (Fig. 3D). ABA and nitrate deliveries were decreased by flooding at all flow rates (Fig. 3, C and D). Calculations of deliveries made at rates of transpiration (Table II) indicate that shoots of flooded plants would have received one-ninth of the ABA and one-fifteenth of the nitrate entering the shoots of their well-drained counterparts.

Entry of Apoplastic Dye from the Soil Water

When pots of well-drained plants were immersed for 15 min in Light Green solution, the apoplastically mobile dye was undetectable in xylem sap at any of the tested flow rates (data not presented). However, dye did enter xylem sap in plants flooded for 24 h (Fig. 4A) and its delivery rate increased with sap flow (Fig. 4B).

Soil Water

Flooding doubled the concentration of ACC in soil water from 0.23 to 0.52 mmol m^{-3}. In contrast, the concentration of phosphate in flood water (0.34 mol m^{-3}) was only half that obtained from well-drained soil. Concentrations of ABA in soil water were approximately 300 times less than those of ACC and increased by 1 μmol m^{-3} after 24 h of flooding (Table III), whereas concentrations of nitrate in soil water declined from 0.28 to 0.03 mol m^{-3}, a decrease of 90%.

DISCUSSION

Dilution problems beset attempts to estimate solute concentrations in the transpiration stream of whole plants. For example, the rate of osmotically driven flow from detopped plants was only 8.8 to 12.5% of the whole-plant

Table II. *Effect of 24 h of soil flooding on concentrations and delivery rates of various solutes in xylem sap flowing at the rates of whole-plant transpiration*

Means of eight replicates (well-drained) and nine replicates (flooded) with SES are given. Means for well-drained plants were significantly different ($P < 0.001$) from flooded means. Deliveries were calculated by multiplying the concentration by a sap flow rate equivalent to the whole-plant transpiration rate. The effects of flooding, as a percentage of well-drained values, are shown in parentheses.

Solute	Concentration in Xylem Sap		Delivery Rate per Plant	
	Well-drained	Flooded	Well-drained	Flooded
ACC	0.1 ± 0.018 mmol m^{-3}	1.03 ± 0.093 mmol m^{-3} (1030)	0.35 ± 0.067 pmol s^{-1}	1.08 ± 0.097 pmol s^{-1} (308)
Phosphate	0.23 ± 0.011 mol m^{-3}	1.83 ± 0.151 mol m^{-3} (796)	0.84 ± 0.042 nmol s^{-1}	1.91 ± 0.157 nmol s^{-1} (227)
ABA	6.95 ± 0.521 μmol m^{-3}	2.83 ± 0.264 μmol m^{-3} (41)	25.8 ± 1.93 fmol s^{-1}	2.94 ± 0.275 fmol s^{-1} (11)
Nitrate	0.39 ± 0.069 mol m^{-3}	0.096 ± 0.012 mol m^{-3} (25)	1.46 ± 0.257 nmol s^{-1}	0.1 ± 0.013 nmol s^{-1} (7)

transpiration rate (Table I). We have shown that this slower flow would concentrate xylem sap solutes through a lessening of dilution. Consequently, measurements of hormone concentrations made in the slowly flowing sap from detopped plants would grossly overestimate those in the transpiration stream of whole plants. To overcome this, and related difficulties, we pressurized detopped roots of flooded and well-drained plants to generate a range of sap flows that included whole-plant transpiration rates of both flooded and well-drained plants. We then examined the effects of these different sap flows on concentrations of ACC, ABA, phosphate, nitrate, and pH. The resulting changes in concentrations are considered to be those that would occur in xylem sap of whole plants as transpiration rates fluctuate naturally. These concentrations and their associated sap flow rates were also used to estimate delivery rates of solutes from roots to shoots in whole plants. Any adverse effects of shoot removal on root metabolism were minimized by making measurements within 80 min of shoot excision. Tests showed that output of ACC from well-drained roots was unchanged for up to 10 h after the shoots were removed.

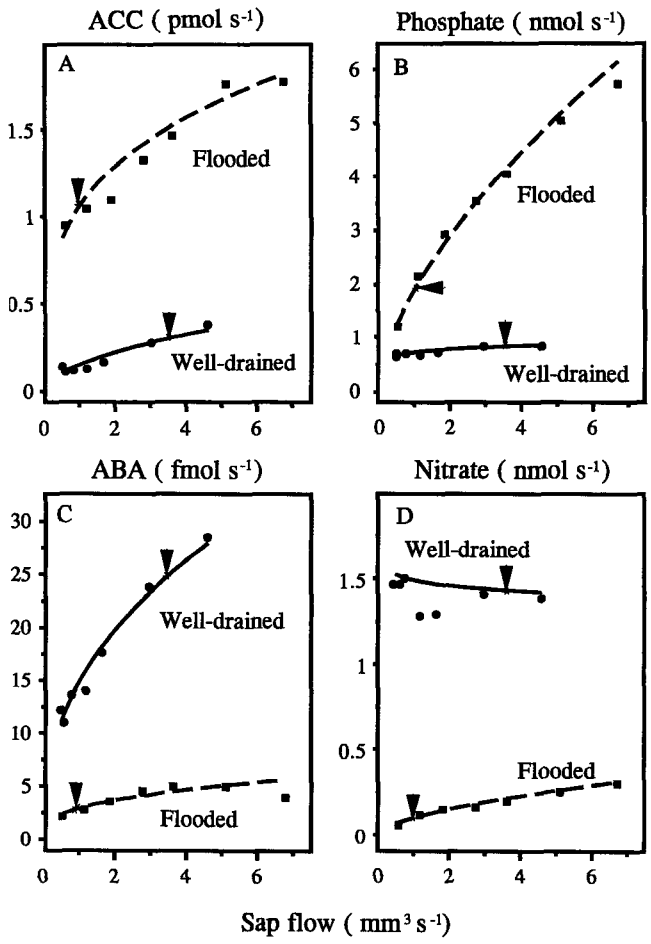

Figure 3. Effect of sap flow rate on delivery of ACC (A), phosphate (B), ABA (C), and nitrate (D) in xylem sap expressed from roots of well-drained tomato plants and plants flooded for 24 h. Back-transformations and mean curves are as described for Figure 2. Arrowheads show transpiration rates of comparable intact plants.

Explaining the Pressure/Flow Response Curves

Sap flow was greater from flooded roots than from well-drained roots at pressures between 0.06 and 0.4 MPa (Fig. 1). Yet, hydraulic conductivities, indicated by the slopes of the linear portions of the curves, were similar, as recently

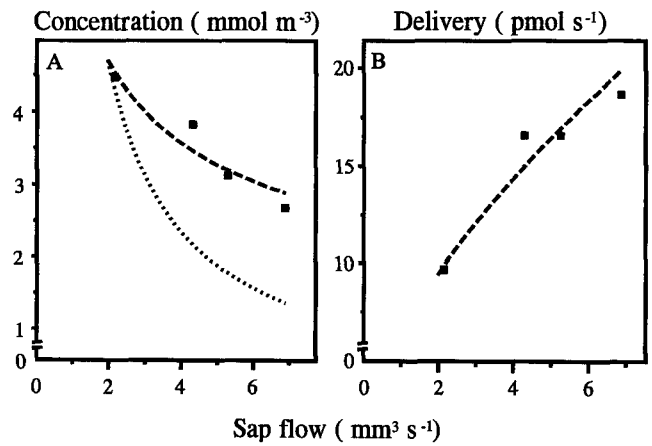

Figure 4. Effects of sap flow on concentration (A) and delivery (B) of apoplastic Light Green dye in xylem sap expressed from root systems of tomato plants flooded for 24 h in a solution of Light Green dye. Concentration of Light Green in soil water was 252.3 mmol m^{-3} at the start and 82.4 ± 2.91 mmol m^{-3} after 24 h of flooding. Back transformations and curves are as described for Figure 2. Dye was not detectable in xylem sap expressed from roots of well-drained plants immersed in 252.3 mmol m^{-3} Light Green solution for 15 min.

Table III. *Effect of 24 h of soil flooding on concentrations of various solutes in soil water*

Means of seven replicates, except for phosphate and nitrate in well-drained soil (five replicates), with SES are given.

Solute	Concentrations in Soil Water	
	Well-drained	Flooded
ACC (mmol m^{-3})	0.23 ± 0.049	0.52 ± 0.062
Phosphate (mol m^{-3})	0.69 ± 0.117	0.34 ± 0.042
ABA (μmol m^{-3})	0.63 ± 0.067	1.67 ± 0.117
Nitrate (mol m^{-3})	0.28 ± 0.050	0.03 ± 0.011

shown by Reece and Riha (1991). This paradox may be explained, in part, by a flood-induced reduction in the reflection coefficient of roots for soil solutes and by a release of solutes into the symplast by cells suffering from oxygen deprivation. These two developments would eliminate the curved flow response (Fiscus, 1977) to low pressures (Fig. 1) by decreasing the water potential of xylem sap, thereby pulling more soil water into the xylem of those flooded roots retaining full semipermeability. The appearance of Light Green dye in expressed xylem sap after 24 h of flooding, its partial dilution as flow rates increased, and its absence from sap of control plants support these conclusions. A more complete explanation must await detailed analysis of pressure-flow curves obtained at various stages of flooding.

Explaining the Dilution Curves

In well-drained and flooded plants, ABA and ACC were diluted less than predicted as sap flow rate was increased, possibly because smaller concentrations in faster-flowing xylem sap steepened the diffusion gradient from source cells into the xylem stream. Faster-flowing sap may also have steepened the diffusion gradient by acting increasingly as an alkaline trap (Hartung et al., 1988), since pH increased with flow rate (Fig. 1). The increasing alkalinity with flow was probably an effect of hydrogen ion dilution. In contrast to ABA and ACC, dilution of phosphate and nitrate was in proportion to sap flow rate (i.e. exponential) in well-drained plants. This may be linked to the dominant role of carrier proteins rather than diffusion or electrical gradients regulating phosphate and nitrate uptake (Lüttge and Clarkson, 1987).

In the oxygen-deficient roots of flooded plants, all measured solutes were diluted less than exponentially (Fig. 2). One contributing factor was probably the entry of ACC, phosphate, ABA, and a little nitrate directly from soil water by mass flow. The presence, in xylem sap, of Light Green dye added to the flood water supports this interpretation. A second contributory influence may have been a flushing-out of solutes from cells suffering loss of osmotic integrity as a consequence of oxygen deprivation.

Concentration versus Delivery

Regression equations describing the relationship between sap flow rate and concentration were used to establish the concentrations in sap flowing at transpiration rates of similar, intact plants. Concentrations of ACC and phosphate in the putative transpiration fluid were increased considerably by 24 h of flooding. In contrast, flooding decreased concentrations of ABA and nitrate. These concentrations are, however, considered to be poor guides to the amounts actually exported by roots into shoots. This is because the reduced rate of transpiration, caused by soil flooding, could have affected concentrations independently of any real change in output from roots. To obtain a better estimate of hormone export, delivery rates were obtained by multiplying sap flow rates by concentration. Surprisingly, few authors have done this (see the introduction). In ideal circumstances, any conveniently contrived sap flow will suffice, since, if concentration decreases in proportion to flow, calculations of delivery at various flow rates will yield the same answer. Unfortunately, ACC and ABA were diluted less than expected in both well-drained and flooded plants and all solutes were diluted less than predicted in flooded plants. Therefore, only deliveries in sap flowing at the rate of transpiration gave close estimates of those taking place in the whole plant. Adopting this approach, we showed significantly greater deliveries of ACC and phosphate from roots flooded for 24 h than from well-drained roots. Conversely, deliveries of ABA and nitrate from oxygen-deficient roots were decreased by flooding. In each instance, the scale of change in output caused by flooding would have been considerably overestimated for ACC and phosphate or underestimated for ABA and nitrate had concentration rather than delivery rate been used. We consider that changes in deliveries occurring as sap flow rates were altered would be those taking place in whole plants when transpiration rates change naturally in response to a wide range of environmental influences.

Explaining the Effects of Flooding on Delivery Rates

Increased delivery of ACC is consistent with details of the biosynthetic pathway and its regulation. ACC oxidation to ethylene in roots is arrested by anaerobiosis, thus leading to an accumulation of unoxidized ACC that finds its way into both the transpiration stream (Bradford and Yang, 1980) and presumably into soil water (Table III). Furthermore, levels of the rate-limiting enzyme ACC synthase and transcription of an ACC synthase gene are enhanced by oxygen shortage (Wang and Arteca, 1992; Zarembinski and Theologis, 1993), which in turn may increase the accumulation of ACC. The presence of mmol m^{-3} levels of ACC in flood water and the permeability of flooded roots to apoplastically mobile Light Green dye suggest that soil water also contributes to the ACC flux in xylem sap. Assuming that the permeability of flooded roots to ACC and Light Green dye is similar, we estimate that 3% of ACC carried in the transpiration stream entered directly from flood water by mass flow.

Decreases in ABA delivery from flooded roots are compatible with the requirements for oxygen in ABA biosynthesis (Zeevaart et al., 1989). The hormone carries oxygen atoms at four positions and is formed from a carotenoid-derived precursor, violaxanthin. Oxygen is required for carotenoid synthesis, for the cleavage of violaxanthin into

xanthoxin and the immediate precursor ABA aldehyde and for the final step generating the acid from the aldehyde (Walton, 1987; Parry and Horgan, 1992). Since pots of flooded soils such as those used in our experiments can become anaerobic within 24 h (Jackson, 1979), root synthesis of ABA would be curtailed after this time. This result is contrary to two previous reports of ours (Jackson, 1993; Jackson et al., 1993) showing an increase in output of ABA from flooded roots. These earlier data are incorrect and arose because of nonspecific binding in the immunoassay used for ABA analyses in flooded plants.

Increased delivery of phosphate in xylem sap of flooded plants may be due to release of stored phosphorus from root cell vacuoles (Lee and Ratcliffe, 1983) as tonoplast integrity is lost because of ATP starvation and cell acidosis (Zhang and Greenway, 1994). Consequently, phosphate accumulates in leaves of flooded plants (Jackson, 1979). The small nitrate delivery in xylem sap of flooded plants reflects both reduced capacity for uptake by flooded roots and decreased nitrate availability in flood water, because of microbial denitrification (Gambrell et al., 1991) and dilution by flood water.

SUMMARY

Our evidence shows that account should be taken of the diluting effect of sap volume flow when estimating solute concentrations in the transpiration stream of whole plants. We also show that delivery rates are preferable to concentrations for estimating the amount of a hormone or other solute entering the shoot from the root system. Since delivery rates are seldom independent of volume flow, particularly in flooded plants, they are best calculated from concentrations in sap flowing at rates of whole-plant transpiration and expressed in terms of the amount of hormone delivered in the transpiration stream per unit time (e.g. mol s^{-1}). Such delivery rates will need to increase or decrease if positive or negative messages sent to shoots by root systems of stressed plants are to be established unambiguously. By these means, we have shown that delivery of ACC and phosphate from roots into shoots is increased by 24 h of soil flooding and delivery of ABA and nitrate is strongly inhibited.

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