

RESEARCH PAPER

# Root water potential integrates discrete soil physical properties to influence ABA signalling during partial rootzone drying

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## Abstract

To investigate the influence of different growing substrates (two mineral, two organic) on root xylem ABA concentration ( $[ABA]_{\text{root}}$ ) and the contribution of the drying root system to total sap flow during partial rootzone drying (PRD), sunflower (*Helianthus annuus* L.) shoots were grafted onto the root systems of two plants grown in separate pots. Sap flow through each hypocotyl was measured below the graft union when one pot ('wet') was watered and other ('dry') was not. Each substrate gave unique relationships between dry pot matric potential ( $\Psi_{\text{soil}}$ ), volumetric water content ( $\theta_v$ ) or penetrometer resistance ( $Q$ ) and either the fraction of photoperiod sap flow from roots in drying soil or  $[ABA]_{\text{root}}$ . However, decreased relative sap flow, and increased  $[ABA]_{\text{root}}$ , from roots in drying soil varied with root water potential ( $\Psi_{\text{root}}$ ) more similarly across a range of substrates. The gradient between  $\Psi_{\text{soil}}$  and  $\Psi_{\text{root}}$  was greater in substrates with high sand or peat proportions, which may have contributed to a more sensitive response of  $[ABA]_{\text{root}}$  to  $\Psi_{\text{soil}}$  in these substrates. Whole plant transpiration was most closely correlated with the mean  $\Psi_{\text{soil}}$  of both pots, and then with detached leaf xylem ABA concentration. Although  $\Psi_{\text{root}}$  best predicted decreased relative sap flow, and increased  $[ABA]_{\text{root}}$ , from roots in drying soil across a range of substrates, the inaccessibility of this variable in field studies requires a better understanding of how measurable soil variables ( $\Psi_{\text{soil}}$ ,  $\theta_v$ ,  $Q$ ) affect  $\Psi_{\text{root}}$ .

**Key words:** ABA, grafting, irrigation scheduling, partial rootzone drying, penetrometer resistance, root water potential, sap flow, soil matric potential.

## Introduction

Although irrigation is widely used in many agricultural systems to stabilize yield, there is increasing concern about its sustainability in the face of changing rainfall patterns, over-abstraction from many watersheds, and competition with other users. In the future, irrigated crops will probably receive less water than considered optimal and thus be grown with 'deficit irrigation'. Partial rootzone drying (PRD) was conceived as a deficit irrigation technique specifically to manipulate plant root-to-shoot signalling to improve agricul-

tural water use efficiency (Dry *et al.*, 1996; Stoll *et al.*, 2000, Kang and Zhang, 2004). PRD distributes water unevenly to the root system such that only part is irrigated to supply sufficient water to the shoots to prevent water deficits (Sobeih *et al.*, 2004), while the remainder dries the soil to produce chemical signals such as abscisic acid, ABA (reviewed in Dodd, 2005), that are transported in the xylem to the shoot partially to close the stomata, thus restricting transpiration and limiting vegetative growth. To sustain

Abbreviations:  $F_{\text{dry}}$ ,  $F_{\text{wet}}$ , fraction of total sap flow from the dry and wet parts of the root system;  $\Psi_{\text{leaf}}$ ,  $\Psi_{\text{root}}$ ,  $\Psi_{\text{soil}}$ , leaf, root, and soil water potential;  $\Psi_{\text{dry}}$ ,  $\Psi_{\text{wet}}$ , soil water potential of the dry and wet pots; PRD, partial rootzone drying;  $Q$ , penetrometer resistance;  $Q_f$ , heat loss by convection by the sap;  $\theta_g$ ,  $\theta_v$ , gravimetric and volumetric soil water content;  $[X\text{-ABA}]_{\text{leaf}}$ ,  $[X\text{-ABA}]_{\text{root}}$ , leaf and root xylem ABA concentration;  $[X\text{-ABA}]_{\text{dry}}$ ,  $[X\text{-ABA}]_{\text{wet}}$ , root xylem ABA concentration from the dry and wet parts of the root system.

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effective chemical signalling to the shoots, it is necessary to maintain water uptake from, and sap flow through, the roots in drying soil, otherwise the fraction of total sap flow derived from these roots in drying soil decreases with soil water potential (Yao *et al.*, 2001; Dodd *et al.*, 2008a, b), limiting signal transport to the shoots.

There is little quantitative information on the dependence of sap flow from the roots in drying soil during PRD and its influence on xylem ABA concentration. Consequently, a novel grafting procedure was developed (Dodd, 2007) to determine the contributions of different parts of the root system to total sap flow and leaf xylem ABA concentration. Sap flow from roots in drying soil declined with soil matric potential ( $\Psi_{\text{soil}}$ ) during PRD. When soil water status of the wet part of the root system remained high ( $\Psi_{\text{soil}} > -1$  kPa), there was an optimal soil water status of the dry part of the root system to maximize ABA export from the entire root system (Dodd *et al.*, 2008a). Further soil drying, although increasing the ABA concentration of xylem sap emanating from those roots, decreased total ABA export to the shoot as the fraction of sap flow from roots in drying soil became less than 20% of total sap flow (Dodd *et al.*, 2008a, b). Practical mitigation of this problem relies on alternating the wet and dry parts of the rootzone (Dodd *et al.*, 2006), although the soil water status at which such alternation events should occur has not been defined physiologically.

While this framework of analysis may prove useful to schedule PRD irrigation within a particular substrate, horticultural industries commonly use a range of substrates both mineral and organic. Since previous work used two different substrates with high organic matter contents (Dodd *et al.*, 2008a, b), the work reported here also grew plants in predominantly mineral substrates with low organic matter contents, to maximize variation in soil physical properties. Debate continues as to the utility of soil volumetric water content and matric potential in describing plant responses to drying soil (Sinclair, 2005, and references therein). Soil drying also increases soil strength, and a recent study suggested that the yield of wheat grown under factorial combinations of soil compaction and soil drying was better explained by season-accumulated penetrometer resistance than matric potential (Whalley *et al.*, 2008). The use of organic substrates that are thought to remain relatively weak as they dry provided an opportunity to assess the impacts of soil strength and matric potential in determining physiological variables. This study aimed to determine whether relationships between sap flow from roots in drying soil or root xylem ABA concentration and various soil physical properties (volumetric water content, matric potential, and penetrometer resistance) or plant variables (root water potential) were unified across different substrates.

## Materials and methods

### Characterization of growing substrates

Four different substrates were sourced for these experiments: sand (Redhill-T, J Wylie and sons, UK), a peat-based substrate

(Levingtons M3, Levington Horticulture Ltd., Ipswich, UK; Dodd *et al.*, 2008a) hereafter designated 'peat', an organic loam (John Innes No. 2, J Arthur Bowers, UK; Dodd *et al.*, 2008b), and a field soil (Warren Field; Whalley *et al.*, 2008) hereafter designated 'clay loam'. Moisture release curves were determined via the pressure plate technique and van-Genuchten models (Table 1) fitted to each curve (Fig. 1a, b), enabling direct measurements of gravimetric soil water contents ( $\theta_g$ ) to be converted into soil matric potentials ( $\Psi_{\text{soil}}$ ). Volumetric water content ( $\theta_v$ ) was determined by multiplying gravimetric soil water content by bulk density.

To estimate soil strength, 0.43 l pots of each substrate were prepared by watering to the drip point. These were equilibrated on tension tables held at 0, 10, 30, and 50 kPa pressure. Penetrometer resistance was measured with a 2 mm diameter penetrometer which had a 60° cone angle (Whalley *et al.*, 2005). Penetrometer resistance ( $Q$ ) for each substrate (Fig. 1c, d) was described by the relationships:  $Q = 1.05317 - 1.78052\theta_g$  ( $P = 0.005$ ;  $r^2 = 0.56$ ) for clay loam,  $Q = 1.11867 - 2.85625\theta_g$  ( $P < 0.001$ ;  $r^2 = 0.95$ ) for sand,  $Q = 1.01764 - 0.99180\theta_g$  ( $P = 0.007$ ;  $r^2 = 0.41$ ) for the organic loam, and  $Q = 1.30234 - 0.22540\theta_g$  ( $P = 0.03$ ;  $r^2 = 0.30$ ) for peat. These equations allowed  $Q$  to be estimated from direct measurements of  $\theta_g$  (see below). In common with all soils, the penetrometer pressure decreases with increasing water content (Whalley *et al.*, 2007). The range in penetrometer pressures was similar for all substrates reported in this paper, but relationships with soil moisture differed. For the mineral soils (clay loam, sand, and organic loam) the scatter in penetrometer pressure is similar to previously published data (Whalley *et al.*, 2005, 2007), but for peat the scatter appears greater, which is presumably an intrinsic characteristic of the material due to compressed fibrous material and bark/twigs.

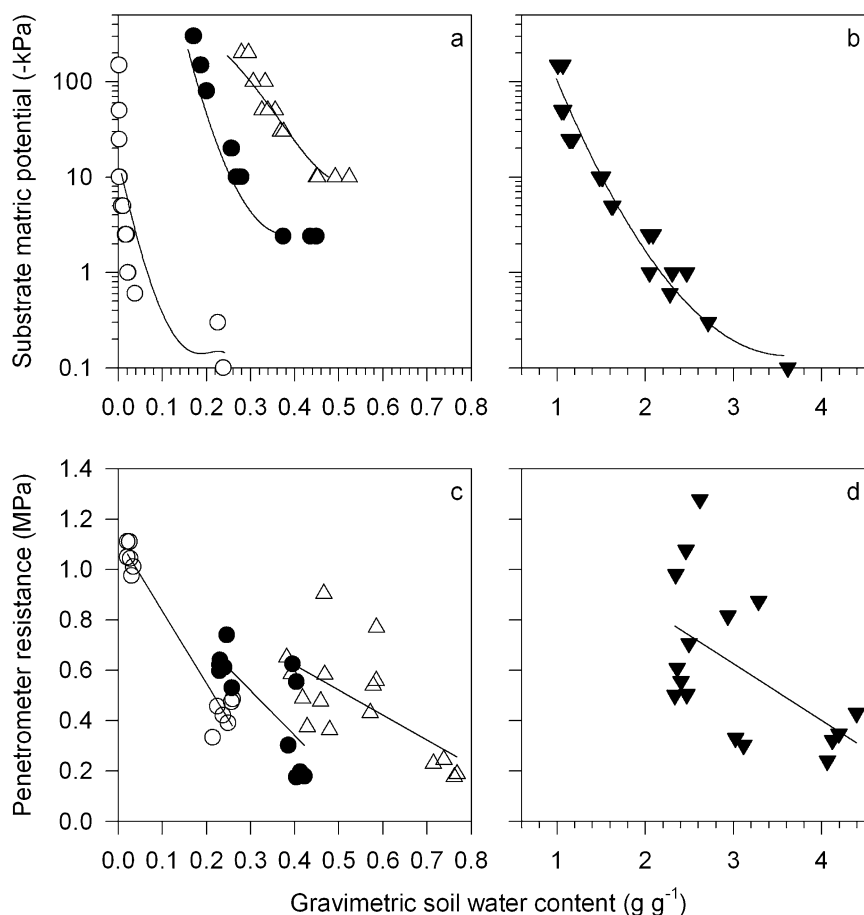
### Plant culture

'Two root-one shoot' sunflower (*Helianthus annuus* L. cv. Tall Single Yellow) plants were created using the grafting procedure previously described (Dodd, 2007; Dodd *et al.*, 2008a, b). Plant culture was as previously described (Dodd *et al.*, 2008a, b) with plants grown in different substrates (Table 1). Seeds were planted into 0.43 l pots (13 cm height, 6.5 cm diameter), then placed into a container, the top of the container covered with aluminium foil (to exclude light and promote hypocotyl extension), and the container placed in a single walk-in controlled environment room (3×4 m) at the Lancaster Environment Centre under the environmental conditions described previously (Kudoyarova *et al.*, 2007). After 1 week, the aluminium foil was removed and the plants allowed to grow for a further 2 weeks before 'two root-one shoot' grafting was implemented as described previously (Dodd, 2007). The grafted plants, resembling an inverted 'Y', were placed in a plastic bag, which was secured around the base of the pots with a rubber band, and allowed to establish for 2 weeks. At the end of this period, plastic bags were removed and the plants allowed to grow for a further 2 weeks. Plants were not watered for 1 week

**Table 1.** Substrate physical parameters including van-Genuchten equation parameters (derived from curves fitted in Fig. 1a, b)

where  $\rho$  is the bulk density,  $\theta_s$  is the saturated water content,  $\theta_r$  is the residual water content,  $\alpha$ ,  $n$ , and  $m$  are parameters specific to the van-Genuchten equation, and  $r^2$  indicates the goodness-of-fit of the van-Genuchten equation

Substrate	$\rho$ (g cm <sup>-3</sup> )	$\theta_s$ (g g <sup>-1</sup> )	$\theta_r$ (g g <sup>-1</sup> )	$\alpha$	$n$	$m$	$r^2$
Sand	1.577	0.2412	0.0072	2.3350	6.3092	0.8415	0.99
Peat	0.165	4.7096	0.5738	26.1532	1.2773	0.2171	0.97
Organic loam	1.004	0.5527	0.0416	0.1227	1.0842	0.0777	0.89
Clay loam	1.180	0.4126	0.1456	0.2977	1.6843	0.4063	0.96



**Fig. 1.** Relationship between soil matric potential (a, b) and penetrometer resistance (c, d) and gravimetric soil water content for clay loam (filled circles), sand (open circles), peat (filled inverted triangles), and organic loam (open triangles). Each point represents a single soil sample. Data in (a) for the clay loam and organic loam are replotted from Whalley *et al.* (2008) and Dodd *et al.* (2006), respectively.

after planting and while enclosed in plastic bags, but otherwise were watered daily with tap water. Plants grown in clay loam or sand were irrigated with a commercial nutrient solution (16:10:27 N:P:K ratio, Wonder-Gro, Wilkinson's, UK) while those in organic substrates received no additional fertilizer. Plants from all treatments grew well, with no visible symptoms of nutrient deficiency. Two batches of plants per substrate (comprising 10–12 plants per batch) were sequentially produced in the same environmental conditions. Partial rootzone drying was imposed by allowing one pot to dry while the other was watered at different frequencies (minimum of twice a day) in different plants, whilst aiming to keep this pot well watered.

Sap flow through each hypocotyl (below the graft union) of the 'two root-one shoot' plants was measured using the heat balance technique with commercially available sensors (Model SGA-5, Dynagage®, Dynamax Inc, Houston, TX, USA) as previously described by Dodd *et al.* (2008a). Sap flow of each hypocotyl was expressed as a fraction of the initial values obtained. Since each plant was placed on a balance which continuously logged pot weight, the effect of PRD on transpiration was determined as the ratios of the slopes of the pot weight versus time relationship at the beginning and end of each experiment (Fig. 2b). Soil water content of each pot, root water potentials, and (root) xylem ABA concentration, whole plant transpiration rate, leaf water potential, and (leaf) xylem ABA concentration were measured as described previously by Dodd *et al.* (2008a). Xylem sap ABA concentration was determined using a radioimmunoassay (Quarrie *et al.*, 1988). To collect xylem sap, an overpressure of 0.4 MPa or 0.5 MPa was applied to the leaves or root systems, respectively (following

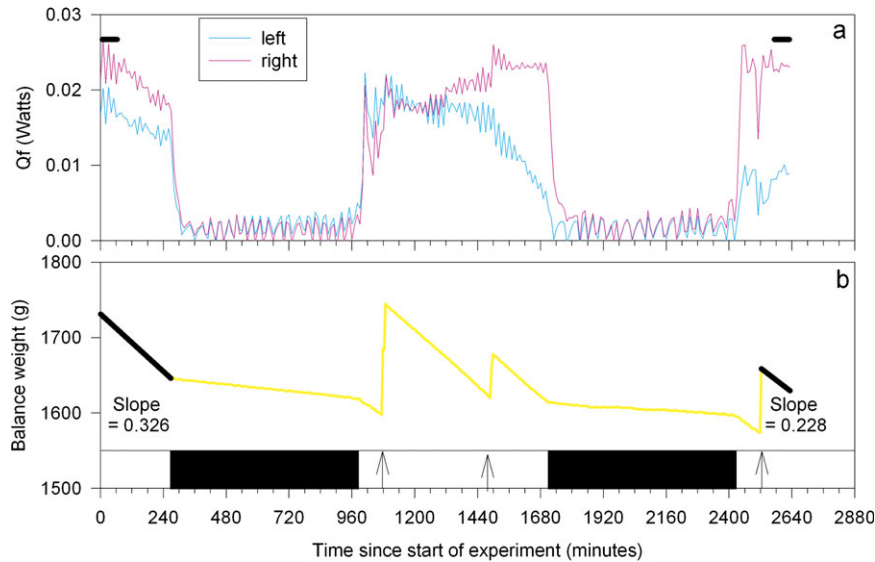
measurement of leaf and root water potential, respectively), allowing xylem sap to be collected in pre-weighed Eppendorf vials to determine sap flow rates. Pressure-induced sap flow rates from roots were compared with those calculated from the product of whole plant transpiration rate and the fraction of sap flow rate through each hypocotyl. Although pressure-induced and calculated *in vivo* sap flow rates did not exactly match, the relative error was statistically equivalent across all substrates (data not shown). Across all plants in this study, the sum of pressure-induced flow rates (from both wet and dry root systems) was 95% of the whole plant transpiration rate. After removal of each pot from the chamber, the substrate (including roots) was removed from the pot, weighed, oven-dried, then weighed again to estimate gravimetric soil water content.

#### Statistical analysis

Regression analysis determined the significance of the relationships between soil and plant variables (Table 2). Analysis of parallelism determined the probabilities that relationships between soil and plant variables had different intercepts and slopes (Table 3).

## Results

In a typical 'two root-one shoot' plant grown in clay loam and subjected to partial rootzone drying (Fig. 2a), sap flow through the two hypocotyls was most similar at the



**Fig. 2.** Absolute sap flow through the two hypocotyls (a) and pot weight (b) of a ‘two root-one shoot’ grafted plant grown in clay loam and exposed to partial rootzone drying. In (a), horizontal black bars on the upper x-axis indicate the periods when sap flow was measured to calculate the fraction of the initial sap flow. In (b), periods of the weight versus time relationship used to calculate relative transpiration rate are indicated by thicker lines, irrigation events supplied to the right pot are indicated as arrows, and horizontal black bars on the lower x-axis indicate the night periods.

**Table 2.** Characterizing relationships between the fraction of initial sap flow through the dry part of the root system and  $\Psi_{dry}$  (Fig. 3b) Peat and organic loam data were replotted from Dodd et al. (2008a) and Dodd et al. (2008b), respectively.

Substrate	$\Psi_{wet}$ (kPa)	Intercept	Slope	P value	$r^2$
Sand	$-0.4 \pm 0.0$	$0.76 \pm 0.16$	$-0.77 \pm 0.45$	0.14	0.37
Peat	$-0.6 \pm 0.2$	$0.57 \pm 0.07$	$-0.0058 \pm 0.0020$	0.016	0.45
Organic loam	$-54 \pm 12$	$0.88 \pm 0.05$	$-0.0225 \pm 0.003$	0.007	0.87
Clay loam	$-10 \pm 1.4$	$1.02 \pm 0.22$	$-0.0087 \pm 0.0034$	0.062	0.62

beginning of the experiment when soil water content of both pots was similar (data collection began after both pots had been watered to the drip point). In the hour after installation of the sap flow gauges, heat losses by convection by the sap ( $Q_f$ ) from the left and right hypocotyls were 18.1 mW and 23.4 mW, respectively. During the night-time,  $Q_f$  was only 12% of that obtained during the photoperiod, and oscillated with changes in air temperature (and vapour pressure deficit) within the controlled environment room (data not shown). A significant divergence in sap flow between the two hypocotyls became apparent after the second irrigation of the wet pot, with sap flow from the dry part of the root system sharply declining. This difference was still apparent at the beginning of the third photoperiod, with  $Q_f$  from the left and right hypocotyls at harvest being 9.1 mW and 23.0 mW, respectively. Thus the fractions of initial sap flow through dry and wet root systems were 0.50 and 0.98, respectively (Fig. 2a). The effect of PRD on transpiration was determined as the ratios of the slopes of the pot weight versus time relationship at the beginning and end of the experiment (Fig. 2b). Since initial and final transpiration

**Table 3.** Statistical analysis of the relationships between relative sap flow (Fig. 3) and xylem ABA concentration (Fig. 5) and soil variables

Analysis of parallelism was used to determine the probabilities that relationships between soil and plant variables had different intercepts and slopes. For the sap flow data (Fig. 3), the variance was stable with substrate and relevant x-axis, while for the ABA data (Fig. 5), a  $\log_{10}$  transformation was used to stabilize variance to make them approximately additive. Differences in relationships in Fig. 5d were entirely due to the organic loam.

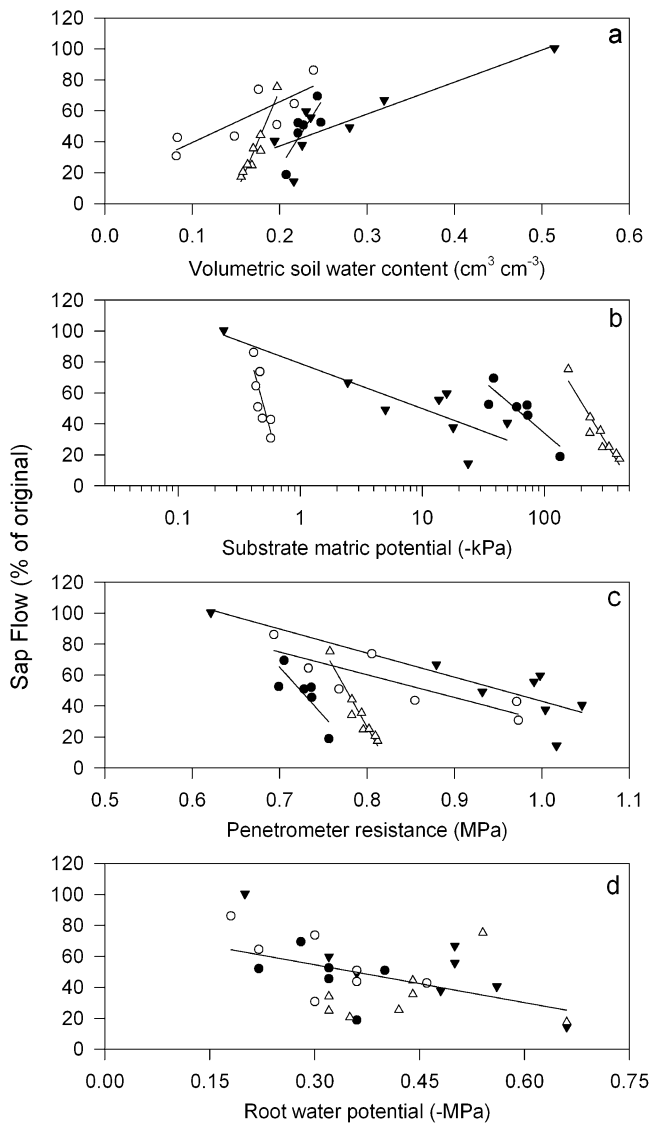
	x-variable	Probability of different intercepts	Probability of different slopes	Conclusion
Fig. 3a	$\theta$	0.006	0.005	Separate relationships
Fig. 3b	$\text{Log}(\Psi_{soil})$	0.007	0.001	Separate relationships
Fig. 3c	Q	<0.001	0.005	Separate relationships
Fig. 3d	$\Psi_{root}$	0.19	0.1	Common relationship
Fig. 5a	$\theta$	0.01	<0.001	Separate relationships
Fig. 5b	$\text{Log}(\Psi_{soil})$	<0.001	<0.001	Separate relationships
Fig. 5c	Q	<0.001	<0.001	Separate relationships
Fig. 5d	$\Psi_{root}$	0.08	0.014	Separate relationships

rates were  $0.326 \text{ g min}^{-1}$  and  $0.228 \text{ g min}^{-1}$ , the relative transpiration rate for this plant (see Fig. 6) was 0.70.

At harvest, a range of soil and plant variables were quantified for each plant. For the plant in Fig. 2, soil water contents of the wet and dry pots were 0.26 and 0.18  $\text{g g}^{-1}$  respectively, root water potentials were  $-0.16$  and  $-0.40$  MPa, respectively, root xylem ABA concentrations were 15.2 and 64.7 nM, respectively, and leaf water potential ( $\Psi_{leaf}$ , the average of two leaves) was  $-0.95$  MPa, and  $[\text{X-ABA}]_{leaf}$  (average of two leaves) was 8 nM. This type of experiment was repeated with 7, 8, 8, and 6 plants grown in

sand, peat, organic loam, and clay loam, respectively, thus developing relationships between soil and plant variables (Figs 3–7).

Each substrate showed a unique, relatively tight relationship between decreasing sap flow through roots in drying soil and decreasing soil volumetric water content (Fig. 3a) or  $\Psi_{\text{soil}}$  (Fig. 3b) and increasing penetrometer resistance (Fig. 3c). Fitting linear regressions allowed the sensitivity of sap flow to soil drying to be assigned a threshold and a slope: sap flow declined most sensitively with  $\Psi_{\text{soil}}$  in sand and least sensitively in organic loam (Table 2). The relationship between decreasing sap flow through roots in drying



**Fig. 3.** Relationships between the percentage of initial sap flow from the dry part of the root system and volumetric soil water content (a), soil matric potential (b), penetrometer resistance (c) and root water potential (d) for PRD plants grown in clay loam (filled circles), sand (open circles), peat (filled inverted triangles), and organic loam (open triangles). Each point represents a single hypocotyl and linear regressions were fitted in SigmaPlot for Windows 2.0. Data for peat and organic loam are replotted from Dodd *et al.* (2008a) and (2008b), respectively.

soil and  $\Psi_{\text{root}}$  was unified across different substrates (Fig. 3d), as determined by analysis of parallelism (Table 3).

Root water potential declined with  $\Psi_{\text{soil}}$  in all substrates (Fig. 4), although the soil–root  $\Psi$  gradient (as mean  $\pm$ SE) was less in organic loam ( $0.16 \pm 0.03$  MPa,  $n=16$ ), clay loam ( $0.19 \pm 0.02$  MPa,  $n=12$ ) and sand ( $0.23 \pm 0.03$  MPa,  $n=14$ ) than in peat ( $0.31 \pm 0.04$  MPa,  $n=16$ ).

Root xylem ABA concentration ( $[X\text{-ABA}]_{\text{root}}$ ) increased with decreasing soil volumetric water (Fig. 5a) or  $\Psi_{\text{soil}}$  (Fig. 5b) or  $\Psi_{\text{root}}$  (Fig. 5d) and increasing penetrometer resistance (Fig. 5c). Analysis of parallelism (Table 3) showed that the relationship between  $[X\text{-ABA}]_{\text{root}}$  and  $\Psi_{\text{root}}$  (Fig. 5d) was more uniform across different substrates than with soil variables.

The transpiration rate of each plant at harvest was expressed as a fraction of the transpiration rate determined prior to imposing PRD (Fig. 2b). Within each substrate, there was no significant relationship between mean (of both pots)  $\Psi_{\text{soil}}$  and relative transpiration rate, but across all substrates, relative transpiration rate tended to decrease ( $P=0.09$ ) with  $\Psi_{\text{soil}}$  (Fig. 6a). Relative transpiration rate was not correlated with either mean  $\Psi_{\text{root}}$  or  $\Psi_{\text{leaf}}$  (Table 4) but seemed to decrease with increasing leaf xylem ABA concentration (Fig. 6b).

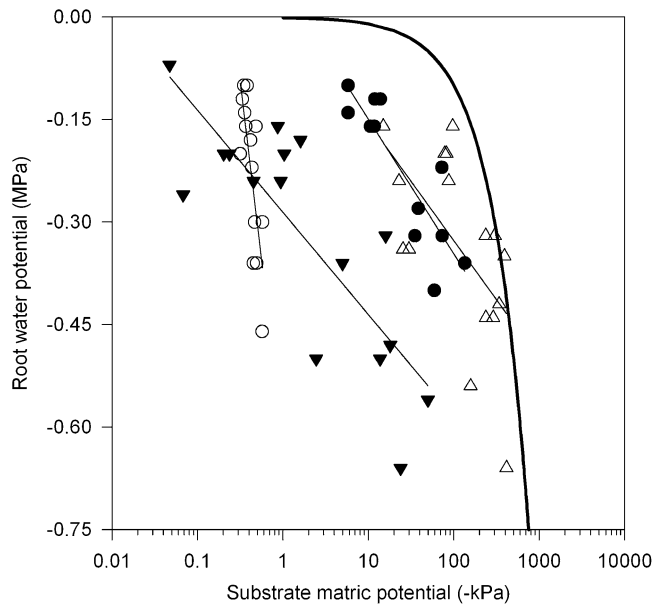
Although leaf xylem ABA concentration ( $[ABA]_{\text{leaf}}$ ) increased as the mean (of both pots)  $\Psi_{\text{soil}}$  decreased (Fig. 7a), it was not correlated with either average  $\Psi_{\text{root}}$  or  $\Psi_{\text{leaf}}$  (Table 4). Leaf xylem ABA concentration was also correlated with values predicted from a model (Fig. 7b), that incorporated the actual xylem ABA concentrations from the wet and dry root systems and the fractions of sap flow from each root system, according to the equation (Dodd *et al.*, 2008a, b):

$$[X\text{-ABA}]_{\text{leaf}} = F_{\text{dry}}[X\text{-ABA}]_{\text{dry}} + F_{\text{wet}}[X\text{-ABA}]_{\text{wet}} \quad (1)$$

where  $F_{\text{dry}}$ ,  $F_{\text{wet}}$  are the fractions of total sap flow from the dry and wet root systems and  $[X\text{-ABA}]_{\text{dry}}$ ,  $[X\text{-ABA}]_{\text{wet}}$  are the root xylem ABA concentrations from the dry and wet root systems. Since equation 1 uses the actual fractions of total sap flow (not the percentages of initial sap flow plotted in Fig. 3), it was assumed that  $F_{\text{dry}}=F_{\text{wet}}=0.5$  in a well-watered plant prior to imposing PRD, thus a 50% decline in sap flow (relative to initial values) from roots in drying soil resulted in  $F_{\text{dry}}$  equalling 0.25. This assumption has been validated previously (Dodd *et al.*, 2008a).

## Discussion

Although partial rootzone drying has been applied to various crops grown in a range of substrates (Dodd, 2009), each trial usually adopts a unique irrigation schedule, often with little consideration of the physiological consequences of decreasing sap flow from the dry part of the root system on the entirety of root-to-shoot signalling. Effective signalling can impact on sap flow, stomatal conductance, and shoot water relations, but these effects can be obscured by environmental variations such as light intensity,

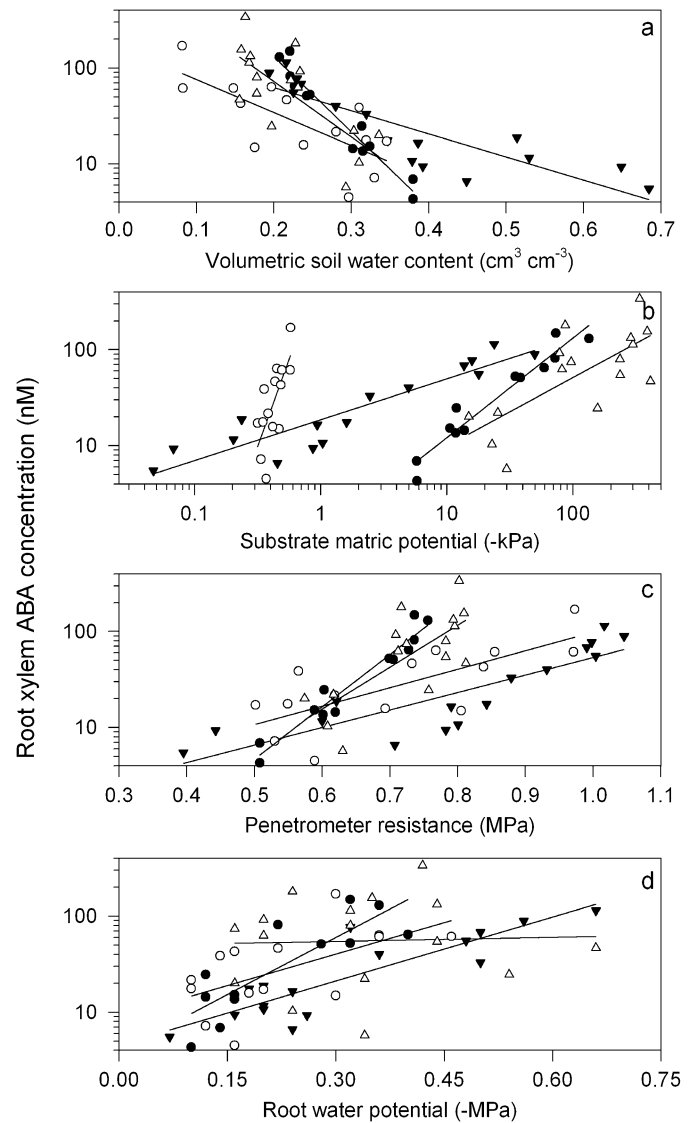


**Fig. 4.** Relationships between root water potential and soil matric potential for PRD plants grown in clay loam (filled circle), sand (open circles), peat (filled inverted triangles), and organic loam (open triangles). Each point represents a single hypocotyl and linear regressions were fitted in SigmaPlot for Windows 2.0. The thick, curved line is the 1:1 relationship.

temperature, and VPD. Consequently, under fluctuating field conditions it can be difficult to detect stomatal closure (Yao *et al.*, 2001; Liu *et al.*, 2007) using instantaneous measurements (even when season-long measurements indicate significant effects of PRD on plant growth and development), thus hindering the usefulness of stomatal conductance measurements in an irrigation schedule.

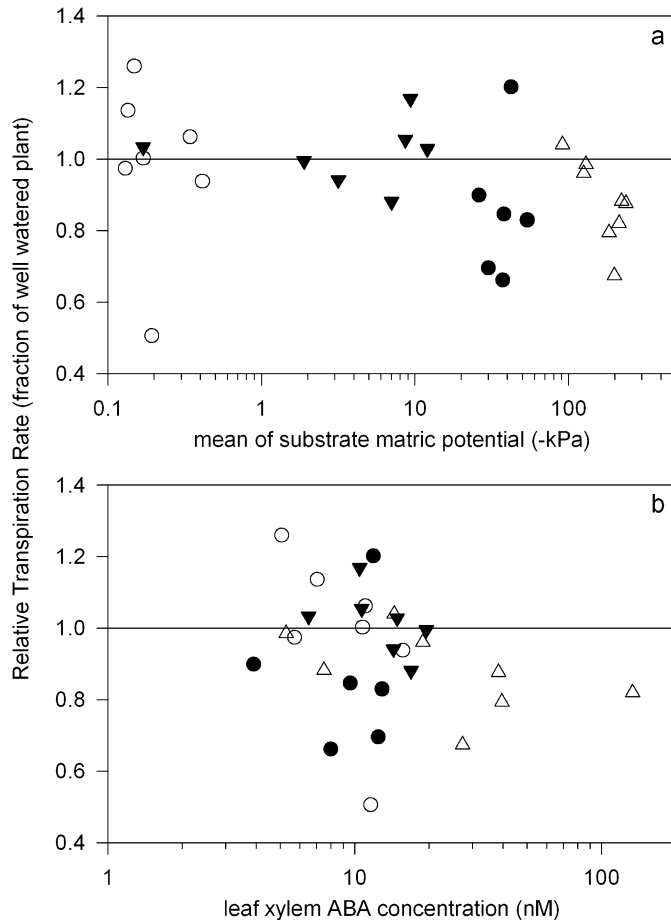
An alternative approach is to schedule irrigation according to measurements of soil water status, implicitly assuming proportional relationships between soil drying, signalling, and shoot physiology. However, since soil drying not only generates root-to-shoot signals (Fig. 5), but also decreases sap flow from that part of the root system in contact with drying soil (Fig. 3), the effects of PRD on signalling can be difficult to predict. This has stimulated the development of models to predict signal (usually ABA) concentrations in the shoot (Dodd, 2008), showing that when part of the root system is well irrigated ( $\Psi_{\text{soil}} > -1$  kPa), signal output from the entire root system is maximized when the dry part of the root system is at an intermediate soil water status, such that further soil drying actually decreases root-to-shoot signalling (Dodd *et al.*, 2008a, b; Liu *et al.*, 2008). Since these models are specific to individual substrates, this study aimed to determine whether sap flow from roots in drying soil or root xylem ABA concentration showed unifying relationships with particular soil or plant variables across a divergent range of substrates.

Sap flow through the dry part of the root system declined with  $\theta_v$  or  $\Psi_{\text{soil}}$  of the dry pot in all substrates, but this relationship apparently varied with the substrate (Fig. 3a, b; Table 3), with sap flow declining even when the soil water



**Fig. 5.** Relationships between root xylem ABA concentration and volumetric soil water content (a), soil matric potential (b), penetrometer resistance (c), and root water potential (d) for PRD plants grown in clay loam (filled circles), sand (open circles), peat (filled inverted triangles), and organic loam (open triangles). Each point represents a single hypocotyl and linear regressions were fitted in SigmaPlot for Windows 2.01. Data for peat and organic loam are replotted from Dodd *et al.* (2008a) and Dodd *et al.* (2008b), respectively.

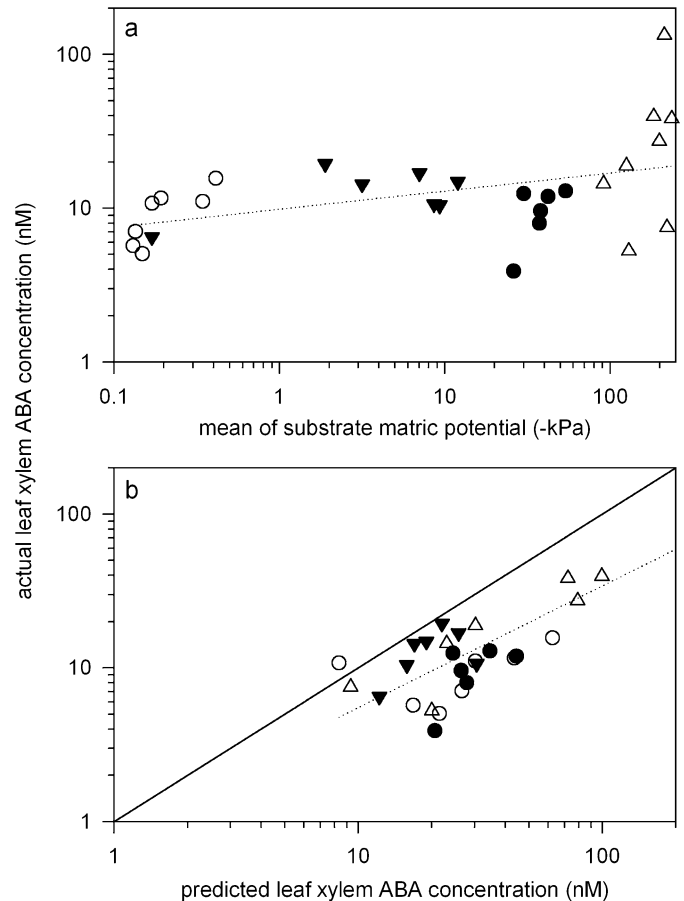
potential of the dry pot ( $\Psi_{\text{dry}}$ ) was relatively high ( $> -1$  kPa) when plants were grown in sand, but at a much lower threshold  $\Psi_{\text{dry}}$  when plants were grown in organic loam. However, in a single substrate, soil water potential of the wet pot ( $\Psi_{\text{wet}}$ ) determines the threshold  $\Psi_{\text{dry}}$  at which sap flow from roots in drying soil decreased, but not the slope of this decrease (Dodd *et al.*, 2008b). Since relatively few plants ( $n=6-8$ ) defined the relationship for each substrate here (Fig. 3), it is difficult to ascertain whether the threshold  $\Psi_{\text{dry}}$  was truly variable between substrates (Table 2) or varied because of differences in  $\Psi_{\text{wet}}$ . Likewise, substrate variability in the sensitivity of sap flow to drying soil (the



**Fig. 6.** Relationships between whole plant relative transpiration rate and mean (of both pots) soil matric potential (a) and detached leaf xylem ABA concentration (b) for PRD plants grown in clay loam (filled circles), sand (open circles), peat (filled inverted triangles), and organic loam (open triangles). Each point represents a single plant. Data for peat and organic loam are replotted from Dodd *et al.* (2008a) and Dodd *et al.* (2008b), respectively.

slope of the relationship between sap flow and  $\Psi_{\text{soil}}$ ) may be attributed to intrinsic differences in substrate properties or variation in  $\Psi_{\text{wet}}$ .

However, substrate certainly influenced the relationship between  $\theta_v$  or  $\Psi_{\text{soil}}$  and root xylem ABA concentration (Fig. 5a, b). Although plants grown in a sand and a loam had a similar relationship between leaf ABA concentration and  $\Psi_{\text{soil}}$  (Jensen *et al.*, 1998), the differences in substrate physical properties in that study were much less extreme than the highly divergent substrates selected here (Fig. 1; Table 1). Although there were unique relationships between  $[\text{X-ABA}]_{\text{root}}$  and  $\theta_v$  or  $\Psi_{\text{soil}}$  for different substrates, there was a more unifying relationship between  $\log [\text{X-ABA}]_{\text{root}}$  and  $\Psi_{\text{root}}$  (Fig. 5d), albeit with some degree of scatter. While the relationship between  $\log [\text{X-ABA}]_{\text{root}}$  and  $\Psi_{\text{root}}$  did not differ between clay loam, sand, and peat, including organic loam in the analysis resulted in significant differences in slope ( $P=0.014$ ), albeit less significant than with other soil variables ( $P<0.001$ , Table 3). It seems that across a range of different soil physical environments,  $\Psi_{\text{root}}$  was



**Fig. 7.** Relationships between detached leaf xylem ABA concentration and mean (of both pots) soil matric potential (a) and predicted xylem ABA concentration (b) for PRD plants grown in clay loam (filled circles), sand (open circles), peat (filled inverted triangles), and organic loam (open triangles). Each point represents a single plant and regression lines were fitted to all data. In (b), the 1:1 relationship is given by a straight line. Data for peat and organic loam are replotted from Dodd *et al.* (2008a) and Dodd *et al.* (2008b), respectively.

**Table 4.** Statistical analysis of relationships between relative transpiration rate (Fig. 6) and xylem ABA concentration (Fig. 7) and potential controlling variables

$P$  values are reported where 'nd' means 'not determined'.

Substrate	$\Psi_{\text{soil}}$ (mean of both pots)	$\Psi_{\text{root}}$ (mean of both pots)	$\Psi_{\text{leaf}}$ (mean of two leaves)	Actual $[\text{X-ABA}]_{\text{leaf}}$	Predicted $[\text{X-ABA}]_{\text{leaf}}$
Relative transpiration	0.09	0.81	0.64	0.24	nd
Actual $[\text{X-ABA}]_{\text{leaf}}$	0.005	0.38	0.24	nd	< 0.001

the best predictor of  $[\text{X-ABA}]_{\text{root}}$ . This merits further investigation but implies variation in the relationship between  $\Psi_{\text{soil}}$  and  $\Psi_{\text{root}}$  between substrates (Fig. 4), with the lower  $\Psi_{\text{root}}$  possibly mediated by a build-up of solutes

at the root–soil interface as the plant transpires (Stirzaker and Passioura, 1996) and/or a decrease in root–soil contact due to the appearance of a vapour phase as the soil dries, which may be exacerbated in sandy soils (Bristow *et al.*, 1984). Although the greatest gradient between  $\Psi_{\text{soil}}$  and  $\Psi_{\text{root}}$  was present in peat (a highly porous substrate), the sensitivity of  $[\text{X-ABA}]_{\text{root}}$  to decreasing  $\Psi_{\text{soil}}$  was most pronounced in sand (Fig. 5b), probably due to poor root–soil contact as directly demonstrated in very sandy soils (North and Nobel, 1997).

A recurrent experimental concern with PRD, especially when  $\Psi_{\text{wet}} > -1$  kPa (Yao *et al.*, 2001; Liu *et al.*, 2007), is that whole plant transpiration rate is scarcely affected (e.g. in peat; Fig. 6a). Effects of PRD on transpiration rate became apparent as the entire root system started to dry out (e.g. in clay loam and organic loam), indicating that PRD-induced restriction of plant water use may not only be attributed to where water is placed within the rootzone, but also the water deficit the entire root system is exposed to. Consequently, it has been advocated that effects of PRD should be assessed, relative to conventional deficit irrigation, across a range of soil water availabilities.

Another potential explanation for the sometimes minimal impacts of PRD on plant gas exchange may simply be inter-plant variation. For plants grown in clay loam, relative transpiration varied from 1.0 to 0.6 at similar entire pot  $\Psi_{\text{soil}}$  (Fig. 6a), apparently independently of either mean  $\Psi_{\text{root}}$  or leaf xylem ABA concentration (Fig. 6b). Other root-to-shoot signals such as cytokinins (Kudoyarova *et al.*, 2007) or xylem pH (Wilkinson *et al.*, 1998) may also directly alter transpiration rate, thus producing apparent variations in stomatal sensitivity to ABA (Dodd *et al.*, 1996). Interestingly, the slope of the relationship between stomatal conductance and xylem ABA concentration varied by more than an order of magnitude in replicate sunflower plants grown under controlled environment conditions (Schurr *et al.*, 1992). Such variation will make impacts of PRD difficult to detect in the absence of extensive replication, and may influence the choice of soil- or plant-based variables in irrigation scheduling, especially if it is impractical to measure these variables in a large number of plants.

Since PRD has been applied to plants grown in a range of substrates with different moisture release curves (Fig. 1a, b), this study aimed to determine a unified basis for irrigation schedules to maximize ABA signalling during PRD. Previous experiments indicated that relative transpiration rate in a range of soil types was best explained by relative available soil volumetric water content (Sinclair, 2005), stomatal closure was best correlated with soil matric potential (Jensen *et al.*, 1998), and shoot dry weight of wheat was best correlated with soil penetrometer resistance (Whalley *et al.*, 2008). By contrast, comparing all the substrates used here revealed that root xylem ABA concentration and sap flow from roots in drying soil varied with  $\Psi_{\text{root}}$  (Figs 3d, 5d), which is not routinely accessible to an irrigation manager (but see Simonneau and Habib, 1991). Since pre-dawn  $\Psi_{\text{leaf}}$  (as a surrogate for  $\Psi_{\text{root}}$  and  $\Psi_{\text{soil}}$ ) generally equilibrates with the wettest part of the soil profile

when plants are exposed to heterogeneous soil moisture (Donovan *et al.*, 2003), it may not always provide useful information for determining when the dry part of the root system should be re-watered during PRD (Ameglio *et al.*, 1997). Furthermore, the degree of scatter in the relationships between  $\Psi_{\text{root}}$  and both sap flow and  $[\text{X-ABA}]_{\text{root}}$  (Figs 3d, 5d), suggests that soil moisture measurements may offer more precise irrigation scheduling during PRD. However, due to unique relationships between sap flow through the dry part of the root system and  $\theta_v$  or  $\Psi_{\text{soil}}$  (Fig. 3a, b) and the  $[\text{X-ABA}]_{\text{root}}$  response to  $\theta_v$  or  $\Psi_{\text{soil}}$  (Fig. 5a, b) between different substrates, maximizing ABA signalling based on soil moisture measurements must consider local (site-specific) variation in soil moisture release characteristics.

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