ROOT PRESSURISATION MAY CAUSE STOMATAL RE-OPENING IN PLANTS GROWING IN DRYING SOIL VIA A MECHANISM, WHICH IS ABA DEPENDENT

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ABSTRACT

Stomatal closure was induced in two cultivars of tomato (*Lycopersicon esculentum* L. cv. Solairo and Alisa Craig) by allowing the plants to dry the soil in which they were rooted. Sealing the roots within a root pressure vessel and applying a pneumatic pressure reversed this restriction in stomatal conductance. Pressurisation resulted in a significant decline the xylem sap concentration of abscisic acid within one of the cultivars, which was consistent with stomatal reopening. We propose a mechanistic explanation of stomatal re-opening based on a pressurisation-induced decrease in abscisic acid concentrations around the active sites for stomatal closure, and an appreciation of the existence of apoplastic bypasses for ABA. Our results cast doubt on the conclusions of similar investigations using root pressurisation, which argue for a dominant role for leaf water status in the control of gas exchange in plants.

Keywords: abscisic acid, chemical signaling, soil drying, stomatal conductance, root pressurization

INTRODUCTION

Recent reports have demonstrated that environmentally induced stomatal closure can be reversed by the application of pressure around the root system of intact plants (Comstock & Mencuccini 1998; Mencuccicini et al. 2000), even when plants are growing in drying soil (Saliendra et al. 1995; Fuchs & Livingstone 1996). In these investigations, root pressurisation is used to restore a favourable water status in the shoot (Passioura 1980; Passioura & Tanner 1985), and it has been argued that this technique allows the investigator to assess the role of changing leaf water status in regulating stomatal aperture. Using this technique, it is also possible to collect xylem sap, which will exude from cut vessels if a slight overpressure is applied to the root system (Schurr 1998).

Re-opening of stomata following pressurisation has been used as evidence to support the case for hydraulic regulation of stomatal behaviour and these interpretations of the experimental data have raised some doubts about the importance of chemical regulation of stomatal behaviour of plants in drying soil

(Zhang & Davies 1990; Khalil & Grace 1993; Schurr & Schulze 1996; Tardieu et al. 1996; Wilkinson & Davies 1997). Earlier work with root pressurisation was used to support the argument for chemical regulation (Gollan, Passioura & Munns 1986; Schurr et al. 1992), as at least in some species, stomatal closure induced by soil drying could not be reversed by root pressurisation. There is now some uncertainty over why stomatal re-opening can be achieved by pressurisation of roots of some species and not others.

Most of the work on chemical regulation of gas exchange of plants in drying soil has focused on the role of abscisic acid (ABA). This suggests that there is increased loading into the root xylem in response to changes in soil water status and increased transport to the shoot where ABA elicits stomatal closure via a direct effect on stomatal guard cell ion channel activity (Davies & Zhang 1991). Initial criticisms of such a signaling mechanism (Munns & King 1988; Munns & Sharp 1993) concerned unrealistic concentrations of applied ABA required to simulate such a signaling response. This criticism has now been addressed in recent work, which demonstrates that even the endogenous levels of ABA present within a well-watered plant can be sufficient to cause significant stomatal closure (Wilkinson et al. 1998).

An investigation of genotypic variation in chemical signalling capacity in tomato, using root pressurisation techniques to gain access to the xylem stream, resulted in the observations reported here. We report the effects of root pressurisation on the stomatal conductance and xylem borne abscisic acid concentrations in plants subjected to soil drying. In doing so, we propose that data previously interpreted as demonstrating hydraulic regulation of stomatal aperture, even in the short term, can be re-interpreted by measurement of the effect of root pressurisation on ABA concentration within the xylem stream in contact with stomatal guard cells. Such information casts doubt on interpretation of the results of previous investigations, which use root pressurisation to modify plant gas exchange.

MATERIALS AND METHODS

Plant material

Tomato (*Lycopersicon esculentum* L. cv. Solairo and Alisa Craig) seeds were sown and germinated in John Innes No. 2 potting compost (Keith Singleton's Seaview Nurseries, Cumbria, UK) in a growth cabinet with a photoperiod of 12 hours (PPFD = 600 μmol m^{-2} s^{-1}), a day/night time temperature of 27/20°C and a day/night relative humidity of 40/60%.

Two-week-old seedlings were transplanted into PVC cylinders (78.5cm high and 25.5cm I.D.) designed to fit inside a root pressurisation vessel (Figure 1) of similar design to those used in other pressurisation studies (e.g. Saliendra et al. 1995; Fuchs & Livingstone; 1996 Comstock & Mencuccini, 1998; Mencuccicini et

al. 2000). The cylinders were filled with John Innes No. 2 potting compost and watered daily via a reservoir system. The cylinders had a solid plastic top with a 10mm aperture in the centre to allow the transplanted plant to protrude. Plants were established in the growth cabinet and watered every day until the stem diameter had increased to a point at which the plant stem made a tight seal with the surrounding aperture.

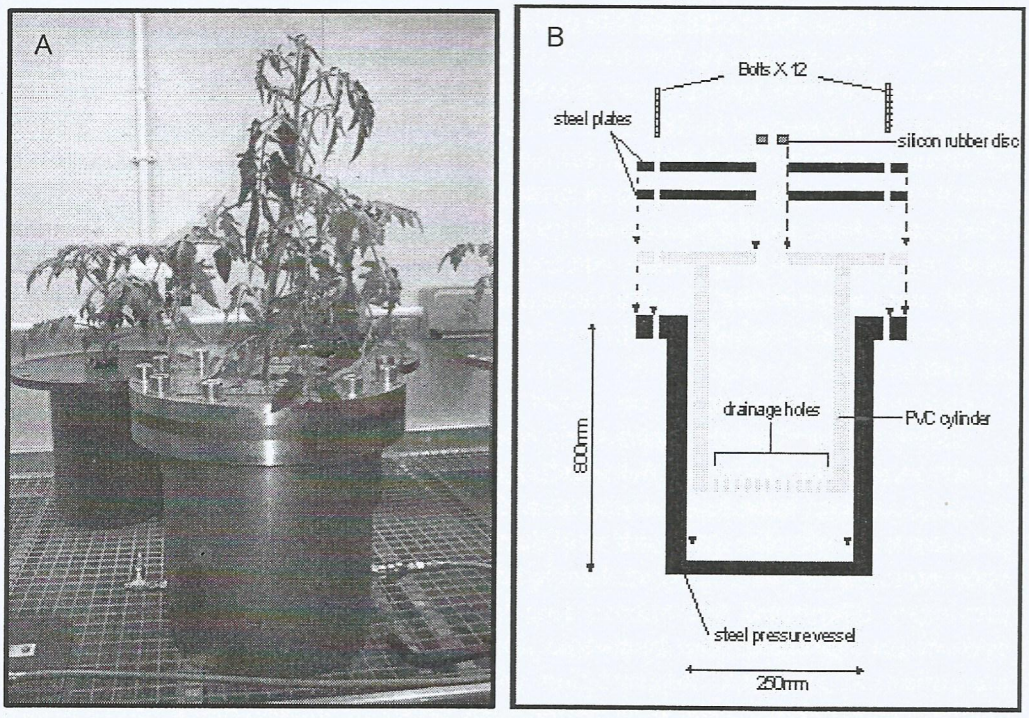

Figure 1. Assembly of the root pressurisation vessel (RPV). (A) by original visual and (B) schematic presence.

Soil drying treatment

After establishment of the plants in PVC sleeves (3 to 4 weeks), water was withheld and the soil was allowed to dry. Volumetric soil water content was measured daily using a Theta Probe (Delta-T Devices, Cambridge, UK) inserted through drilled holes in the side of the PVC pot. Measurements were taken at days 1, 3 and 5 after withholding water. Stomatal conductance (g_s) of the youngest fully expanded leaf was measured twice daily in control and soil drying treatments using a diffusion porometer (Delta-T Devices, Cambridge, UK). Plants were loaded into the root pressurisation vessel (RPV) when a significant decrease in stomatal conductance was measurable (*c.*50-70% restriction in stomatal conductance).

Root pressurization

The pressure chamber consists of a stainless steel cylinder (28cm long, 71.5cm I.D. and 86cm O.D.). Circular grooves machined into the top of the cylinder accommodate rubber O-rings (0.5cm thick and 77.0cm O.D.) to provide a pressure seal with carbon steel plates, which are bolted onto the top of the chamber. The bottom of the cylinder is sealed with a single plate (0.5cm thick and 77.0cm in diameter). Two pairs of plates (each 1.0cm thick and 112.0cm in diameter) seal the top of the cylinder. Each of the plates has a 1.70cm diameter central hole to accommodate the stem of the plant within the PVC cylinder. The two plates split into two to allow the plates to be mounted around the stem of the plant. The hole in the lower plate is threaded to accommodate a silicon rubber disc. A pressure seal around the seedling stem is formed by a soft silicon rubber disc (Xanthopren VL. Plus. Bayer, Germany), which is secured around the stem as close to the base of the shoot as possible. Plants were loaded into the chamber 24 hours before measurements began. 30 minutes prior to pressurization and at 15 minute intervals throughout the experiment stomatal conductance was determined.

Pressure was applied at a rate of 0.1MPa per minute until a balancing pressure was achieved using compressed nitrogen gas (Fuchs & Livingstone 1996). Balancing pressure was defined as the point at which xylem sap could be seen to exude from a cut petiole of a leaflet, close to the position at which stomatal conductance was being monitored. The pressure applied was taken as an integrated measurement of plant water potential. Pressure was monitored with a PV2-LECH 0001 pressure gauge (Luneside Engineering Co., Halton, UK) with a 0 – 3 MPa range. Pressure was controlled manually by the operator and held just above the balancing point for 45 minutes after the initial exudation was recorded.

After initial air drying of the cut surface, xylem sap exuding from the cut petiole close to the site of measurement of stomatal conductance, was collected into eppendorf tubes on ice using a micropipette. Subsequently, tubes were re-sealed and stored at -20°C for subsequent determination of abscisic acid concentrations using radioimmuinoassay (Quarrie et al. 1988). A single pressurisation/sampling episode was completed within 45 minutes. After this time, plants were discarded as a re-pressurisation was found to result in anomalous stomatal behaviour.

RESULTS AND DISCUSSION

In both cultivars, soil drying resulted in a decline in stomatal conductance at 3 DAT. Conductances increased significantly upon pressurisation from 160-180 mmol m^{-2} s^{-1} to 250-300 mmol m^{-2} s^{-1} in both cultivars over a period of 30-45 minutes, although conductances shortly after pressurisation (15 min) where transiently decreased (Figure 2). The reversal of stomatal closure via an application of pneumatic pressure around the roots is well reported and has received renewed attention recently (Saliendra et al. 1995; Fuchs & Livingstone 1996; Comstock & Mencuccini, 1998; Mencuccicini et al. 2000). It has been argued that this response

demonstrates a dominant role for leaf water status in the regulation of stomatal aperture (Comstock 2002). Such work casts doubt on the significance of chemical regulation of gas exchange via the sensing of soil water status and transmission of chemical messages such as ABA to the shoot (Zhang & Davies 1990; Khalil & Grace 1993; Schurr & Schulze 1996; Tardieu et al. 1996; Wilkinson & Davies 1998), even though most of the re-pressurisation work reported to date does not include a quantification of potential chemical signals moving through the xylem stream.

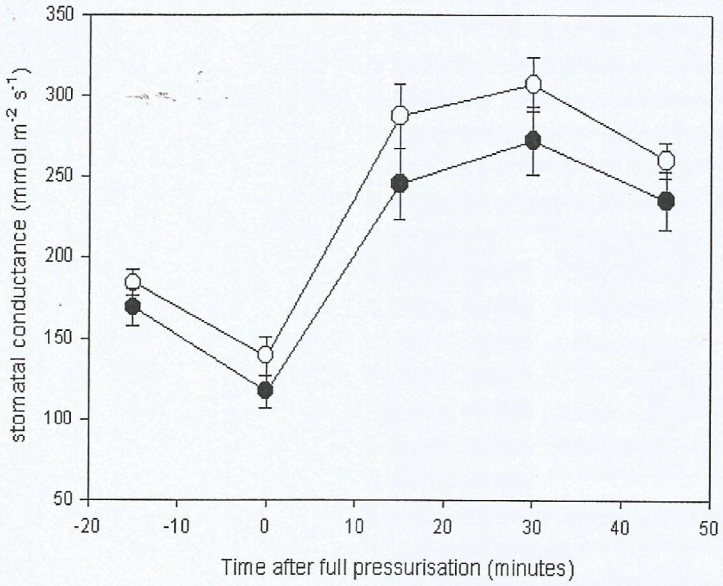

Figure 2. Stomatal conductance response of Ailsa Craig (○) and Solairo (•) cultivars of tomato (*Lycopersicon es culentum* L.) to root pressurisation, after exposure to a soil drying treatment. Each point is the mean of 3 determinations of stomatal conductance during 5 pressurisation experiments using 5 different plants ±SE).

Pressurisation caused a steady reduction in the concentration of xylem ABA (from *c.* 48 nmol dm^{-3} to 25 nmol dm^{-3} in Alisa Craig at 3DAT, but no significant change in xylem ABA concentrations in the Solairo cultivar (Figure 3), which remained constant at *c.*320-340 nmol dm^{-3}. Mean leaf water potential declined in response to soil drying from –0.32 MPa in Ailsa Craig and –0.42 MPa in Solairo, to –0.9 MPa and –1.09 MPa respectively, approximately 3 days after withholding water (Table 1).

The observed re-opening of stomata reported in this paper is consistent with many published observations. However, the determination of the concentration of

ABA within the xylem sap in this study allows us to suggest an alternative to the hydraulic explanation of stomatal control in drying soil.

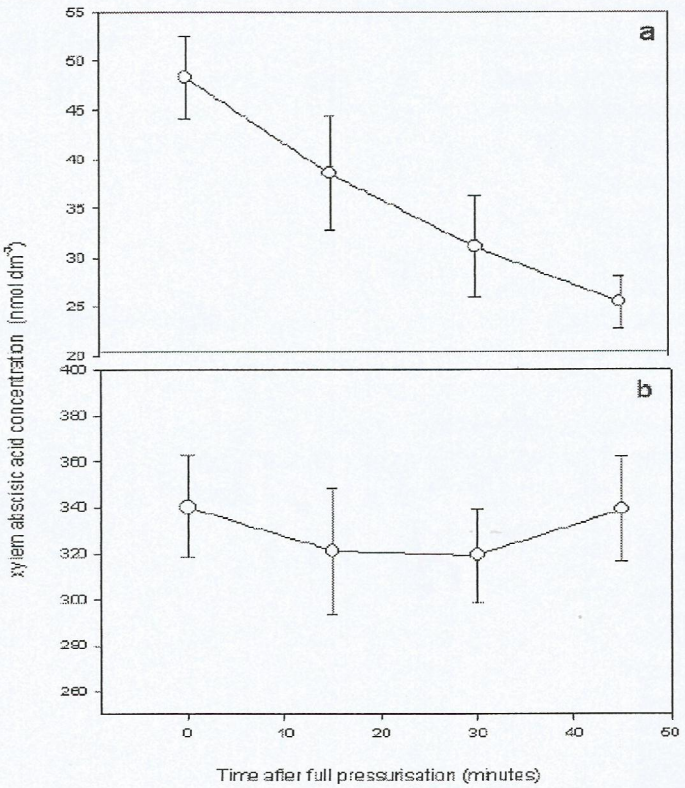

Figure 3. The concentration of abscisic acid measured in the xylem sap expressed from tomato plants cv. Ailsa Craig (a) and Solairo (b) by pressuring the root system for 45 minutes. Each point is the mean of concentrations determine from at least 15 xylem sap samples per sample time, obtained from 5 repeats of the experiments with 5 different plants ±SE.

In the Alisa Craig cultivar of tomato we have observed a decrease in xylem sap ABA concentration due to pressurisation (Figure 4). We would propose that this will be mirrored by similar changes in concentration in the apoplastic compartment of the leaf, (Wilkinson & Davies 1997; Wilkinson et al. 1998) as a function of the continuum which exists between the two. We therefore predict that such a decline in ABA concentration is responsible for the stomatal re-opening observed here and elsewhere (e.g. Comstock & Mencuccini 1998; Mencuccini et al. 2000). Similar changes were not however, observed in the Solairo cultivar. One difference between these two cultivars which may explain the differing response of

xylem ABA concentrations to root pressurisation is the degree of apoplastic bypass for ABA in these cultivars. An apoplastic bypass describes the phenomenon whereby flow of ABA across the root endodermis is possible. The measured reflection co-efficient for ABA of the root endodermis (Freundl et al. 1998) indicates that substantial amounts of ABA can be dragged with water across the endodermis (by solvent drag) directly into the xylem, buffering ABA fluctuations caused by increased transpirational water loss. Such an increase in transpirational water flow would be mimicked by root pressurisation. The concentration of substances within the xylem would, therefore, be expected to fall, if water flux increased suddenly (as with root pressurisation), in the absence of an apoplastic bypass. The presence of a bypass would provide the potential for ABA concentrations to be maintained in the xylem as water flux increased, providing there were sufficient concentrations of ABA outside of the root. We would, therefore, speculate that the Solairo cultivar posses an apoplastic bypass, while the Ailsa Craig cultivar does not. In such a way, pressure induced increases in water flux would not dilute xylem ABA in Solairo, as the existence of a bypass would permit the passage of ABA across the root and into they xylem, at a rate directly proportional to water flux within the xylem. In the absence of any change in ABA within the xylem of Solairo, it is difficult to argue that an ABA-deponent mechanism is governing the stomatal re-opening response observed.

Table 1. Leaf water potential of Alisa Craig and Solairo cultivars of tomato (*Lycopersicon esculentum*) in well-watered plants and those experience soil drying. Measurements are the mean of 5 determinations of potential $\pm$ S.E.

	Well watered plants	**Soil drying plants**
Alisa craig	0.3 ± 0.01	0.9 ± 0.1
Solario	0.42 ± 0.01	1.09 ± 0.1

We can speculate that the mechanism of stomatal re-opening by root pressurisation remains the same, when stomata are closed by other environmental perturbations (e.g. Comstock & Mencuccini 1998; Mencuccini et al. 2000) as other related work suggests that ABA can regulate gas exchange, even in well watered plants (Jarvis & Davies 1998; Wilkinson et al. 1998). The ABA-based mechanism proposed, does not require any change in the synthesis of ABA, although this may occur.

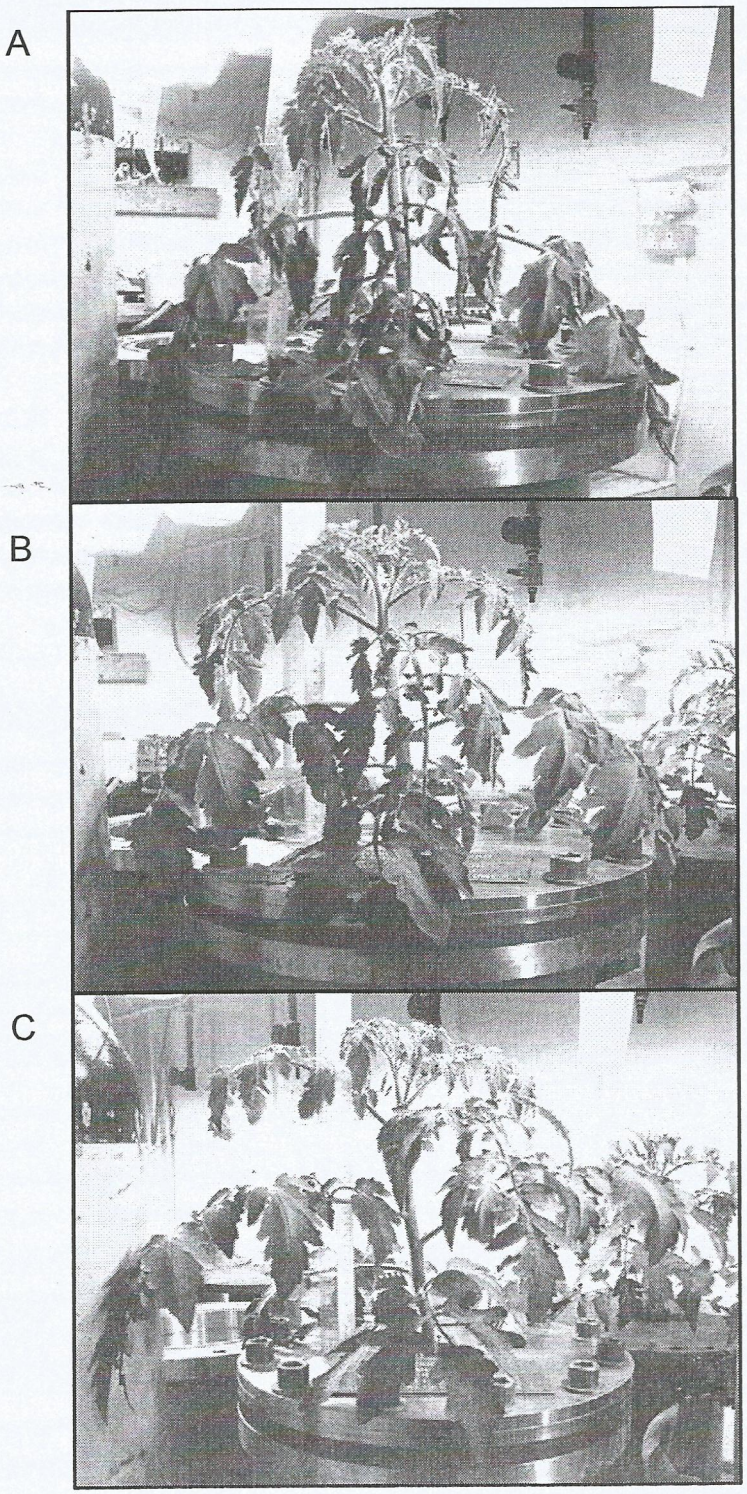

Figure 4. Leaves changes as pressurized at (A) 0 to 10 minutes, stomata closed and leaves wilted (B) 15-25 minutes, stomata slowly opened and (C) 30-45 minutes, stomatal fully opened and the leaves back to WW condition (cv. Solairo).

In a recent paper, Mencuccini et al. (2000) have speculated that root pressurisation may affect the distribution of ABA within leaf compartments (Mencuccini et al. 2000) and explain the observed response to changes in leaf water status in the absence of any increase in leaf ABA concentrations. The observations reported in this paper may support this proposal and allow us to re-interpret reported changes in the sensitivity of stomatal re-opening to root pressurisation (Mencuccini et al. 2000). Root pressurisation may cause a change in the loading of ABA into the xylem, in such a way that although increased ABA accumulation in the leaf may not be detected (or occur), its effective concentration at the active site for stomatal closure may be significantly altered. In the absence of any increased synthesis, we would propose that root pressurisation simply causes a redistribution of ABA within the plant, manifested by a decline in concentrations within the xylem compartment.

Differences in response to root pressurisation between species have often been interpreted in terms of potential differences between woody and herbaceous species (Salendra et al. 1995; Mencuccini et al. 2000). Herbaceous species that have been used in root pressurisation experiments seem relatively unresponsive to root-pressurisation (e.g. Gollan et al. 1986; Schurr et al. 1992). However, the observations reported here on tomato, a herbaceous species, would cast doubt on this potential division in response.

It is also interesting to note that the endogenous levels of ABA within the xylem stream prior to pressurisation vary enormously between cultivars. Endogenous concentrations of ABA vary enormously between species (Dodd et al. 1996) and we may therefore speculate that in the current investigation, the change in ABA concentrations with soil drying rather than the absolute concentration is the variable of interest in the context of control of stomatal behaviour.

CONCLUSION

Authors of many studies using root pressurisation techniques argue for a dominant role for leaf water potential in the regulation of stomatal aperture have been happy to make the suggestion that such responses provide a good insight into *in planta* responses. We must therefore argue for the validity of an alternative explanation based on an assessment of the delivery to shoots of potential chemical regulators of gas exchange in plants.

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