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AGRONOMY AND SOILS

Physiological Response of Cotton to a Root Zone Soil Moisture Gradient: Implications for Partial Root zone Drying Irrigation.

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ABSTRACT

Partial Root Zone Drying (PRD) is an irrigation strategy which involves the alternate drying and wetting of sub-sections of the plant root zone. Savings in crop water use productivity from PRD is a result of changes in the plant biochemical and physiological response from the imposed soil moisture gradient. However, an understanding of the relationship between the soil moisture gradient and plant response is required before PRD can be used to improve crop water use productivity. The objective of this study was to investigate the response of cotton (*Gossypium hirsutum* L.) to a soil moisture gradient imposed across the root zone. Cotton plants were grown under greenhouse conditions in split pots. Control treatment pots were well watered on both sides of the split pots and a Non-alternated PRD treatment had water applied to only one side of the split pot with the other allowed to dry over a 24 day period. Soil moisture potential on each side of the split pots was measured along with changes in plant stem sap abscisic acid, sap pH, and stomatal conductance. The imposed soil moisture gradient resulted in a four-fold increase in xylem sap abscisic acid concentration, peaking at a soil moisture potential of -2360 kPa. However, this soil moisture gradient did not produce any significant (P<0.05) difference in either xylem sap pH or stomatal conductance. These findings suggest that it may not be possible to maintain the plant water status of cotton grown under commercial field conditions and simultaneously impose a sufficiently large soil moisture gradient across the root zone to induce a PRD response.

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It is now widely accepted that both hydraulic and biochemical signals are involved in regulating plant growth rates and stomatal responses to changes in the abiotic environment (Davies et al., 1994; Davies and Zhang, 1991). Recent research (Bahrun et al., 2002; Davies et al., 2002; Davies and Zhang, 1991; Dodd et al., 1996; Loveys et al., 1997; Sobeih et al., 2004; Stoll et al., 2000; Wilkinson and Davies, 2002) has identified that changes in both sap pH and abscisic acid (ABA) concentration in the root and xylem sap correspond to changes in root zone soil moisture availability can result in reduced stomatal conductance and vegetative growth.

Reductions in stomatal conductance in the presence of elevated ABA are due to a net loss of potassium salt from the guard cells, with a consequent reduction in turgor, cell shrinkage and closure of the stomatal pore (MacRobbie, 1991). Alkalinization of sap pH has been found to reduce stomatal conductance, even under conditions where ABA has not changed (but been present) and therefore acts via an ABA dependent mechanism (Bacon et al., 1998; Dodd et al., 2003; Holbrook et al., 2002; Wilkinson et al., 1998; Wilkinson and Davies, 1997). The sensitivity of stomatal response to ABA has been found to increase under nitrogen deprivation and is also associated with an increase in alkalization of sap pH (Bahrun et al., 2002; Radin et al., 1982; Wilkinson, 1999; Wilkinson and Davies, 2002). Leaf elongation rate is also reduced as sap pH is alkalinized and xylem sap ABA elevated (Bacon et al., 1998).

Partial Root Zone Drying (PRD) is an irrigation strategy which involves the alternate drying and wetting of sub-sections of the plant root zone to maintain elevated biochemical signalling (Dry et al., 1996; Loveys et al., 2000; Loveys et al., 1998; Stoll et al., 2000). This strategy attempts to simultaneously maintain water availability and plant water status while elevating the biochemical signalling (increasing ABA levels and alkalization of sap pH) within the plant. The elevated ABA has been found (Dry et al., 1996; Loveys et al., 2000; Stoll et al., 2000) to coincide with a partial reduction in stomatal conductance and a decrease in vegetative growth rate, both

of which lead to an improvement in crop water use efficiency (WUE). Investigations of the biochemical signalling and physiological responses using split-root experimental systems (Ayoub et al., 1993; Gowing et al., 1990; Holbrook et al., 2002; Loveys et al., 2000; Sabb and Sharp, 1989; Stoll et al., 2000) generally do not alternate the side of the plant root system that is allowed to dry. The other side of the plant root zone is kept well watered to maintain plant water status. The biochemical signalling (i.e. elevated ABA and alkalinization of sap pH) and physiological responses observed during the drying cycle are then used to schedule the alternation period for PRD so that an elevated biochemical signal is maintained.

Regulated Deficit Irrigation (RDI) aims to reduce the moisture availability throughout the entire plant root zone. It is a strategy which improves crop WUE by maintaining plant water status within prescribed limits of deficit with respect to maximum water potential (Kriedemann and Goodwin, 2003). Hence, the major distinguishing factors between PRD and RDI are the differences in implementation and the effects on localized soil moisture conditions and plant water status. Partial root zone drying aims to maintain plant water status and create a favorable physiological response due to biochemical signalling only. Regulated deficit irrigation does not maintain plant water status and reportedly does not achieve the same level of biochemical signalling as PRD (Kriedemann and Goodwin, 2003). There is on-going debate (Bravdo et al., 2004; Pudney and McCarthy, 2004) over (a) whether perceived PRD responses and associated WUE benefits are due to PRD or are a form of RDI and (b) whether PRD conditions can be effectively implemented under commercial field conditions.

Cotton is a major irrigated crop commonly grown in areas experiencing limited water supply. There has been some research to investigate the benefits associated with implementing PRD irrigation in cotton under field conditions (Tang et al., 2005; Topcu et al., 2002). However, the soil moisture conditions imposed and the arbitrary period selected for irrigation alternation in these trials cast some doubt over whether the observed changes in water use were due to a PRD biochemical response or a deficit irrigation response. Hence, there is a need to clarify whether the plant physiological responses are different when a soil moisture gradient is imposed across a root zone compared to when a more general root zone deficit is present. The aim of this paper was to identify whether it is possible to maintain plant water status in cotton while inducing a physiological PRD response by imposing a soil moisture gradient across the root zone.

MATERIALS AND METHODS

Agronomic management. This trial was conducted at the University of Southern Queensland, Australia during the 2004-05 summer season. Greenhouse temperature was maintained between 20-30º C with mean daily day degrees equal to 13. Four to five cotton seed of variety Sicot 80 (Cotton Seed Distributors, Wee Waa, New South Wales) were planted into well-watered Peat 80 Plus (Searles Pty Ltd, Kilcoy, Queensland) potting mix in 140 mm round seedling pots at a depth of 50 mm. Pots were irrigated daily to drainage and thinned to one plant per pot 10 days after planting.

Seedlings were transplanted 38 days after planting into split-pots consisting of two joined square 4.5 L pots (175 mm by 175 mm wide by 240 mm deep) with a U shaped gap (70 mm deep by 30 mm wide) between the two pots to accommodate the plant base. Geofabric® (i.e. woven polypropylene fabric) was placed in the base of each pot to minimize soil loss and root growth out of the pot. The seedlings were immersed in water prior to removal from the seedling pots. The roots were then gently hand washed before being transplanted into the split-pots with visually equal roots in each pot except for the taproot bias to one side. A course soil mix of Coal Fines Paunch Mix (Superior sand and gravel landscaping, Toowoomba, QLD) with a low soil moisture holding capacity was used in the split-pots. Osmocote (Scotts Australia Pty Ltd, Baulkham Hills, New South Wales) slow release fertilizer was used in all pots at a rate equivalent to 100 kg N ha^{-1}. No nutrient deficiencies were noted during the trial period. Geofabric® was also placed over the top of the soil surface to reduce soil moisture evaporation.

The irrigation trial consisted of a Control (both joined pots maintained well watered) and Non-alternated (Non-alt.) PRD treatment (i.e. only one of the joined pots maintained well watered). A low soil moisture potential (~ 2 kPa) was maintained in the well watered (i.e. "wet") pots by equilibration with a free water table at the base of the pot. The air filled porosity of the soil at equilibrium was assessed greater than 0.10 cm^3 cm^{-3} which is above the threshold for water logging responses in cotton (Hodgson and Chan, 1982). The plants were allowed to recover for 21 days after being transplanted to

allow for equal root distribution in both joined pots for each plant. The trial was initiated by removing the free water table at the base of the non-taproot side of the Non-alt. PRD pots.

Soil and plant measurements. Destructive plant sampling was conducted every three to four days throughout the duration of the trial to measure stem water potential (Ψ_s), xylem sap, and soil water content. Four plants were sampled from both the Non-alt. PRD and Control treatments, except on the last sampling day when the remaining 8 plants in each treatment were sampled.

Stomatal conductance was measured between 1200 and 1300 hours on five occasions during the trial using a Li-6400 portable photosynthesis system (LI-COR, Inc, Lincoln, Nebraska). Quantum flux was set to match cloudless midday conditions in the greenhouse, CO_2 reference gas was set at 380 µmols mol^{-1} and block temperature was set at 30 ^{0}C. The measurement system was recalibrated after every 10th sample measurement. Measurements were conducted on the youngest fully expanded leaf on all plants remaining at each measurement date. For 10, 12 and 17 days after trial initiation (DAT), one sample per leaf was taken. For 19 and 21 DAT, 2 and 3 samples per leaf were taken, respectively.

Stem water potential was measured with a Scholander pressure chamber. These measurements were conducted on the lowest main stem leaf at solar noon on the day before each plant was to be destructively sampled. The leaf was covered and sealed with aluminium foil for approximately 2 hours before measurement. Stem water potential was measured instead of leaf water potential to account for whole plant evaporative demand (plant water status) and overcome variance in leaf water potential which can arise due to differences in individual leaf conditions, position, exposure and rates of water loss. Stem water potential is considered a better indicator of plant water status than leaf water potential (Remorini and Massai, 2003).

Xylem sap was collected by destructive sampling using a technique similar to that of Bahrun et al. (2002). Root over pressure was not applied to obtain the sap sample due to the suggested inaccuracies as outlined by Bacon et al. (1998) and Wilkinson et al. (1998). Hence, xylem sap was collected at dawn to ensure sufficient root pressure was present to collect approximately 0.5 cm^3 of sap. Plants were de-topped 20 - 30 mm from the soil surface and the cut surface cleaned to remove any contaminants originating from cut cells. Disposable plastic eye droppers with a graduated tip were used to collect the sap samples. The eye dropper was placed over the plant stump and sealed with parafilm before being covered with aluminium foil to minimize contamination, photo degradation and radiant heat. Sufficient sap was collected within 1 hr of being de-topped. Sap samples were transferred into pre-cooled micro tubes (1 cm^3) and placed in an ice packed dark box for transport before being stored in a –75 ^{0}C cold room until analysis.

Abscisic acid and sap pH were measured on the stem sap samples. Abscisic acid concentration was measured using an enzyme linked immunosorbent assay (ELISA) Phytodetek®ABA Enzyme Immunoassay Test Kit, supplied by Agdia (Elkhart, Indiana, USA). Measurements were made using the test kit instructions plus an additional preliminary dilution of each sap sample to bring the concentrations within the measurement range for maximum accuracy.

Variation in daily photosynthetically active radiation, temperature and vapor pressure deficit (VPD) can influence plant water potential, stomatal conductance, ABA production and ABA removal within plants (Gutschick, 2002; Trejo et al., 1995; Trejo et al., 1993; Wilkinson and Davies, 2002). Hence, comparison and interpretation of raw results between these measurements taken on different days is difficult. To overcome this, all data is presented as relative to the Control at each sampling date.

Sap pH was measured with an Orion combination needle pH electrode (Orme Scientific LT., Manchester, UK) fitted to a pH meter (Hanna Instruments, Melbourne). The pH electrode was cleaned between each sap sample by placing it in a cleaning solution (0.1N HCL) for 2 minutes before being rinsed with reverse osmosis water. The electrode was also re-calibrated after every 10 sap samples measured.

Soil moisture was obtained from samples taken at a depth of 125 mm in each pot after each plant was de-topped. Soil samples were weighed, placed in an oven at 105 °C for 48 hours and then left to cool in a desiccator vacuum for 4 hours before being re-weighed to calculate the volumetric soil moisture content. Soil water potential at sampling was calculated using the soil moisture characteristic for the potting medium, which was measured using repacked soil cores and the pressure plate method (McKenzie et al., 2002).

Statistical methods. The trial consisted of 84 split-potted plants laid out in a randomized block design consisting of 3 blocks each with 14 Control and 14 Non-alt. PRD plants. Data were analysed by

one-way ANOVA (using SPSS for Windows v12.0.1, Chicago, Illinois). All data sets were tested for compliance with the underlying ANOVA assumptions. Data sets found to violate the ANOVA assumption of normality were transformed to improve the symmetry of the distribution prior to analyses. Levene's test for homogeneity was used to test the equal variance assumption. Where the Levene's test found heterogeneity (Coakes, 2005), but the ratio of the largest to smallest sample standard deviation was less than two, the data set was considered suitable for ANOVA as the p-values for ANOVA are only mildly distorted (Ott, 1988). However, where the ratio of the largest to smallest sample standard deviation exceeded two, the P = 0.01 significance level was used to limit the occurrence of Type 1 errors. Unless otherwise stated, the level of significance was tested at P = 0.05.

RESULTS

Soil moisture on the 'dry' side of the Non-alt. PRD treatment was found to dry down from an initial 29.3 +/- 1.1 % to 9.3 +/- 0.4 % over the 24 days of the trial (Fig. 1). Saturated water content was 31.6% and residual water content (i.e. at -1500 kPa) was 13.0% for the soil media used. The soil moisture extraction on the 'dry' side of the Non-alt. PRD treatment reached a plateau by 15 DAT at 11.6 +/- 0.4 % (-2360 kPa).

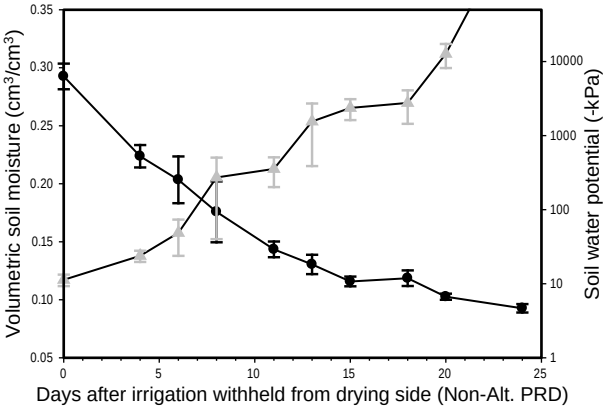

Figure 1. Volumetric soil water (●) and soil water potential (▲) on drying side of Non-alternated PRD treatment. Data are means (n = 4) with standard errors.

There was no significant difference in Ψ_s between the Control and Non-alt. PRD treatment at any sampling date (n = 4), except 21 DAT when the Non-alt. PRD treatment was significantly higher than the Control (n = 8) (Fig. 2). Two distinct peaks in xylem ABA levels were observed during the trial (Fig. 3). The first elevation in ABA was greater than a two-fold increase over the concentration of ABA in the Control and occurred during 6 to 8 DAT corresponding to an average soil moisture of 17.6 +/- 2.6 to 20.3 +/- 2.0 % (-271 to -354 kPa). The second peak in ABA represented a four-fold increase over the Control and occurred at 15 DAT with an average soil moisture of 11.6 +/- 0.4% (-2360 kPa). There were no significant differences in either stomatal conductance (Fig. 4) or stem sap pH measurements (Fig. 5) found between any of the Control and Non-alt. PRD treatments. While there was a variance observed in sap pH, there was no significant difference or trend in sap pH over the sampling period.

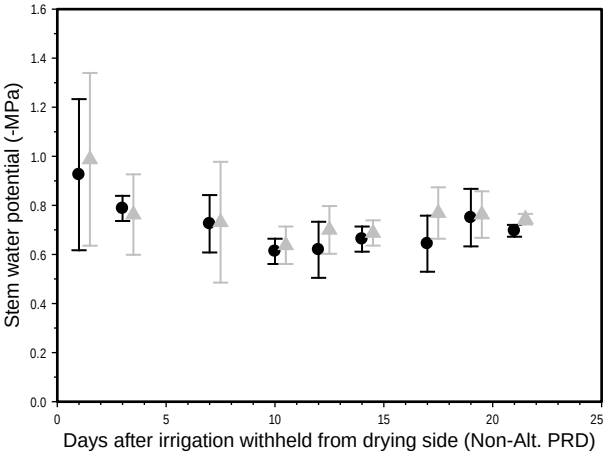

Figure 2. Plant water status measured by stem water potential. Control (●) and Non-alternated PRD treatment (▲) sampled on same day, Non-alternated PRD treatment offset ½ day ahead for graph clarity. Data are means (n = 4) with 95% confidence intervals (n = 8 for day 21).

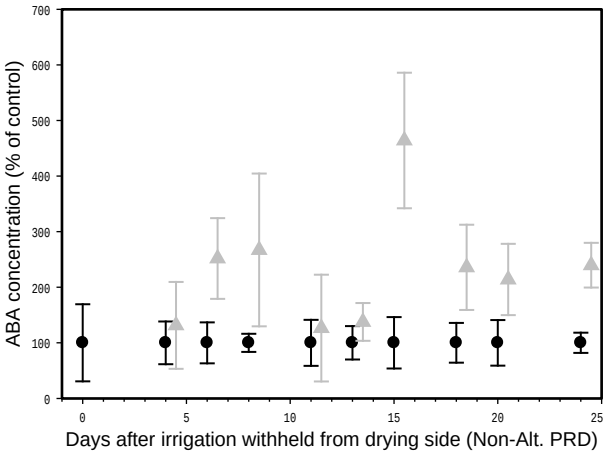

Figure 3. Abscisic acid concentration of xylem sap. Control (●) and Non-alternated PRD treatment (▲) sampled on same day, Non-alternated PRD treatment offset ½ day ahead for graph clarity. Data are means (n = 4) with 95% confidence intervals.

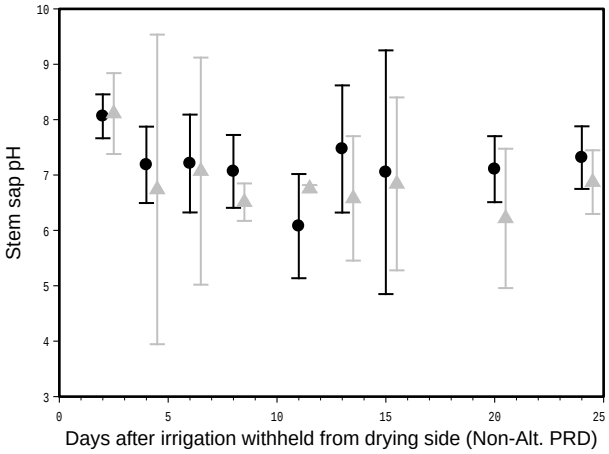

Figure 4. Stomatal conductance. Control (●) and Non-alternated PRD treatment (▲) sampled on same day, Non-alternated PRD treatment offset ½ day ahead for graph clarity. Data are means (n = 28 - 24 over sampling period) with 95% confidence intervals.

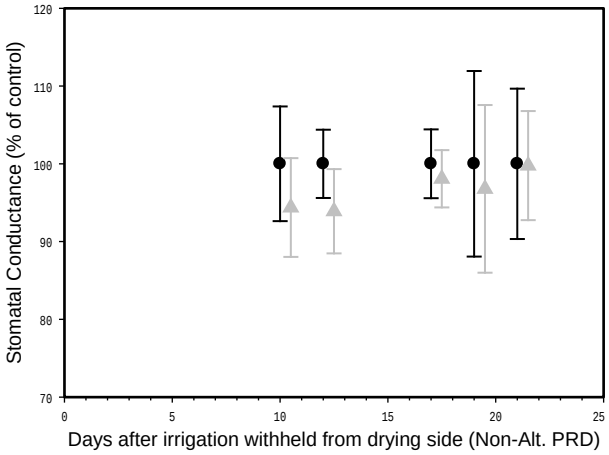

Figure 5. Stem sap pH. Control (●) and Non-alternated PRD treatment (▲) sampled on same day, Non-alternated PRD treatment offset ½ day ahead for graph clarity. Data are means (n = 4) with 95% confidence intervals (n = 8 for day 21).

DISCUSSION

Plant water status was maintained throughout a majority of the trial (Fig. 2). The main difference between PRD and RDI is the maintenance of plant water status under PRD (unlike RDI) while causing a biochemical (not hydraulic) change within the plant. Given that an ABA response was found (Fig. 3) while plant water status (Fig. 2) was maintained, this confirms that a biochemical response due to non-alternated drying under PRD conditions as opposed to deficit irrigation was achieved. The small but significant difference in plant water status found on 21 DAT (Fig. 2) was not expected (given that the 'wet' side of the Non-alt. PRD treatment and both sides of the Control had water available at <2 kPa) and can be attributed to the reduced variance associated with an increased sample size (n = 8).

ABA response. Xylem sap ABA was found to increase significantly in the Non-alt. PRD treatment over time as soil moisture decreased. This is consistent with previous work that has identified a strong correlation between elevated xylem sap ABA and soil drying under PRD (Dry et al., 1996; Loveys et al., 2000; Loveys et al., 1998; Sobeih et al., 2004; Stoll et al., 2000). However, the presence of two peaks in ABA elevation during the trial has not been reported previously. The first ABA elevation and subsequent decline occurred at a relatively low soil moisture deficit of -271 to -354 kPa and may be a 'false' response induced by the drying of roots which had grown into the Geofabric® lining in the bottom of each pot. When the water was removed from the saucers below the pots on trial initiation, roots in the Geofabric® would have dried rapidly. This created an elevated synthesis and release of ABA and mobility into the xylem was relatively easy through the still active transpiration stream.

The second peak in ABA under PRD occurred after 15 days and coincided with a volumetric soil moisture level of 11.6% (-2360 kPa). This response under PRD conditions to soil moisture coincided with a more extreme soil water deficit, similar to that found in previous studies (Dry et al., 1996; Loveys et al., 2000; Loveys et al., 1998; Sobeih et al., 2004; Stoll et al., 2000). However, what must also be considered are the method and timing of sap extraction used and the origin of the elevated ABA levels measured in the stem sap given the extremes in soil moisture deficit present. Night time re-hydration and hence movement of sap into the drying roots most likely occurred and therefore a degree of catabolism of the drying root-derived ABA may have been present. To add to this is the uncertainty in the degree of contribution to sap flow at the surface of the cut stem originating from the drying roots due to the extreme in soil water potential.

Stomatal response. The elevation of xylem sap ABA under PRD has been reported (Dry et al., 1996; Loveys et al., 2001; Loveys et al., 1998; Stoll et al., 2000) to cause a partial reduction in stomatal aperture. This trial found that imposing a soil moisture gradient across the root zone elevated the stem sap ABA by four-fold compared to that in the Control treatment (Fig. 3). However, increases in stem sap

ABA did not correspond to any reduction in stomatal conductance (Fig. 4) for the same measurement period. Unfortunately, stomatal response was not measured on the same day as the maximum ABA elevation (15 DAT). However, on 18 DAT and 20 DAT twice the ABA level was measured without any stomatal response measured on 19 DAT.

The absence of a stomatal response may partly be attributed to the trial being conducted under comparatively low evaporative conditions within a glassed greenhouse environment, reducing the transport of ABA to, and the sensitivity of, the stomata (Gutschick, 2002; Radin, 1992; Trejo et al., 1995; Wilkinson and Davies, 2002). Additionally, the concentration of ABA measured in the stem sap may not accurately reflect the actual amount of ABA present in the leaf (Wilkinson and Davies, 1997). The ABA carried in the transpiration stream of cotton has been found to be almost ten times that found in the leaves (Kefu et al. 1991). This suggests that leaf metabolism plays a major role in reducing ABA delivered to stomata in cotton.

The ABA in the leaf apoplast (i.e. site of action) is influenced by the sequestration and release of ABA by the symplast and metabolism by mesophyll cells (Radin, 1992; Trejo et al., 1995; Trejo et al., 1993; Wilkinson and Davies, 2002). Hence, the increases in xylem ABA concentrations may not have been sufficient to produce changes in stomatal behavior (on measured DAT). Other factors (e.g. sap pH) have also been linked to stomatal responses (Wilkinson, 1999; Wilkinson and Davies, 1997; Zhang and Davies, 1990).

Sap Ph. There was no change in stem sap pH associated with the soil moisture gradients imposed in this trial. Wilkinson and Davies (2002) suggest that species which exhibit a very low stomatal sensitivity to increases in ABA may do so because the xylem/apoplastic pH does not alkalinize with soil drying. As previously found in cotton (Radin et al., 1982), the lack of sap alkalization results in greater partitioning of ABA away from the guard cell apoplast. Stomatal response in cotton therefore requires sufficient ABA to overload and/or sufficient alkalinization to reduce partitioning of ABA away from the guard cell apoplast. Hence, the lack of stomatal response in cotton to elevated xylem sap ABA may be also partly explained by the lack of stem sap pH response.

Implications for imposing PRD under commercial conditions. This work raises serious doubts regarding the potential to impose a PRD strategy under commercial field conditions. Although an increase in ABA was found due to the imposed soil moisture gradient, this was achieved only when the dry side of the root zone reached approximately -2360 kPa. However, as cotton is normally grown on heavy clay soils in Australia, it would be difficult to simultaneously maintain plant water status and to create this level of soil moisture gradient across the plant row using existing irrigation application systems.

CONCLUSIONS

The application of a soil moisture gradient across the root zone of cotton grown under greenhouse conditions produced an elevation in xylem sap ABA after 15 days of soil drying (i.e. equivalent to a soil moisture potential on the dry side of -2360 kPa). Plant water status was maintained throughout the trial and therefore non-alternated PRD conditions were successfully applied. However, the application of the soil moisture gradient did not produce a reduction in stomatal conductance or any change in sap pH during the trial. The lack of a measured stomatal response may be due to insufficient elevation in xylem ABA, limited delivery to guard cells, lack of change in xylem sap pH, and/or comparatively low evaporative conditions imposed within the greenhouse environment.

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