

CAN WE DELIVER ENHANCED WATER USE EFFICIENCY, SUSTAIN YIELDS AND ENHANCE YIELD QUALITIES BY EXPLOITING ROOT-TO SHOOT SIGNALLING MECHANISMS IN CROP PLANTS?

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INTRODUCTION

Controversy still surrounds the regulation of plant growth and gas exchange by chemical and hydraulic influences in drying soils. What is clear is that restrictions in growth and gas exchange can occur with relatively small changes in soil water availability, in the absence of any change in plant water status (see Henson *et al.*, 1989). This observation has been used to suggest that non-hydraulic regulation of stomatal water loss pre-empts and in fact prevents a change in plant water status.

Root to Shoot Signalling

A range of putative root-to-shoot signals are implicated in communicating information regarding the water status of the soil from the roots to the shoots. These signals include the plant growth regulators abscisic acid (ABA) and ethylene, numerous ionic signals and the pH of the xylem sap itself.

Abscisic Acid

ABA concentrations in the xylem sap increase in a linear manner in response to a decline in soil water availability, providing good evidence for its role as a putative signalling molecule. Particularly in field condition, measured values of stomatal conductance show a closer relationship to the ABA concentration in the xylem sap than the water status of the leaf (Tardieu & Davies 1992). Much of the evidence to support a role for ABA has however relied on simple correlations between changes in xylem sap concentrations of ABA and changes in stomatal conductance and leaf expansion rates. Little data, until the late 1990's provided any convincing evidence to suggest a causal relationship. See Davies & Zhang (1991) for a review.

Xylem sap pH

The pH of the xylem sap has also been shown to correlate well with soil water status, within an increasing alkalinisation of sap as soil dries being reported in numerous species. How this change in xylem sap pH is generated is unclear, although as the soil dries numerous changes occur to the ionic composition of sap, which may result in changes to pH directly or via changes in sap buffering capacity (see Gollan *et al.*, 1992). By artificially increasing the xylem sap pH in bioassay systems, we have shown that increased xylem sap pH can induce stomatal closure and inhibit leaf expansion, mimicking the effect of soil drying. By using a range of naturally occurring mutants which produce low levels of ABA under both well watered and soil drying conditions, we have been able to demonstrate that the pH induced responses are ABA-dependent. Mutants with little or no endogenous ABA do not respond to an increase in xylem sap pH in bioassay systems. However, the re-introduction of a very low concentration of ABA back into the xylem stream (which in itself cannot elicit stomatal closure or a restriction in leaf growth rate when fed under more acidic 'well watered' xylem sap pH values) restores the stomatal and leaf

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growth response of mutant plants to increased xylem sap pH. See Wilkinson *et al.* (1998) and Bacon *et al.*, (1997).

The ABA-dependent pH response can be explained by the properties of the ABA molecule as a weak acid. Under 'well-watered' conditions, the pH of the xylem sap is below that at which the ABA molecule will dissociate (its pKa value). In its undissociated form, ABA carries no net charge and is readily taken up into the symplast, away from its active sites for growth regulation and stomatal closure in the apoplast. However, as the pH of the sap increases, ABA readily dissociates. The resulting charged molecule is not so readily taken up into the symplast and resides for longer in the apoplastic compartment of the leaf, close to its active site. This increased residency time is sufficient to allow relatively low concentrations of ABA (those typically found in well watered plants) to be physiologically active. This is some of the first data to provide evidence of a causal relationship between ABA and growth regulation and the control of gas exchange and some of the first evidence to suggest that increased ABA concentrations are not always required for it to have a physiologically important role.

Using Chemical signalling to explain hydraulic responses

An understanding of how ABA may redistribute within the leaf or plant can be used to explain apparently hydraulically regulated phenomenon (see Mega Wahab, 2004).

In some species, stomatal closure in response to soil drying, can be reversed by the artificial application of pneumatic pressure around the root systems (see Comstock & Mencuccini, 1998). The ability to reverse stomatal closure in this way has been used to suggest that stomatal function is regulated by leaf water potential. However, measurement of ABA concentrations at the active site for stomatal closure during pressurisation, has demonstrated that ABA concentrations decline and the xylem sap becomes less alkaline. Removal of these inhibitors, via pressurisation, can therefore be used to explain the stomatal re-opening response.

Ethylene as a plant growth regulator?

There is increasing interest in the role of ethylene in the regulation of plant growth. We have collected data which demonstrates that removal of endogenous ethylene levels via use of the ACO₁ anti-sense tomato transgenic line, reduces the sensitivity of the leaf growth process to soil drying.

Corroborative evidence from a different biological system also supports the role of ethylene in restricting leaf expansion. Certain plant growth promoting rhizobacteria (PGPR) such as *Variovorax paradoxus* contain an enzyme which deaminates 1-amino-cyclopropane-1-carboxylic acid (ACC). ACC is xylem mobile and is thought to act as a signal from the root to the shoot, at which point it is hydrolysed to form ethylene within the leaf where it restricts cell expansion. Inoculation with PGPR is therefore thought to prevent the generation of this ACC signal via its deamination and use as a bacterial metabolite in the rhizosphere. Using this approach and also via metabolic inhibition of ethylene synthesis using chemical inhibitors of ethylene biosynthesis, it is possible to show that both PGPR and ethylene biosynthesis inhibition, promotes plant growth. Elevation of growth restriction using both of these approaches is particularly dramatic in plants experiencing soil drying, providing very persuasive evidence of a role for ACC and ethylene in mediating the plant growth response to changes in soil water availability (Belimov *et al.*, 2003).

Ethylene levels would appear to be regulated by ABA. In ABA-deficient mutants of tomato, endogenous ethylene levels are significantly raised and plants express clear signs of

dramatically increased endogenous ethylene levels, including advantageous root development. This evidence has been used to suggest that by restricting ethylene levels, ABA is actually acting as a growth promoting plant growth regulator, rather than in its classic role as a growth inhibitor (see Sharp, 2002).

USING AN ACADEMIC TOOL TO DELIVER WATER SAVING IN AGRICULTURE

The Split Pot Experiment

Some of the early experiments to demonstrate a non-hydraulic regulation of growth involved the splitting of plant root systems between two pots (the split pot system). In this way it was possible to allow a proportion of the roots to dry the soil in which they were rooted, while supplying sufficient water to the other proportion of the root system to ensure that plant water status was maintained at values comparable to well-watered controls. Despite successful maintenance of plant water status, exposure of part of the root system to drying soil, resulting in a decline in leaf growth rate and stomatal closure. Watering the roots in the drying soil or removing them via excision reversed this effect and led to the conclusion that a positive, root-sourced signal must have been provided to the shoot, via the xylem stream, by those roots in contact with drying soil. See Gowing *et al.* (1990).

Partial Root Drying (PRD)

The ability to restrict stomatal water loss and excessive vigour remain two of the goals of commercial viticulture. Brian Loveys and colleagues at the Waite Institute, CSIRO in Adelaide took the concept of the split-pot technique and developed 'Partial Root Drying' (PRD). In the field, the 'split pot' principle is applied by supplying irrigation to a proportion of the roots while a proportion of the roots are allowed to dry the soil in which they are rooted. Via regular alternation of the proportion of the roots that are allowed to dry the soil, the entire root system is maintained in a viable state. PRD offers the ability to better control the water relations of the crop, when compared to conventional deficit irrigation practices. While these deficit practices are well established in commercial agriculture, unregulated deficits can lead to changes in plant water status, which depending upon when they occur, may have negative impacts on yield quantity and quality.

The early experiments at CSIRO, demonstrated that by using PRD, the total amount of water applied to the crop during the season could be significantly reduced, the amount of water lost through transpiration decreased as a result of partial stomatal closure and vegetative vigour was reduced. Importantly, crop yield was not affected and the quality characteristics of the berries and subsequent wine, were significantly improved. The reasons for this increase in quality are not fully understood, but an increase in the exposure of developing berries to the sun, achieved via reduced vegetative vigour, is seen as an important factor in determining ideal concentrations of a range of metabolites in grapes for use in high quality red wine production.

The IRRISPLIT European Research Consortium

The success of the Australian story has led to increased interest in this technique across many different sectors of global agriculture. The IRRISPLIT research consortium, funded by the European Union's Framework V Research Programme, were tasked with trailing the technique in a range of different European and Mediterranean countries in a range of crops, including cotton, maize, field vegetables, grapes, citrus, olives and soft fruit. PRD and other deficit regimes were compared against conventional irrigation approaches, with varying degrees of

success. Some crops such as pepper seemed wholly unsuited to irrigation via PRD, with no increases in water use efficiency (WUE) evident, while other crops such as raspberry appeared remarkably resilient to quite severe levels of deficit irrigation, with significant increases in WUE. Crops such as grape, cotton and tomato, exhibited only marginal declines in yield, for significant increases in water use efficiency, but with some interesting and positive effects on yield quality. In a majority of cases, PRD was shown to effectively control plant water status.

In a detailed series of experiments, we have demonstrated that PRD prevents the damaging changes in cellular water relations in developing tomato fruit which are responsible for the production of poor quality fruit under poorly controlled traditional deficit regimes (see also Davies *et al.*, 2000).

Does PRD offer something that traditional deficit techniques do not?

Using a cellular micro-pressure probe it is possible to detect changes in the water status of individual cells of tomato fruits in the outer tissue layer that regulate fruit growth rate and quality. Under PRD irrigation, the water relations of these cells are maintained, while deficit irrigation causes a gradual loss of cellular turgor, a key component of cellular water relations in determining the rate of fruit growth. While the rate of fruit growth in deficit-irrigated plants was shown to correlate well with plant cell water relations, this was not evident in PRD irrigated plants. Growth rates in both PRD and deficit irrigated plants were associated with increases in the pH of the apoplastic compartment bathing these expanding cells. In PRD experiments in grapes growing in Portugal (see Chaves, *et al.*, 2003), there were clear differences in the effect of PRD and deficit irrigation on the level of stomatal closure achieved via the application of an identical, but reduced volume of water throughout the season (See Mingo *et al.*, 2003). These observations provide some evidence therefore that PRD is exacting a particular, chemically-based regulation of physiology, in the absence of any changes in plant water relations.

In another series of experiments examining the performance of tomato plants exposed to PRD irrigation, a significant increase in biomass allocation to the roots, at the expense of all other parts of the plant was observed (Mingo *et al.*, 2004). This phenomenon appeared to relate to the increased rate of biomass accumulation by re-watered roots that had previously been allowed to dry the soil, rather than enhanced root growth in drying soil.

CONCLUSIONS

Despite the large amount of work now being carried out on the physiological basis to PRD there is clearly a lot we do not understand. While we are probably confident in the ability of PRD in maintaining the water relations of a crop while applying a reduced amount of water, the chemical signalling hypothesis remains poorly supported by any wealth of data. There are also several indirect effects of PRD, most notably the changes to yield quality, root biomass allocation and subsequent effects on nutrient use efficiency. In some cases these may be more important than the primary effect of PRD on plant water status. At the most basic level, application of PRD and other deficit techniques may simply be coupling water supply more closely to water use. To determine whether PRD actually delivers an increase in water use efficiency – or more crop per drop – in a way that other more conventional deficit regimes cannot, will require a more developed experimental framework. This framework will need to consider the response of crop yield and quality to a variety of levels of deficit irrigation, applied via conventional and PRD means and the effect the severity of any type of deficit treatment. It will also be important to consider deficit regimes against both crop water use and conventional

application rates. Using this framework we will be able to generate yield response surfaces and determine whether PRD provides a clear modification to a plants physiology different from responses to traditional deficit irrigation. This framework will also provide the basis to a thorough physiological interrogation of the underlying growth regulatory mechanisms.

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