Has extrapolation from biochemistry to crop functioning worked to sustain plant production under water scarcity?

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Abstract
This paper considers the impact on crop productivity of an enhanced understanding and subsequent manipulation of plant drought stress biochemistry and physiology. Emphasis is placed on possible exploitation of our recent increase in understanding of long-distance chemical signalling in plants, particularly in relation to soil drying. We review evidence for a variety of signalling cascades involving hormones and nutrient ions in the xylem sap. We also assess the importance of changes in the pH of the leaf cell apoplast as influenced both by edaphic and climatic variation, as a regulator of shoot growth and functioning. The sensitivity of the sensing and signalling mechanism is emphasised.

A number of possible manipulations of the long-distance signalling process are discussed but emphasis is placed on the development of a novel deficit irrigation system: partial rootzone drying (PRD). This system has been much researched and introduced into a variety of plant production systems. Substantial increases in the efficiency of water use can result from the use of PRD, along with increases in crop quality. The modification of plant performance (most commonly reduced vegetative growth with only minimal effects on reproductive development) is commonly attributed to the effects of drought signalling. We assess the evidence for this assertion and argue for more understanding of the nature of the signalling processes in the droughted plant. This can help us further develop both high-technology and low technology modifications of production systems to allow for more sustainable use of resources.

Media Summary
The commercial exploitation of recent advances in our understanding of fundamental plant biology. A low technology irrigation solution which allows the growth of crops with significant reductions in water use without substantial yield penalty (i.e. increased water use efficiency).

Key Words
Long distance drought signalling, abscisic acid, ethylene, pH, deficit irrigation, partial rootzone drying (PRD), water use efficiency (wue)

Introduction
The growth, development and yielding of crop plants is highly sensitive to a reduction in water availability in the soil (e.g. Boyer, 1982) and even a few days of drought stress at a critical period during the production of yield components can result in complete crop failure (e.g. Boyle et al. 1991). There is general agreement among plant breeders that much reduction of yield due to 'drought stress' occurs at the wet end of the spectrum, well before plants are themselves stressed in the conventional sense of the word (e.g. Richards, 1991). This is because plants can sense and respond to changes in their immediate environment (e.g. soil drying) and then regulate growth and functioning, for example, to avoid shoot dehydration stress. To sustain yielding as soil dries, which will be necessary as demands grow for judicious water management in agriculture, the plant biologist must initially address these regulatory processes, rather than focussing on processes that contribute to desiccation resistance. For example, the limitation of leaf growth as soil dries around the roots is often highly tuned to a reduction in soil water availability with the result that biomass production is often substantially reduced when there is still plenty of water in the soil. In this case, reducing the sensitivity of the plant's environmental sensing mechanisms or its regulatory response to the stress can act to stabilise vegetative crop yield between years and enhance yield per unit cropping area, which will be necessary to sustain food production on an ever-decreasing stock of cultivatable land. In contrast to this, we show below how sensitive long-distance signalling mechanisms can be manipulated in some crops to reduce un-necessary vegetative growth while allowing maintenance of fruit production with a reduced supply of water. Crop quality can also be sustained under water deficits by the manipulation of signalling processes. Such manipulations will be necessary if we are

maintain food production while reducing the amount of irrigation water used in agriculture. Currently, 70% of the world's water is used in agriculture. If substantial water savings in agriculture can be achieved without substantial yield penalty, then the use of this water elsewhere can bring substantial benefits to society.

In our view, an understanding of the plant's <u>regulation</u> of growth and development as the soil dries can aid substantially in water management in dryland environment. We will show below how long distance chemical signalling in plants in drying soil can close stomata to sustain shoot water status which can itself be a benefit for crop quality, even if growth is not sustained. An understanding and manipulation of the processes limiting shoot growth and development of plants in drying soil (hydraulic and chemical manipulation) may enable us to produce more biomass per unit of water used in agriculture.

Water use can be reduced by more precision in water delivery. New technologies are available to achieve this but even if these are used by farmers, there is still the opportunity to save yet more water while sustaining crop production. For many crops there is still an incomplete understanding of just how much water is required for yielding. Advances in understanding in this area can save water but this understanding also focusses attention on the possibilities for the use of so-called deficit irrigation (the application of only a predetermined percentage of potential water loss). If this type of management practice is not to damagingly reduce crop yielding, then the kind of plant manipulations proposed above will be necessary. Water management practices such as deficit irrigation and protected cropping will not only greatly reduce water use, they also offer the opportunity to enhance crop quality and enhance the yielding of stress-responsive, plant-sourced chemicals which may have positive benefits for human health.

Although plant biologists have given an enormous amount of attention to plant desiccation resistance, these processes are largely irrelevant for crop yielding. If plant cells desiccate, crop yielding will be negligible and even if yield is doubled by plant manipulation, then it is still negligible! One exception to this situation is the combination of responses that allow a perennial crop plant to stay alive under desiccating conditions. This capacity to 'live to fight another day' can be highly advantageous for yield in succeeding growth seasons. The capacity to survive is largely irrelevant in an annual crop plant where a stress-induced delay in development can result in a complete loss of yield (e.g. if the crop is growing in a relatively short frost-free season).

We argue here that by focussing our attention on understanding the processes which contribute to the regulation of crop growth when there is plenty of water in the soil or when soil moisture deficits are relatively mild, then there are prospects of maintaining yield while using substantially reduced quantities of water in agriculture, a highly desirable combination of deliverables. In our view, this can be achieved most effectively by exploiting our understanding of the plant's drought stress physiology. In this review we place emphasis on the gains that can be achieved by an understanding and exploitation of the long distance chemical signalling processes in plants.

Long distance Abscisic acid signalling
It is now well known that stomatal behaviour can be closely related to changes in soil water availability even when the leaf water status is not affected by soil drying. Using spit root techniques, with one drying and one well watered root half (later referred to as partial root drying, PRD) (Blackman and Davies, 1985) showed that stomata close despite an unchanged leaf water potential. Later it turned out (for review see Davies and Zhang, 1991) that abscisic acid was the major chemical root-to-shoot stress signal. Transgenic plants that have a low capacity for ABA biosynthesis show a disturbed stomatal behaviour and grafting ABA-deficient roots to ABA-sufficient shoots cannot restore the stomatal response (Borel et al., 2001). Some rather different results have been obtained with other species (Holbrook et al. 2002), but from the balance of evidence it has been concluded that roots can 'measure' the water availability in the soil which results in increased ABA biosynthesis, a stimulated ABA transport and release into the xylem vessels. Abscisic acid can be synthesised in the cytosol of all root cell types with comparable rates in the different cell types. High ABA concentrations in root tips are a result of the low vacuolisation of the root tip cells (shown for maize and runner bean roots, Hartung et al., 1999). Besides drought stress, other soil factors that are characteristic of soils of arid climates, such as special nutrient relations (variation in nitrate and ammonium supply, phosphate and potassium deficiency) soil strength, high salt concentrations

of the soil solution, all stimulate ABA formation in roots, release to the xylem and translocation to the leaves. In only some cases does this extra ABA accumulate in the leaves. It has been shown that at least in the case of P-deficiency (Jeschke et al, 1997) and K^+- deficiency (Peuke et al., 2002) ABA is metabolised to phaseic acid after action on stomata. Therefore, although ABA acts in these situations as an effective root-to-shoot signal, no accumulation in the leaves can be observed.

Besides ABA being synthesised in the roots, much of the ABA found in the xylem actually originates in the leaves. ABA synthesised in the leaves and can be loaded into the phloem and transported to the roots. Here one part of it may be deposited in the tissue or metabolised, another part, however, may be loaded to the xylem vessels and recirculated to the shoots. Salt stress, phosphate deficiency and ammonium nutrition enhance the percentage of ABA arriving in the phloem that is recirculated from the roots to the shoots (Jeschke et al., 1997; 1990; Peuke et al., 1994). Most interestingly, recent work by Soar et al. (2004) suggests that much of the ABA found in grapevine leaves is made *in situ* and stays there.

Both free and conjugated ABA also can be taken up by the roots from sources external to the plant and then loaded into the xylem vessels. Both free ABA and ABA-glucose ester (ABA-GE) have been detected in the soil solution under a range of crop plants in concentrations up to 10 nM or 30 nM respectively (Hartung et al., 1996; Sauter and Hartung, 2000). External, free ABA can be readily taken up by roots. It also participates in maintaining an ABA equilibrium between roots and the external medium and this can be crucial in ensuring that long distance ABA signalling operates optimally (Hartung et al., 1996). The uptake from the soil of conjugated ABA is strongly dependent on the extent and properties of apoplastic barriers/bypass in the hypodermis, as discussed later in this paper.

Until recently little information was available regarding the transport paths and transport mechanisms of ABA into, out of and through roots. It was assumed that radial ABA transport occurs exclusively in the symplast (Jackson et al., 1996). In this case the intensity of the ABA signal in the xylem depends strongly on the lateral water flow through roots. ABA would be diluted drastically when transpirational water flow is increased. Computer simulations of Slovik et al. (1995) support such assumptions. Modelling of a symplastic ABA transport pathway of the root-to-shoot-signal has indicated dramatic changes in the ABA concentration of the xylem sap, even on a diurnal basis. In the case of field grown maize, however, Tardieu et al. (1992) found constant xylem ABA concentrations during the diurnal day/light cycles indicating that mechanisms must exist that maintain some ABA homeostasis in the xylem Steudle and co-workers (e.g. see Steudle and Peterson, 1998) have suggested that some water and solutes, including ions, pass into the root exclusively through the apoplast. This resulted in a complex model, the composite-transport model, which adequately accounts for the various physiological demands of roots. The model suggests that ABA could be translocated to the xylem by solvent drag with the transpirational water stream through the apoplast. Such a bypass-flow could buffer the dilution that can be caused by increased water flow. The model of Slovik et al. (1995) predicts that a small contribution (1%) of such an apoplastic bypass-flow will compensate for any ABA-dilution caused by symplastic transport combined with high rates of water transport.

Experimental evidence for the presence of an apoplastic ABA bypass-flow was provided by Freundl et al. (1998, 2000). These workers applied ABA in the physiological concentration range of 5 nM up to 100 nM to media bathing excised root systems of maize (*Zea mays*) and sunflower (*Helianthus annuus*). The xylem sap was then collected by application of subatmospheric pressure to the cut surface of the mesocotyl (Freundl et al., 1998). An intensified radial water flow (J_{Vr}), induced by the pressure gradient, did not reduce the ABA concentration of the xylem sap and even increased it.

Being a weak acid ($pk_a = 4.8$), ABA is distributed within the apoplast and the symplast of root tissues according to the anion-trap concept and the Henderson-Hasselbalch equation. Acidification of the apoplast, as occurs in ammonium-supplied roots (Gerendás and Ratcliffe, 2000), should redirect ABA to the cytosol of root cortical cells. ABA should then be translocated predominantly in the symplast. An increased water flow J_{vr} should therefore dilute the signal. Indeed, as shown by Peuke et al. (1994) the concentration of ABA in the xylem sap is significantly higher in plants supplied by NH_4^+. However, the ABA concentration is reduced whenever water flow is increased (Hose et al., 2002).

In most roots, hypodermal cell layers can build up Casparian bands in their radial cell walls (Peterson, 1988), in addition to those in the endodermis. Hose et al. (2001) demonstrated that Casparian bands in the hypodermis caused a significant reduction in radial ABA flow through exodermal roots, especially at high water flow rates. On the other hand, roots lost significantly smaller amounts of ABA to the surrounding media when a Casparian band was present in the hypodermis. Hose et al. (2002) compared the efflux of ABA from exodermal and non-exodermal maize roots using compartmental efflux-analysis. The half time of isotope exchange from the apoplast of exodermal roots was 2-3 times higher than in the non-exodermal controls. This indicates that the Casparian band of the maize exodermis is an effective barrier for ABA, slowing down ABA efflux to the surrounding medium. Lower efflux results in an increased ABA concentration in the root apoplast. Such "extra ABA" can stimulate the hydraulic conductivity of root cortical plasma membranes (Hose et al., 2000). It is also available for apoplastic translocation by solvent-drag directly into the xylem vessels.

pH as a long distance signal of soil drying
Alkalisation of xylem sap is a common response to soil drying in some plants (e.g. tomato, barley and grapevine) (see e.g. Wilkinson et al., 1998 for tomato data) and supplying detached leaves with neutral or alkaline buffers (pH ≥ 7) via the transpiration stream can restrict transpiration (Wilkinson and Davies, 1997; Wilkinson et al., 1998). These buffers can apparently increase apoplastic pH, which will result in an increase in higher apoplastic ABA concentrations (ABA will distribute between compartments of the leaf according to the Henderson-Hasselbalch equation). pH-induced increases in apoplastic ABA concentration will ultimately close the stomata (Wilkinson and Davies, 1997). Stomatal closure in response to xylem-supplied alkaline buffers was shown to be ABA-dependent, as leaves detached from an ABA-deficient tomato mutant (*flacca*) did not show stomatal closure when fed pH 7 buffers (Wilkinson et al., 1998). In wild-type (WT) plants, it is possible that increased xylem sap pH could elicit ABA-dependent stomatal closure without the need for increased xylem ABA delivery. In other words there will always be enough ABA to close stomata, even in the well watered plant, but the response to that ABA will be highly pH dependent. Temporal changes in xylem sap pH and ABA concentration during a soil drying episode have been measured, and increased xylem sap pH can precede (Bahrun et al., 2002; Sobeih et al., 2004) or occur after (Liu et al., 2003) increased [X-ABA].

Increased xylem sap pH can also correlate with drought-induced leaf growth inhibition in barley and feeding leaves alkaline buffers via the xylem inhibits leaf growth (Bacon et al., 1998). Feeding alkaline buffers to an ABA-deficient barley mutant (*Az34*) did not inhibit leaf growth unless an ABA concentration typical of well-watered plants was also present in the buffer. These responses are directly analogous to the effect of alkaline buffers on detached leaf transpiration. In grass leaf bases, increased sap alkalinity presumably allows ABA access to sites of action within the leaf elongation zone, inhibiting growth. In well-watered plants with a more acidic, apoplastic pH, ABA is presumably partitioned into alkaline compartments in the symplast and away from sites of action regulating leaf growth.

It interesting to note that the pH of xylem sap of all plants is not increased as soil dries. This may particularly be the case in woody plants (see e.g. Wilkinson and Davies, 2002) and in order to understand these differences, it is important that we understand the mechanistic basis of the change in xylem pH as the soil dries. Clearly the buffer capacity of the sap is important here. There is very little information on the relationship between xylem pH and the pH of the apoplast of the leaves. The proposal made above for long-distance pH signalling requires that there should be a fairly tight relationship between the two. Our most recent work highlights the impact of climatic factors on the pH of the apoplast/xylem sap within the shoot and while this may suggest a rather exciting way for the plant to integrate the impacts of climatic and edaphic factors, these observations do complicate rather simple proposals that information on soil water availability can be communicated directly to shoots.

ABA conjugates as root-to-shoot stress signals
Munns and King (1984), Netting et al. (1992) and Munns and Sharp (1993) have postulated that ABA conjugates (sometimes referred to as the ABA adduct) can act as an additional root-to-shoot stress signal. Until now, the chemical structure of this adduct could not be elucidated, but ABA glucose esters have been found in xylem and these compounds may play an important signaling role.

Bano et al. (1993, 1994) identified enhanced concentrations of glucose esters of ABA, phaseic acid and dihydro phaseic acid in the xylem sap of stressed sunflower and rice plants. The concentrations of free and bound ABA decreased again after relief of the stress condition. Dietz et al. (2000) found a 4-5 fold stimulation of ABA-glucose ester (ABA-GE) concentration in the xylem sap of barley under salt stress by 4-5 fold. Five alkali hydrolysable conjugates of ABA were detected in the xylem sap of well-watered sunflower plants (Hansen and Dörffling, 1999). Under stress a sixth conjugate appeared and the concentration of the others was enhanced significantly. Drought stress increased the amount of ABA conjugates with β-glucosidic linkages, predominantly ABA-GE. More data on ABA conjugates in the xylem sap of stressed plants are cited and discussed by Sauter et al. (2002).

External conjugated ABA-GE) cannot be dragged with the water flow across the hydrophobic endodermis, as it is the case for free ABA. Especially aeroponically cultivated maize roots with a complete exodermis are not able to take up external ABA-GE. Both tissues, the exodermis and the endodermis are good barriers for ABA-conjugates. However, hydrolytic enzymes of the root cortex apoplast are able to cleave the conjugate (Sauter and Hartung, 2000). The released free-ABA can then be translocated in the apoplast to the xylem vessels. Translocation of endogenous ABA-GE must occur within the root symplast. Once arrived at the xylem parenchyma cells the conjugate can be released into the xylem elements since the plasma membranes of stelar cells show a five-fold higher permeability than those of the cortical cells. The mechanisms of membrane transport of ABA-GE are still unknown. Since ABA-GE can be loaded into the xylem elements, and transported to an even greater extent under stress conditions, the conjugate can be considered as a long distance signal. Dietz et al. (2000) have investigated the fate of the ABA-GE after having arrived in the leaf apoplast, and results show that esterases in the leaf apoplast release free ABA from its conjugates. The activity of these esterases is increased substantially under salt stress, thus making more free ABA available in leaves and linking the long distance signals in the xylem (ABA-conjugate) with an enzyme activity in the apoplast of the leaves.

Cytokinin signalling
Since the early days of phytohormone research (for references see Kamboj and Baker, 1998) we have known something of the importance of cytokinins as a hormonal signal originating mainly in the roots and transported in the xylem to the shoot. Cytokinins also are discussed as antagonists of ABA action on stomata. A role for cytokinins as a negative root-to-shoot stress signal has been widely discussed (Incoll et al., 1989). Stoll et al. (2001) have found a 50% reduction of zeatin and zeatinriboside both in the shoots and stems of grapevines grown under partial root-zone drying (PRD) conditions. They concluded that cytokinin transport in the xylem also should be reduced. Information about cytokinins in the xylem of plants under drought stress is rare in the literature. Masia et al. (1994) and Sashidar et al. (1996) did not find significant decreases in xylem cytokinins of drought-stressed sunflower plants (perhaps because of the extent of the drought stress applied), while Bano et al. (1994) investigated this problem with sunflowers and under drought stress found a decrease in the xylem sap of sunflower of trans-zeatin, zeatin riboside (ZR), isopentenyl adenine and isopentenyladenosine. Concentration changes of ZR in the xylem were investigated in more detail by Hansen and Dörffling (2003). When the water potential of sunflower hypocotyl decreased, the concentration and the flux of ZR decreased substantially after a transient rise. After rewatering it rose continuously reaching a 60-fold increase after 5 h and declined again afterwards. This increase correlated with an increase of leaf conductance. No information, however, is available about the significance of cytokinins in the rooting medium, although they have been found to be present in the soil solution under a range of different plants (Frankenberger and Arshad, 1995). Until now we also lack experimental data on lateral cytokinin transport through roots and the mechanisms that regulate the intensity of the cytokinin signal in the xylem. Moreover, we only can speculate about the fate of cytokinin conjugates such as ZR. They may be cleaved similarly by apoplastic esterases, as is the case for ABA conjugates. Dietz et al. (2000) have shown that different cytokinin conjugates can serve as substrates for such enzymes.

There are very few reports of studies where combinations of hormones are assayed. This is a clear deficiency in the literature as, for example, cytokinin:ABA ratios presumably will have an important impact on plant development and functioning (see e.g. Stoll et al., 2001).

Ethylene as a regulator of leaf and root growth under drought

Drought also increases root and xylem concentrations of the ethylene precursor ACC (1-aminocyclopropane-carboxylic acid) (Gomez-Cadenas et al. 1996). Feeding both ABA and ACC (the immediate precursor of ethylene) via the xylem to well-watered plants inhibits leaf growth additively (Dodd and Davies, unpublished results), suggesting an important role for ethylene in the regulation of leaf growth under stress. In contrast, in plants at low water potential, these two hormones interact where ABA restricts the extent of runaway ethylene synthesis and ABA accumulation is necessary to minimise ethylene-mediated decreases in root elongation (Sharp, 2001). Although the delivery of ACC from the root system can account for shoot ethylene evolution (Else and Jackson, 1998) and may thus limit leaf growth under drought, the relationship between xylem ACC concentration and leaf growth of plants exposed to drying soil has not been defined. Stomatal behaviour is apparently relatively insensitive to ethylene supply to leaves, except in very specialised circumstances

We have evidence that the plant hormone ethylene can be involved in both the suppression of root growth during soil drying and the suppression of leaf growth via long-distance chemical signalling, again emphasising a key role for this hormone in the regulation of plant production in dryland environments. Partial drying of the soil around the roots (PRD) of tomato plants can maintain leaf water potential at values equivalent to well-watered plants for up to 2 weeks This is largely a function of partial stomatal closure following ABA/pH long distance signalling from roots in drying soil. Ethylene evolution of WT tomato plants increased as soil dried but could be suppressed using transgenic ($ACO1_{AS}$) plants containing an antisense gene for one isoenzyme of ACC oxidase. Most importantly, $ACO1_{AS}$ plants also showed no inhibition of leaf growth when exposed to PRD (Sobeih et al., 2004) (Fig. 1) even though both $ACO1_{AS}$ and WT plants showed similar changes in other putative chemical inhibitors of leaf expansion (xylem sap pH and ABA concentration). It seems likely that the enhanced ethylene evolution under PRD is responsible for leaf growth inhibition of WT plants. $ACO1_{AS}$ plants showed no leaf growth inhibition over a range of soil water contents which significantly restricted growth of WT plants. There is now good evidence in the literature that enhanced ethylene production in plants in compacted soil leads to a limitation of leaf growth (Hussain et al., 2001) and since an increase in soil strength is a key component of soil drying, it seems likely that this hormone is an important component of the changes in long distance signalling capability that is stimulated by changes in water availability.

Exploiting the plants signalling capacity through the use of deficit irrigation

A major issue for the production of many fruit crops is excessive vegetative vigour. Use of assimilates in leaf growth can restrict fruit development while excessive leaf area can lead to wasteful losses of water and the development of damaging water deficits. Too many leaves can predispose plants to fungal diseases and lead to shading of fruits, causing a reduction in fruit colour and quality. With many crops, shoot development is restricted by pruning but this can be an expensive process and can provide infection sites for diseases. Some root crops will also suffer from excessive shoot vigour while by contrast, biomass crops generally require shoot growth to be maximised at the expense of root development, which is a major issue if water supply is restricted.
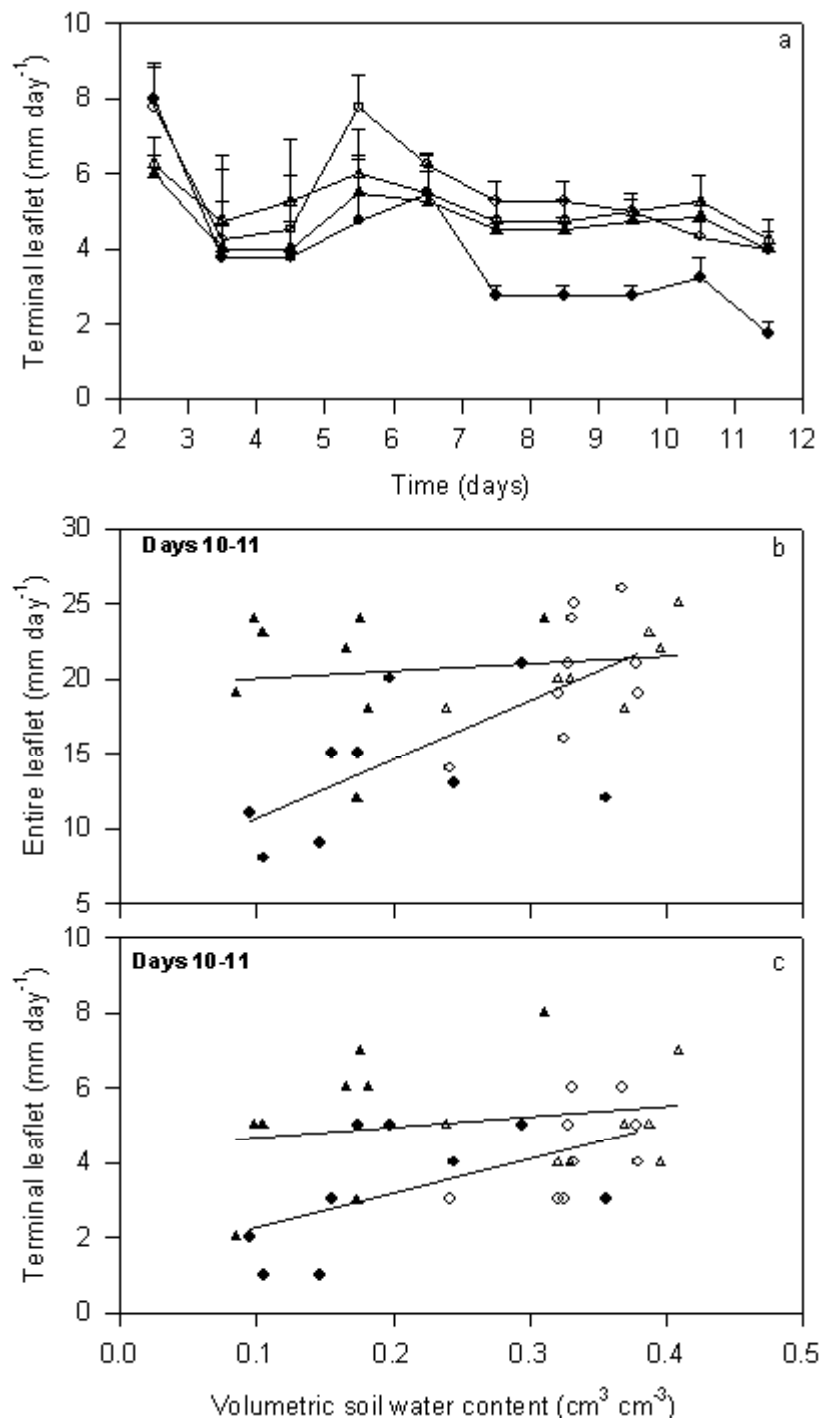
Figure 1. Terminal leaflet (a) elongation rates of leaves at Node 12 from WT tomato (●,○) or ACO1$_{AS}$

plants (▲,△) watered daily on one (●,▲) or both (○,△) sides of the split-pot. Data are means ± S.E. of 7-9 replicates. Entire leaf (b) and terminal leaflet (c) elongation rate (Days 10-11) plotted against the pre-watering volumetric water content of the upper 6 cm of soil on Day 10. Linear regressions were fitted to each genotype in SigmaPlot for Windows 2.01. Modified from Sobeih et al. (2004)

Restricting the supply of water to growing plants will nearly always reduce plant production and so in many production systems it is common to supply excess irrigation water to ensure high crop yields. In many situations, water is used wastefully and where water is in short supply, deficit irrigation (the application of only a predetermined percentage of calculated potential plant water use) is an attractive management option. Used carefully, Regulated Deficit Irrigation (RDI) will restrict water use and can beneficially restrict vegetative growth but it can also commonly reduce fruit yields (e.g., Matthews and

Anderson, 1988) and in hotter, drier years there is the risk of catastrophic crop failure. This approach can also result in shrinking and swelling of fruits, resulting in splitting or other types of fruit blemishing which further reduce the value of the crop. Furthermore, to operate RDI effectively, the grower must monitor plant and soil moisture status and deliver precisely regulated and directed quantities of water on demand and this can require sophisticated, expensive equipment.

Plant growth in drying soil is commonly limited by a combination of chemical and hydraulic influences (e.g., Bacon et al., 1998). Even when turgor of growing cells is sustained, growth can be limited by chemical 'signals' arriving in the shoot via the transpiration stream. Gowing et al. (1990) made a key observation which has now been exploited in deficit irrigation around the world. Namely, that production and expansion of leaves of apple trees could be significantly restricted by watering only half the plant root system (PRD). This restriction occured without any obvious influence of the treatment on shoot water relations and was attributed to the influence of increased delivery to the shoots of chemical regulators in the transpiration stream.

The introduction of the Partial Root Drying (PRD) irrigation technique
In 1991, Loveys suggested that it might be possible to exploit long-distance chemical signalling to regulate vegetative growth of grapevines in the vinyard. The hope was that root-sourced chemical regulators would restrict shoot development of this vegetatively over-vigorous crop, while the maintenance of shoot water status would ensure 'normal' fruit development. Subsequently, Dry and Loveys (1998) described results from experiments where grape vine vigour was significantly reduced without any yield penalty and at the same time, efficiency of water use and quality of fruit were significantly improved. These results were achieved by employing PRD in different experiments over several seasons. In PRD, the two halves of the plant's root system are watered alternately. Roots in contact with wet soil supply water to shoots, while roots in contact with drying soil generate root signals and these move to the shoot to modify growth and functioning. Irrigation must be switched regularly from one side of the root to the other to keep roots in dry soil alive and sustain the supply of root signals (Fig. 2). Effects of PRD on plant growth, yielding and functioning are quantitatively different from those of RDI. For example, when operated properly, plants under PRD will show sustained and even increased shoot and fruit turgor even though a reduced amount of water is applied to roots (Mingo et al., 2003). This is because of a signalling-induced partial closure of stomata and a reduced loss of water. Turgor maintenance and maintenance of total water potential can be important for maintenance of growth and for improved yield quality (de Souza et al., 2003; dos Santos et al., 2003) but most importantly reduces the risk and deleterious effects of using deficit irrigation (above).

In a substantial series of more recent experiments in Australia, the UK and in countries around the Mediterranean (e.g. Stoll et al., 1998; Davies et al., 2002) application of PRD to grape vines and subsequently to a range of other crops has resulted in substantial saving of irrigation water coupled with the maintenance of an economic yield. Previously, in commercial wine grape production in South Australia up to 12 ML water ha^{-1} was routinely applied in some regions. With the introduction of PRD into the commercial sector, some producers are now applying a fraction of this amount and this application rate has been sustained over 4 years without substantial yield penalty. WUE has at least doubled and in some cases increased to a much greater extent. In addition to this, PRD has provided added value in terms of increased yield quality and reduced labour costs with reduced pruning requirement. Increased investment in irrigation hardware is relatively trivial. Reports of positive effects of PRD on other crops are now beginning to appear in the literature. For example, Warner (2002) recently reported that PRD improved background colour in Fuji apples which also contained higher sugar levels than fully irrigated trees. The storage potential of PRD fruit was also increased. Despite some difficulties in operating PRD irrigation techniques in certain soils and in some climatic conditions (Kriedemann, 2003), it seems clear that substantial water savings and increases in crop quality can result. Appropriate attention must be given to plants under PRD to ensure that plant water status is effectively under control. Progress from the first elucidation of the science of long-distance chemical signalling, through the development of systems for its exploitation to substantial take up by industry in Australia, has taken little more than 10 years.

Why does PRD result in an increase in WUE?
We still do not fully understand why chemical signalling induced by drought does not reduce fruit initiation and development in grapes, although we have proposed that restricted hydraulic linkage between fruit and vegetative plant parts may be important (Davies et al., 1998). This and other hypotheses need to be further investigated as experience with a wide range of crops and different genotypes shows a range of yield responses from insensitivity to soil drying through to significant limitation in fruit production and growth. Perhaps most importantly, there is little information on the nature of long-distance chemical signalling in all but a very restricted range of horticultural crops under very specific climatic conditions.
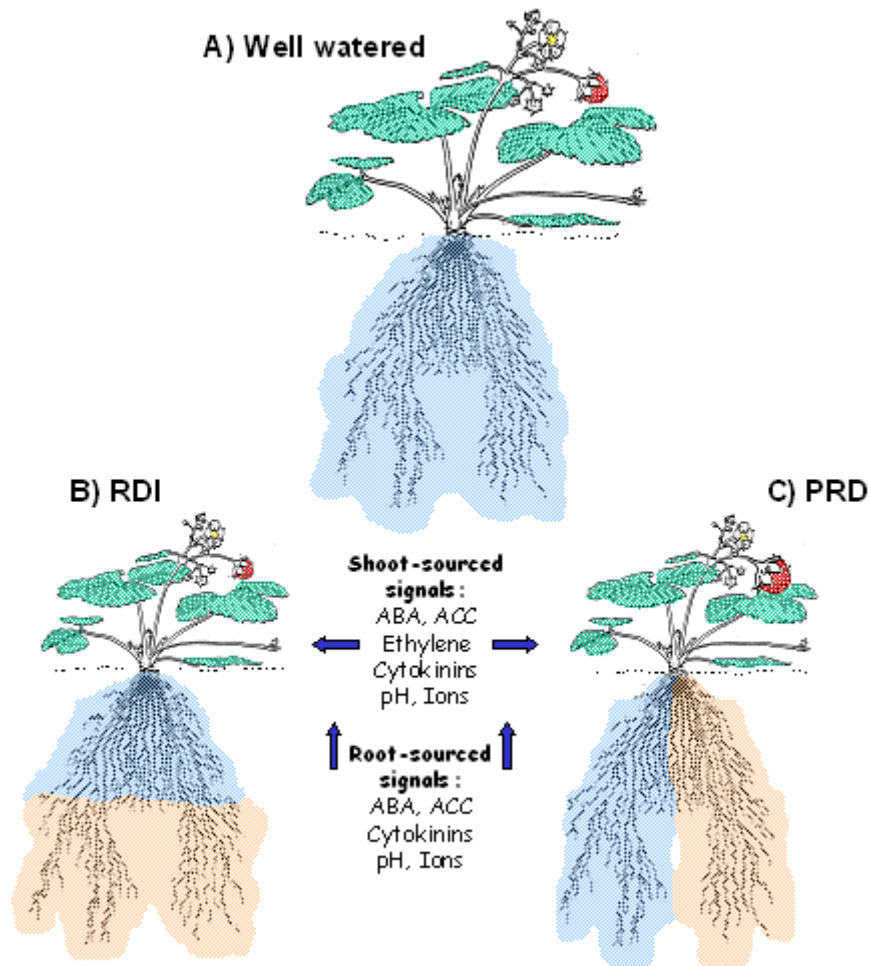

Figure 2. Illustration of the principles behind RDI and PRD, differences in operation and opportunities for research and exploitation. In A), all of the roots are wetted by the applied water. In B), half the volume of water is applied and some roots are exposed to drying soil. These roots, if not rewetted can die and signalling may diminish. If this is the case, shoot water deficit can result. Leaf area is reduced under this treatment while fruit size and quality are often reduced. In C), half the volume of water is applied alternately to each half of the root system. Half of the roots are exposed to drying soil at any one time before the irrigation is switched but roots remain alive and signalling is sustained. If PRD is optimised, shoot turgor can be maintained as a result of restricted stomatal opening in response to signalling. Again, leaf area is reduced but fruit size can be maintained; fruit 'quality' may even be increased. Sustained root growth and deeper rooting can also result in increased exploitation of available soil water. Root- and shoot-sourced signals that may regulate these responses are listed. Drawing courtesy of Mark Else.

Existing technology (furrow, sprinkler and drip irrigation systems) can be utilised to deliver PRD in a cost effective way, although several irrigation companies are now marketing welded twin irrigation lines specifically for use with PRD. This technology may be useful if precision is required in delivering water with respect to distance from the base of the plant. More research is needed into this issue and also into the question of just when irrigation should be changed from one side of the plant to the other. Most logically this decision will be based on some assessment of plant growth or physiology (as signalling to

shoots becomes less intense presumably stomata will re-open and growth rates will increase), but for effective technology transfer this may have to be done empirically.

Possible limitations to the use of PRD could be nutritional or salinity problems associated with the limited soil volume occupied by plant roots in soil that is periodically dried. To overcome this problem fertigation can be applied *via* drip lines and soil profiles can be flushed periodically with excess water. This will provide the additional possibility of optimising nutrient use efficiency and using nutrient treatments to perturb the plant's signalling system to the benefit of the grower and without growing plants under what can be high evaporative demands with reduced amounts of soil water.

Both controlled soil drying and modified nutrient supply can be used to modify plant growth, development and the quality of yield. Important changes include more rapid plant development, enhanced carbohydrate partitioning to fruit and to roots, modified fruit biochemistry (some compounds with positive impacts on consumer health), more flavour and aroma compounds in fruit, modified canopy structure and side shoot development, earlier harvest dates and enhanced shelf life and storage properties (see e.g., de Souza et al., 2003; dos Santos et al., 2003). Plant growth regulation of this type can replace chemical growth regulators and, as such, is consistent with principles of sustainable development. Nevertheless, more research is needed into the science behind these potential changes to optimise deliverables in the different sectors. Many of these changes are attributable to modification of the plant's long-distance signalling patterns and one excellent example of this is the work of Yang et al. (2001a) where mild soil drying treatments are applied after anthesis of cereals to enhance senescence of vegetative plant parts. This in turn results in enhanced redistribution of carbon reserves from the stem to the filling grain. Grain yields can be significantly enhanced by this treatment and this is particularly the case in crops grown with high nitrogen availability, a treatment known to delay senescence. Yang et al. (2001b) have shown how drought-induced changes in hormone signalling can be linked to changes in resource partitioning. Mingo et al. (2004) report a substantial change in drought-induced resource partitioning which can be beneficial to plant growth and functioning under certain dryland conditions. There are clearly circumstances, however, where, for example, enhanced root growth and accelerated senescence may deleteriously influence yielding (the staygreen trait can in fact be highly beneficial). If agriculture is to benefit from a manipulation of plant drought responses, then we will need to tailor our manipulations of the plant for particular soil and environmental conditions. Those operating PRD under commercial conditions are already well aware of this necessity (Kriedemann, 2003).

Conclusions

It should be clear from what is written above that a range of irrigation management options have already been developed to allow exploitation of our understanding of the biochemistry and physiology of crop functioning to sustain plant production under water scarcity. These techniques have been used with success in a range of plant production systems in different regions of the world. While the use of deficit irrigation can be based upon a general desire to enhance water use efficiency via the manipulation of one or another of the plant's drought stress responses, there are very few comprehensive studies which demonstrate clearly the mechanistic basis of the plant response to deficit irrigation. This is certainly the case with PRD (partial rootzone drying), although there is good quality information on the mechanistic basis of the PRD treatments to grapevines and the yield responses of these crops in the field (e.g. Stoll et al., 2002).

Much of the mechanistic basis of long distance chemical signalling in plants has been worked out with a relatively few crop species, largely in the laboratory. Attention has focussed largely on a few hormones, pH and individual ionic components of the xylem sap. Even here, our understanding of the signalling processes is very incomplete. Despite this, those arguing for the principle of manipulation of the signalling process via irrigation practice have moved to the field, often with impressive savings of water achieved and with other associated benefits. PRD is now being applied to an increasing range of crops in the field and these are in some cases the same species which have been the subject of laboratory studies. More detailed studies of PRD effects are beginning to reveal the potential benefits of manipulating different signalling pathways (e.g. Sobieh et al., 2004). More work is needed however, particularly if we are to understand how to apply these treatments in different soils, in different climatic conditions and to genotypes that may differ substantially in signalling capacity. It might be argued that knowledge that there are root signals is enough for the improvement of plant growth and functioning under dryland

conditions. We would suggests that exploitation of the plant's physiology can be further optimised as we understand more about the plant's drought stress physiology and biochemistry.

To date there have been only limited attempts to use transgenesis to modify plant yielding under drought stress (as contrasted with attempts to enhance survival of plants under really severe stress). It seems likely that focussing attention on the processes regulating growth and yielding under very mild water deficits can yield dividends in terms of sustained crop production and enhanced water use efficiency. We discuss here the manipulation of leaf growth under drought stress by the genetic suppression of ethylene synthesis and these results suggest that manipulations of this kind can be important for plant production in dryland environments.

Because it is highly unlikely that we will ever overcome all the lesions induced by desiccation stress, the prospect of engineering a crop to yield under really severe drought is somewhat remote. We must therefore think imaginatively and consider growing our crops in some regions in periods of the year when there is water in the soil profile but when growth may have been limited by some other stress that is potentially easier to overcome. Such an example may be the engineering of crops to expand leaf areas at low temperatures when there may be plenty of radiation around but when radiation interception and biomass accumulation is otherwise limited by leaf area development.

Substantial progress in modifying the sustainability of crop production is likely to be a function of a combination of advances both in crop genetics and management. The history of plant physiology suggests that however powerful the new genetics, modification of random stress resistance traits will be unlikely to result in the development of stress resistant crops. Nevertheless, a thorough understanding of the physiology of crop yielding will suggest opportunities for both genetic and management manipulations and we suggest that a systems approach to understanding and manipulating crop yielding under stress can provide a framework of analysis for geneticists to focus on key crop properties and for managers to devise innovative production systems for dryland environments. The PRD irrigation system is an example of one such system.

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