DISSERTATION

# WATER RELATIONS OF APPLE TREES (*MALUS* X *DOMESTICA* BORKH., CV. GALA) UNDER PARTIAL ROOTZONE DRYING IRRIGATION

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY TODD EINHORN ENTITLED WATER RELATIONS OF APPLE TREES (*MALUS* X *DOMESTICA* BORKH., CV. GALA) UNDER PARTIAL ROOTZONE DRYING IRRIGATION BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

WATER RELATIONS OF APPLE TREES (*MALUS* X *DOMESTICA* BORKH., CV. GALA) UNDER PARTIAL ROOTZONE DRYING IRRIGATION

Single-leaf water relations, fruit growth and quality were monitored from 2001-2003 for field-grown apple (*Malus* x *domestica* Borkh. cv. 'Gala') trees. Trees were subjected to three irrigation treatments: 1) well-watered Control, 2) (DI50) 50 % of irrigation volume applied the same way, and 3) (PRD50) 50 % irrigation volume applied to half the surface area of the Control. In all years, average fruit size, yield and quality were not affected by irrigation treatments. Gas exchange was very low, irrespective of treatments, however leaf water potential ($\psi$) did not attain levels previously suggested to close stomata. Leaf abscisic acid (ABA) concentration did not differ among treatments. Stomata of apple leaves responded sensitively to the vapor pressure deficit (VPD) of the atmosphere regardless of soil moisture status. Water use efficiency (WUE) was not increased by deficit irrigation, but regulation of gas exchange by VPD allowed apple orchards to be irrigated at approximately 30 % of reference evapo-transpiration ($ET_o$).

In 2003, a split-rooted containerized system was developed by approach-grafting, one-year-old 'Gala' apple trees. Four irrigation treatments were imposed in 2004 over a 30-day experimental period: Control (>100 % of $ET_c$) applied to both containers; PRD100 (>100 % $ET_c$) applied to one container only; and two treatments receiving 50 % $ET_c$ applied to either one (PRD50) or both containers (DI50). Both PRD treatments had water alternated between containers when a threshold soil water content was attained. Monitoring of sap flow under fully irrigated conditions showed the dependency of root sap flow on root cross-sectional area. Previously dried roots responded rapidly to irrigation events. Both 50 % $ET_c$ treatments experienced similar declines in predawn $\psi$ ($\psi_{pd}$) and single-leaf gas exchange, relative to Controls. Leaf ABA concentration was similar for PRD50 and DI50, and was significantly

higher than both >100 % $ET_c$ treatments. The PRD100 and Control had similar $\psi_{pd}$, however gas exchange was reduced in PRD100 by 30 % compared to Controls. Leaf ABA concentration was higher in PRD100 than Controls, but could not unequivocally account for the down-regulation of PRD100 stomata. As similarly reported in field experiments, WUE was not increased with deficit treatments.

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# 1 Introduction

Globally, agriculture exceeds all other sectors in both freshwater withdrawals and consumptive use, however the proportion of total water allocated to agriculture has been shrinking from nearly 90 % in 1930 to 66 % in 2000 (Kirda and Kanber, 1999). This decline in allocation to agriculture is in large part a response to the exponential growth rate of the human population and the subsequent competition from the municipal and industrial sectors that have historically viewed agriculture as a source to meet increasing water demands. Regional population trends vary, but globally growth is projected to continue at roughly the same rate over the next 100 years (United Nations, 2004). Currently it is estimated that 450 million people in 29 countries suffer from water shortages, and approximately 800 million people globally face starvation as a direct consequence of decreased water supply (Serageldin, 1999). However, there are limited energy efficient and, hence economically feasible solutions for future development of freshwater resources. Despite water shortages and stiffening competition among water users, irrigated lands currently generate 40 % of the global food supply, and there is the expectancy for agricultural technologies to further improve yields under limited resources.

Considerable production acreage is dedicated to apple (*Malus* x *domestica* Borkh.), with total land area harvested in 2000 estimated to be 5.6 million hectares (FAO, 2001). It is well established that yield is positively correlated to water use, and apple

water use on an orchard basis is high in comparison with other crops (Doorenbos and Pruitt, 1977). Current production practices favoring high-density plantings (upwards to 10,000 trees per hectare) further increase the exploitation of water and nutrients on an orchard land-area basis. During periods of water scarcity, yield sustenance is dependent upon increased water productivity, and this has been the focal point of deficit irrigation strategies. However, apple fruit growth responds sensitively to changes in plant water status, and the simultaneous occurrence of rapid fruit and shoot growth render it difficult to limit irrigation supply without adversely impacting fruit growth. To date there has been relatively little success with traditional deficit irrigation management of apple orchards (based on sub-optimal fruit size and yields). However, a fairly new deficit irrigation strategy, partial rootzone drying (PRD), has shown promise for grapevines (*Vitis* sp.) in Australia, and based on its proposed mode of action, might be a viable alternative for use in apple production systems. The objectives of this study were therefore to; 1) test PRD against an equally deficient traditional deficit irrigation strategy using apple trees under both field and container conditions; 2) determine physiological responses of apple to deficit irrigation strategies under semi-arid conditions; and 3) investigate the effects of deficit irrigation treatments on fruit size, yield and quality.

# 2 Literature Review

## 2.1 Partial Rootzone Drying

Partial Rootzone Drying (PRD) is a deficit irrigation method whereby a portion of the rootzone is maintained at or near its field capacity (FC), while concurrently another portion is subjected to drying. This allows for application of water at a targeted level below the actual water use of the crop. Irrigation is alternated throughout the season between wet and dry zones to achieve an optimal level of plant performance. The necessary duration and extent of the soil drying cycle will be based on physiological and biochemical plant responses to perturbations in hydration status along the soil-plant-atmosphere-continuum (SPAC). The putative operative mechanisms of PRD, contrary to other deficit irrigation strategies, are dependent on the presence of root initiated chemicals in response to soil drying, resulting in growth suppression and/or stomatal regulation in the absence of declining plant water potentials. Although a prescribed duration of the soil drying cycle (10-14 days) has been proposed (Dry et al., 2001; Loveys et al., 2000; Stoll et al., 2000), the strength of the PRD signal would be expected to fluctuate in a species specific manner as a function of a given plant's adaptive strategies to water deficits. Such prescribed protocols are insufficient for broad use since soil texture, structure, depth and irrigation volume will drastically alter the response. Since the objective of PRD is to keep the plant from experiencing water stress, irrigation

timing using threshold water potential values, as is currently practiced with other deficit irrigation protocols (Shackel et al., 1997), is not applicable. How then is PRD implemented? Despite the suggestion that regulated deficit irrigation (RDI) often requires a 'more than basic' understanding of soil and plant monitoring (Dry et al., 2001), it is becoming increasingly apparent from the current body of PRD literature that proper use of the PRD technique in fact requires considerable monitoring of multiple dynamic processes. In addition, optimal signaling may require regulation of irrigation volumes throughout the season based on crop phenological growth stages, sink strength, endogenous chemical concentrations, canopy volume and cultural practices which alter plant development. The focus for this review therefore, will be to elucidate the underpinning principles of PRD, address the widening gap of controversial results and outline effective measurements necessary for evaluation and progression of the PRD technique.

## 2.2 Chemical Signaling: The Role of Abscisic Acid (ABA) and Alternative Agents

With respect to biochemical root-to-shoot signaling in response to water deficit, ABA has received the most attention, and has been directly implicated in the success of PRD. ABA production is ubiquitous in plants serving multiple roles throughout plant development (reviewed by Trewavas and Jones, 1991) however, several lines of evidence point to the central role of this compound in the regulation of gas exchange in response to water deficits. Taylor and Burden (1970) and Taylor et al. (2000) described the metabolic pathway of ABA biosynthesis and demonstrated that ($C_{40}$) carotenoid

precursors could be oxidatively cleaved *in vitro* to yield the ABA precursor xanthoxin (a *cis*-aldehyde). Experiments with ABA mutant wilty tomatoes ('flacca' and 'sitiens') demonstrated an accumulation of ABA-aldehydes (synthesized from xanthoxin, and yielding ABA upon oxidation) due to lesions in the final step of the pathway (Parry et al., 1988). The inability of 'flacca' and 'sitiens' mutants to convert ABA-aldehydes to ABA was also confirmed by feeding mutants deuterium-labeled ABA-aldehyde (Taylor et al., 1988). Similar lesions, and hence reduced capacity to synthesize ABA, resulting in wilting conditions in response to water stress, have also been identified in a mutant of *Nicotiana plumbaginifolia* (Borel et al., 2001; Walker-Simmons et al., 1989) and the *Solanum phureja* mutant 'droopy' potato (Duckham et al., 1989). The link between ABA and its role in stomatal regulation comes from the reversal of wilting in mutants upon applications of exogenous ABA (Sharp et al., 1994; Tal and Imber, 1970). Furthermore, ABA-induced stomatal closure has been demonstrated through exogenous applications of physiological concentrations of ABA to detached leaves and whole plants of non-mutant types to produce similar plant responses to those occurring under water stress (Aasamaa, et al., 2002; Jones, 1992; Trejo et al., 1993; Trewavas and Jones, 1991; Wan and Zwiazek, 2001).

Increased endogenous levels of xylem sap ABA in response to soil drying is largely due to root cell synthesis. Davies and Zhang (1989) provided direct evidence for root cell ABA synthesis of field grown maize plants subjected to a water withholding treatment. Temporally, root ABA levels were well correlated with soil moisture status so that as soil drying increased with depth over time, concomitant increases in root [ABA] were observed in roots extracted from soil depths (Davies and Zhang, 1989). The cellular

sites for ABA synthesis, based on location of key metabolic enzymes, reside in the cytosol, and support for heightened root ABA synthesis comes from anatomical studies showing low vacuolization in the cells of root tips (Hartung et al., 1999). It is now well established that root derived ABA traveling in the xylem arrives at the leaf to initiate a stress response (Davies and Zhang, 1991; Hartung et al., 2002; Sauter et al., 2001). What is not certain, however, is identification of the appropriate compartment for measurement of ABA based on its relationship with stomatal conductance ($g_s$). Data suggests that prevailing xylem sap ABA is more sensitively related with stomatal changes than leaf accumulated ABA (Jia and Zhang, 1999). The authors modeled the rate of accumulation based on the flux of foliar ABA as a function of transpiration rate, xylem [ABA] and ABA degradation, the latter through experiments using an inhibitor of ABA catabolism. Data showed that stomata responded sensitively to xylem [ABA] reaching steady state conditions within 20 minutes while leaves continued to accumulate ABA for an additional 80 minutes (Jia and Zhang, 1999). Zhang and Outlaw (2001) provided data which support the role of intra-leaf redistribution of ABA to the guard cell apoplast in controlling stomatal aperture without detectable changes in either whole leaf or apoplastic pools of ABA. These results along with others (Gowing et al., 1993) have raised concerns regarding the use of whole leaf ABA content as a sensitive determinant of chemical signaling, however, strong correlations between bulk leaf ABA and stomatal response have been reported for split-rooted grapevines (Lovisolo et al., 2002; Stoll et al., 2000), drought stressed soybean (Liu et al., 2003) and several deciduous tree species (Aasamaa et al., 2002). To complicate matters, ABA recirculation and free exchange between phloem and xylem (Sauter et al., 2001) alters concentrations independently of

plant biosynthesis. In addition, leaves are capable of ABA synthesis when subjected to dehydration, although conflicting reports suggest varying levels of dehydration required to induce leaf synthesis of ABA. Soar et al. (2004) demonstrated that ABA synthesis in grapevine leaves occurred at 85% of Control fresh weight, although for several other species, ABA synthesis was not observed until the turgor potential approached zero (Beardsell and Cohen, 1975; Pierce and Raschke, 1980). With respect to PRD, the argument for *de novo* leaf synthesis of ABA and its contribution to chemical signaling may be minimal since guard cell regulation usually occurs under much milder degrees of soil drying, and most PRD reports show the maintenance of leaf water potentials at Control levels.

Although it seems certain that root derived ABA exerts control over stomatal aperture, several factors contribute to the strength of the signal. Not all xylem and apoplastic ABA reaches the guard cells. Trejo et al. (1993) incubated leaf discs, bare mesophyll and isolated epidermal strips in equal concentrations of ABA solutions. Sensitivity of stomata was greatest for isolated epidermis, and epidermal [ABA] was 12 fold greater in epidermal strips than in leaf discs. The authors concluded that rapid metabolism by mesophyll cells of leaf discs exerts control over the amount of ABA reaching the epidermal guard cells. This was confirmed using tetcyclacis, an inhibitor of ABA catabolism, which resulted in accumulation of ABA in epidermal tissue of leaf discs and subsequent increased sensitivity of stomata to lower concentrations of ABA in the incubating solution (Trejo et al., 1993). Further evidence for the role of apoplastic ABA pools (root derived or redistributed) in modulating stomatal behavior comes from the elegant work of Hartung (1983). Stomatal aperture of isolated leaf epidermis

decreased linearly with increasing external [ABA], regardless of the bathing solution pH (5.0 and 8.0), however the uptake rate of radiolabelled ABA into isolated guard cells of the epidermal tissue was 382 pmol $h^{-1}$ and 0 pmol $h^{-1}$ at pH of 5.0 and 8.0, respectively. Therefore, it was demonstrated that the primary site of action must be located on the outer plasmalemma of the guard cells. In addition, ABA-induced stomatal closure was not pH dependant, however, pH does appear to control ABA redistribution and subsequently determines [ABA] at the sites of action.

The role of pH in the regulation of chemical signaling has received much recent attention. Typically, ABA is in equilibrium between soil and roots as has been shown through computer simulations (Slovik et al., 1995) and confirmed experimentally (Hartung et al., 1996). Contribution of ABA to soil pools is through root efflux (either live or dead roots) and/or soil microorganisms. However, because ABA is weakly acidic it partitions based on pH and can be severely depleted from roots in contact with alkaline soils (Degenhardt et al., 2000). The transport of ABA in the plant is similarly affected by pH and strongly adheres to the anion trap concept established by the Henderson-Hasselbach equation. Abscisic acid is dragged along in response to bulk flow in the unstressed apoplasm [generally with pH values neutral to slightly acidic (Taiz and Zeiger, 2000)] and due to its hydrophilic nature freely crosses membranes and becomes sequestered in the more alkaline cellular compartments. Release of sequestered ABA ($ABA^-$) requires either active transport or a 'leveling' of the pH gradient between the apoplast and cytosol. In fact, several plant species have responded to drought stress through an alkalization of the apoplast (Gollan et al., 1992; Wilkinson et al., 1998), although not unanimously (Wilkinson and Davies, 2002). In addition, feeding high pH

solutions to xylem resulted in stomatal closure under steady state ABA levels (Wilkinson and Davies, 1997). In fact, Holbrook et al. (2002) used under-producing ABA mutants of tomato in various approach-grafted configurations with wild types, and showed that increased leaf ABA can occur independently of root derived ABA, suggesting a potential and likely role for altered pH. Two theories have been proposed to explain pH effects on ABA; 1) gradients of pH between the alkaline compartments in the cell and the less alkaline xylem are leveled with an alkalization of the xylem resulting in a release of ABA to the apoplasm from anion traps (Hartung and Radin, 1989), and 2) high pH lengthens the residence time of ABA in the apoplasm of a water stressed plant thereby allowing a greater potential for its arrival at the guard cells (Wilkinson and Davies, 1997). In addition to pH effects on guard cell regulation, an inverse relationship between pH and leaf expansion has been shown to exist (Bacon et al., 1998; Zhang and Davies, 1990), although growth suppression in the presence of high pH requires ABA (Bacon et al., 1998). Contrary to the sites of ABA reception on the outer guard cell plasmalemma (Hartung, 1983), cellular sites controlling expansion growth have not yet been identified. It seems plausible that expansin activity might be suppressed during alkalization of the apoplasm, in contrast to expansive growth processes occurring during acidification of cell walls (Salisbury and Ross, 1992).

In addition to pH regulation of ABA signaling, there exists strong support for calcium as a secondary messenger in the ABA signal transduction cascade, since increased cytosolic levels of free calcium occur during ABA-induced stomatal closure (Atkinson, 1991; DeSilva et al., 1985; Schroeder et al., 2001). Therefore, it is plausible that ion uptake, movement and concentrations will affect ABA signaling. This is

particularly true for potassium ions, since they are implicated in the regulation of guard cell turgor (Salisbury and Ross, 1992). Furthermore, guard cell sensitivity to ABA has been shown to be increased under minimal reductions in water potential (Behl and Hartung, 1984; Tardieu and Davies, 1993). In fact, such small shifts in leaf water potential, and the likely existence of intra-leaf water potential gradients (Shackel et al., 1987) suggests that undetectable, yet important modulators in chemical signaling are continually operative. Clearly, ABA signaling and its potential exploitation under PRD regimes is quite complex, and is a function of multiple dynamic processes, which might offer a partial explanation for the lack of consistency in results reported in the PRD literature.

It should be noted, however, that other biochemical stress signals have been shown to affect gas exchange and growth during periods of soil drying. Stoll et al. (2000) described the interactions between ABA and cytokinins (zeatin and zeatin riboside) on shoot processes. A relationship was found between maximum root derived bulk leaf ABA and minimum $g_s$ in half dried potted grapevines. The effect of endogenous [ABA] on $g_s$ could be reversed by exogenous application of benzyladenine (BA). When assayed, endogenous cytokinin levels of roots, buds and shoot tips were significantly lower than Controls. Others have also shown a relationship between low cytokinin concentrations and reduced $g_s$ for drought stressed plants (Bano et al., 1993; Bano et al., 1994). Toit et al. (2003) examined the relationship between nitrate reductase (NR) concentration and PRD drying cycles. NR was positively related to $g_s$ and decreased with increasing drying cycles. It was speculated that depressed NR activity was due to lower substrate availability in the leaf as a function of limited uptake and delivery of nitrogen during soil

drying. It is of interest that NR activity of etiolated barley leaves has been shown to be inhibited by ABA, and partially reversed by exogenous BA (Lu et al., 1992), although such effects could not be ascertained since neither ABA nor cytokinins were quantified in Toit et al. (2003). In addition to cytokinins, recent evidence implicates ethylene in ABA signaling, as will be discussed further below.

## 2.3 Chemical Signaling and Agricultural Applications: Foundation of PRD

Blackman and Davies (1985) were perhaps the first to show that under split root conditions, a rise in ABA concentration correlated well with stomatal closure during drying of one half of maize root systems. Zhang et al. (1987) also showed marked increases in root ABA four to eight days following partial drying of *Commelina communis* L. root systems. Importantly, both observations occurred prior to any detectable changes in leaf water potential. Tan and Buttery (1982) observed vigor control during split root drying of peach (*Prunus persica* L.) seedlings, and Loveys (1984) presented strong correlations between root derived ABA in grapevines and reduced gas exchange. However, results of Gowing et al. (1990) became the foundation for the development of PRD. Gowing et al. (1990) demonstrated that potted split-rooted apple explants that underwent partial drying (one container not watered and one container well-watered) maintained leaf water potential at Control levels (both containers well-watered) while experiencing 40 % reductions in vegetative growth, due to restriction in both leaf initiation rate and final length of fully expanded leaves. In addition, PRD plants had 5-20 % lower transpiration than Control plants. Indirect evidence of a chemical signal was

based on two observations; 1) transpiration and growth was reduced with partial drying of split-rooted plants without any perturbation in leaf water potential, pressure potential, or osmotic potential, and 2) excision of the dry portion of the root systems after 24 days of drying restored growth to Control levels. It was argued that removal of half of the root system would not be expected to increase water supply, but rather, the elimination of a chemical produced from the dry roots enabled the resumption of normal growth processes. Abscisic acid was speculated to be the chemical signal, but identification and quantification of ABA was not performed, however the work has since been incorrectly cited as having established the direct role of ABA in split-rooted systems (Davies et al., 2002; Wakrim et al., 2005).

## 2.4 The PRD Response: Reduced Gas Exchange and Growth Suppression

Aside from the vast need to improve crop water productivity, PRD was developed as a means to control vigor via altered shoot water relations in grapevines. A given reduction in canopy volume has the potential to increase water use efficiency (WUE), since crop water use is largely a function of leaf area. Competition for carbohydrates between shoots and fruit and deleterious effects on fruit quality due to excessive canopy shading, as well as increased disease incidence, all contributed to the development of PRD (Dry et al., 2001; Loveys et al., 1998). In short, early conclusions were that PRD-induced vigor control resulted in smaller canopies, which positively influenced berry quality due to increased canopy light penetration and distribution. Observations included increased anthocyanin production, lower pH and higher skin: flesh ratios, the latter attributed to

slight, often insignificant reductions in berry size resulting in improved wine quality due to the higher proportion of flavor compounds present in the skin (Dry and Loveys, 1998; Dry et al., 2001; Loveys et al., 1997; Loveys et al., 2002). An advantage often cited in these PRD investigations is the near doubling of WUE, however this measurement is potentially misleading and implores criticism as it is nearly entirely expressed as tons of harvested crop per ML of applied water. As PRD treatments are generally applied at 50 % of Control volumes, a near doubling of WUE will undoubtedly be observed, even when taking into account small reductions in berry size and yield. In fact, yield would have to be more than halved before a reduction in WUE is observed. Slight loss of grape berry size is tolerated and even encouraged while other commercial horticultural crops are penalized for such reductions in fruit size, irrespective of large WUE savings. Instantaneous WUE (assimilation/transpiration) is a better predictor of the mechanistic effects of PRD on WUE, because it is based on the relationship between gas exchange of $CO_2$ and $H_2O$ as a function of stomatal conductance. Crops which have similar slopes for both net assimilation and transpiration for a given change in $g_s$ will not increase WUE under water-limited conditions. Although rarely investigated, several recent reports have attributed depressed rates of photosynthesis under PRD to stomatal limitations and not as a consequence of impairment of the photosynthetic machinery (Centritto et al., 2005; Souza et al., 2003; Souza et al., 2005). Although growth suppression has been shown to occur under PRD regimes despite maintenance of stomatal conductance at Control levels (Turner et al., 1996), there is little evidence for direct growth control via chemical signaling. In several cases hydraulic effects have been ruled out since water potential of PRD plants is often maintained at non-stressed levels, although it is plausible that

undetectable, slight shifts in turgor potential in meristematic tissue could produce such a response. In addition, intraleaf gradients in leaf water potential have been shown to occur through pressure probe techniques (Shackel et al., 1987). The existence of such gradients may suggest the involvement of an integrated signal (Comstock, 2002). The exact mechanisms for the maintenance of fruit growth under deficit irrigation has not been elucidated, but may be due to the higher sink strength of fruit, which has been hypothesized to be a function of its higher osmotic properties (Chalmers, 1989).

## 2.5 Maximizing the PRD Signal

Dry et al. (1996, 2000a) presented data demonstrating recoveries in $g_s$ and growth of grapevines with half dried root compartments five to ten days after the onset of drying. In addition, growth was sustained at Control levels despite negligible soil water extraction from the dry compartment (Dry et al., 2000a). It was hypothesized that despite likely ABA production in dehydrating roots, minimal contribution to the transpiration stream diminished the strength of the signal. Therefore, multiple wet/dry cycles were suggested (Dry et al., 2001) to maintain a steady state chemical signal in the canopy. Stoll et al. (2000), however, showed the effect of nocturnal water redistribution between wet and dry root compartments by feeding isotopically enriched water to the wet sides and recovering the label in dry roots. It was proposed that re-hydration of previously dried roots high in ABA will enable ABA transport to continue, although the effects of such a process on signal longevity, arrival and perception are not clear. Despite the proposal of a 10-14 day drying cycle, several works stand in the literature as evidence

against such a fixed rate of switching between wetting and drying. Wakrim et al. (2005) applied 50 % PRD and 50 % deficit irrigation (DI) treatments to common bean (*Phaseolus vulgaris* L.) and observed similar water potential declines for both treatments. Alternation of irrigation between wetting and drying root compartments occurred every ten days, and although soil moisture data was not presented for individual PRD containers, the fraction of transpirable water (FTSW) was shown to be severely depleted equally under both deficit treatments. Concomitantly, leaf [ABA] and xylem pH were not affected by deficit treatments. It is likely that soil moisture levels in the wet container of the 50 % PRD were not sufficient to maintain adequate plant production as evidenced by reduced shoot growth, pod weight and water potential of PRD plants (Wakrim et al., 2005). O'Connell and Goodwin (2004) observed consistent and marked reductions in stem water potential and $g_s$ of pear (*Pyrus communis* L.) throughout a 14 day PRD drying cycle. Measured sap velocity (transpiration) also did not suggest the existence of a transient stomatal signal throughout the cycle. In addition, Poni et al. (2005) did not observe a recovery in $g_s$ or assimilation of grape leaves during a steady drying of one side of split-rooted vines. Soil moisture content in the dry compartment had reached the permanent wilting point on day 3 of the experiment and remained constant for an additional 11 days. Further work is required to validate the transient nature of a signal under PRD conditions.

The effects of altering both length of drying cycle and irrigation volume on soil moisture between wetted and drying sides compared to a well-watered Control were investigated on olives (*Olea europaea* L.) by Wahbi et al. (2005). Switching between dry and wet sides following each irrigation event resulted in drastic water deficit in both the

wet and dry soil profiles. Similar data were also recently reported for a 50 % PRD treatment applied to potato (*Solanum tuberosum* L.) (Liu et al., 2006), in which severe water depletion on the wet side resulted in substantial reductions in tuber biomass. Wahbi et al. (2005) improved soil moisture status under a four week irrigation switch, although an increase in stomatal resistance for both 50 % treatments could not maintain plant water status at Control or 100 % PRD levels. Interestingly and in compliance with the PRD theory, the 100 % PRD plants exhibited lower $g_s$ and shoot growth relative to Controls, without noticeable differences in water potential between the two treatments. While such results lend support for a root-shoot signal, direct evidence was not provided. Irrespective of irrigation amount, PRD did not adversely affect fruit size or yield components, although the xeric nature of olive trees should allow greater water-use-efficiency under such deficits than other commercial horticultural crops. For crops which are more sensitive to hydraulic mechanisms these results imply that optimal PRD regimes probably fall somewhere between 50-100 % of ET.

Dry and Loveys (1999) reported that split root drying led to significant decreases of $g_s$ and shoot growth of container-grown 'Shiraz' and 'Chardonnay' grapevines without changes in water potential compared to a well-watered Control. Although reported growth and $g_s$ for half-dried treatments was 70-80 % of Control levels, these were only significant on two separate occasions. The half-dried treatment was also compared to a fully-dried treatment to emphasize the effects of PRD relative to both fully-watered and non-watered treatments. As expected, fully-dried treatments had significant reductions in water potential, $g_s$ and growth and it was concluded that growth-suppressing hydraulic signals override chemical signaling when deficits are severe. The water saving benefits

associated with reduced shoot growth and/or $g_s$ from 50 % PRD treatments relative to 100 % well-watered Controls have been reported in additional grape studies (Dry et al., 2000a,b; Loveys et al., 1998), raspberry (*Ribus idaeus* L.) (Grant et al., 2004), olive (Wahbi et al., 2005) and tomato (*Lycopersicon esculentum* Mill.) (Stikic et al., 2003). However the lack of comparison to an equally water deficient DI treatment limits the ability to unequivocally relate the observed effects to irrigation placement (PRD) as opposed to irrigation volume. Recently, however, experimental designs have incorporated such equally water deficient comparisons to address whether or not irrigation placement or volume is a better predictor of plant response to deficit irrigation. Souza et al. (2003) and Santos et al. (2003) published compendium papers showing non-significant results between 50 % DI and PRD for their effects on gas exchange (photosynthesis and $g_s$), photochemical efficiency, electron transport rate, non photochemical quenching, yield and cluster weight. There were, however significant vegetative growth reductions associated with PRD, although it is difficult to determine the cause for this since ABA quantification and soil moisture data were lacking. Gu et al. (2004) showed that PRD lacked distinct advantages over DI on grape berry growth, quality and physiological processes while comparing DI and PRD each at 2 levels of $ET_c$ (0.8 and 0.4). Hence it was shown that fruit and vegetative growth responded to irrigation amount and not irrigation placement, as corroborated by other grapevine studies (Bravdo et al., 2004; Chalmers et al., 2004; Pudney and McCarthy, 2004). Similar conclusions were attained for leaf size and initiation rate for rapeseed (*Brassica napus* L.) (Wang et al., 2005). Goldhamer et al. (2002) using a 14 day switch for a PRD treatment receiving 50 % $ET_c$ observed a significant water potential reduction in peach trees. This

is the first account in which PRD irrigation volume was regulated throughout the season based on phenological crop development. Application of 50 % $ET_c$ applied during dry weight stage I and/or II (DW I and DW II) was relieved at the onset of the rapid fruit growth phase (DW III) by supplying 120 % $ET_c$. However, 50 % $ET_c$ applied during DW II by PRD resulted in similar midday stem water potentials as 50 % $ET_c$ applied through RDI (both of which were drastically reduced from Control levels), suggesting that PRD in fact behaved no differently than RDI. However, there weren't any negative effects from this practice since it is well established that reduction of water potential during DW II, the pit hardening stage of peach fruit development, leads to vigor control without adverse effects on final fruit size, since only roughly 33 % of the harvestable fruit size is attained at the completion of DW II (Chalmers, 1989). Zegbe-Dominguez et al. (2003) compared a well-watered Control treatment to 50 % DI and 50 % PRD using processing tomato without observable differences in either gas exchange parameters or yield components among the three treatments on two given dates. Although leaf water potentials did not vary among treatments, volumetric soil moisture content of the deficit treatments was half that of Control values. A higher proportion of carbohydrates were partitioned to vegetative tissues and fresh weight was reduced in both deficit treatments, however the latter is of less importance in processing tomatoes than fresh market tomatoes. In a similar study with pepper (*Capsicum annuum* L.), Dorji et al. (2005) arrived at similar conclusions. However, analysis of their data suggests that low croploads resulting from increased flower abscission in both deficit irrigation treatments relative to the Control ameliorated any adverse treatment effects on fruit size. In addition, percentage of fruit with blossom end rot (a calcium related disorder that can be

induced by water stress) was shown to be up to three-fold higher than Controls. Although both deficit irrigation regimes were promoted as being successful, it is likely that yield reductions due to the above mentioned phenomena might weaken commercial acceptance of either deficit regime. Using greenhouse-grown fresh market tomato, Kirda et al. (2004) compared a full irrigation treatment to several DI and PRD treatments: 30 % and 50 % of full irrigation (FI) applied under both PRD and DI. The 50 % PRD treatment was divided into two distinct treatments based on the time elapsed between switches in irrigation events (switching sides with each irrigation event or every other irrigation). Although statistically insignificant, large reductions in fruit size and yield were observed under all deficit treatments relative to Controls. Review of their data shows that soil moisture levels were reduced similarly among all deficit treatments, including the wet side of PRD. As discussed above, but worthy of reemphasis, original PRD work was based on the premise that the wetted rooting volume be sufficiently moist to meet the demand of the whole plant at its new steady state of water use. If deficits are allowed to occur in the wetted soil profile (brought about by rapid switching between dry and wet sides using 50 % $ET_c$) the overall effect will resemble traditional plant water stress inducing deficit irrigation practices. Reported soil moisture data is therefore a critical component of PRD studies in order to accurately assess the potential merit of PRD. Although a direct plant measure of water stress was not employed, it is likely that PRD imposed a substantial water stress. Abscisic acid was quantified, but was not significantly different among deficit treatments. Leaf tissue ABA content was only slightly increased on one date, although equally for both deficit treatments relative to the Control. The authors concluded that partitioning of carbohydrates predominantly to fruit

occurred under PRD and DI, however recalculation of their data suggests otherwise (Kirda et al., 2004).

## 2.6 PRD Effects on Growth

Dry et al. (2000b) demonstrated an increase in root area relative to Control for the container soil profile >20 cm in depth, although total root dry mass was unaffected by treatment. Others have shown rapid increases in root biomass over Control plants after three days of a partial drying treatment (Jokhan et al., 1996). Mingo et al. (2004) reported a disproportionate biomass allocation to roots of PRD tomato plants at the expense of shoot growth, although total biomass was unaffected by treatment. The maintenance of total biomass by PRD treatments was attributed to the nonlinearity between photosynthesis and $g_s$. This has been suggested by others as support for PRD, however results for crops such as apple would be expected to be quite different since strong linear relationships between net assimilation and $g_s$ have been reported under non-stressed conditions (Lakso, 2003). The increase in root biomass was most notable following re-watering of the previously dried compartment, though a small increase during the drying period was observed. In a container study void of root compartment barriers, Wang et al. (2005) showed that rapeseed plants selectively foraged in wet portions of containers by increasing root biomass at the expense of biomass in dryer portions of the container. Apple roots appeared to have shorter survivorship under dry portions of a PRD soil profile as indicated by increased pigmentation (< 10 days) under 50 % PRD (Palanjian et al., 2005). Despite increased carbon costs, rapid turnover in a

defined soil volume might be advantageous for nutrient and water acquisition and management. Kang et al. (2001) showed increased root:shoot ratios for PRD half dried pepper plants under alternating dry/wet cycles. The fact that PRD might increase root:shoot ratios is not surprising given the strength of the well established inverse relationship between soil moisture and root:shoot ratios. Although ABA was not quantified in the above experiments (Dry et al., 2000b; Kang et al., 2001; Mingo et al., 2004) several reports stand in the literature as testament to an ABA-dependent root biomass increase in response to short-term soil drying (Saab et al., 1990; Saab et al., 1995; Sharp et al., 1994). Future root turnover and activity studies under PRD regimes as well as incorporation of ABA monitoring/quantification in agricultural experiments will likely be necessary if relationships are to be strengthened between ABA controlling mechanisms elucidated in the basic science literature and their validity in crop production studies.

Although a large proportion of PRD studies demonstrate the maintenance of fruit growth and yield under PRD regimes, or show relatively small reductions as a function of stomatal limitations, previously observed PRD-induced reductions in tomato fruit growth prompted Mingo et al. (2003) to investigate the mechanism. Results showed the maintenance of fruit cell turgor at Control levels throughout the experiment, despite a reduction in growth rate under PRD. Although transpiration data was not provided, the authors concluded that lower water use in response to PRD acted favorably on cellular pressure potentials. A weak negative correlation between pH and fruit growth was shown, and the authors suggested a role for ABA signaling, or perhaps more specifically, pH mediated ABA signaling, in the direct control of growth, although this argument is

slightly weakened due to the lack of ABA detection/quantification. In addition, other work with tomatoes has shown limited xylem connections to maturing fruit, suggesting a diminished role for a xylem derived signal imparting an effect on fruit during late development (Davies et al., 2000). As pointed out above, pH does appear to regulate ABA concentration within the apoplasm surrounding expanding cells, and support for the theory comes from Bacon et al. (1998) having shown that barley leaf expansion required both high pH and ABA in the apoplasm to induce a growth response. The mechanism is partly due to reduced activity of expansins (cell wall loosening enzymes) in response to a high pH environment, however high pH buffers alone (without ABA) were unable to limit growth (Bacon et al., 1998). Further work on tomato by Sobeih et al. (2004) tested the interactions among pH, ABA and ethylene on both stomatal regulation and inhibition of vegetative growth under PRD. Hydraulic signals were ruled out since both PRD and well-watered Control treatments maintained equal leaf water potentials. Results suggested that xylem pH and ABA played a synergistic role in partial stomatal closure under PRD, however, xylem pH and ABA effects could not account for the observed regulation of vegetative growth. Instead PRD induced leaf growth inhibition was correlated to increased ethylene emission. A role for ethylene induced suppression of vegetative growth is supported from work by LeNoble et al. (2004) which demonstrated through the use of two single Arabidopsis mutants (ethylene insensitive and ABA deficient) and a double mutant (ethylene insensitive/ABA deficient), that growth is dependent upon ABA in the presence of ethylene. Others have also shown the interrelationship of ethylene and ABA, and their effect on growth (Sharp, 2002 and citations therein). Presently, ethylene has not been evaluated in the PRD literature and

such interactions add complexity in evaluation of results and application of a seemingly 'easy' irrigation technique.

## 2.7 PRD Effects on Fruit Quality

Very few PRD investigations have analyzed fruit quality attributes. The early assertions that PRD led to increased pigment content of grape berries (Dry et al., 2001; Loveys et al., 1998) have more recently been shown to be better related to irrigation volume rather than irrigation placement (Chalmers et al., 2004; Santos et al., 2003). Effects on fruit color appear to be largely a function of increased incident light levels in response to decreased canopy volume. In fact, grapevines under a non-irrigated treatment increased anthocyanin content above both 50 % DI and PRD treatments significantly while exhibiting the greatest growth reductions (Santos et al., 2003). Severe canopy growth suppression in high light environments can over-expose fruit to radiation load resulting in greatly increased fruit temperatures culminating in a higher degree of sunburn, although this has not been shown under PRD. In addition, early claims of positive PRD-induced effects on pH, acidity and sugars (Brix) have not been consistently related to PRD in grapevine (Gu et al., 2004; Santos et al., 2003), pepper (Dorji et al., 2005), apple (Hooijdonk et al., 2004; Leib et al., 2006) and tomato (Davies et al., 2000; Kirda et al., 2004).

## 2.8 PRD and Apple

Historically, DI regimes applied to apple have resulted in significant fruit size and yield reductions (Assaf et al., 1974; Beukes and Weber, 1982; Irving and Drost, 1987; Kilili et al., 1996; Mpelasoka et al., 2000; Mpelasoka et al., 2001a-c), the subject of which will receive thorough attention in subsequent chapters. With respect to PRD and apple, only one known report other than work from Caspari's group (as discussed in later chapters; Caspari et al., 2004a,b; Einhorn and Caspari, 2004; Leib et al., 2006; Lombardini et al., 2004) exists in the literature. Hooijdonk et al. (2004) compared a well-watered Control to PRD (50 % of Control irrigation volume) and a non-irrigated season long treatment. Carbon discrimination was used as an integrative measure of factors contributing to photosynthesis and $g_s$ processes, however significant differences were not detected between Control and PRD plants. Non-irrigated treatments showed less discrimination of carbon isotopes and had significant reductions in late season leaf water potentials, however, fruit growth and yield parameters were not compromised. While the soil moisture of the non-irrigated treatment was cumulatively reduced at the measurement sites, a combination of low VPD, adequate early-season soil moisture and the likelihood of large root systems (MM. 106) resulted in a significant water savings (100 % of Control) without deleterious effects on fruit relations. It would be worthwhile to investigate if multiple seasons under this regime would lead to different results. Green et al. (1997) showed preferential root uptake in response to localized wetting events to previously drying apple roots. These results provide strong evidence for rapid modification of root system function and activity between shallow and deep roots. It was

also established that root uptake processes are not impaired when roots inhabit drying soil, and data such as this supports investigation of PRD regimes for apple. It is possible that apple, due to its strong osmotic regulation (Lakso et al., 1984; Wang et al., 1995) and inherently high WUE (Lakso, 2003), may be somewhat insensitive to ABA, or some other non-hydraulic signal (Gowing et al., 1990) under cropping conditions. It has been demonstrated that most plant species possess non-stress ABA levels in their leaf tissues sufficient to promote stomatal closure (Sauter et al., 2001), including apple (Powell, 1975). Whether or not consistent regulation of $g_s$ is achievable with PRD remains to be established.

# 3 Partial Rootzone Drying and Deficit Irrigation of Gala Apples: I. Effects on Fruit Size, Yield and Quality

## 3.1 Introduction

Deficit irrigation (DI) broadly encompasses irrigation regimes that limit water supply at some level below the actual, or most often estimated, water use of the crop. Its application serves two purposes: mitigation of agricultural consumptive water use, and as a means to control excessive vegetative vigor, which is often in direct competition with fruit for the allocation of assimilates. Initially, timing and volume of DI strategies such as regulated deficit irrigation (RDI) were established according to the developmental and phenological stages unique to the crop (Chalmers et al., 1981; Mitchell and Chalmers, 1982). Success of RDI could be ascribed to the clear separation between accelerated vegetative and reproductive growth stages, and the differential sensitivity between these compartments to reduced plant water potentials. Induction and relief of soil moisture deficits were timed accordingly with the onset of rapid vegetative and fruit growth, respectively. Suppression of shoot growth appears to be due to heightened sensitivity of cell wall expansion and cell growth processes in the presence of small reductions in water potential (Bradford and Hsiao, 1982; Salisbury and Ross, 1992). Fruit growth, however, is less affected due to increased fruit osmolyte concentrations, which have been proposed to establish a favorable energy gradient for water movement into developing fruits

(Chalmers, 1989) resulting in the necessary turgor maintenance for cell growth (Jerie et al., 1989). It is imperative that DI regimes impose moderate and controlled water deficits and that severe conditions are not allowed to develop.

However, for apple the literature demonstrates that even moderate to mild water deficits adversely impact fruit size (Ebel et al., 1993, 1995; Lötter et al., 1985; Mills et al., 1994; Mpelasoka et al., 2000, 2001a-c). Based on the physiological principles underpinning RDI it is plausible to suggest that apple would not be a suitable candidate. There are two lines of evidence which support this: (1) phenological stages, especially those pertaining to rapid shoot and fruit growth, are nearly inseparable (Forshey and Elfving, 1989), and (2) fruit growth rate declines linearly with decreasing plant water potential (Lakso, 2003). Rapid canopy development early in the season coincides with exponential fruit growth rates characteristic of the cell division stage, and water deficits imposed during this period have resulted in markedly smaller fruit (Assaf et al., 1974; Beukes and Weber, 1982; Irving and Drost, 1987; Kilili et al., 1996; Mpelasoka et al., 2000). On the other hand, DI regimes which allowed for adequate irrigation volumes necessary for fruit growth maintenance during the cell division stage did not lead to suppression of vegetative growth. Furthermore, late season deficits imposed following completion of vegetative growth have also been shown to decrease fruit size (Mpelasoka et al., 2000, 2001a), suggesting that even in the absence of competing shoot growth, apple fruit growth still remains quite sensitive to water stress.

Partial rootzone drying (PRD) appears to be a viable alternative DI strategy, based on its underlying mechanism of growth control. With PRD, a portion of the rootzone is maintained at or near its field capacity (FC), while concurrently another portion is

subjected to drying conditions (Dry et al., 1996; Loveys et al., 2000). Irrigation is alternated throughout the season between wet and dry zones to achieve an optimal level of plant performance. The suggested PRD-mechanism is the production of root-initiated chemicals in response to soil drying (Davies and Zhang, 1991) which in turn may lead to inhibition of growth (Turner et al., 1996), stomatal regulation (Augé and Moore, 2002) or some combination of the two (Dry and Loveys, 1999; Gowing et al., 1990; Wahbi et al., 2005). The main advantage of PRD over other DI strategies is that stomatal regulation and suppressed growth often occur in the absence of any changes in water potential. Therefore growth processes which are sensitive to small perturbations in water potential might benefit under PRD, so long as the degree of stomatal closure does not significantly limit $CO_2$ diffusion and hence photosynthetic efficiency, and the inhibition of vegetative growth isn't too severe. In apple, the degree of stomatal regulation resulting from PRD will be of the greatest importance since PRD studies to date have found no effect on vegetative growth (Caspari et al. unpublished; Hooijdonk et al., 2004; Lombardini et al., 2004). While early PRD studies failed to compare PRD treatments to an equally water limited DI treatment, recent work has shown little or no advantages of PRD regimes over DI for grape (Bravdo et al., 2004; Chalmers et al., 2004; Gu et al., 2004; Pudney and McCarthy, 2004; Santos et al., 2003; Souza et al., 2003), apple (Caspari et al., 2004; Hoojidonk et al., 2004; Leib et al., 2006; Lombardini et al., 2004), peach (Goldhamer et al., 2002), tomato (Kirda et al., 2004) and common bean (Wakrim et al., 2005).

The most commonly reported effect of RDI and DI on apple fruit quality is an increase in soluble solids (SS) (Ebel et al., 1993; Irving and Drost, 1987; Kilili et al., 1996; Mills et al., 1994; Mpelasoka et al., 2000; Mpelasoka et al., 2001b,c; Proebsting et

al., 1984). Starch hydrolysis is generally delayed in DI fruit (Ebel et al., 1993) and appears to be dependent on the level of SS and on the timing of DI (Mpelasoka et al., 2000). Fruit firmness (FF) is negatively correlated to apple fruit size (Volz et al., 2003), and Ebel et al. (1993) found that an apparent increase in fruit firmness with DI was removed when taking into account the smaller fruit size of DI compared to a well-watered Control. However, Mpelasoka et al. (2000) compared fruit of similar size and reported that early or late DI treatments increased fruit firmness over the Control. In grape berries, increased fruit quality has also been associated with PRD studies, although this appears to be largely dependent on the extent of vigor control, since canopy reduction leads to increased light penetration and distribution and has been positively related to color and flavor compounds in the skin of grape berries (Dry et al., 2001). Effects of PRD on apple FF, SS and starch pattern index (SPI) are largely lacking in the literature. The objectives of this study, therefore, were to compare two equal deficit irrigation regimes, PRD and DI, to a well-watered Control to determine the effects of PRD on apple fruit growth, yield and quality.

## 3.2 Materials and Methods

The experiment was carried out over a three-year period (2001-2003) at the Colorado State University's Western Colorado Research Center (108° 28' 01" W, 39° 02' 33" N, elevation 1,450 m) on two separate orchard blocks: 'Royal Gala'/M9 (2001-2003) planted in 1997 at a density of 1281 trees/ha (1.8 x 4.3 m) and 'Gale Gala'/M26 (2002-2003) planted in 1995 at a density of 673 trees/ha (2.4 x 6.1 m). The two different

orchard sites will be regarded as M9 and M26 for the remainder of the paper. The experimental design and measurements discussed below pertain to both orchards, unless otherwise stated. Soil type is a Hinman clay loam, although an alluvial, coarse gravel layer 0.6 m in depth and approximately 0.3-0.6 m below the soil surface extends through the M26 site. Field capacity (FC) and permanent wilting point (PWP) are 0.29 $m^3$ $m^{-3}$ and 0.11 $m^3$ $m^{-3}$, respectively. A separate drip irrigation system was added to the existing micro-sprinkler system in 2001 and 2002 for the M9 and M26 orchards, respectively. Micro-sprinklers were used in spring until the end of the cell division stage [approximately 40 days after full bloom (DAFB)] and also after harvest, close to senescence to leach salts and refill the soil profiles. Irrigation was by drip at all other times.

The experimental design was a randomized complete block design (RCB) with three treatments and five replicates. At the start of the experiments, trees were selected for canopy uniformity and blocked on trunk cross-sectional area (TCA). Replicates were comprised of three-tree-plots with the center tree utilized as the experimental unit. Drip irrigation was supplied through parallel drip lines spaced 0.6 m either side of the tree line with inline emitters (3.8 L/hr for both Control and PRD treatments and 1.9 L/hr for DI) spaced at 0.3 m. Irrigation treatments consisted of a Control, which was intended to represent typical rather than non-limiting conditions (irrigated when approximately 25 % of available water (AW) had been depleted), and two deficit irrigation treatments each receiving 50 % of Control volumes but differing in their wetted surface area. With PRD50, irrigation was supplied by one line only per irrigation event allowing a portion of the profile to experience similar soil moisture levels to Control conditions while

simultaneously allowing another portion to dry. The decision for switching irrigation lines (rewetting the drying profile and drying down the wetted profile) varied each year and was based on interpretation of soil moisture and physiological plant response data. With DI50, irrigation was applied using both drip lines and wetting an approximately equivalent area to the Control, but delivering 50 % of the Control's volume by halving the emitter output. Irrigation was scheduled to deliver approximately 25 mm to the Control per event. This volume was based on the soil water holding capacity, depth of the wetting front, emitter flow rates, and soil infiltration rates. Automatic shutoff flow meters were inserted inline at the head of all experimental orchard rows.

**Table 3.1.** Irrigation volumes applied prior, during and following treatment periods to 'Royal Gala' on M9 and 'Gale Gala' on M26 apple trees, 2001-2003. $ET_o$ and precipitation was recorded between May 1-October 31 for all years.

| Rootstock | Year | Treatment | Pre-Treatment (mm) | Treatment Period (mm) | Rewetting (mm) | Total (mm) | Precipitation (mm) | ETo (mm) |
|---|---|---|---|---|---|---|---|---|
| M9 | 2001 | Control | 369 | 98 | 131 | 597 | 81 | 1013 |
| M9 | 2001 | PRD,DI | 369 | 49 | 131 | 548 | 81 | 1013 |
| M9 | 2002 | Control | 117 | 85 | 90 | 292 | 127 | 1008 |
| M9 | 2002 | PRD,DI | 117 | 45 | 90 | 252 | 127 | 1008 |
| M9 | 2003 | Control | 78 | 152 | 83 | 313 | 90 | 1057 |
| M9 | 2003 | PRD,DI | 78 | 76 | 83 | 236 | 90 | 1057 |
| M26 | 2002 | Control | 83 | 152 | 51 | 287 | 127 | 1008 |
| M26 | 2002 | PRD,DI | 83 | 76 | 51 | 210 | 127 | 1008 |
| M26 | 2003 | Control | 50 | 152 | 171 | 374 | 90 | 1057 |
| M26 | 2003 | PRD,DI | 50 | 76 | 171 | 297 | 90 | 1057 |

Volumetric soil moisture content ($m^3$ $m^{-3}$) was measured using time-domain reflectometry (TDR) (Tektronix Model 1502C, Beaverton, OR). Two sets of stainless steel parallel waveguides 0.3 m in length and 6 mm in diameter were inserted 0.45 m from the tree trunk on opposite sides of the tree for all replicates. Measurements of electrical conductivity (EC) taken throughout the orchards to assess soil salinity indicated that EC was not sufficiently high to alter the dielectric properties of the profile. In 2003, TDR waveguides were removed in order to accommodate a TDR access tube system,

however measurement error was unacceptable and consequently the 2003 soil moisture data is excluded from this report.

Fruit were thinned in accordance with commercial practices, although fruit number per tree was adjusted based on TCA (fruit $cm^{-2}$) in order to reduce croploading effects on fruit growth rate and size. However, in 2002 fruit counts necessary for formulating cropload levels were incorrectly recorded and as a result a wide range of croploads within a given treatment existed. Ten fruit per replicate (50 fruit per treatment) were tagged following the June drop period and measured weekly using a Cranston fruit gauge (Cranston Machinery Co., Oak Grove, OR). Measurements were taken at the same time of morning to reduce the effect of diurnal fluctuations in expansion and contraction of the fruit. Diameter was converted to fruit volume assuming the fruit to be a sphere. Harvest dates were based on maturation protocols performed on randomly selected non-experimental trees throughout the orchard. Harvest dates for the orchards were as follows: August 14 and 21, 2001, August 19, 2002 and August 14 and 21, 2003 for M9, and August 20, 2002 and August 19 and 21, 2003 for M26. Total fruit weight per tree was recorded and individual fruit weight, circumference, SS, flesh firmness, and SPI were immediately determined on ten randomly selected fruit per tree (50 fruit per treatment). In 2002 and 2003, an additional ten fruit per tree were collected, weighed and held at 1 °C for 12 weeks in order to assess treatment effects on fruit quality following cold storage. Fruit firmness was measured on opposite sides of the fruit with a penetrometer (model FT 327, Wagner Instruments, Greenwich, CT) fitted with an 11.1 mm head, and the juice generated was pipetted onto a digital refractometer (model PR101, Atago Co, Tokyo, Japan) for determination of SS. Fruit were cut through the

equatorial region and halves were sprayed with a potassium iodide solution (10 g KI + 2.5 g I in 500 ml of DI water) to determine SPI. In order to eliminate variability resulting from subjectivity, SPI ratings were conducted by one experimenter using colored plates specific to 'Royal Gala'.

In each year, TCA was measured at a height of 0.15 m above the graft union just prior to full bloom, and again in the fall after leaf senescence and used as an indicator of vegetative growth. Meteorological data was collected by an official weather station of the United States National Weather Service located within 100 m of the trial site.

Statistical analysis was performed using the SAS system software (SAS Institute, Cary, N.C.). Treatment means were compared using ANOVA (PROC GLM) and significance was tested at $P \leq 0.05$. Mean separation was determined by Student-Newman-Keuls test (SNK).

## 3.3 Results

Seasonal irrigation volumes, cumulative potential evapotranspitation ($ET_o$) and precipitation for 2001-2003 are provided in Table 3.1. Season-long M9 irrigation volumes of PRD50 and DI50 were 54 %, 25 % and 22 % of $ET_o$ for the 2001, 2002 and 2003 season, respectively, while season-long M26 irrigation volumes for PRD50 and DI50 were 21 % and 28 % of $ET_o$ for the 2002 and 2003 season, respectively. Soil moisture data for 2001 shows a clear and significant difference between the wetted and dry profile of the PRD treatment (Fig 3.1A). The mean soil moisture content throughout the 2001 experimental period for the 'dry' PRD50, 'wet' PRD50 and Control profiles was 0.21 $m^3 m^{-3}$, 0.25 $m^3 m^{-3}$ and 0.25 $m^3 m^{-3}$, respectively.

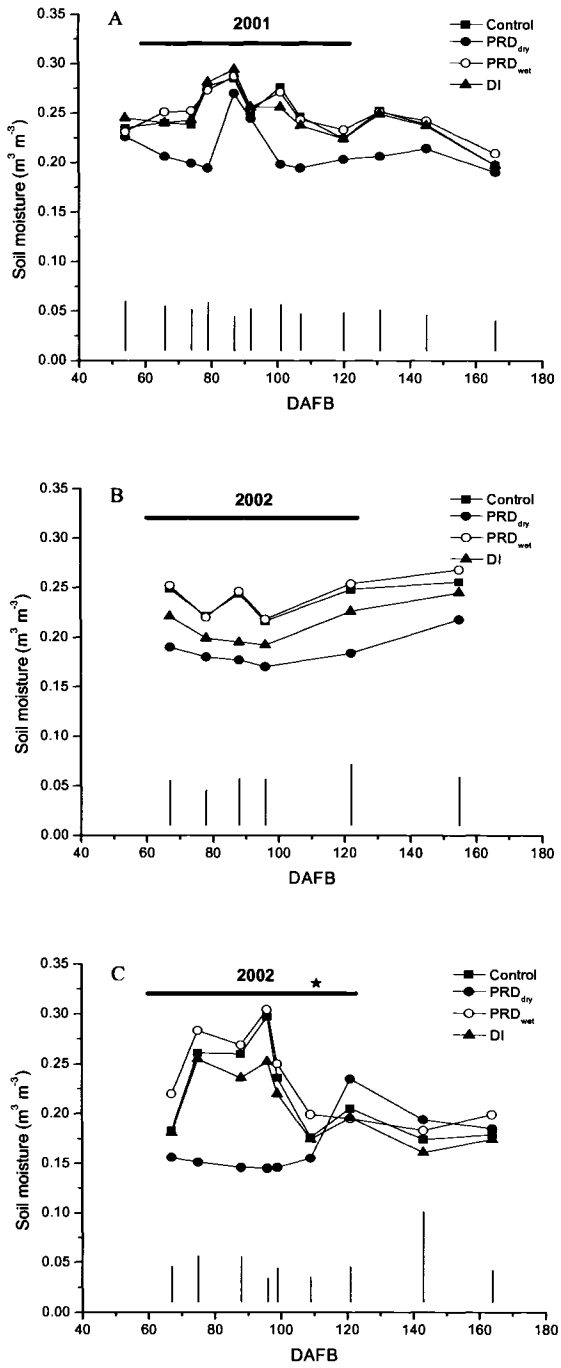


**Fig 3.1.** The effects of irrigation treatments on mean soil moisture content in the top 30 cm of 'Royal Gala' on M9 apple trees in 2001 (A) and 2002 (B), and 'Gale Gala' on M26 apple trees during 2002 (C). Horizontal bar at top indicates duration of the treatment irrigation period. The asterisk at the top of (C) signifies an irrigation switch between dry and wet zones for the PRD treatment. Vertical bars indicate LSD ($P < 0.05$).

A rather rare 22.6 mm rainfall event that occurred on 83 DAFB, just prior to a planned switch, re-wetted the dry side of PRD50. Consequently, irrigation lines were not switched and the soil profile was allowed to re-dry. The mean soil moisture of DI50 (0.25 $m^3$ $m^{-3}$) did not differ from Control levels, even though it received 50 mm less irrigation than the Control.

In 2002, total irrigation volume for M9 trees was reduced by decreasing early-season irrigation volume to allow for greater soil moisture depletion. TDR measurements began 7 days following the first treatment period irrigation, which explains the treatment differences observed on the first measurement date (Fig 3.1B). The mean soil moisture content throughout the M9 2002 experimental period for the Control, 'dry' PRD50, 'wet' PRD50 and DI50 profiles was 0.24 $m^3$ $m^{-3}$, 0.18 $m^3$ $m^{-3}$, 0.24 $m^3$ $m^{-3}$ and 0.21 $m^3$ $m^{-3}$, respectively (Fig 3.1B). On 96 DAFB, when the 'dry' side of PRD50 was at 33 % of AW, a decision was made to continue drying the dry side since soil water extraction, although minimal, was still evident and changes in physiological measurements were undetectable. Soil moisture monitoring in 2002 at the M26 site began on 67 DAFB following instrumentation, which was seven days after the previous irrigation event. The mean soil moisture content throughout the M26 2002 experimental period for the Control, 'dry' PRD50, 'wet' PRD50 and DI50 profiles was 0.23 $m^3$ $m^{-3}$, 0.16 $m^3$ $m^{-3}$, 0.25 $m^3$ $m^{-3}$ and 0.22 $m^3$ $m^{-3}$, respectively. A significant difference in soil moisture between the PRD 'wet' and 'dry' profiles was established and maintained prior to 111 DAFB at which point soil water depletion from the 'dry' PRD50 side had ceased and irrigation lines were switched (Fig 3.1C). The 2003 soil moisture measurements are not included as discussed above, however soil moisture was assumed to be similar to 2002 data as irrigation

regimes (i.e. volume and timing) were nearly identical to 2002 for both the M9 and M26 orchards (Table 3.1), and there was no appreciable precipitation during the treatment period.

For all years (2001-2003), there were no significant differences in fruit diameter at any given measurement point (Fig 3.2A,C,E and Fig 3.3A,C). There was, however, a high-temperature effect, regardless of treatment, on fruit growth rate (FGR). In each year, periods of high temperatures (> 35 °C) in the second and/or third week of July (data not shown) correlated well with reductions in mean FGR of 16 to 31 % for M9 and 20 to 38 % for M26 relative to the period preceding or following the heat spells (Fig 3.2B,D,F and Fig 3.3B,D). Mean daily maximum temperatures during these high-temperature periods were 37 °C, 39 °C and 39 °C for 2001, 2002 and 2003, respectively. Fruit size was larger in 2001 and 2003 than in 2002 (Table 3.2). Trunk growth was not affected by treatment for either rootstock and was similar between years (data not shown).

Yield components did not significantly differ among treatments (Table 3.2). In 2002, total yields were higher than in 2001 and 2003 as a direct consequence of higher croploads (Table 3.2). When data was expressed as yield efficiency (kg $cm^{-2}$ TCA), both the M9 and M26 orchards were low (1.26 to 4.45 kg $cm^{-2}$ TCA), although yield efficiency of the M9 site was approximately threefold that of the M26 orchard, and is reflective of the much larger M26 tree sizes and very low croploads. The low fruit set was a result of the interaction between weather and over-application of thinning agents. Fruit quality parameters at harvest and after cold storage, in all years, were also not significantly influenced by irrigation treatment (Table 3.3). In 2003, fruit

**Table 3.2.** The effects of irrigation treatments on average fruit size and yield for 'Royal Gala' on M9 and 'Gale Gala' on M26 apple trees, 2001-2003.

| Year | Rootstock | Treatment | Treatment Period Irrigation[x] (mm) | Cropload (fruit $cm^{-2}$) | Fruit Size (g $fruit^{-1}$) | Yield (kg $tree^{-1}$) |
|---|---|---|---|---|---|---|
| 2001 | M9 | Control | 97.8 | 3.2 | 162.1 | 6.2 |
| | | PRD50 | 48.8 | 3.6 | 154.5 | 6.3 |
| | | DI50 | 48.8 | 3.7 | 157.5 | 7.3 |
| *Pr >F* | | | | *0.59* | *0.52* | *0.67* |
| *LSD (α=0.05)* | | | | *1.36* | *17.27* | *3.44* |
| 2002 | M9 | Control | 84.9 | 6.6 | 128.3 | 11.8 |
| | | PRD50 | 45.1 | 5.9 | 124.8 | 10.5 |
| | | DI50 | 45.1 | 5.2 | 137.3 | 10.4 |
| *Pr >F* | | | | *0.56* | *0.32* | *0.75* |
| *LSD (α=0.05)* | | | | *3.48* | *21.75* | *5.31* |
| 2003 | M9 | Control | 152.4 | 2.2 | 151.3 | 6.6 |
| | | PRD50 | 76.2 | 2.0 | 146.5 | 5.1 |
| | | DI50 | 76.2 | 1.9 | 158.8 | 6.6 |
| *Pr >F* | | | | *0.86* | *0.22* | *0.78* |
| *LSD (α=0.05)* | | | | *1.42* | *17.81* | *6.64* |
| 2002 | M26 | Control | 152.4 | 2.4 | 130.2 | 27.7 |
| | | PRD50 | 76.2 | 2.2 | 131.9 | 24.7 |
| | | DI50 | 76.2 | 1.9 | 129.2 | 20.9 |
| *Pr >F* | | | | *0.54* | *0.78* | *0.37* |
| *LSD (α=0.05)* | | | | *1.03* | *9.95* | *12.32* |
| 2003 | M26 | Control | 152.4 | 1.4 | 157.9 | 19.1 |
| | | PRD50 | 76.2 | 1.2 | 161.6 | 18.6 |
| | | DI50 | 76.2 | 1.3 | 160.8 | 18.4 |
| *Pr >F* | | | | *0.76* | *0.68* | *0.99* |
| *LSD (α=0.05)* | | | | *0.73* | *11.72* | *11.53* |

[x] Irrigation treatment periods were as follows; 2001 M9 59-122 DAFB, 2002 M9 60-124 DAFB, 2003 M9 46-118 DAFB, 2002 M26 60-123 DAFB and 2003 M26 47-116 DAFB.

appeared to be slightly more advanced than in prior years for a given rootstock based on small increases in SS and SPI for both M9 and M26, and reduced FF for M26 only.

## 3.4 Discussion

Scheduling irrigation based on soil moisture, fruit growth, and physiological plant responses allowed for the application of very low seasonal $ET_c$ values, as similarly reported by Leib et al. (2006). The reason for this rather low irrigation requirement appears to be the high daily vapor pressure deficit (VPD) characteristic of Colorado's fruit growing region. Stomatal conductance ($g_s$) of apple leaves is tightly coupled to

evaporative demand (Flore et al., 1985; Lakso, 2003; Powel, 1976; West and Gaff, 1976), and in our study $g_s$ declined rapidly whenever VPD values reached 2.5 to 3 kPa. At our site during the summer months, those threshold values are reached mid morning resulting in very low $g_s$ and transpiration for the remainder of the day (see Chapter 4).

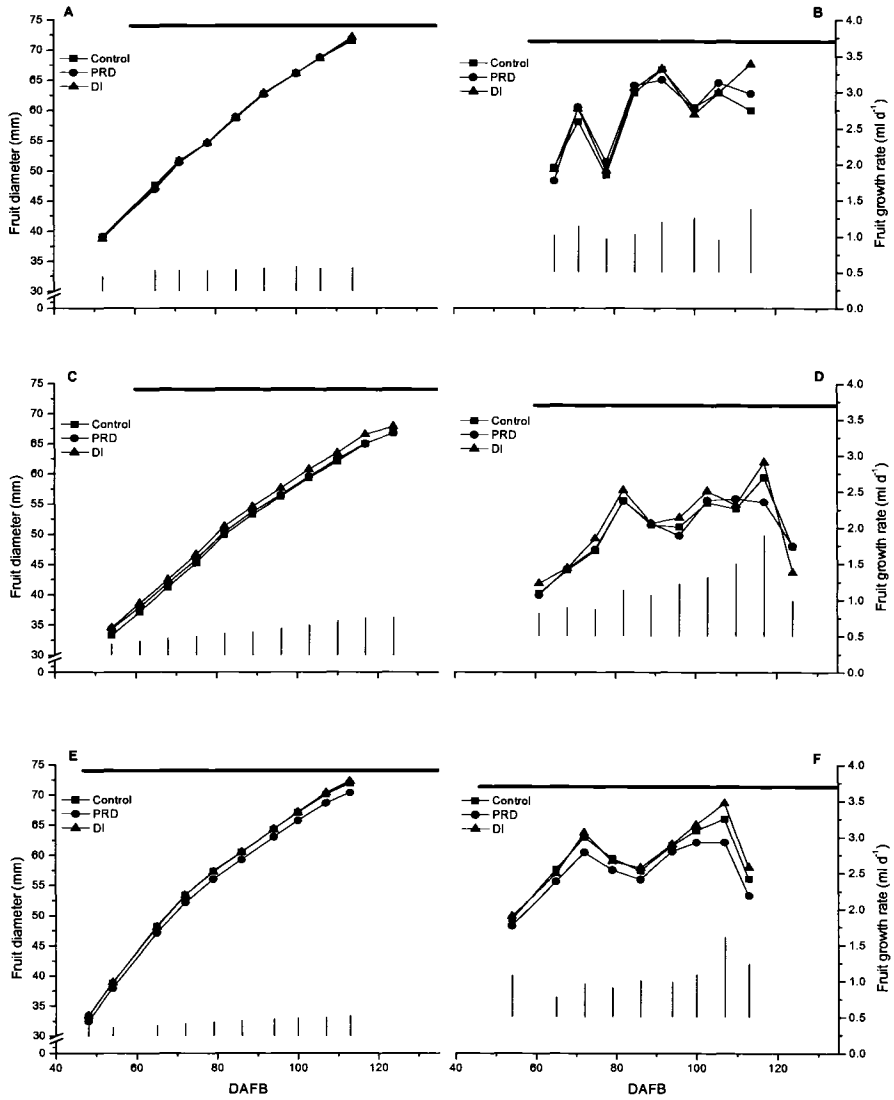


**Fig 3.2.** The effects of irrigation treatments on mean fruit diameter (A, C, E) and fruit growth rate (B, D, F) for 'Royal Gala' on M9 apple trees for 2001-2003. Horizontal bar at top indicates duration of the treatment irrigation period. Vertical bars indicate LSD ($P< 0.05$).

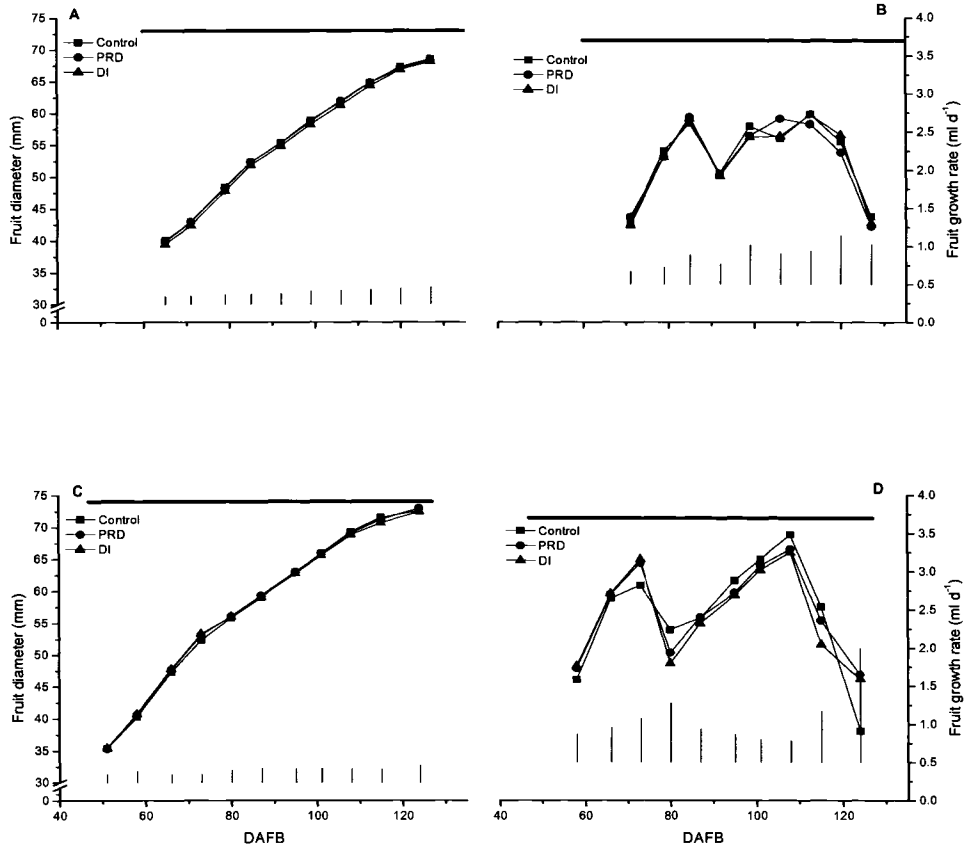


**Fig 3.3.** The effects of irrigation treatments on mean fruit diameter (A, C) and fruit growth rate (B, D) for 'Gale Gala' on M26 apple trees for 2002 and 2003. Horizontal bar at top indicates duration of the treatment irrigation period. Vertical bars indicate LSD ($P< 0.05$).

Atkinson (1980) reviewed the literature on apple root systems and noted that over a wide range of experiments approximately 70 % of root weight occurs in the top 0-0.3 m of soil, although distribution and depth will vary based on tree age, density, scion-rootstock interaction, soil moisture, soil type and management. Our soil moisture measurement depth was intended to assess soil moisture conditions for that portion of the rootzone which is most active and easily manipulated, but it fails to address the potential existence of deeper soil moisture reserves and the influence and activity of deeper roots on ameliorating the effects of shallow soil water deficits. De Silva et al. (1999) showed

root length density (RLD) of similarly aged 'Fuji' on M26 to be highest in the top 0.2-0.4 m of soil, although horizontally, RLD increased with increasing radial distances up to 1.8 m from the trees. This occurred under micro-sprinkler irrigation and higher tree densities than those reported here. Based on the prior micro-sprinkler management and low planting densities of both the M9 and M26 orchards, it is possible that roots were not adequately confined to the herbicide strips and may have had access to soil moisture reserves between rows, although the presence of a non-irrigated grass cover crop should have limited water available for tree roots. In fact, in each season the grass cover crop stopped growth shortly after the switch to drip irrigation and turned brown during the later part of the season, presumably due to water stress. Nonetheless, average M9 and M26 seasonal irrigation volumes (2001-2003) were approximately 33 % of $ET_o$ (Table 3.1). The PRD treatment clearly showed differential wetting/drying, although a leveling off of soil moisture extraction in the 'drying' side did not coincide with any noticeable changes in leaf gas exchange or water potential (see Chapter 4). In addition, extraction ceased when soil moisture was at 55.6 % and 33.3 % of AW in 2001 and 2002 for the M9 and 15.6 % in 2002 for the M26 and did not continue to approach the PWP, suggesting the potential for shifts in root activity and preferential uptake of water from wetted fractions of the profile, as has been previously observed with apple (Caspari et al., unpublished; Green et al., 1997). Such shifts towards water uptake from the wettest part of the profile might also benefit root maintenance under drying portions of the profile due to transferal of water from wet to dry roots, as suggested by Dry et al. (2000b) and demonstrated experimentally with grapevines (Stoll et al., 2000).

**Table 3.3.** The effects of irrigation treatments on fruit quality and maturity parameters (Average Fruit Size, FF=Fruit Firmness, SS=Soluble Solids, SPI=Starch Pattern Index and % Weight Loss following cold storage) for 'Royal Gala' on M9 and 'Gale Gala' on M26 apple trees, 2001-2003. LSD ($P< 0.05$).

| Year | Rootstock | Treatment | Average Fruit Size (g fruit$^{-1}$) | FF (kg mm$^{-2}$) | SS (%) | SPI[x] | Weight Loss[y] (%) |
|---|---|---|---|---|---|---|---|
| 2001 | M9 | Control | 176.0 | 9.44 | 15.44 | 3.11 | - |
| | | PRD50 | 172.9 | 9.34 | 15.44 | 2.95 | - |
| | | DI50 | 174.7 | 9.34 | 15.32 | 2.84 | - |
| *Pr >F* | | | *0.91* | *0.97* | *0.94* | *0.52* | - |
| *LSD(α=0.05)* | | | *18.94* | *1.20* | *1.06* | *0.62* | - |
| 2002 | M9 | Control | 155.5 | 9.57 | 16.65 | 3.89 | 1.62 |
| | | PRD50 | 140.0 | 9.41 | 16.02 | 4.04 | 1.68 |
| | | DI50 | 148.2 | 9.79 | 16.92 | 4.15 | 1.63 |
| *Pr >F* | | | *0.30* | *0.62* | *0.12* | *0.86* | *0.91* |
| *LSD(α=0.05)* | | | *26.37* | *1.05* | *1.13* | *1.29* | *0.43* |
| 2003 | M9 | Control | 173.0 | 9.14 | 16.48 | 5.13 | 3.76 |
| | | PRD50 | 156.6 | 9.33 | 16.66 | 5.13 | 4.10 |
| | | DI50 | 171.1 | 9.20 | 16.88 | 4.69 | 3.78 |
| *Pr >F* | | | *0.20* | *0.88* | *0.64* | *0.22* | *0.83* |
| *LSD(α=0.05)* | | | *25.01* | *1.05* | *1.11* | *0.73* | *1.63* |
| 2002 | M26 | Control | 159.1 | 9.26 | 15.85 | 3.96 | 2.11 |
| | | PRD50 | 146.4 | 9.33 | 15.70 | 4.12 | 2.04 |
| | | DI50 | 143.8 | 9.31 | 16.31 | 4.55 | 2.22 |
| *Pr >F* | | | *0.12* | *0.98* | *0.24* | *0.37* | *0.47* |
| *LSD(α=0.05)* | | | *19.31* | *0.89* | *0.95* | *1.10* | *0.39* |
| 2003 | M26 | Control | 187.3 | 8.14 | 16.70 | 4.84 | 4.17 |
| | | PRD50 | 181.6 | 8.55 | 16.44 | 4.64 | 3.47 |
| | | DI50 | 186.3 | 8.65 | 16.87 | 4.56 | 4.20 |
| *Pr >F* | | | *0.67* | *0.21* | *0.53* | *0.52* | *0.51* |
| *LSD(α=0.05)* | | | *17.50* | *0.75* | *1.00* | *0.65* | *1.87* |

[x] SPI Rating Scale; 1= full starch, 6= no starch.
[y] Fruit weight loss following 12 weeks of cold storage at 1 °C.

Irrigation scheduling based on historical and/or current season fruit growth rate has been previously applied to apple (Assaf et al., 1982; Ebel et al., 1995), although the constant linear slope of the fruit expansion phase renders it difficult to adjust irrigation volume accordingly in response to decreases in FGR. Ebel et al. (1995) demonstrated irreversible loss in fruit size under RDI for croploads greater than 4.6 fruit cm$^{-2}$ TCA, however, under light croploads (1.7 fruit cm$^{-2}$ TCA) small reductions in fruit size were reversible with irrigation adjustment. In the current study, weekly fruit growth measurements did not reveal any deficit-induced reductions in fruit size (Fig 3.2 and Fig

3.3), nor any noticeable differences between rootstocks in a given year. The latter is in agreement with results attained using similarly aged 'Gala' on a range of rootstocks and at two different cropload levels (Al-Hinai and Roper, 2004). Year to year variation, however, was evident and noticeable size reductions in 2002 had already manifested < 65 DAFB (Fig 3.2 and Fig 3.3). Al-Hinai and Roper (2004) concluded that warmer temperatures in the first five weeks from full bloom affected growth by increasing the cell division rate. Although our study was not designed to explain an anatomical basis for the observed differences in fruit size, temperatures were cooler and less growing degree days (GDD) accumulated throughout the first 45 DAFB in 2002 than in other years (data not shown). Other investigations have also demonstrated an increase in fruit size by 42 DAFB as a function of higher temperatures resulting in increased cortical cell divisions (Bergh, 1990; Greybe et al., 1998).

Goffinet et al. (1995) determined that for 'Empire' fruit, delaying post-bloom thinning resulted in smaller fruit with fewer cells, and the impacts of final cell size and the proportion of intercellular space on final fruit size was less important than cell number. These authors concluded that fruits thinned early in the cellular division stage were larger due to the absence of nearby competing fruit. The inverse relationship between fruit size and cropload for apple is well established (Palmer et al., 1997), and Naor et al. (1997) demonstrated that increased demand for assimilates from higher croploads reduced fruit size and this limitation increased with decreasing irrigation level. Increased sensitivity to deficit irrigation under high croploads has also been demonstrated by Mpelasoka et al. (2001b). In our study, it appeared that higher 2002 croploads in the M9 (approximately 6 fruit $cm^{-2}$ TCA) may have compounded early season low-

temperature effects on fruit growth, however this does not explain differences in size observed in the M26 orchard. Although M26 croploads in 2002 were nearly double those in 2001, we have found in this and other studies (Caspari, unpublished) that crop load does not affect fruit size in the range of 1 to 4 fruit $cm^{-2}$ TCA. Hence the smaller fruit size in 2002 is most likely the result of lower temperatures during initial fruit growth. In both rootstocks smaller fruit size and lower growth rates were maintained throughout the 2002 season, and the average FGR for M26 and M9 was about 20 to 25 % lower than during the other two years. As a result, final fruit size in 2002 was reduced by 17.9 % and 14.6 % for M9 compared to 2001 and 2003, and 18.3 % for M26 in comparison with 2003 data.

The effects of high-temperature stress on FGR was observed in all years, irrespective of irrigation regime, during extremely warm weeks as can be seen by sharp depressions in FGR (Fig 3.3). Francesconi et al. (1997) showed that net carbon exchange rate of four-year-old, potted 'Royal Gala' trees was negatively related to increasing temperature from 15-34 °C. During mid-season in the semi-arid climate of Colorado, temperatures often exceed 35 °C, and latent heat of vaporization is compromised due to the high VPD negatively affecting $g_s$, resulting in an approximate 2-4 °C increase in leaf temperatures over ambient temperatures. Apple fruit are even less able to dissipate heat than leaves, and skin temperature of a sun-exposed apple might be as high as 13-14 °C above ambient (Parchomchuk and Meheriuk, 1996). In our climate, saturating levels of photosynthetically active radiation (PAR) are generally observed by mid-morning and it is suggested that some combination of photoinhibition, increased respiratory costs, and stomatal limitations on gas exchange are responsible for a reduced carbon supply during

weeks at high temperatures. Dissipation of excess heat from evaporative cooling via overhead misting is used to enhance color and size and reduce sunburn on fruit in semi-arid regions, however this practice often results in excessive water usage and has not been implemented on the experimental site. Fruit were rated for severity of sunburn in 2003, and were not impacted by treatment (data not shown). Overall ratings were also low, and this may be due to the suggestion that 'Gala' appears to be less sensitive to sunburn than other cultivars (Palmer et al., 2003).

As has been previously shown, providing ample irrigation volumes throughout the cell division stage did not result in suppression of vegetative growth (Assaf et al., 1974; Beukes and Weber, 1982; Irving and Drost, 1987; Mills et al., 1994). As discussed above, deficit irrigation was not expected to restrict vegetative growth of 'Gala' due to adequate early season soil moisture, its relatively low vigor, and its short vegetative growth period at our semi-arid site. Likewise, in a companion study with the more vigorous 'Fuji' in Washington State, Lombardini et al. (2004) found no effect of a DI and PRD treatment on shoot number and mean shoot length.

The difference in yield among years was a function of fruit number, and the low yield for the M26 site is reflective of over-thinning in both years. Starch pattern index is a consistently used indicator of maturity due to its qualitative assessment of starch hydrolysis. Starch degradation has been shown to be delayed by DI (Ebel et al., 1993), however in our study there were no significant differences among treatments in SPI. Higher values of SPI and SS in 2003 were due to a later pick as fruit were marketed for immediate fresh consumption rather than cool storage as in previous years. Fruit firmness is negatively related to fruit size (Volz et al., 2003), and firmer fruit reported in DI

studies are often an artifact of smaller fruit with DI (Ebel et al., 1993). In our study, there was no treatment effect on fruit size or FF, yet decreased FF in the M26 in 2003 compared to 2002 appears to be related to the increased size. The lack of treatment effects on SS and SPI after storage was expected since there was no treatment effect on fruit quality parameters tested at harvest.

## 3.5 Conclusion

Deficit irrigation regimes did not negatively affect yield, fruit growth or fruit quality of 'Gale Gala' and 'Royal Gala' apples, even under extremely low irrigation inputs, and occurred regardless of irrigation placement. Therefore, no apparent benefits of PRD over DI existed, as has been recently demonstrated for apple comparing PRD to a non-irrigated treatment under the very different environmental conditions of New Zealand (Hooijdonk et al., 2004). The reason that DI and PRD were successful might be due to the control exerted by VPD on gas exchange processes resulting in less water use and conservation of soil moisture under deficit regimes. In addition, abscisic acid (ABA) data collected from these trees (see Chapter 4) indicate that all treatments had relatively low [ABA]. The low [ABA] of DI and PRD leaves suggests that either ABA is not a front line response to water stress in apple, as previously postulated by Davies and Lakso (1978), or that the level of water stress achieved was insufficient to trigger a significant increase in ABA.

# 4 Partial Rootzone Drying and Deficit Irrigation of Gala Apples: II. Effects on Gas Exchange and Water Relations

## 4.1 Introduction

*Malus* x *domestica* Borkh., apple, employs many of the classical drought tolerance and avoidance mechanisms proposed by Turner (1979) (see later review by Lakso, 1994), most notably the maintenance of turgor at low water potentials through osmotic adjustment (Lakso, 1979; Wang et al., 1995). During slowly developing water stress apple osmotically adjust allowing the maintenance of stomatal conductance ($g_s$) and photosynthesis during periods of water deficit. Water potential does not impact gas exchange of apple leaves until relatively low water potential values are attained (Lakso, 1979; Warrit et al., 1980; West and Gaff, 1976). In spite of the broad ecological benefits of such mechanisms, research related to cropping efficiency has demonstrated that the lowering of water potential in apple adversely impacts potential fruit size (Lakso, 2003).

Stomatal sensitivity to water potential is greatly influenced by the vapor pressure deficit (VPD) of the atmosphere (Flore et al., 1985; Lakso, 2003; Powel, 1976). The effects of VPD on gas exchange of apple leaves contribute to the maintenance of a favorable plant water balance under conditions of high evaporative demand through partial stomatal closure. The VPD response of apple leaves is consistent with the feed-forward hypothesis of Farquhar (1978) and has been determined experimentally (Thorpe

et al., 1980; West and Gaff, 1976), although most likely some degree of hydraulic feedback control occurs concomitantly (Landsberg and Jones, 1981). In addition to hydraulic mechanisms in the regulation of gas exchange, Gowing et al. (1990) have demonstrated that apple leaves also are responsive to non-hydraulic signals. While imposing water deficit on half of the root system of split-rooted apple explants, noticeable reductions in shoot growth and gas exchange were observed even though plant water potential was maintained at similar levels to well-watered Controls (Gowing et al., 1990). Although the signal remained unidentified, abscisic acid (ABA) was implicated based on previous correlative evidence for its regulatory role in the control of $g_s$, and heightened ABA levels found in plant roots subjected to soil drying (Davies and Zhang, 1991; Loveys, 1984; Zhang and Davies, 1989; Zhang et al., 1987). In fact, it is this type of plant response to soil drying that formed the basis of Partial Rootzone Drying (PRD) (Dry et al., 1996; Loveys et al., 2000), although direct quantification of ABA remains largely lacking in the vastly growing body of PRD literature.

In spite of the increasing amount of PRD reports only a few are related to apple (Caspari et al., 2004a,b; Leib et al., 2006; Lombardini et al., 2004; Hooijdonk et al., 2004), and of these only Lombardini et al. (2004) and Hooijdonk et al. (2004) presented physiological data. However, Hooijdonk et al. (2004) did not observe differences among a well-watered Control, a PRD regime irrigated at 50 % of Control and a season-long non-irrigated treatment for both physiological responses and fruit relations despite large differences in soil moisture prior to harvest. Lombardini et al. (2004) presented results from one season in which a deficit irrigation (DI) and a PRD treatment, both irrigated at 50 % of Control, had similar reductions in leaf water potential and single-leaf and whole-

canopy gas exchange, although on several occasions PRD was more negatively affected than DI. It stands to reason that, in theory, an irrigation method mediated by a non-hydraulic signal might be advantageous to apple production, since fruit size is sensitive to reductions in water potential (Lakso, 2003). It was of interest to determine whether or not apple will produce ABA in response to partial rootzone drying (PRD) and if so, to what extent is the signal perceived under semi-arid growing conditions? Much of the VPD-effect on apple has been determined under cool, humid conditions or through modified leaf chamber micro-environments. When operating in semi-arid climates further reductions in gas exchange might be detrimental to fruit sizing since apple already appear to possess good water use efficiency (WUE) under non-stressed conditions (Lakso, 2003), and further limitations in transpiration would likely result in equivalent reductions in assimilation. The current study was designed to evaluate the effects of PRD and DI on the physiology and water relations of apple.

## 4.2 Materials and Methods

The experimental site and design were described elsewhere (see Chapter 3). Experimental data from the M9 site in 2001 was similar to other years and is omitted for ease of data presentation, and because the M26 site was not instrumented until 2002. Briefly, the treatments, each with five replicates, consisted of a Control, which received irrigation via parallel drip lines with 12 inline emitters (six per line) per tree delivering 3.8 L/hr, and two deficit irrigation treatments (PRD50, DI50) each receiving 50 % of irrigation volumes applied to the Control but differing in their wetted surface area. With

PRD50, irrigation was supplied by one line (six emitters per tree) per irrigation event while with DI50 irrigation wetted an approximately equivalent area to the Control by halving the emitter output (two lines of six emitters each, rated at 1.9 L/hr). Irrigation events were scheduled to deliver approximately 25 mm to Control trees based on the soil water holding capacity, depth of the wetting front, emitter flow rates and soil infiltration rates. Irrigation application decisions were based on volumetric soil moisture measurements ($m^3$ $m^{-3}$) obtained using time-domain reflectometry (TDR) (Tektronix model 1502C, Beaverton, OR) to a depth of 30 cm as described previously (see Chapter 3), and physiological measurements outlined below. The PRD50 wet and dry sides were switched for the M26 site in 2002 on 110 days after full bloom (DAFB), and were maintained without alternating wet and dry sides in the M9 in 2002 and for both rootstocks in 2003.

Gas exchange data was collected in 2002 using a factory calibrated Licor 1600 steady state porometer (Licor, Lincoln, NE) and in 2003 using a PP systems Ciras-2 gas analyzer (PP systems, Amherst, MA). Measurements were conducted on the first fully mature sunlit leaves of extension shoots, between 12:00-14:00 HR on days with similar climatic conditions. Diurnal measurements were also conducted on selected days in both the 2002 and 2003 growing seasons. Five replications for each treatment (two leaves per replicate) were utilized for 2002 measurements, and three replications (two leaves per replicate) were utilized for 2003, as this represented the maximum number of measurements achievable within the physiological time-frame of interest.

Predawn and midday leaf water potential measurements were collected using a pressure chamber (model 600, PMS, Corvallis, OR) according to the Scholander method (Scholander et al., 1965) on three replications (two leaves per replicate) per treatment.

Extraction and purification procedures for ABA necessary for comparable results between GC and ELISA have been previously reported for apple tissue (Soejima et al., 1990), and have been slightly modified herein. Briefly, leaf tissue was collected and immediately dipped in liquid N and held at -80 °C for several weeks prior to lyophilization. Following freeze drying tissues were ground and sieved through a 150 μm mesh screen. Extraction ratio was 10 mg tissue per 1 ml 80 % cold methanol (80 % MEOH : 20 % DI $H_2O$). Samples were rotated for 24 hours in the dark at 4 °C, removed and centrifuged at 6000 g for 10 min at 4 °C. Supernatant was vacuum evaporated to remove MEOH. The water fraction was filtered using a 0.2 micron nylon filter and added to polyvinyl-polypyrrolidone (PVPP) at a ratio of 20 mg PVPP: 100 mg tissue, in order to remove phenolic compounds. The pH was adjusted to 8.3 and centrifuged at 6000 g for 10 min at 4 °C. Supernatant was collected, readjusted to pH 8.3 and partitioned with an equal volume of ethyl acetate to remove neutral and basic organic-soluble compounds. The organic fraction was collected and set aside. The lower water phase was adjusted to pH 2.5 and partitioned three additional times against equal volumes of ethyl acetate. Partitioning against acid conditions brings ABA into the organic layer (ethyl acetate). All organic phases were combined for analysis of the physiologically active form of ABA (2-*cis*-(S)-ABA). The water phase containing conjugates of ABA was not examined. One ml of the ethyl acetate fraction was vacufuged and reconstituted in 1 ml TBS buffer. The above procedures enabled the use of very low quantities of leaf

tissue (150 mg) and all separatory stages were performed in 5 cc syringes. Efforts were made to work rapidly, under dimly lit conditions due to photo-degradation of ABA.

Quantification of the physiologically active, 'free' ABA (2-*cis*-(S)-ABA) was by a competitive ELISA assay (Agdia, Elkhart, IN), using a spectrophotometer (Spectramax 384 Plus, Molecular Devices Corp., Sunnyvale, CA) equipped with a 96-well micro-titre plate reader. Procedures are outlined by Agdia (Agdia Inc., Elkhart, Indiana). Internal standards (2-*cis*-(S)-ABA) (Sigma-Aldrich, St. Louis, Missouri) were added to sample tissue prior to the extraction protocol in order to calculate recovery rates. Recovery rates were always higher than 75 %. Standards and samples were run in duplicate and were re-assayed if their coefficient of variability (cv) was > 0.10. Standard curves were prepared fresh for each plate. Log-logit transformations of standard curves were performed and sample optical densities (OD) always fell within the linear range of the standard curves. The $R^2$ values for standard curves of individual plates were between 0.980 and 0.998.

Meteorological data was collected by an official weather station of the United States National Weather Service located 100 meters from the trial site.

Statistical analysis was performed using the SAS system software (SAS Institute, Cary, N.C.). Treatment means were compared using ANOVA (PROC GLM) and significance was tested at $P \leq 0.05$. Mean separation was determined by Student-Newman-Keuls test (SNK). PROC REG was used for regression analysis.

## 4.3 Results

Midday leaf water potential ($\Psi_{md}$) measurements taken throughout the 2002 and 2003 seasons did not decline with decreasing soil water contents of the DI treatments (Table 4.1 and 4.2). The M9 seasonal mean $\Psi_{md}$ values were -2.0, -1.98 and -1.84 MPa for 2002, and -1.87, -1.79 and -1.76 MPa for 2003, for Control, PRD50 and DI50, respectively. The M26 seasonal mean $\Psi_{md}$ were similar to M9 values and were -1.93, -1.99 and -1.92 for 2002, and -1.94, -1.98 and -1.95 MPa for 2003, for Control, PRD50 and DI50, respectively. Predawn leaf water potential ($\Psi_{pd}$) taken during 2003 for both rootstocks was only significantly different for the M26 DI50 treatment on 92 DAFB (Table 4.3), however $\Psi_{pd}$ for all treatments was similar by midday (Table 4.1). The M9 $\Psi_{pd}$ measurements on 86 and 109 DAFB were recorded 12 and 7 days following the previous irrigation event, respectively, and the M26 $\Psi_{pd}$ measurement dates of 81, 92 and 115 DAFB were 7, 6 and 14 days from the last irrigation event, respectively.

**Table 4.1.** The effects of irrigation treatments on midday leaf $\Psi$ (MPa) for 'Gale Gala' on M26 apple trees during 2002 and 2003.

| Treatment | M26 2002 DAFB | | | | | | | M26 2003 DAFB | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 79 | 86 | 96 | 101 | 117 | 121 | 162 | 92 | 101 | 110 | 115 |
| Control | -2.13 | -2.18 | -1.77 | -2.15 | -1.52 | -2.11 | -1.70 | -2.04 | -2.09 | -1.73 | -1.90 |
| PRD50 | -2.12 | -2.22 | -2.10 | -2.07 | -1.58 | -2.13 | -1.71 | -2.28 | -1.84 | -1.81 | -1.98 |
| DI50 | -2.10 | -2.14 | -1.91 | -1.91 | -1.63 | -1.95 | -1.79 | -2.05 | -1.86 | -1.90 | -1.97 |
| *Pr >F* | *0.96* | *0.75* | *0.06* | *0.23* | *0.20* | *0.15* | *0.65* | *0.50* | *0.09* | *0.25* | *0.56* |
| *LSD(α=0.05)* | *0.19* | *0.23* | *0.27* | *0.29* | *0.13* | *0.20* | *0.23* | *0.47* | *0.25* | *0.20* | *0.16* |

**Table 4.2.** The effects of irrigation treatments on midday leaf $\Psi$ (MPa) for 'Royal Gala' on M9 apple trees during 2002 and 2003.

| Treatment | M9 2002 DAFB | | | | M9 2003 DAFB | | |
|---|---|---|---|---|---|---|---|
| | 120 | 122 | 163 | | 89 | 100 | 109 |
| Control | -2.31 | -2.23 | -1.48 | a[z] | -2.17 | -1.93 | -1.50 |
| PRD50 | -2.12 | -2.14 | -1.67 | b | -2.10 | -1.75 | -1.53 |
| DI50 | -2.08 | -1.96 | -1.48 | a | -2.08 | -1.68 | -1.53 |
| *Pr >F* | *0.22* | *0.16* | *0.01* | | *0.67* | *0.18* | *0.95* |
| *LSD(α=0.05)* | *0.29* | *0.30* | *0.12* | | *0.22* | *0.27* | *0.18* |

[z] Means within columns for each date followed by different letters are significantly different by SNK at $P$=0.05 level.

**Table 4.3.** The effects of irrigation treatments on predawn leaf Ψ (MPa) for 'Gale Gala' on M26 and 'Royal Gala' on M9 apple trees during 2003.

| Treatment | M26 2003 DAFB | | | | M9 2003 DAFB | |
|---|---|---|---|---|---|---|
| | 81 | 92 | | 115 | 86 | 109 |
| Control | -0.29 | -0.26 | a[z] | -0.33 | -0.31 | -0.38 |
| PRD50 | -0.32 | -0.25 | a | -0.32 | -0.27 | -0.38 |
| DI50 | -0.29 | -0.32 | b | -0.33 | -0.33 | -0.43 |
| *Pr >F* | *0.26* | *0.01* | | *0.91* | *0.46* | *0.11* |
| *LSD(α=0.05)* | *0.036* | *0.045* | | *0.046* | *0.112* | *0.060* |

[z] Means within columns for each date followed by different letters are significantly different by SNK at $P$=0.05 level.

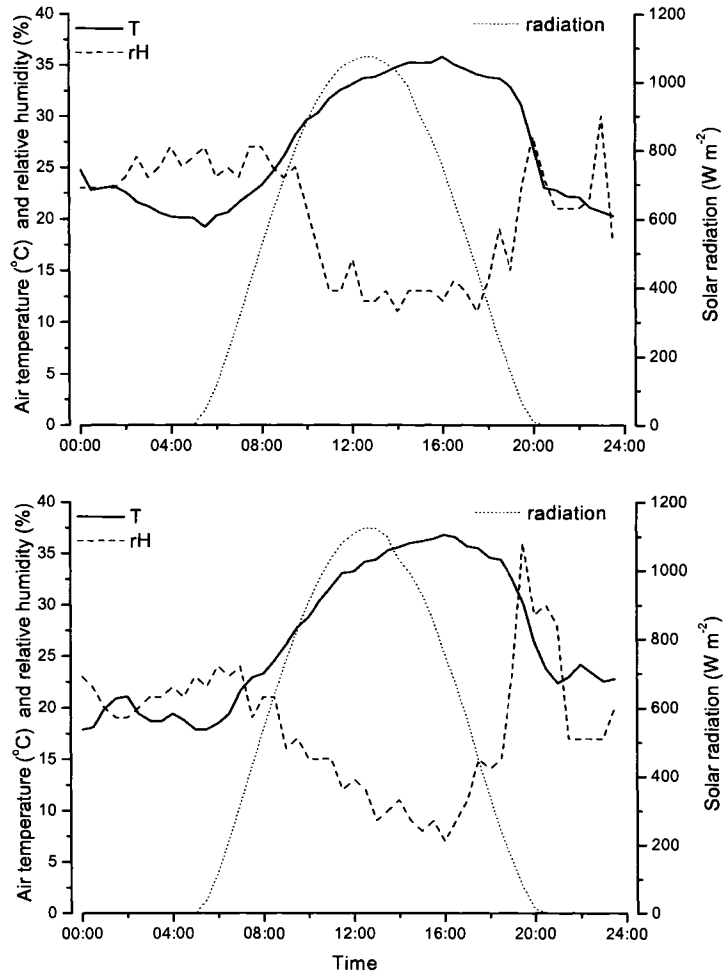


**Fig 4.1.** Diurnal trends of air temperature, relative humidity and solar radiation for 78 DAFB (top), and 81 DAFB (bottom), 2003.

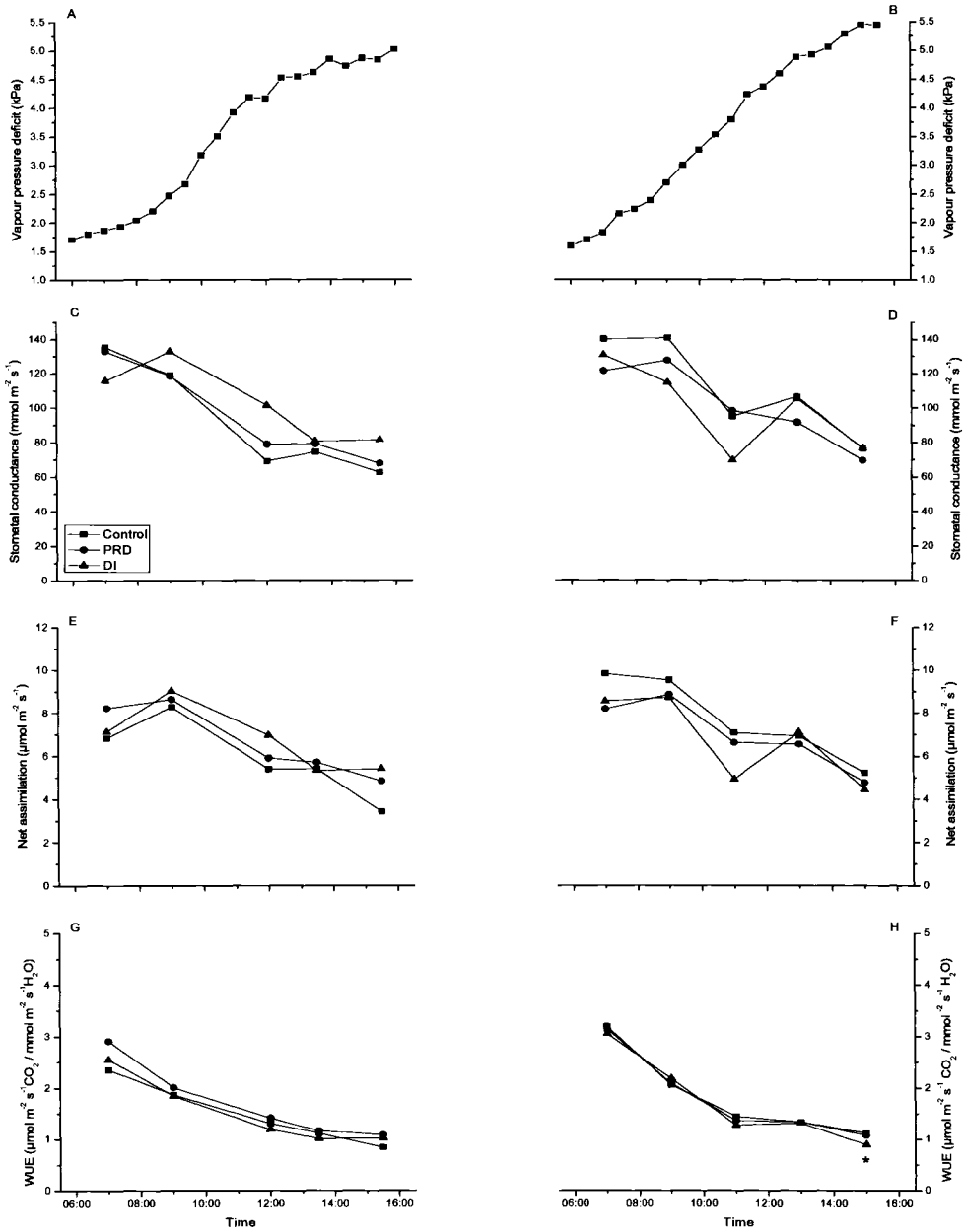


**Fig 4.2.** Vapor pressure deficit (VPD) and irrigation effects on single-leaf gas exchange ($g_s$, $A$, $WUE_{A/E}$) for 'Royal Gala' on M9 apple trees, 78 DAFB (A, C, E, G) and 'Gale Gala' on M26 apple trees, 81 DAFB (B, D, F, H), 2003. Asterisk represents significance at $P < 0.05$.

Typical midseason diurnal curves are provided for solar radiation, temperature and relative humidity (RH), for 78 DAFB and 81 DAFB, 2003 (Fig 4.1). Climatic

conditions were similar between dates with daily high temperatures and low RH occurring near 16:00 HR. The corresponding VPD and gas exchange parameters are shown for 78 DAFB and 81 DAFB in 2003 for the 'Royal Gala'/M9 and 'Gale Gala'/M26 sites, respectively (Fig 4.2A-H). Results were similar between rootstocks and showed no significant differences among treatments for $g_s$ and $A$. The instantaneous $WUE_{(A/E)}$ was significantly different on only one measurement, 15:30 HR, for the M26 site (Fig 4.2H). A clear depression in $g_s$ was apparent following the 9:00 HR measurement in all treatments and for both rootstocks. The reduction in $g_s$ was paralleled by similar declines in $A$ which were larger than that of E and subsequently resulted in a steady decline throughout the day in $WUE_{(A/E)}$ for all treatments. Diurnal measurements taken for both orchards and across several dates during 2003 yielded similar trends. The VPD is provided for both dates (Fig 4.2A,B), and similar peak values between 4-6 kPa are attained daily throughout the summer months. Seasonal midday gas exchange measurements are provided for both sites in 2002 (Fig 4.3A-D) and 2003 (Fig 4.4A-F). No consistent patterns emerged from the data. In 2002, differences in $g_s$ occurred only on 162 DAFB for the M26 site and 87 DAFB for the M9 site. In 2003, $g_s$ was only significantly different on 115 DAFB for the M26 site. Treatments responded similarly on all other measurement dates.

The 2003 $g_s$ data taken from midday and diurnal measurements throughout the season and from both rootstocks were pooled due to the lack of significant differences among treatments and plotted against VPD allowing a broad range of VPD values under natural conditions (Fig 4.5). Natural light levels for apple leaves were saturating for all data points (PAR >1000 $\mu$mol $m^{-2}$ $s^{-1}$) and the regression of VPD on $g_s$ was highly

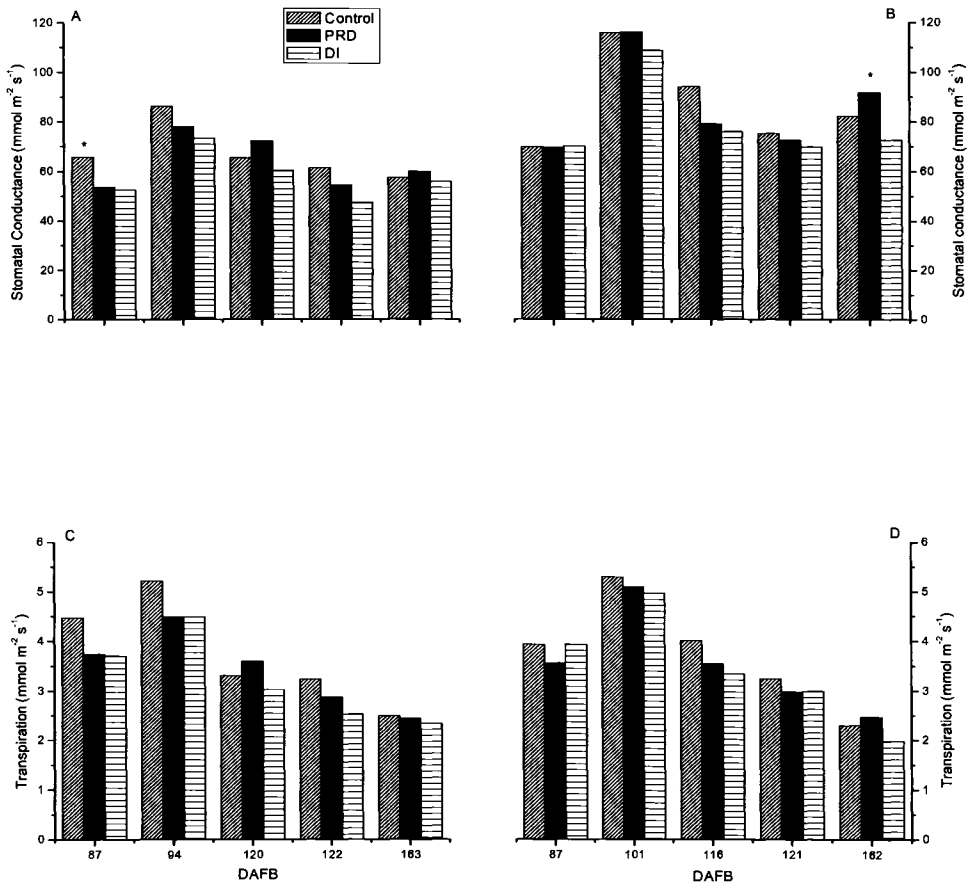


**Fig 4.3.** The effects of irrigation treatments on single-leaf gas exchange ($g_s$, E) for several dates in 2002 for 'Royal Gala' on M9 (A, C) and 'Gale Gala' on M26 (B, D) apple trees. Asterisks represent significance at $P < 0.05$.

significant ($P < 0.0001$) (Fig 4.5). The relationship for 2002 data was similar (data not shown). However, 2002 data had a distinct shift across the season in the $g_s$ response to VPD, and differences in the absolute VPD values were most likely in response to the different instrumentation used between years. The 2002 VPD-$g_s$ relationship was highly significant ($P < 0.0001$) over four separate occasions in early-mid July ($g_s$ = -27.62 VPD + 166.17 with $R^2$ = 0.86), and again for four dates taken in early-mid August ($g_s$ = -26.95 VPD + 199.23 with $R^2$ = 0.94). The VPD for a given $g_s$ shifted by approximately 1 kPa

from July to August, and the slopes for the 2002 curves were slightly higher than in 2003. There were, however, only two data points taken in August for the 2003 data collection and therefore a similar conclusion cannot be drawn.

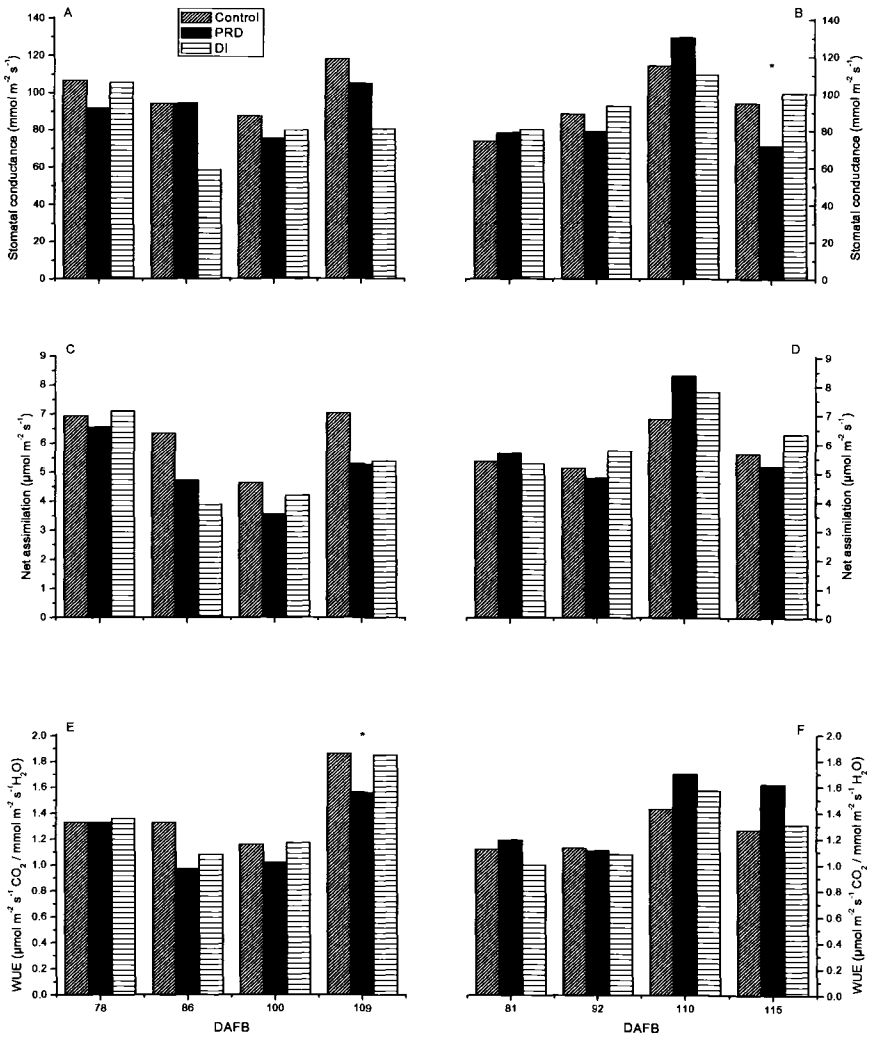


**Fig 4.4.** The effects of irrigation treatments on single-leaf gas exchange ($g_s$, $A$, $WUE_{A/E}$) for several dates in 2003 for 'Royal Gala' on M9 (A, C, E) and 'Gale Gala' on M26 (B, D, F) apple trees. Asterisks represent significance at $P < 0.05$.

The VPD effects on gas exchange can also be observed when regressing $g_s$ on E, and is shown for diurnal measurements taken on 'Royal Gala'/M9 on 56 DAFB, 2003

(Fig 4.6A). Two distinct data populations for the E/$g_s$ relationship emerged depending on time of measurement. The apparent difference observed between slopes of morning and afternoon measurements, indicate that for an equivalent $g_s$, afternoon E will be two-fold that in the morning. This is directly a function of the energy gradient, since $g_s$ is empirically derived from E, using the leaf-atmosphere gradient in vapor pressure measured in the leaf chamber. The rapid increase in both the VPD and solar radiation between early morning and solar noon leads to increased rates of E, followed by a leveling off of E with further increasing VPD beyond solar noon. The result of a broadening VPD and a concomitant, relatively stable rate of E is a reduced $g_s$. However, the linear relationship for $g_s$ on $A$ remains constant throughout the day (Fig 4.6B). In fact, the strength of the relationship for $g_s$ on $A$ existed for all data points (n= 410) collected throughout 2003 and was highly significant ($P$ <0.0001) ($A$ = 0.057 $g_s$ +0.706, $R^2$ = 0.79) (data not shown).

Bulk leaf ABA concentration was quantified in 2002 on 87 DAFB for both rootstocks (Table 4.4). Volumetric soil moisture for the PRD dry side at the time of ABA sampling was 0.18 and 0.15 $m^3$ $m^{-3}$ for M9 and M26, respectively, which equates to 37 % and 20 % AW remaining in the dry profile. The M9 had higher absolute leaf ABA concentration than M26, and the Controls exhibited lower ABA leaf concentration than deficit treatments, albeit non-significantly, in both rootstocks. The 2003 ABA concentration was not significantly different across treatments in either rootstock or sampling date (Table 4.4). However, in the M9 site, ABA concentration of DI50 was highest on both measurement dates, while in the M26 site the PRD50 treatment was observed to have the highest ABA concentration on both dates. The absolute ABA

concentration was lower on 110 DAFB for both sites. Irrigations were supplied for both rootstocks 6 and 8 days prior to the 93 DAFB and 110 DAFB sampling dates, respectively.

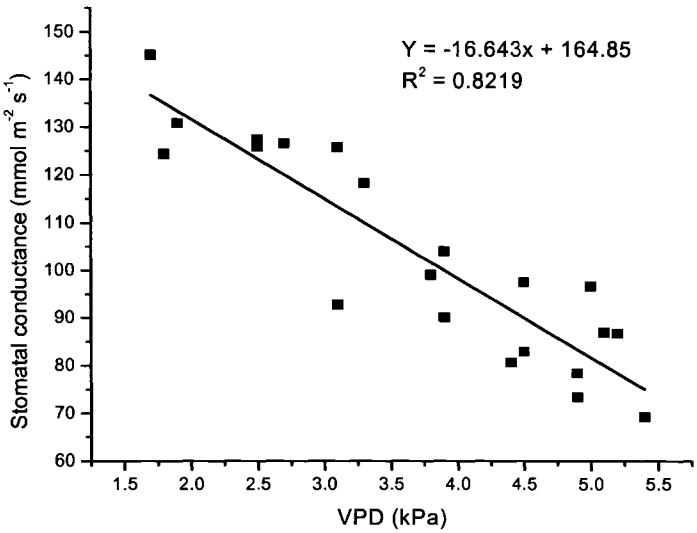


**Fig 4.5.** Effect of vapor pressure deficit (VPD) on stomatal conductance of apple leaves. Each data point is the mean of 30 $g_s$ and 2 VPD measurements collected in a 1 HR time-frame (see text). *P* <0.0001.

## 4.4 Discussion

It is well established that fruit tree $\Psi_{leaf}$ is responsive to environmental conditions (Jones et al., 1985; Lakso, 2003), and good correlations have been reported between $\Psi_{stem}$ and VPD for *Prunus domestica* L. cv 'French' (McCutchan and Shackel, 1992) and apple (Peretz et al., 1984). In the present study, $\Psi_{leaf\ (md)}$ measurements taken over 2 seasons and both rootstocks (Table 4.1 and 4.2) are not indicative of $\Psi$-induced limitations on $g_s$. We have previously shown diurnal trends for apple leaf $g_s$ and $\Psi_{leaf}$, with mid-morning depressions in $g_s$ occurring at $\Psi_{leaf}$ values of approximately -1.5 MPa (Einhorn and Caspari, 2004). Reported threshold $\Psi_{leaf}$ values on $g_s$ vary, but have been shown to occur

near -2.0 MPa (Warrit et al., 1980), -1.8 to -2.4 MPa (West and Gaff, 1976), and downwards to -3 MPa (Lakso, 1979), with differences dependent on the degree of osmotic adjustment, leaf age, and environmental factors (Lakso, 1979; Landsberg and Jones, 1981).

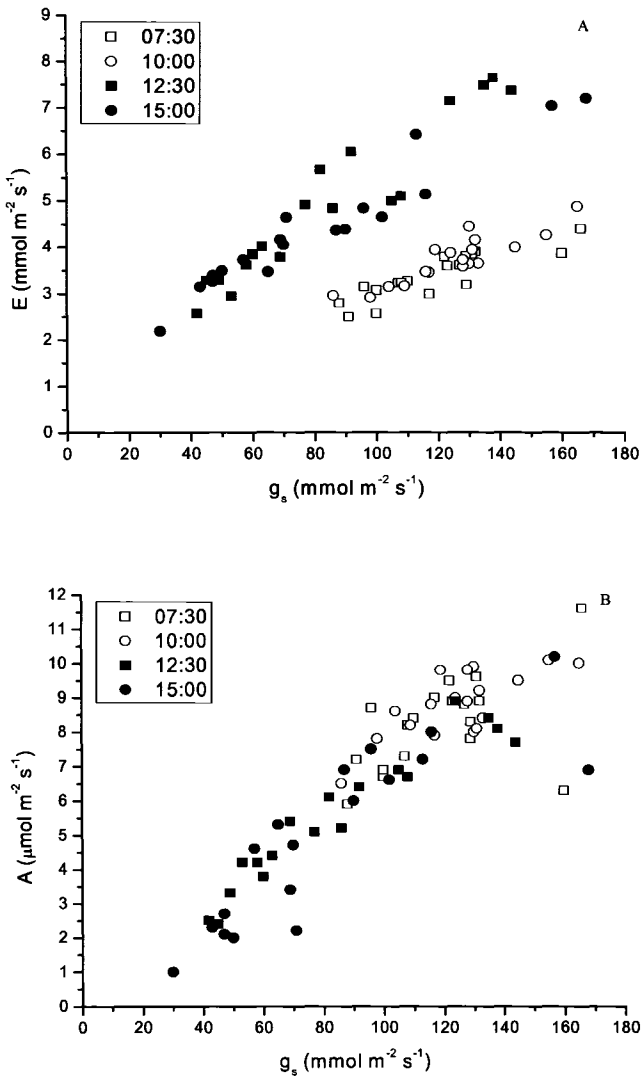


**Fig 4.6.** Diurnal relationship of transpiration (E) and stomatal conductance ($g_s$) (A), and net assimilation (*A*) and stomatal conductance ($g_s$) (B) of single leaves of 'Royal Gala' on M9 apple trees taken on 56 DAFB, 2003. Data points for treatments were pooled due to the lack of a treatment effect on the relationships.

Atkinson et al. (2000) subjected several ungrafted clonal rootstocks to water deficit and found that M9 and M26 demonstrated a stomatal insensitivity to decreasing $\Psi_{leaf}$. Our $\Psi_{leaf}$ measurements were not rigorous enough to exclude feedback inhibition on $g_s$ and it is probable that some combination of feedback and feedforward mechanisms might be occurring, as has been previously reported for apple (Landsberg and Jones, 1981). It is likely that the large resistance encountered in the apple hydraulic pathway (Lakso, 1994), and high evaporative demands of our region, allowed for adequate soil moisture reserves by limiting plant water use, as evidenced by extremely low $g_s$ and E values. This is also evident from the high (less negative) $\Psi_{leaf\ (pd)}$ values recorded on several dates in 2003 which do not indicate that PRD50 or DI50 trees ever achieved stress levels (Table 4.3). It is important to note that predawn water potential readings are reflective of the wetter portions of the soil profile (Jones, 1990). This might explain the apparent maintenance of plant water status at Control levels as reported in the PRD literature in cases where the wetted PRD profile is maintained near field capacity (FC), as is the case in the current study based on extrapolation from 2002 soil moisture data.

**Table 4.4.** The effects of irrigation treatments on leaf abscisic acid (ABA) concentration (ng $g^{-1}$DW) for 'Royal Gala' on M9 and 'Gale Gala' on M26 apple trees during 2002 and 2003.

| Rootstock | Treatment | 2002 | 2003 | |
|---|---|---|---|---|
| | | 87 DAFB | 93 DAFB | 110 DAFB |
| M9 | Control | 197 | 128 | 70 |
| | PRD50 | 245 | 131 | 69 |
| | DI50 | 231 | 145 | 87 |
| *Pr >F* | | *0.14* | *0.16* | *0.08* |
| *LSD (α=0.05)* | | *90* | *19* | *18* |
| M26 | Control | 126 | 128 | 85 |
| | PRD50 | 145 | 157 | 128 |
| | DI50 | 192 | 142 | 74 |
| *Pr >F* | | *0.35* | *0.25* | *0.13* |
| *LSD (α=0.05)* | | *105* | *35* | *56* |

The low root-length density and sparse nature of apple root systems (Atkinson, 1980; DeSilva et al., 1999) presents difficulty in effectively evaluating soil moisture in

proximity to active roots. However, withstanding the likelihood of soil moisture heterogeneity along the profile and the potential that some roots are exposed to increased levels of soil moisture not detected by our TDR probes (see 2002 data Chapter 3) provides further support for the tight control exerted by VPD on apple leaf gas exchange.

The strong correlation between VPD and apple $g_s$ (Fig 4.5) fits well within the model predictions of Dragoni et al. (2004), and demonstrates the accuracy of the model at the high end of the VPD spectrum, which is rarely reported for apple under natural conditions. Due to the high VPD in our region and general occurrence of maximal VPD values between 16:00 HR and 17:00 HR, a recovery in $g_s$ following mid-morning depression is not observed (data not shown). The lack of recovery of $g_s$ under our climatic conditions is exacerbated by the marked elevations in leaf temperature (~2-4 $^{\circ}$C over ambient temperatures) as a consequence of decreased evaporative cooling. The increasing leaf temperatures expand the gradient for leaf-air VPD beyond the point at which atmospheric VPD begins falling. This apparent 'feedforward' response of decreasing transpiration under increasing VPD has been observed to consistently occur in early-mid morning when VPD is approaching 2.5 to 3 kPa. These results are consistent with those of Yoon and Richter (1991) for apple and similar to those reported for other species (Franks et al., 1997). While experimentally controlling the VPD, Franks et al. (1997) demonstrated that declining $g_s$ during high VPD was irreversible when the VPD was then decreased, and it was suggested that imposition of high VPD sets in motion a closing reaction which cannot be interrupted by reducing the evaporative demand. It is of interest to note that under midday conditions leaves of all trees in the orchard demonstrated classic drought avoidance mechanisms by assuming vertical positions and

rolling their leaf blades, presumably to lessen the incident radiant load. This strategy appears to be successful as evident from the maintenance, and at times slight recovery, in water potential beyond solar noon, despite an increase in evaporative demand due to continued rise in both temperature and VPD. As pointed out above, the tight coupling between VPD and $g_s$ acts favorably on the maintenance of soil moisture in both deficit treatments even though these trees received approximately 50-75 mm less irrigation volume than Controls in both 2002 and 2003. The shift in stomatal sensitivity to declining VPD observed in 2002 deserves further investigation, since it suggests that apple may be able to adapt seasonally by maintaining a threshold level of $g_s$ during episodes of high VPD. Previous work with apples has demonstrated that high VPD can induce leaf stomatal responses similar to those occurring under moisture stress, even though soil moisture was maintained at FC (Flore et al., 1985). These authors found that previously stressed trees responded less sensitively than well-watered Controls to increasing VPD (a 1-2 kPa increase, with maximum values attained < 3 kPa), suggesting stomatal adaptation to VPD (Flore et al., 1985). It seems apparent that the much higher VPD range with which our field plants are exposed to will lead to large restrictions in gas exchange.

It should also be noted that for crops to benefit from PRD it would be necessary that their response curves for $CO_2$ assimilation and transpiration differ in terms of their saturation points relative to $g_s$. Due to the tight coupling already established between tree canopies and atmospheric conditions (Jarvis, 1985; Jones et al., 1985), and the high WUE displayed by non-stressed apple plants (Lakso, 2003) it is evident that WUE can not be increased under conditions which limit $g_s$ (Fig 4.2 and 4.4), at least within the range of $g_s$

observed in our climate. Furthermore, as previously discussed, the apparent doubling of E for a given level of $g_s$, results in a decrease in $WUE_{A/E}$ across the day, since A was shown to decline linearly with $g_s$ (Fig 4.2G,H and Fig 4.5AB). However, WUE expressed as $WUE_{A/gs}$, inherently incorporates the effect of VPD on stomata, and under such conditions, the $WUE_{A/gs}$ will remain relatively constant across the measurement period, and suggests that effects on A are dependent upon $g_s$, which is an indirect measure of the stomatal aperture. In fact, internal carbon ($C_i$) was not observed to have increased with declining $g_s$ (data not shown), further suggesting that reduction in *A* is primarily a function of decreased pore size, and not due to non-stomatal limitations. It is fairly common for PRD work to cite large gains in WUE, where WUE is defined as unit of yield per unit of water applied. However, WUE expressed as tons of harvested crop per ML of irrigation water applied, does not provide insight into the mechanism(s) controlling carbon gain under water deficits. The declining WUE demonstrated here would most likely be deleterious to fruit sizing under heavy croploading conditions, but did not adversely affect fruit growth in the current study due to the light croploads (see Chapter 3).

The development of PRD was based on research elucidating the relationship between stomatal regulation and apparent production of root derived signals in the presence of soil drying (Blackman and Davies, 1985; Davies and Zhang, 1991; Loveys, 1984). Gowing et al. (1990) demonstrated the occurrence of a non-hydraulic signal culminating in the reduction of growth and water use of half dried apple explants. In spite of the overwhelming implication of ABA-induced regulation of gas exchange under PRD, very few PRD reports have actually quantified the compound. In fact, we are

unaware of any field studies that have reported significant changes in ABA concentration under controlled PRD. Although the similarity of $g_s$ among treatments for both rootstocks in all years was not suggestive of an ABA-mediated PRD signal, ABA concentration was determined for both M9 and M26 trees on three different dates. The much higher LSD values reported in 2002 (Table 4.4) were due to small sample sizes (three leaves per treatment) selected for ABA analysis. We did not observe any significant treatment effects on leaf ABA concentration for either rootstock on any given sampling date, although the DI treatments often had higher ABA concentration relative to the Controls (Table 4.4). In addition, ABA values reported here are consistent with other published work from non-stressed apple leaves (Fernandez et al., 1997; Powell, 1975; Robinson and Barritt, 1990). However, in contrast to other investigations, our soil drying treatments did not lead to marked differences in ABA leaf concentrations among treatments. It is currently accepted that root derived ABA traveling in the xylem arrives at the leaf to initiate a stress response (Davies and Zhang, 1991; Sauter et al., 2001; Hartung et al., 2002). However, ABA delivery from the transpiration stream is subject to metabolism and sequestration by mesophyll cells (Trejo et al., 1993) en route to the physiological site of action located on the outer guard cell plasmalemma (Hartung, 1983). In addition, the electro-chemical status of various tissue zones can facilitate intra-leaf ABA redistribution (Wilkinson and Davies, 2002), which can affect stomatal regulation in the absence of detectable changes in whole leaf ABA content (Zhang and Outlaw, 2001; Zhang et al., 1987). It has also been demonstrated that stomata respond sensitively to xylem [ABA], reaching steady state conditions within 20 minutes while the accumulation of leaf ABA content continued for an additional 80 minutes (Jia and Zhang,

1999). These results along with others (Gowing et al., 1993) have raised concerns regarding the use of whole leaf ABA concentration as a sensitive determinant of chemical signaling. This may explain the difficulty in finding significance in the present study due to the fairly high within-treatment variability in bulk leaf ABA concentrations, as observed in other investigations (Fernandez et al., 1997), although good correlative evidence between bulk leaf ABA and stomatal response has been reported for water stressed split-rooted grapevines (Lovisolo et al., 2002; Stoll et al., 2000), drought stressed soybean (Liu et al., 2003) and soil drying of several deciduous tree species (Aasamaa et al., 2002).

Bravdo (2005) has recently proposed that drip irrigation is essentially a form of PRD due to localized irrigation events that only wet a portion of the total rhizosphere. This suggestion could potentially explain the lack of ABA treatment differences, however, if the PRD mechanism is mediated by ABA, then our data do not support this contention since all treatments had comparable ABA concentrations to well-watered one-year-old 'Gala' apple trees (Fernandez et al., 1997) and non-stressed approach-grafted 'Gala' apple trees (see Chapter 5). The latter study has quantified an increase in ABA leaf concentration relative to the degree of imposed water deficit for PRD50 and DI50 treatments in the order of five to sevenfold of those values reported herein. An alternative explanation for the potential involvement of ABA in the stomatal regulation observed in the current work might be through interaction with high VPD. Franks et al. (1997) suggested that the lack of recovery in $g_s$ under high evaporative conditions could be due to additional hormonal control over gas exchange induced by leaf synthesis of ABA. Although leaf synthesis can most likely be ruled out since it has been

demonstrated in several species that ABA biosynthesis does not begin until turgor potentials approach zero (Pierce and Raschke, 1980), others have shown that amplification of the ABA signal is dependent upon evaporation rate from the guard cells (Zhang and Outlaw, 2001). This concept is supported by work that has shown increased signal perception of the guard cells to steady-state ABA concentration under small changes in water potential (Tardieu and Davies, 1992). It would be expected that slight perturbations in epidermal water relations should be readily perceived by species which display feed-forward stomatal control, of which apple has been categorized (Farqhuar, 1978; West and Gaff, 1976). The implication is that under high evaporative demand, measurements of bulk leaf water potential and leaf ABA concentration may be incapable of detecting the actual hydraulic/chemical signaling mechanism.

In addition to an unresolved role for ABA in the regulation of gas exchange, the PRD treatment did not exhibit noticeable recoveries in $g_s$ or E during extended drying of half the root zone as previously observed in split-rooted grapevines (Dry et al., 2000a). This is most noticeable between 85 and 110 DAFB, 2002 for the M26 site when soil moisture extraction had ceased (Fig 3.1C), and is seen by the similar $g_s$ values for all treatments (Fig 4.3AB and 4.4AB). Poni et al. (2005) has also demonstrated a lack of recovery in gas exchange during a steady PRD drying cycle using container grown grapevines. It was shown that the dry compartment had reached the permanent wilting point after 3 days of water withholding and maintained this level for an additional 11 days without concomitant changes in gas exchange (Poni et al., 2005). Although a 10-14 day switch has been proposed for PRD regimes based on the previously observed transient nature of the PRD signal (Dry et al., 1996; Loveys et al., 2002), in our case,

consistent gas exchange values and the season-long drying of the M9 in 2002 and both the M9 and M26 orchards in 2003 suggests that any PRD signal might be muted in the presence of a much higher atmospheric signal.

## 4.5 Conclusion

The explorative nature of apple roots has been proposed to diminish the rate of stressing thereby granting additional time for adaptive processes (Lakso, 1979). However, under semi-arid growing climates, such as those of Colorado, environmental factors assert strong control over apple leaf gas exchange, irrespective of a wide range in soil moisture availability. Therefore a short-term root-derived water stress signal such as ABA may not have as important a role in our semi-arid climate as in a more temperate climate, or in different species. This is in contrast to the putative role of ABA, although others have demonstrated a greater influence of osmotic adjustment than ABA in the regulation of gas exchange for apple (Davies and Lakso, 1978) and apricot, *Prunus armeniaca* L., (Loveys et al., 1987) when subjected to water deficits. Therefore, PRD has no distinct advantages over DI. In addition, both deficit strategies were unable to increase WUE as a result of equally compromised $CO_2$ and $H_2O$ diffusion, although a minimal level of $A$ was maintained under extremely high VPD. Under such conditions, croploads may require downward adjustments.

# 5 An Approach-grafted Split-rooted Apple System to Test the Effects of Partial Rootzone Drying on Tree Physiology and Water Relations

## 5.1 Introduction

A growing body of literature is providing compelling evidence that roots located in drying soil have the ability to 'measure' soil moisture and relay this information to the shoots in the form of chemical signals, prior to a detectable energy drop in leaf water status (see reviews by Davies and Zhang, 1991; Dodd, 2003; Hartung et al., 2002; Sauter et al., 2001). Abscisic acid (ABA) is the chemical agent predominantly ascribed to the regulation of vegetative growth and stomatal conductance during episodes of soil drying. Gowing et al. (1990) demonstrated the effect of an unidentified 'positive' root sourced signal on growth and transpiration of half dried apple (*Malus* x *domestica* Borkh.) plants. The work by Gowing et al. (1990) was extrapolated to agricultural systems as a potential deficit irrigation strategy to control excessive vegetative vigor of grapevines, *Vitis vinifera*, and termed partial rootzone drying (PRD) (Dry et al., 1996, 2000a,b; Loveys et al., 2000). In short, early conclusions were that vigor control induced by PRD positively influenced berry quality due to increased light penetration and distribution within canopies (Dry and Loveys, 1998; Dry et al., 2001; Loveys et al., 1997; Loveys et al., 2002). Suppression of vegetative growth was often accompanied by the maintenance of fruit size, potentially as a result of hydraulic isolation of the grape berry following

veraison (Shackel et al., 1987). Although in early literature, PRD was operated at 50% of ET and compared to a well-watered Control, more recent comparisons including deficit treatments that received similar amounts of irrigation to PRD suggest that plants respond to irrigation volume and not placement when applied to apple (Caspari et al., 2004; Einhorn and Caspari, 2004; Leib et al., 2006), common bean (*Phaseolus vulgaris* L.) (Wakrim et al., 2005), potato (*Solanum tuberosum* L.) (Liu et al., 2006), grapes (Bravdo et al., 2004; Chalmers et al., 2004; Gu et al., 2004; Poni et al., 2005; Pudney and McCarthy, 2004), pear (*Pyrus communis* L.) (O'Connell and Goodwin, 2004) and tomato (*Lycopersicon esculentum* Mill.) (Zegbe-Dominguez et al., 2003).

Aside from the dynamic environmental stimuli influencing physiological plant responses, additional difficulties associated with successful implementation of PRD in the field are a function of several factors: differences among species in adaptive mechanisms employed during water deficits (Turner, 1979), soil type, depth and heterogeneity, the inability to confine and dry a known portion of the total root system, cropload effects, etc. In the case of apple, previous work using seedlings has shown that leaf ABA concentration correlated well with turgor potential, but not stomatal conductance, and although plants responded to water stress by synthesizing ABA, the higher [ABA] did not improve water use efficiency (Davies and Lakso, 1978). Because the original work of Gowing et al. (1990) demonstrated the capacity for apple to produce chemical signals, and the inability of apple field studies to show theoretical PRD responses (Caspari et al., 2004), a container experiment was designed, using two-year old split-rooted apple trees growing under ambient conditions, to investigate the effect of PRD on leaf water relations, gas exchange and ABA production.

## 5.2 Materials and Methods

The experiment was conducted in a retractable-roof greenhouse at Colorado State University's Western Colorado Research Center, in Grand Junction, Colorado, USA. One year old 'Gale Gala' apple trees grafted onto M7 rootstocks were potted into 30 L containers into a mixture of a native soil : peat mix (~50:50 by volume). The measured bulk density (Bd) of the soil mix was 0.75 g/cm$^3$. Thirty split-rooted units were formed by approach-grafting together two trees approximately 120 cm above their scion-rootstock graft unions in early summer of 2003. All split-rooted systems were allowed to callus and grow for the remainder of the year. Following bud-break in spring of 2004, one canopy was removed above the approach graft and twenty similar units were chosen for the application of treatments. At the start of the growing season, all trees were fully watered until the beginning of the experimental period. The experiment was conducted as a complete randomized design (CRD) comprised of four irrigation treatments each with five replications. Individual treatments were as follows: 1) Control (C), both containers irrigated once daily with > 100 % of their daily $ET_c$ (see below); 2) PRD100, one container irrigated once daily with > 100 % of daily $ET_c$, the other receiving no irrigation; 3) DI50, both containers irrigated once daily with 50 % of daily $ET_c$, distributed evenly between the two containers; and 4) PRD50, one container irrigated once daily with 50 % of daily $ET_c$, the other receiving no irrigation. Irrigation of PRD treatments was alternated between wet and dry root compartments when soil moisture extraction was observed to have leveled off in the dry compartment. Unless otherwise noted, trees were watered by hand once per day (at dusk) using watering cans filled with

the exact percentage of $ET_c$. The experimental period was from 18 July to 31 August, 2004 [day of year (DOY) 200-246]. On DOY 215, the DI50 was returned to full irrigation (C) and the PRD50 became a PRD100. Leaf area (LA) was calculated prior to (mass:area calculation using every $100^{th}$ leaf) and following [10 % sample (w:w) with a calibrated leaf area meter (Licor LA 3100, Lincoln, Nebraska)] the experimental period.

Soil moisture content was determined both gravimetrically and volumetrically in the morning and again at dusk. Gravimetric measurements were performed using a transportable 4 m high wooden tripod utilizing three S-type load cells (34 kg load capacity per cell with an accuracy ± 0.01% of applied load) and wired to a digital display for manual recording. Tripod height was designed to limit interference with canopies while centered over units during weighing. Plywood sheets (0.6 $m^2$) were fabricated with circumferential cutouts, and were attached to container rims in order to provide support to split-rooted units during weighing. Eyebolts were attached to the platform at equidistant points to facilitate lifting via a ratcheting hand crank. Semi-transparent polyethylene covers were placed in containers to reduce evaporative loss from the soil. Volumetric measurements were performed using time-domain reflectometry (TDR) (Tektronix Model 1502C, Beaverton, Oregon). Stainless steel wave-guides, 6 mm in diameter, 300 mm in length, were inserted into each container of a split-rooted unit for all replicates. A mixing ratio was calculated using the measured Bd of the soil mix along with known Bd's for media components to generate a calibration curve, based on the equations of Topp et al. (1980). Several days of TDR data were regressed against gravimetric measurements in order to verify the calibration, and the line equation describing the relationship was TDR (L) =1.028 weight (kg), $R^2$ of 0.85 ($P< 0.0001$), (n= 167).

Daily water use ($ET_c$) was calculated from the difference between morning and evening gravimetric and volumetric soil moisture measurements. Changes in soil water between morning and evening readings were considered to be entirely due to tree water use, since trees were irrigated at dusk and drainage from C and PRD containers (receiving > 100 % of $ET_c$ on two or one container) ceased over night, container surfaces were covered and shoot growth had ceased prior to the start of the experiment. The $ET_c$ of C trees determined gravimetrically was used to calculate irrigation volumes.

Water use was also calculated from measurements of sap flow using the T-max heat pulse method (Cohen et al., 1981; Green et al., 2003). Miniature stem flow sensors were installed into parallel holes drilled radially into the stems and root shanks of two replications of C, PRD100 and PRD50 trees. Three sets of heat pulse probes, each comprising of a line heater and two temperature sensors (Tranzflow, Palmerston North, New Zealand), were inserted into each root shank (below the approach-graft union but above the original rootstock/scion graft) and in the stem above the approach-graft union. Sap velocity was measured at two radial depths (5 and 10 mm) in the stem and one depth (5 mm) in the root shanks. A 0.7 second heat pulse was applied every 30 minutes and data were collected via a data logger (Campbell Scientific, Logan, UT). Heat pulse velocity was converted to sapflow based on trunk cross-sectional area (TCA). Correction factors for wounding were derived according to Green et al. (2003).

An additional three heat pulse probes were installed into the stems of three single-stem apple trees. These single-stem trees were the same as the trees described above, except for the approach-graft. Single-stem trees were used to compare water use estimated by the heat pulse technique to actual rates of transpiration measured

gravimetrically. One tree was placed onto a 32-kg scale (1 g resolution; Model SB 32000, Mettler Toledo, Greifensee, Switzerland) and weighed using the dynamic weighing function every 30 seconds. The scale was connected to a laptop computer and each measurement was immediately transferred into an Excel spreadsheet. With the exception of a few minutes in the morning, when the scale was used to weigh all three single-stem trees, and a period of about 90 minutes in the evening, when trees were weighed again and then irrigated, any given tree remained on the scale for 24 hours. The larger interval in the evening allowed for the bulk of excess irrigation water to drain as the single-stem trees received >100 % of $ET_c$ before returning the tree back onto the scale. The tree on the scale was changed once a day following the morning weighing.

Gas exchange parameters [$A$ = photosynthesis (μmol $m^{-2}$ $s^{-1}$); $g_s$ = stomatal conductance (mmol $m^{-2}$ $s^{-1}$); E = transpiration (mmol $m^{-2}$ $s^{-1}$); $WUE_{A/E}$ = water use efficiency] were measured with a PP systems Ciras-2 gas analyzer (PP systems, Amherst, MA) on three replications (two leaves per replicate). Measurements were taken on the first fully mature sunlit leaves of extension shoots, between 12:00-14:00 HR on days with fairly similar climatic conditions. Diurnal measurements were also conducted on various days throughout the experimental period. All measurements were made under natural saturating PAR for apple leaves (> 1000 μmol $m^{-2}$ $s^{-1}$), unless otherwise stated.

Predawn leaf water potential ($\Psi_{pd}$) measurements were taken on three replications (two leaves per replicate) using a pressure chamber (model 600, PMS, Corvallis, OR) according to the Scholander method (Scholander et al., 1965).

ABA extraction and purification procedures necessary for comparable results between GC and ELISA have been previously reported for apple tissue (Soejima et al.,

1990), and have been slightly modified herein. Briefly, leaf tissue was collected from three replications (two leaves per replicate) and immediately dipped in liquid N and held at -80 °C for several weeks prior to lyophilization. Following freeze drying, tissues were ground and sieved through a 150 μm mesh screen. Extraction ratio was 10 mg tissue/ 1 ml 80 % cold methanol (80 % MEOH : 20 % DI $H_2O$). Samples were rotated for 24 hours in the dark at 4 °C, removed and centrifuged at 6000 g for 10 min at 4 °C. Supernatant was vacuum evaporated to remove MEOH. The water fraction was filtered using a 0.2 micron nylon filter and added to polyvinyl-polypyrrolidone (PVPP) at a ratio of 20 mg PVPP : 100 mg tissue in order to remove phenolic compounds. The pH was adjusted to 8.3 and centrifuged at 6000 g for 10 min at 4 °C. Supernatant was collected, readjusted to pH 8.3 and partitioned with an equal volume of ethyl acetate to remove neutral and basic organic-soluble compounds. The organic fraction was collected and set aside. The lower water phase was adjusted to pH 2.5 and partitioned three additional times against equal volumes of ethyl acetate. Partitioning against acid conditions brings ABA into the organic layer (ethyl acetate). All organic phases were combined for analysis of the physiologically active form of ABA (2-cis' S-ABA). One ml of the ethyl acetate fraction was vacufuged and reconstituted in 1 ml TBS buffer. The above procedures enabled the use of very low quantities of leaf tissue (150 mg), and all separatory stages were performed in 5 cc syringes. Efforts were made to work rapidly, under dimly lit conditions due to photo-degradation of ABA.

Quantification of the physiologically active, 'free' ABA (2-*cis*-(S)-ABA) was by a competitive ELISA assay (Agdia, Elkhart, IN), using a spectrophotometer (Spectramax 384 Plus, Molecular Devices Corp., Sunnyvale, CA) equipped with a 96-well micro-titre

plate reader. Procedures are outlined by Agdia (Agdia Inc., Elkhart, Indiana). Internal standards (2-*cis*-(S)-ABA) (Sigma-Aldrich, St. Louis, Missouri) were added to sample tissue prior to the extraction protocol in order to calculate recovery rates. Recovery rates were always higher than 75 %. Standards and samples were run in duplicate and were re-assayed if their coefficient of variability (cv) was > 0.10. Standard curves were prepared fresh for each plate. Log-logit transformations of standard curves were performed and sample optical densities (OD) always fell within the linear range of the standard curves. The $R^2$ values for standard curves of individual plates were between 0.980 and 0.998.

Meteorological data were collected by an official weather station of the United States National Weather Service located 50 meters from the retractable roof greenhouse.

Statistical analysis was performed using the SAS system software (SAS Institute, Cary, N.C.). Treatment means were compared using ANOVA (PROC GLM) and significance was tested at $P \leq 0.05$. Mean separation was determined by Fisher's Protected LSD. PROC REG was used for regression analysis.

## 5.3 Results

Soil moisture in the PRD100 'wet' root compartment was maintained at similar levels to C from DOY 201-215, while the PRD100 non-irrigated compartment was progressively depleted to a minimum total water content of 3.5 L, following 7 days of drying (Fig 5.1A). Irrigation was then alternated between sides. Subsequent switches were conducted when approximately 3.5 L of total soil water remained in the 'dry' profile, which constituted drying periods of 7 and 11 days following the first and second switch,

respectively. The 'wet' compartment of the PRD100 treatment only recovered to 70 % of C levels between the second and third switch (DOY 215-226). The mean soil moisture content of DI50 declined to 3.6 L on DOY 215, after which it was returned to a C treatment (Fig 5.1B). The ensuing daily application of >100 % $ET_c$ to DI50 required an additional 7 days for soil moisture to recover to C levels. The PRD50 'dry' compartment leveled off at 3.5 L on DOY 208, similar to the PRD100 'dry' compartment on that date. The 'wet' portion of the PRD50, however, steadily declined over the first deficit cycle reaching 35 % of the soil moisture content of C on DOY 208. Mean soil moisture content of both containers of the PRD50 treatment remained approximately 15 % higher than DI50 throughout the eight day drying cycle. A similar trend continued throughout the second deficit cycle, although by DOY 215, mean soil moisture content was similar for both treatments. On DOY 215, the PRD50 was returned to a PRD100, and the wet compartment reached and sustained C soil moisture levels by DOY 219.

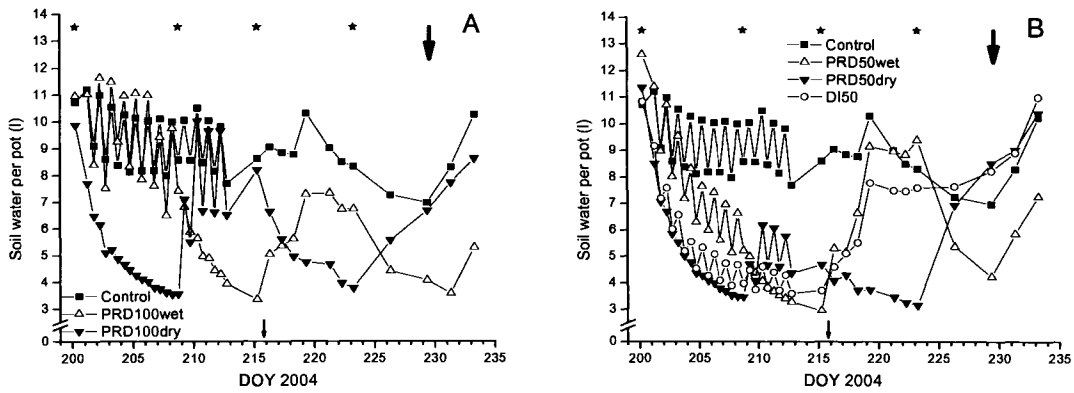


**Fig 5.1.** The effects of irrigation treatments on soil water content for individual containers of split-rooted, two-year-old 'Gale Gala' apple trees; C and PRD100 (A), and DI50 and PRD50 (B). Asterisks signify switches in irrigation between PRD 'wet' and 'dry' compartments. Bold arrow at top indicates termination of treatment period and small arrow below indicates the change of PRD50 and DI50 to PRD100 and C levels, respectively.

Prior to the start of the experiment, the soil water content in all containers was brought up to pot capacity. When both containers were near pot capacity, relative root sap flow between compartments of all trees was dependent on trunk cross-sectional area (TCA), as is shown for one of the Control trees in Fig 5.2A and is also evident for the days immediately preceding and following the initiation of the PRD regime (see DOY 198-202, Fig 5.2B). This dependency of sap flow on TCA confirmed that the approach-graft union did not alter hydraulic properties of the split-rooted units. Root activity of individual compartments of PRD split-rooted units responded rapidly to wetting events by increasing sap flow from the irrigated side while reducing sap flow from the drying side (Fig 5.2B). The rapid shift in root water uptake of the previously dry root compartment is also evidenced by the marked increase in sap flow within hours of an irrigation event, equaling that of the previously irrigated compartment within 24 hours (Fig 5.3).

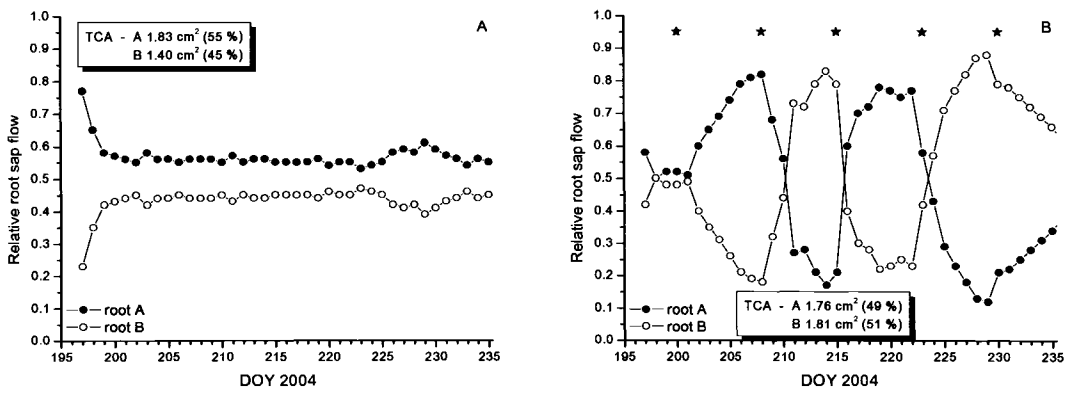


**Fig 5.2.** Effect of irrigation treatment and the trunk cross-sectional area (TCA) of individual root shanks (insets) on relative root sap flow arising from two root systems of a two-year-old apple split-rooted, well-watered Control (A), and PRD50 (B). Asterisks signify switches in irrigation between PRD 'wet' and 'dry' compartments (B).

The $\Psi_{pd}$ of PRD100 was not significantly different than C throughout the entire experimental period, although PRD100 values were slightly lower than C on several

occasions (Fig 5.4A). Both deficit treatments had lower $\Psi_{pd}$ than C and PRD100 starting on the fifth day into the drying cycle (DOY 206), but were not significant until DOY 209 (Fig 5.4A). A slight recovery in the water status of PRD50 was observed several days following the alternation in irrigation on DOY 208, and both deficit treatments had higher $\Psi_{pd}$ following the change of PRD50 and DI50 on DOY 215 to PRD100 and C levels, respectively. The observed shifts in the $\Psi_{pd}$ values of the well-watered C are a direct function of fluctuating atmospheric demands throughout the experimental period.

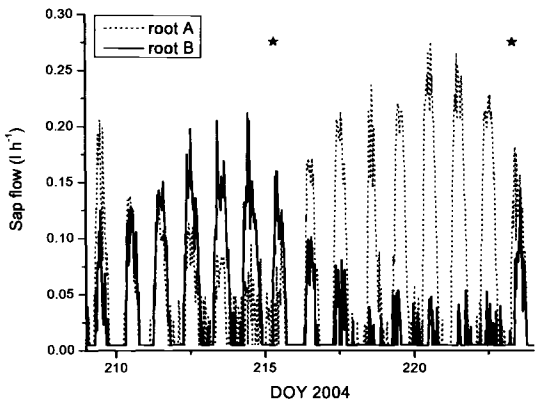


**Fig 5.3.** Effect of an irrigation switch between wet and dry root compartments on daily sap flow of individual root systems of a split-rooted two-year-old PRD100 apple tree as measured by the T-max method. Asterisks signify switches in irrigation between PRD 'wet' and 'dry' compartments.

Midday $g_s$, E, and *A* of PRD100, PRD50 and DI50 trees were progressively reduced during the first 10 days of the experimental period, relative to C (Fig 5.4B-D). The percent reduction in $g_s$ relative to C across all measurement dates was 30 %, 45 % and 54 % for the PRD100, PRD50 and DI50, respectively (Fig 5.4B). Similar reductions were observed for E (Fig 5.4C) and *A* (Fig 5.4D), although PRD50 and DI50 were severely water stressed between DOY 209-212 (Fig 5.4A), and as a consequence, respiration equaled and at times exceeded assimilation during solar noon measurements (Fig 5.4D). Instantaneous $WUE_{A/E}$ was decreased in all treatments relative to C, with the

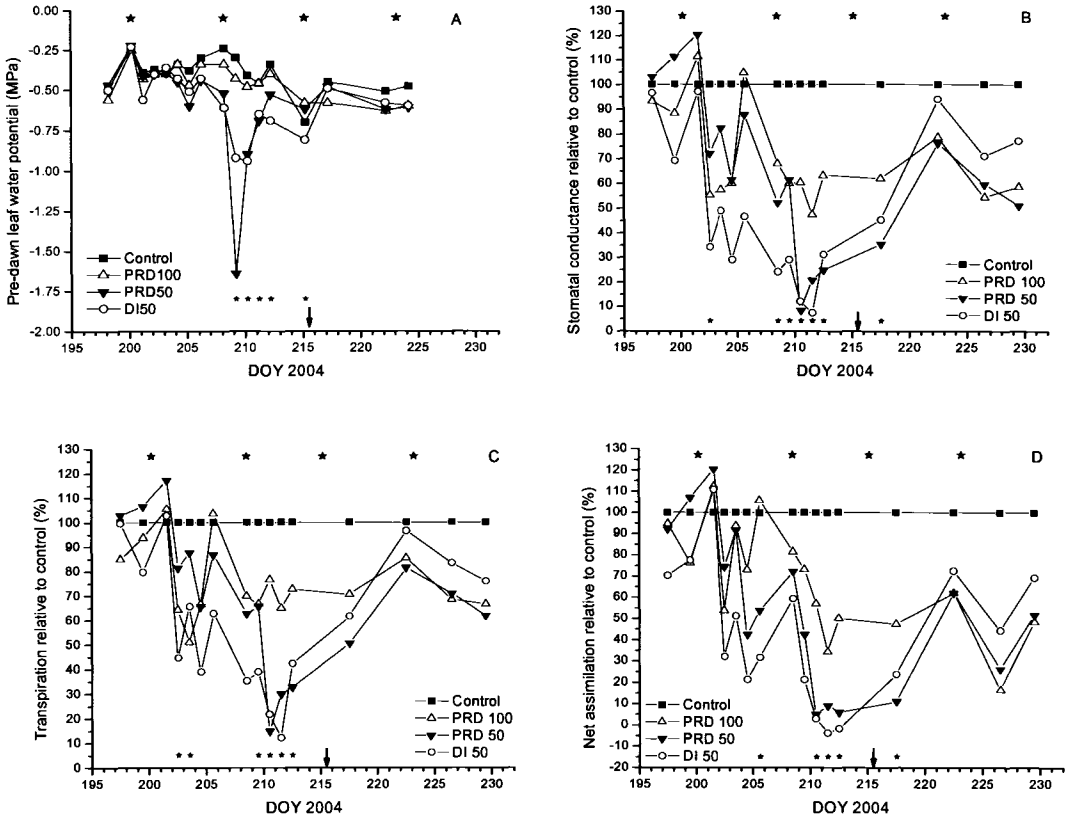


**Fig 5.4.** The effects of irrigation treatments on predawn leaf water potential and single-leaf gas exchange of split-rooted, two-year-old containerized 'Gale Gala' apple trees measured at solar noon. Water potential (A), stomatal conductance (B), transpiration (C) and net assimilation (D) are expressed as percent of C. Arrow indicates the change of PRD50 and DI50 to PRD100 and C levels, respectively. Asterisks on top signify switches in irrigation between PRD 'wet' and 'dry' compartments. Asterisks on bottom represent significance at $P < 0.05$. Each point is the mean of 6 leaves.

level of reduction dependent on treatment (data not shown). Gas exchange of PRD50 recovered to PRD100 levels within one week from its change to a PRD100 regime however, DI50 never reached C values for $g_s$ or $A$ following its return to full irrigation. The marked depression in $A$ observed in all treatments relative to Control on DOY 226 was most likely in response to temperatures exceeding 40 °C during solar noon, which is well beyond the photosynthetic temperature maxima for apple leaves (Palmer et al., 2003). Compared to C, DI50 trees experienced a much greater depression in $A$ despite

possessing equivalent soil moisture status as C, presumably due to a lag time in recovery following stress.

Diurnal curves are presented for DOY 204 and DOY 210 (three and nine days into the experimental period) for $g_s$, $A$, and $WUE_{A/E}$ (Fig 5.5A-F). Irrespective of soil moisture status (Fig 5.1) all treatments experienced declining values for gas exchange parameters throughout both diurnal periods, although the magnitude of the depression varied with treatments. On DOY 204, the average daily $g_s$ for PRD100, PRD50 and DI50 expressed as a percentage of C was 79 %, 68 % and 55 %, respectively. The $g_s$ values of both PRD50 and DI50 were significantly different than C for the mid-morning measurement. Solar noon ($P$= 0.07) and late afternoon ($P$= 0.06) measurements were not significant (Fig 5.5A). Although DI50 values were lower than PRD50, the differences were not significant. The diurnal trends of $A$ for all treatments behaved similarly to $g_s$, although values for PRD100, PRD50, and DI50 were not significantly different to C (Fig 5.5C). In contrast, E remained fairly constant until solar noon, after which it decreased to a greater extent than $A$ for the remainder of the day. Consequently, $WUE_{A/E}$ decreased after the first measurement in the morning with a slight recovery in the latter part of the day, irrespective of treatment (Fig 5.5E). Both deficit treatments had lower $WUE_{A/E}$ throughout the day with significant differences only occurring at solar noon. Substantial soil water deficits had accrued by DOY 209 (Fig 5.1), resulting in marked and significant reductions of $g_s$ for deficit treatments (Fig 5.5B). Although DOY 209 was one day after the irrigation switch between 'wet' and 'dry' compartments, the $g_s$ of PRD100 was approximately 77 % of C, as similarly observed on DOY 204. The average daily $g_s$ for the PRD50 and DI50 were 26 % and 28 % of C, respectively. The diurnal trend in

photosynthesis was similar to that of $g_s$ during the morning, however all treatments experienced very low *A* values at solar noon as a direct result of cloud cover and subsequent reductions in PAR (from 1600 $\mu$mol $m^{-2}$ $s^{-1}$ to 200 $\mu$mol $m^{-2}$ $s^{-1}$) (Fig 5.5D). However, the very low *A* of the PRD50 and DI50 during early-mid morning increased by mid-late day, and as a result $WUE_{A/E}$ increased for these treatments in the afternoon (Fig 5.5F).

Bulk leaf ABA concentration (ng $g^{-1}$ DW) for PRD100 and C was not significantly different on several dates throughout the experimental period although PRD100 generally had higher values than C (Table 5.1). In contrast, ABA concentrations of deficit treatments increased sharply by DOY 204 and remained significantly elevated over C until DOY 222, which was one week following the return of these treatments to their > 100 % $ET_c$ counterparts (Table 5.1). The absolute ABA values varied substantially across the measurement period, however, the effects of soil drying remained fairly consistent when values for both PRD and the DI regime are expressed as percent of C (Table 5.1).

The relationship between ABA concentration and soil moisture content in the wet container across all measurements was poor (Fig 5.6A). However, the same relationship was highly significant ($P < 0.0001$) during the first eight days of the drying cycle (Fig 5.6B). Similarly, there was no clear relationship between ABA and $g_s$ when using all data points throughout the entire experimental period (Fig 5.7A), but an improvement in the relationship was observed when plotting the data for the first eight days of soil drying (Fig 5.7B,C).

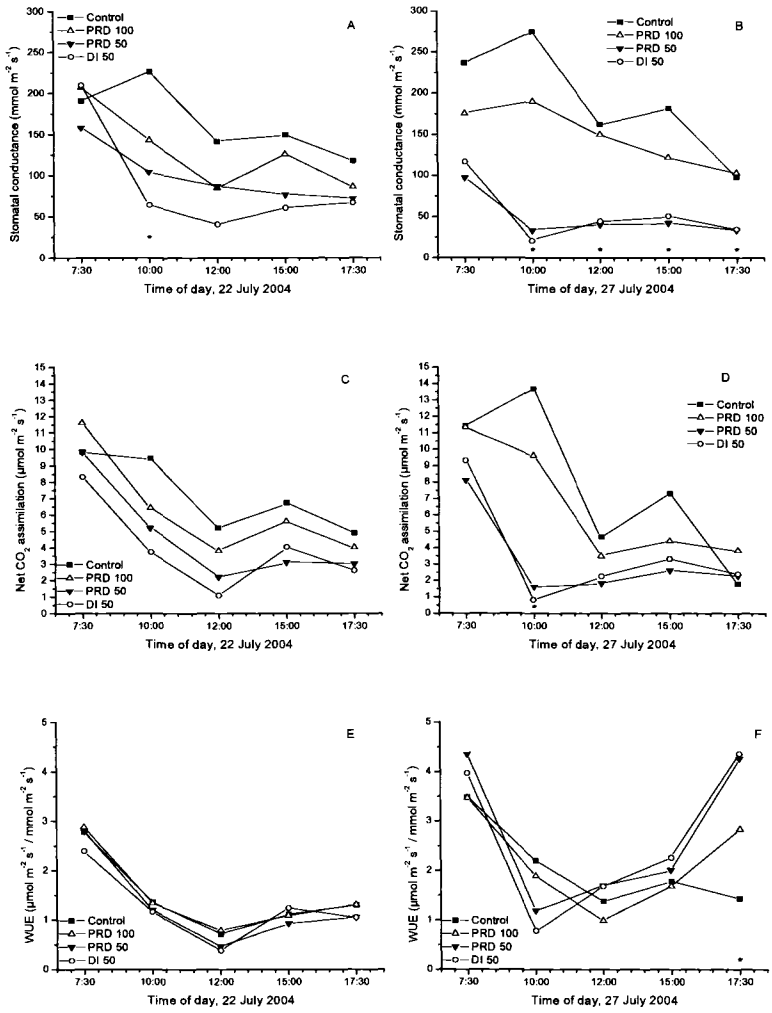


**Fig 5.5.** The effects of irrigation treatments on single-leaf gas exchange of split-rooted, two-year-old containerized 'Gale Gala' apple trees, on DOY 204 (A, C, E), and DOY 209 (B, D, F), 2004. Stomatal conductance (A, B), net assimilation (C, D) and WUE (E, F). Asterisks on bottom represent significance at $P < 0.05$. Each point is the mean of 6 leaves.

**Table 5.1.** The effects of irrigation treatments on leaf ABA concentration (ng $g^{-1}$DW) of split-rooted, two-year-old containerized 'Gale Gala' apple trees. Leaves were collected directly following measurements of gas exchange at solar noon.

| | DOY | | | | | |
|---|---|---|---|---|---|---|
| Treatment | 201 | 204 | 208 | 212 | 217 | 222 |
| C | 97[x] | 107 b[z] | 282 b | 73 b | 58 | 106 |
| PRD100 | 110 (114)[y] | 157 b (147) | 296 b (105) | 88 b (120) | 76 (132) | 92 (87) |
| PRD50 | 107 (110) | 261 ab (244) | 438 ab (155) | 221 a (302) | 100 (173) | 93 (87) |
| DI50 | 108 (111) | 421 a (395) | 774 a (274) | 139 b (190) | 82 (141) | 93 (87) |

[x] Values are means from six leaves (two leaves each from three replications).

[y] Values in parenthesis are treatments expressed as percent of C

[z] Means within columns for each date followed by different letters are significantly different at $P < 0.05$.

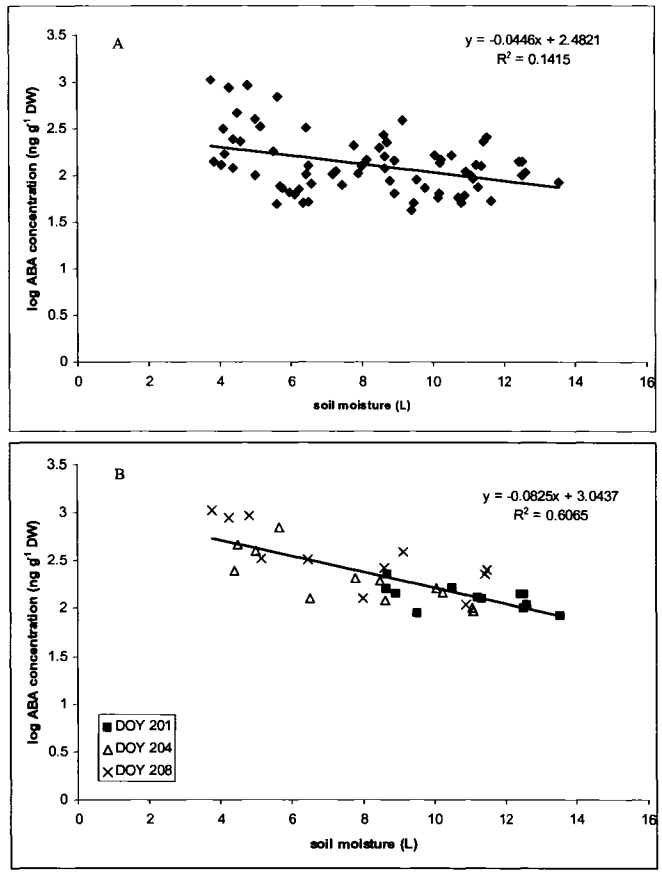


**Fig 5.6** The relationship between soil moisture content of the 'wet' container of split-rooted apple trees and abscisic acid (ABA) concentration in leaves throughout a 21-day experimental period (A), and for day 1, 3 and 7 of the first drying cycle (B). Each data point represents one split-rooted unit and is the mean of 2 leaves for ABA concentration.

## 5.4 Discussion

Stomatal conductance of PRD100 trees was consistently 30 % lower than C throughout the experimental period despite receiving >100 % $ET_c$ (Fig 5.4B). The maintenance of leaf water status (Fig 5.4A) while fully drying one half of the root zone, is indicative of root to shoot communication (Davies and Zhang, 1991). Although the leaf ABA concentration of PRD100 increased to 147 % of C levels on DOY 204 (Table 5.1), it was

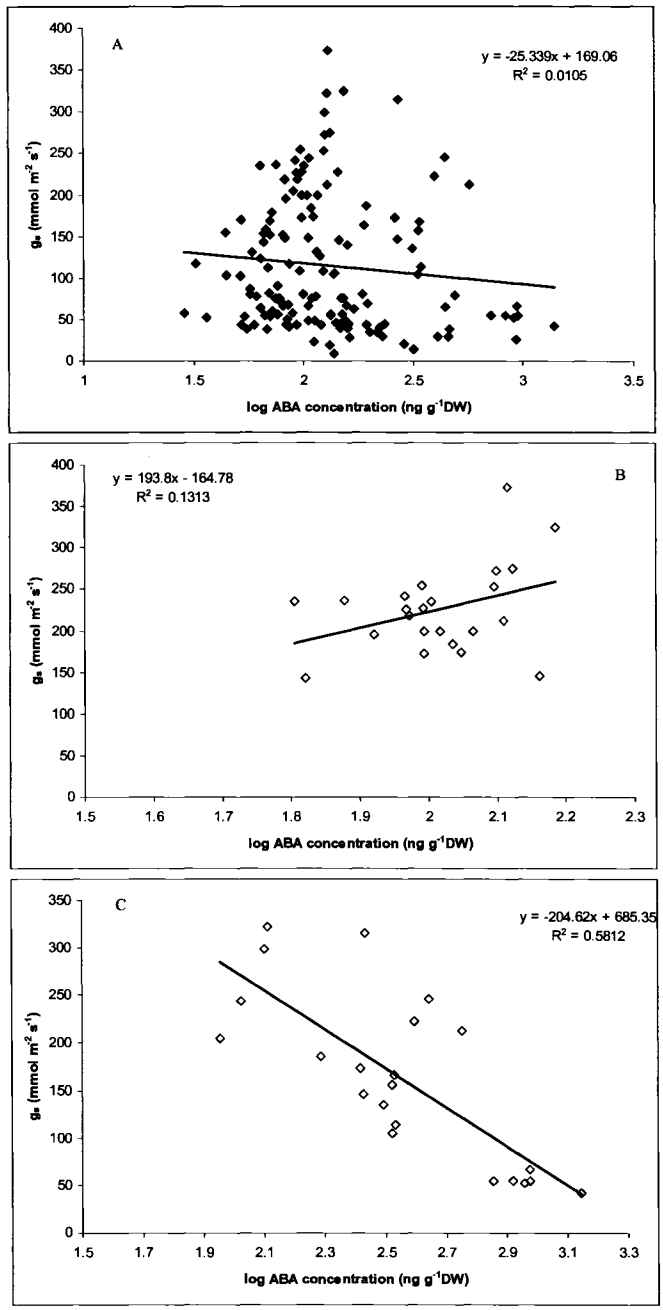


**Fig 5.7.** The relationship between abscisic acid (ABA) concentration in leaves and stomatal conductance of split-rooted, two-year-old apple trees throughout a 21-day experimental period (A), on day 1 (DOY 201) (B) and day 7 (DOY 208) (C) of the first drying cycle. Each data point represents one leaf.

only slightly higher than C on all but the last sampling date, when it was in fact lower than C, and thus there was only a weak relationship between [ABA] and $g_s$ (data not shown). Others have shown similarly poor relationships between whole leaf ABA concentration and $g_s$ (Jia and Zhang, 1999; Zhang and Outlaw, 2001). Jia and Zhang

(1999) demonstrated that the prevailing xylem sap ABA concentration, and not the ABA accumulated in the leaf, is more sensitively related to $g_s$. Zhang and Outlaw (2001) showed that intra-leaf redistribution of ABA to the guard cell apoplast controlled stomatal aperture in the absence of detectable changes in either whole leaf or apoplastic ABA concentrations (Zhang and Outlaw, 2001). In addition, an alkalization of the apoplast has been observed for several plant species either subjected to drought or provided with artificial xylem sap (Gollan et al., 1992; Wilkinson et al., 1998), allowing for both an extended presence of 'free' ABA in the apoplasm, and release of conjugated and sequestered symplastic pools of ABA (Hartung and Radin, 1989; Hartung et al., 1998; Wilkinson and Davies, 1997). Sobeih et al. (2004) observed no difference in xylem ABA between PRD and well-watered tomato genotypes. The authors did, however, observe a decline in $g_s$ when the soil water content of the dry side of PRD decreased by 45 %. This decline in $g_s$ coincided with an increase in xylem pH, and the authors suggested that an increase in xylem sap ABA was not required to alter $g_s$, but that xylem pH interacts with ABA in the control of $g_s$. Likewise, pH changes in the epidermal tissue due to increases in vapor pressure deficit (VPD) have been shown to account for increased penetration of ABA to the guard cells, resulting in depressed rates of $g_s$ with concomitantly slight and/or no alterations in plant water potential (Davies et al., 2002; Tardieu and Davies, 1992). Using the pressure probe technique, Shackel et al. (1987) have shown small gradients in the energy status of water across the leaf surface, which potentially could lead to differences in ABA responsiveness across the leaf. Indeed, stomatal patchiness has been demonstrated (Düring and Stoll, 1996a,b).

The VPD during the experimental period in the present study often reached 5-6 kPa by late afternoon, and we have previously shown the tight control of VPD on $g_s$ for cropping apple trees under field conditions (see Chapter 4). The effects of VPD in the present study can be indirectly observed through the strong diurnal depression in $g_s$ for C trees (Fig 5.5AB). Leaf water potential was not measured at solar noon, although based on the slightly lowered $\Psi_{pd}$ of the PRD100 compared to C, it is possible that $\Psi$ was further reduced by midday, although the reduced gas exchange of the PRD100 plants may have maintained a favorable leaf water status. The fact that previous work has shown an increase in guard cell sensitivity to ABA under small perturbations in water potential (Behl and Hartung, 1984; Tardieu and Davies, 1993), suggests that a potentially minimal shift in hydraulic status may have amplified the response of PRD100 trees to an ABA signal. All of the above are suggestive of the potential role of ABA in guard cell regulation despite non-fluctuating ABA leaf concentrations, and it is therefore not surprising that, at best, only 58 % of the differences in $g_s$ could be ascribed to ABA leaf concentration (Fig 5.7C). Still others have observed good relationships between leaf ABA concentration and $g_s$ (Aasamaa et al., 2002; Lovisolo et al., 2002; Stoll et al., 2000).

Although the identity of the signal remains elusive, others have found similar reductions in gas exchange using PRD100 compared to well watered Controls in tomato (Mingo et al., 2004), grapes (Poni et al., 2005), and olive (Wahbi et al., 2005). A rather simple explanation for the discord in gas exchange between the two > 100 % $ET_c$ treatments in the present study is that reduced $g_s$ is merely proportional to the percentage of roots inhabiting dry soil. In fact, the total soil water of PRD100 pots for the period shown in Figure 5.4 was 71 % of C. This 29 % reduction in total soil water is roughly

equal to the 30 % reduction in gas exchange at solar noon throughout this period (Fig 5.4B). Because apple have been shown to possess high WUE under non-stressed conditions (Lakso, 2003), they would not be expected to assume the role of a 'water spender' when subjected to water deficits. Accordingly, the already established high hydraulic resistance of apple roots (Atkinson, 1980) would increase for roots adjacent to drying soil, thereby limiting flow to the canopy. As discussed above, the increased hydraulic resistance in the pathway may not have translated to a reduction in $\Psi$ since water use was proportionally restricted. Despite the capacity of apple roots to rapidly alter their activity in response to wetting events (Fig 5.2 and 5.3; Green et al., 1997), absolute sap flow rates of PRD100 root compartments never exceeded C levels when maximum flow was recorded, in either root compartment, regardless of previous conditions (data not shown). This suggests that wet roots of PRD100 did not compensate for the reduction in total soil water by operating at supra-optimal rates of hydraulic conductance.

Results for PRD50 and DI50, however, were quite different. The large increases observed in ABA concentration for both of these treatments, and particularly for DI50, may have been the result of leaf ABA synthesis. Soar et al. (2004) have recently shown that leaves of grapevines began synthesizing ABA when their relative water content fell to 85 %. Stoll et al. (2000) observed a five-fold difference in leaf ABA concentration between DI and PRD in grape, and this difference was related to the reduced leaf water status of the DI compared to the PRD treatment. In our case, both deficit treatments had similarly reduced $\Psi_{pd}$, with the exception of a few observations (Fig 5.4A). Others, however, have shown that leaf synthesis does not occur until turgor approaches zero

(Pierce and Raschke, 1980). The fact that Ψ of PRD50 plants was compromised runs counter to PRD objectives, since ideally $g_s$ should adjust to reduced soil moisture and thereby prevent a decline of plant water status. In fact, under conditions of mild stress after only 3 days of pot drying (DOY 204), the ABA concentration of PRD50 was 244 % while $g_s$ was reduced to 68 % of C, however $\Psi_{pd}$ was sustained at C levels. This suggests that on DOY 204, PRD50 was operating according to PRD theory, and it is possible that the elevated ABA concentration in leaves on this date was root derived since $\Psi_{pd}$ was not lowered. In contrast, on DOY 209, one day following the first switch for PRD regimes, $\Psi_{pd}$ was significantly lower than C, and gs was reduced by 74 % (Fig 5.4A,B).

The differences observed in ABA concentration between PRD50 and PRD100 early in the experimental period (DOY 204 and DOY 208) may have been partly attributable to dilution effects. Loveys et al. (2000) have shown a fivefold increase in ABA concentration of roots in drying soil following three to five days of soil water depletion in PRD grapevines. However, these concentrations returned to those of well-watered grapevine roots on day six, even though soil moisture remained depleted in the dry containers. Similar results have also been reported for maize roots under field drying conditions (Davies and Zhang, 1989). Because soil moisture depletion in the dry containers was nearly identical for PRD50 and PRD100 over the first nine days of the experiment (Fig 5.1), it is likely that any ABA synthesis occurring in these roots would have also been similar. However, diurnal trends in gas exchange for DOY 204 show that $g_s$ and E were reduced early in the day for PRD50 compared to PRD100 (Fig 5.5), and the higher availability of water in the wet container and the resulting higher water use of

PRD100 compared to PRD50 may have been sufficient to dilute root derived ABA, as has been previously postulated (Dry et al., 2000a; Mingo et al., 2004), and demonstrated experimentally (Jokhan et al., 1996). On DOY 208, the $g_s$ of PRD50 was 33 % of PRD100, and lower sap flow for PRD50 was similarly recorded (data not shown). In addition, the drying roots in the 'wet' compartment of PRD50 on DOY 208 (Fig 5.1) may have contributed considerable ABA to the leaves. Indeed, there was a good relationship between leaf ABA concentration and the soil moisture content of the 'wet' container for DOY 201-208 (Fig 5.6B), and the potential dilution effects as a function of higher E from plants with access to increased soil moisture may partly explain why the soil moisture content of 'dry' containers was more poorly related to leaf ABA than that of 'wet' containers.

The operating principal of PRD is the creation of a chemical root-derived stress signal without a hydraulic signal. This requires a dry or drying profile to generate the root signal while maintaining a wet profile to avoid a reduction in plant water status. Given the importance of knowing the soil water status of the wet and dry side it is rather surprising that only approximately 20 % of published PRD studies have included measurements of soil water status. In the majority of PRD studies to date - with or without a DI regime for comparison - the PRD regime has been irrigated at 50 % of the Control. In two separate field studies with apple in the temperate climate of New Zealand and the semi-arid climate of Washington State, USA, we have recently shown that irrigation volumes of 50 % relative to a Control were insufficient to maintain a wet profile with PRD (Caspari et al., 2004a,b; Leib et al., 2006), as was also confirmed in the present container study (Fig 5.1B). In fact, this lack of maintaining the wet profile when

PRD was irrigated at 50 % of a Control is evident in studies with grapes (Santos et al., 2005), tomatoes (Kirda et al., 2004; Zegbe et al., 2006; Zegbe-Dominguez et al., 2003), hot peppers (Dorji et al., 2005), potato (Liu et al., 2006), maize (Kirda et al., 2005), common bean (Wakrim et al., 2005), and olive (Wahbi et al., 2005). In most of the afore-mentioned studies, the leaf water potential of PRD plants was lower than that of the well-watered Control, indicating that the lack of a sufficiently wet PRD side caused a decline in the plant water potential. In contrast, in studies where the wet side of the PRD treatment was indeed maintained wet and near Control levels, such as in our PRD100, there was no or only a minor, non-significant effect on plant water potential (Dry and Loveys, 1999; Mingo et al., 2003, 2004; Poni et al., 2005; Sobeih et al., 2004; Toit et al., 2003) while significantly reducing $g_s$ (Dry and Loveys, 1999; Mingo et al., 2004; Poni et al., 2005; Sobeih et al., 2004; Toit et al., 2003).

Based on the similarity in response between PRD50 and DI50 for nearly all measured parameters in our study, it is clear that fixing PRD regimes at 50 % of actual plant water use is inappropriate, since cumulative drying on the wet portion of the root system caused severe plant water stress and drastically depressed gas exchange (Fig 5.4). Just how much irrigation input can be reduced with PRD needs to be addressed in future studies. Measurements of $g_s$ and E of various species generally show reductions in the order of 20-40 % with PRD, again indicating that water savings of 50 % can not be achieved unless there is a concomitant reduction in leaf area, as has indeed been found with grape vines (Dry and Loveys, 1999; Santos et al., 2005; Toit et al., 2003). Further, single-leaf measurements of gas exchange may indicate larger reductions of $g_s$ and E than measurements on a canopy basis. Poni et al. (2005) reported 23 and 25 % reductions of

single leaf $g_s$ and E, yet when water use was calculated on a canopy basis the reductions were only 15 %. In species such as apple, where deficit irrigation is avoided during the first 30-40 days after full bloom and shoot growth may cease even before the start of a PRD regime, water savings with PRD need to be proportionate to the reductions in E. Based on the 25-30 % reduction of $g_s$ and E achieved with PRD100 in our study, it is likely that the optimal level of PRD lies between 70 % and 80 % $ET_c$, although in our climate WUE does not increase when water use is replaced below actual $ET_c$ (Fig 5.5 E,F). In fact, when irrigation volumes in an apple orchard in the semi-arid region of Washington State were applied to maintain the wet side of a PRD regime, rather than a fixed 50 % of Control, we were able to reduce the seasonal irrigation input by 30 % without any negative effects on yield or fruit quality (Leib et al., 2006; Caspari et al., unpublished data).

## 5.5 Conclusion

We have shown that apple trees receiving >100 % $ET_c$ to one container of a split-rooted system, while the other was allowed to dry, were capable of reducing gas exchange relative to well-watered Controls by roughly 30 %. This depression in gas exchange could not exclusively be explained by a difference in leaf ABA concentration between the two treatments. There are, however, many modulators of the ABA signal which were not investigated in this study. One tenet of PRD is the increase in WUE under water supply limitations. It is clear from our results that for apple in our climate, WUE is not increased when gas exchange is reduced. In addition, 50 % $ET_c$ applied via PRD has no

advantages over DI50, because progressive drying of the 'wet' soil profile leads to similar degrees of plant water stress. However, care is required extrapolating these results to field conditions, since our potted apple trees were not preconditioned, and apple leaves have been shown to substantially osmotically adjust during long-term drying (Lakso, 1979).

# 6 Conclusion

The aim of this research was to investigate fruit growth, quality, yield, and water relations of apple trees in response to partial rootzone drying (PRD), a novel deficit irrigation technique. The first experiments were administered under field conditions over a three-year period (2001-2003). Comparison of PRD to a well-watered Control and an equally water-deficient treatment (DI) enabled the evaluation of both water deficit and irrigation placement on the parameters investigated. Fruit size, quality, and yield were not affected by irrigation treatment. The most apparent explanation for the lack of a treatment effect on fruit relations is the tight control over water use exerted by the VPD of our climate. The literature contains very few examples of apple leaf or canopy behavior at such high absolute VPD, although our results do confirm modeled predictions of stomatal response of apple leaves to VPD. The down-regulation of stomatal conductance, and hence transpiration, by VPD acted favorably on the maintenance of soil moisture for deficit treatments, despite receiving half the irrigation volume of the Controls. However, water use efficiency was not improved, and this was a consequence of the tight relationship between stomatal conductance and leaf gas exchange which caused roughly equal reductions of both transpiration and photosynthesis. Although net assimilation declined with stomatal conductance it was sufficient to maintain fruit size at the derived croploads. However, such limitations in carbon supply could potentially

result in decreased fruit size under higher croploads, and further work is required to define the maximal cropload levels achievable under deficit regimes.

The fact that PRD did not alter gas exchange compared with other treatments, as previously theorized, casts doubt on the contribution of a chemical signal in the modulation of leaf conductance under field conditions. In fact, leaf ABA concentrations were very low and did not significantly differ among treatments, which suggests that in our climate, either ABA is not a frontline response to drought, or that our treatments did not create sufficient stress due to the overriding influence of VPD. However, these conclusions are likely specific to our environmental conditions, since delivering approximately 30 % of $ET_0$ in more temperate climates would likely lead to excessive water stress and reduced fruit size. Because of the difficulty of estimating actual root volume and our inability to define the necessary proportion of soil volume to dry in order to elicit a signal, it seemed worthwhile to investigate the potential for ABA signaling under more controlled conditions.

A container project using approach-grafted, split-rooted apple trees was developed in 2003, and treatments commenced in 2004 to test if PRD would indeed lead to increased ABA concentrations in apple leaves under both 50 % and 100 % $ET_c$. Monitoring of sap flow under fully irrigated conditions showed the dependency of root sap flow on root cross-sectional area and confirmed the function of our approach-grafted, split-rooted model. Previously dried roots responded rapidly to irrigation events, and the agricultural implication for these rapid switches in root activity is that development of PRD-fertigation regimes could synchronize nutrient delivery around irrigation switches, potentially resulting in improved nutrient uptake. Our results show that supplying 50 %

$ET_c$ to PRD is inadequate as both 50 % $ET_c$ treatments (PRD50 and DI50) experienced similar declines in predawn $\psi$ ($\psi_{pd}$) and single-leaf gas exchange, relative to Controls. The reason for the reduction in water potential of PRD50 was a reduced availability of soil moisture in the 'wet' compartment. Although leaf ABA concentration was markedly elevated in PRD50 relative to both Control and PRD100, it was generally similar to DI50 levels. The higher leaf ABA concentrations of the deficit treatments could have been root derived, leaf synthesized, or some combination of the two.

The PRD100 and Control had similar $\psi_{pd}$, however gas exchange was reduced in PRD100 by 30 % compared to Controls. Leaf ABA concentration was higher in PRD100 than Controls, but could not unequivocally account for the down-regulation of PRD100 stomata. However, the increase in ABA concentration associated with a reduction in stomatal conductance in PRD100 without any negative effect on plant water status is in support of the PRD theory. Therefore, it is likely that the optimal irrigation level of PRD for apples lies somewhere between 50 and 100 % $ET_c$ of a well-watered Control. Although application of $ET_c$ requires consistency during the linear fruit cellular expansion phase, it may be possible to apply even less during post-harvest. Apple leaves show long photosynthetic ageing, however, care must be taken so as to not reduce net assimilation substantially during this period since carbohydrate reserves are necessary for spring growth and fruit set. The development of effective PRD protocols will then require several years of testing under field conditions.

# 7 Literature Cited

Aasamaa, K., A. Sõbr, W. Hartung and Ü. Niinemets. 2002. Rate of stomatal opening, shoot hydraulic conductance and photosynthetic characteristics in relation to leaf abscisic acid concentration in six temperate deciduous trees. Tree Phys. 22: 267-276.

Al-Hinai, Y.K. and T.R. Roper. 2004. Rootstock effects on growth, cell number, and cell size of 'Gala' apples. J. Amer. Soc. Hort. Sci. 129: 37-41.

Assaf, R., B. Bravdo and I. Levin. 1974. Effect of irrigation according to water deficit in two different soil layers, on the yield and growth of apple trees. J. Hort. Sci. 49: 53-64.

Assaf, R., I. Levin and B. Bravdo. 1982. Apple fruit growth as a measure of irrigation control. HortScience 17: 59-61.

Atkinson, C.J. 1991. The flux and distribution of xylem sap calcium to adaxial and abaxial epidermal tissue in relation to stomatal behaviour. J. Exp. Bot. 42: 987-993.

Atkinson, C.J., A.D. Webster, S.P. Vaughan, L. Taylor and G. Kingswell. 2000. Interactions between root restriction, irrigation and rootstock treatments on 'Queen Cox' apple trees: Effects of soil and plant water relations. J. Hort. Sci. Biotech. 75: 376-382.

Atkinson, D. 1980. The distribution and effectiveness of the roots of tree crops. Hort. Rev. 2: 424-490.

Augé, R. and J. Moore. 2002. Stomatal response to nonhydraulic root-to-shoot communication of partial soil drying in relation to foliar dehydration tolerance. Env. Exp. Bot. 47: 217-229.

Bacon, M.A., S. Wilkinson and W.J. Davies. 1998. pH-Regulated leaf cell expansion in droughted plants is abscisic acid dependent. Plant Physiol. 118: 1507-1515.

Bano, A., K. Dorffling, D. Bettin and H. Hahn. 1993. Abscisic acid and cytokinins as possible root-to-shoot signals in xylem sap of rice plants in drying soil. Aust. J. Plant Physiol. 20: 109-115.

Bano, A., H. Hansen, K. Dorffling and H. Hahn. 1994. Changes in the content of free and conjugated abscisic acid, phaesic acid and cytokinins in the xylem sap of drought-stressed sunflower plants. Phytochemistry 37: 345-347.

Beardsell, M.F. and D. Cohen. 1975. Relationship between leaf water status, abscisic acid levels, and stomatal resistance in maize and sorghum. Plant Physiol. 56: 207-212.

Behl, R. and W. Hartung. 1984. Transport and compartmentation of abscisic acid in roots of *Hordeum distichon* under osmotic stress. J. Exp. Bot. 35: 1433-1440.

Bergh, O. 1990. Effect of temperature during the first 42 days following full bloom on apple fruit growth and size at harvest. S. Afr. J. Plant Soil 7: 11-19.

Beukes, D.J.and H.W. Weber. 1982. The effects of irrigation at different soil water levels on the water use characteristics of apple trees. J. Hort. Sci. 57: 383-391.

Blackman P.G. and W.J. Davies. 1985. Root to shoot communication in maize plants of the effects of soil drying. J. Exp. Bot. 36: 39-48.

Borel, C., A. Frey, A. Marion-Poll, F. Tardieu and T. Simonneau. 2001. Does engineering abscisic acid biosynthesis in *Nicotiana plumbaginifolia* modify stomatal response to drought? Plant, Cell and Environ. 24: 477-489.

Bradford, K.J. and T.C. Hsiao. 1982. Physiological responses to moderate water stress, p. 263-324. In: Lange OL et al., (Eds.) Encyclopedia of Plant Physiology, New Series. Physiological plant ecology. II. Water relations and carbon assimilation. Berlin: Springer-Verlag.

Bravdo, B. 2005. Physiological mechanisms involved in the production of non-hydraulic root signals by partial rootzone drying - a review. Acta Hort. 689: 267-276.

Bravdo, B., A. Naor, T. Zahavi and Y. Gal. 2004. The effect of water stress applied alternately to part of the wetting zone along the season (PRD - partial rootzone drying) on wine quality, yield and water relations of red wine grapes. Acta Hort. 664: 101-109.

Caspari, H.W., S. Neal and P. Alspach. 2004a. Partial rootzone drying – a new deficit irrigation strategy for apple? Acta Hort. 646: 93-100.

Caspari, H.W., T.C. Einhorn, B.G. Leib, C.A. Redulla, P.K. Andrews, L. Lombardini, T. Auvil and J.R. McFerson. 2004b. Progress in the development of partial rootzone drying of apple trees. Acta Hort. 664: 125-132.

Centritto, M., S. Wahbi, R. Serraj and M.M. Chaves. 2005. Effects of partial rootzone drying (PRD) on adult olive tree (*Olea europaea*) in field conditions under arid climate. II. Photosynthetic responses. Agric., Eco. and Environ. 106: 303-311.

Chalmers, D.J. 1989. A physiological examination of regulated deficit irrigation. N.Z. J. Agric. Sci. 23: 44-48.

Chalmers, D.J., P.D. Mitchell and L. van Heek. 1981. Control of peach tree growth and productivity by regulated water supply, tree density, and summer pruning. J. Amer. Soc. Hort. Sci. 106: 307-312.

Chalmers, Y.M., G. Kelly and M.P. Krstic. 2004. Partial rootzone drying of *Vitis vinifera* cv. 'Shiraz' winegrapes in a semi-arid climate. Acta Hort. 664:133-138.

Cohen, Y., M. Fuchs and G.C. Green. 1981. Improvement of the heat pulse method for determining sap flow in trees. Plant, Cell and Environ. 4: 391-397.

Comstock, J.P. 2002. Hydraulic and chemical signaling in the control of stomatal conductance and transpiration. J. Exp. Bot. 53: 195-200.

Davies, F.S. and A.N. Lakso. 1978. Water relations in apple seedlings: changes in water potential components, abscisic acid levels and stomatal conductances under irrigated and non-irrigated conditions. J. Amer. Soc. Hort. Sci. 103: 310-313.

Davies, W.J. and J. Zhang. 1989. Chemical regulation of growth and physiology in drying soil: the case for abscisic acid. Curr. Top. Plant Biochem. Physiol. 8: 100-109.

Davies, W.J. and J. Zhang. 1991. Root signals and the regulation of growth and development of plants in drying soil. Ann. Rev. Plant Physiol. Plant Mol. Bio. 42: 55-76.

Davies, W.J., S. Wilkinson and B. Loveys. 2002. Stomatal control by chemical signaling and the exploitation of this mechanism to increase water use efficiency in agriculture. New Phytol. 153: 449-460.

Davies, W.J., M.A. Bacon, D.S. Thompson, W. Sobeih and L. González Rodriguez. 2000. Regulation of leaf and fruit growth in plants growing in drying soil: exploitation of the plants' chemical signaling system and hydraulic architecture to increase the efficiency of water use in agriculture. J. Exp. Bot. 51: 1617-1626.

Degenhardt B., H. Gimmler, E. Hose and W. Hartung. 2000. Effect of alkaline and saline substrates on ABA contents, distribution and transport in plant roots. Plant and Soil 225: 83-94.

De Silva, D. L. R., A. M. Hetherington and T. A. Mansfield. 1985. Synergism between calcium ions and abscisic acid in preventing stomatal opening. New Phytol. 100: 473-482.

De Silva, H.N., A.J. Hall, D.S. Tustin and P.W. Gandar. 1999. Analysis of distribution of root length density of apple trees on different dwarfing rootstocks. Ann. of Bot. 83: 335-345.

Dodd, I.C. 2003. Hormonal interactions and stomatal responses. J. Plant Growth Reg. 22: 32-46.

Doorenbos, J.and W.O. Pruitt. 1977. Guidelines for predicting crop water requirements. Food and Agricultural Organisation of the United Nations, Rome.

Dorji, K., M.H. Behboudian and J.A. Zegbe-Dominguez. 2005. Water relations, growth, yield, and fruit quality of hot pepper under deficit irrigation and partial rootzone drying. Scientia Hort. 104: 137-149.

Dragoni, D., A.N. Lakso and R.M. Piccioni. 2004. Transpiration of an apple orchard in a cool humid climate: measurement and modeling. Acta Hort. 664: 175-180.

Dry, P.R.and B.R. Loveys. 1998. Factors influencing grapevine vigour and the potential for control with partial rootzone drying. Aus. J. Grape Wine Res. 4: 140-148.

Dry, P.R.and B.R. Loveys. 1999. Grapevine shoot growth and stomatal conductance are reduced when part of the root system is dried. Vitis 38: 151-156.

Dry, P.R., B.R. Loveys and H. Düring. 2000a. Partial drying of the rootzone of grape. I. Transient changes in shoot growth and gas exchange. Vitis 39: 3-7.

Dry, P.R., B.R. Loveys and H. Düring. 2000b. Partial drying of the rootzone of grape. II. Changes in the pattern of root development. Vitis 39: 9-12.

Dry, P. R., B. R. Loveys, D. Botting and H. Düring. 1996. Effects of partial rootzone drying on grapevine vigour, yield, composition of fruit and use of water. In: C.S. Stockley, A.N. Sas, R.S. Johnstone, T.H. Lee (Eds.): Proc. 9th Aus. Wine Ind. Tech. Conf.: 126-131.

Dry, P.R., B.R. Loveys, M.G. McCarthy and M. Stoll. 2001. Strategic irrigation management in Australian vineyards. J. Int. Sci. Vigne Vin. 35: 129-139.

Duckham, S.C., I.B. Taylor, R.S.T. Linforth, R.J. Al-Naieb, B.A. Marples and W.R. Bowman. 1989. The metabolism of cis ABA-aldehyde by the wilty mutants of potato, pea and *Arabidopsis thaliana*. J. Exp. Bot. 40: 901-905.

Düring, H. and M. Stoll. 1996a. Stomatal patchiness of grapevine leaves. I. Estimation of non-uniform stomatal apertures by a new infiltration technique. Vitis 35: 65-68.

Düring, H. and M. Stoll. 1996b. Stomatal patchiness of grapevine leaves. II. Uncoordinated and coordinated stomatal movements. Vitis 35: 69-71.

Ebel, R.C., E.L. Proebsting and M.E. Patterson. 1993. Regulated deficit irrigation may alter apple maturity, quality, and storage life. HortScience 28: 141-143.

Ebel, R.C., E.L. Proebsting and R.G. Evans. 1995. Deficit irrigation to control vegetative growth in apple and monitoring fruit growth to schedule irrigation. HortScience 30: 1229-1232.

Einhorn, T. and H.W. Caspari. 2004. Partial rootzone drying and deficit irrigation of 'Gala' apples in a semi-arid climate. Acta Hort. 664: 197-204.

FAO Crop Production Statistics Web site [online], http://apps.fao.org/page/collections?subset=agriculture (2001).

Farquhar, G.D. 1978. Feedforward responses of stomata to humidity. Aust. J. Plant Physiol. 5: 787-800.

Fernandez, R.T., R.L. Perry and J.A. Flore. 1997. Drought response of young apple trees on three rootstocks. II. Gas exchange, chlorophyll fluorescence, water relations, and leaf abscisic acid. J. Amer. Soc. Hort. Sci. 122: 841-848.

Flore, J.A., A.N. Lakso and J.W. Moon. 1985. The effect of water stress and vapor pressure gradient on stomatal conductance, water use efficiency, and photosynthesis of fruit crops. Acta Hort. 171: 207-218.

Forshey, C.G. and D.C. Elfving. 1989. The relationship between vegetative growth and fruiting in apple trees. Hort. Rev. 11: 229-287.

Francesconi, A.H.D, A.N. Lakso and S.S. Denning. 1997. Light and temperature effects on whole-canopy net carbon dioxide exchange rates of apple trees. Acta Hort. 451: 287-294.

Franks, P.J., I.R. Cowan and G.D. Farquhar. 1997. The apparent feedforward response of stomata to air vapour pressure deficit: information revealed by different experimental procedures with two rainforest trees. Plant, Cell and Environ. 20: 142-145.

Goffinet, M.C., T.L. Robinson and A.N. Lakso. 1995. A comparison of 'Empire' apple fruit size and anatomy in unthinned and hand-thinned trees. J. Hort. Sci. 70: 375-387.

Goldhamer, D.A., M. Salinas, C. Crisosto, K.R. Day, M. Soler and A. Moriana. 2002. Effects of regulated deficit irrigation and partial root zone drying on late harvest peach tree performance. Acta Hort. 592: 343-350.

Gollan, T., U. Schurr and E.-D. Schulze. 1992. Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. I. The concentration of cations, anions, amino acids in, and pH of, the xylem sap. Plant, Cell and Environ. 15: 551-559.

Gowing, D.J.G., W.J. Davies and H.G. Jones. 1990. A positive root-sourced signal as an indicator of soil drying in apple, *Malus* x *domestica* Borkh. J. Exp. Bot. 41: 1535-1540.

Gowing, D.J.G., H.G. Jones and W.J. Davies. 1993. Xylem-transported abscisic acid: the relative importance of its mass and its concentration in the control of stomatal aperture. Plant, Cell and Environ. 16: 453-459.

Grant, O.M., M. Stoll and H.G. Jones. 2004. Partial rootzone drying does not affect fruit yield of raspberries. J. Hort. Sci. and Biotech. 79: 125-130.

Green, S.R., B.E. Clothier and D.J. McLeod. 1997. The response of sap flow in apple roots to localised irrigation. Agric. Water Man. 33: 63-78.

Green, S., B. Clothier and B. Jardine. 2003. Theory and practical application of heat pulse to measure sap flow. Agron. J. 95: 1371-1379.

Greybe, E., O. Bergh and D.I. Ferreira. 1998. Fruit growth and cell multiplication of Royal Gala apples as a function of temperature. Togepaste Plantwetenskap 12: 10-14.

Gu, S., D. Guoqiang, D. Zoldoske, A. Hakim, R. Cochran, K. Fugelsang and G. Jorgensen. 2004. Effects of irrigation amount on water relations, vegetative growth, yield and fruit composition of Sauvignon blanc grapevines under partial rootzone drying and conventional irrigation in the San Joaquin Valley of California, USA. J. Hort. Sci. and Biotech. 79: 26-33.

Hartung, W. 1983. The site of action of abscisic acid at the guard cell plasmalemma of *Valerianella locusta*. Plant, Cell and Environ. 6: 427-428.

Hartung, W. and J.W. Radin. 1989. Abscisic acid in the mesophyll apoplast and in the root xylem sap of water-stressed plants: the significance of pH gradients. Curr. Top. Plant Biochem. and Physiol. 8: 110-124.

Hartung, W., S. Wilkinson and W.J. Davies. 1998. Factors that regulate abscisic acid concentrations at the primary site of action at the guard cell. J. Exp. Bot. 49: 361-367.

Hartung, W., A.D. Peuke and W.J. Davies. 1999. Abscisic acid - a hormonal long distance stress signal in plants under drought and salt stress, p. 731-747. In: M. Pessarakali (Ed.). Handbook of Crop Stress, 2nd edition. Marcel Decker, New York, NY.

Hartung, W., A. Sauter and L. Hose. 2002. Abscisic acid in the xylem: where does it come from, where does it go? J. Exp. Bot. 53: 27-32.

Hartung, W., A. Sauter, N.C. Turner, I. Fillery and H. Heilmeier. 1996. Abscisic acid in soils: what is its function and which factors and mechanisms influence its concentration? Plant and Soil 184: 105-110.

Holbrook, N.M., V.R. Shashidhar, R.A. James and R. Munns. 2002. Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. J. Exp. Bot. 53: 1503-1514.

Hooijdonk, B.M., K. Dorji and M.H. Behboudian. 2004. Responses of 'Pacific Rose' apple to partial rootzone drying and to deficit irrigation. Europ. J. Hort. Sci. 69: 104-110.

Irving, D.E. and J.H. Drost. 1987. Effects of water deficit on vegetative growth, fruit growth and fruit quality in Cox's Orange Pippin apple. J. Hort. Sci. 62: 427-432.

Jarvis, P.G. 1985. Coupling of transpiration to the atmosphere in horticultural crops: the omega factor. Acta Hort. 171: 187-205.

Jerie, P.H., P.D. Mitchell and I. Goodwin. 1989. Growth of Williams' Bon Chretien pear fruit under regulated deficit irrigation (RDI). Acta Hort. 240: 271-274.

Jia, W. and J. Zhang. 1999. Stomatal closure is induced rather by prevailing xylem abscisic acid than by accumulated amount of xylem-derived abscisic acid. Physiol. Plant. 106: 268-275.

Jokhan, A.D., M.A. Else and M.B. Jackson. 1996. Delivery rates of abscisic acid in xylem sap of *Ricinus communis* L. plants subjected to part-drying of the soil. J. Exp. Bot. 47: 1595-1599.

Jones, H.G. 1990. Plant water relations and implications for irrigation scheduling. Acta Hort. 278: 67-76.

Jones, H.G. 1992. Plants and microclimate. Cambridge University Press, Cambridge.

Jones, H.G., A.N. Lakso and J.P. Syvertsen. 1985. Physiological control of water status in temperate and subtropical fruit trees. Hort. Rev. 7: 301-344.

Kang, S., Z. Lu, H.X. Tao, L.Z. Jun and P. Jerie. 2001. An improved water use efficiency for hot pepper grown under controlled alternate drip irrigation on partial roots. Scientia Hort. 89: 257-267.

Kilili, A.W., M.H. Behboudian and T.M. Mills. 1996. Composition and quality of 'Braeburn' apples under reduced irrigation. Scientia Hort. 67: 1-11.

Kirda, C. and R. Kanber. 1999. Water, no longer a plentiful resource, should be used sparingly in irrigated agriculture, p. 1-20. In: C. Kirda et al. (Eds.). Crop yield responses to deficit irrigation. Dordrecht, Boston.

Kirda, C., M. Cetin, Y. Dasgan, S. Topcu, H. Kaman, B. Ekici, M.R. Derici and A.I. Ozguven. 2004. Yield response of greenhouse grown tomato to partial root drying and conventional deficit irrigation. Agric. Water Man. 69: 191-201.

Kirda, C., S. Topcu, H. Kaman, A.C. Ulger, A. Yazici, M. Cetin and M.R. Derici. 2005. Grain yield response and N-fertiliser recovery of maize under deficit irrigation. Field Crops Res. 93: 132-141.

Lakso, A.N. 1979. Seasonal changes in stomatal response to leaf water potential in apple. J. Amer. Soc. Hort. Sci. 104: 58-60.

Lakso, A.N. 1994. Apple, p. 3-42. In: B. Schaffer and P.C. Andersen (Eds.). Handbook of Environmental Physiology of Fruit Crops Volume 1. Temperate Crops. CRC Press, Boca Raton.

Lakso, A.N. 2003. Water relations of apples, p. 167-194. In: D.C. Ferree and I.J. Warrington (Eds.). Apples: Botany, Production and Uses. CAB International, Oxon.

Lakso, A.N., A.S. Geyer and S.G. Carpenter. 1984. Seasonal osmotic relations in apple leaves of different ages. J. Amer. Soc. Hort. Sci. 109: 544-547.

Landsberg, J.J. and H.G. Jones. 1981. Apple orchards, p. 419-469. In: T.T. Kozlowski (Ed.). Water deficit and plant growth. Academic Press, New York.

Leib, B.G., H.W. Caspari, C.A. Redulla, P.K. Andrews and J. Jabro. 2006. Partial rootzone drying and deficit irrigation of 'Fuji' apples in a semi-arid climate. Irrig. Sci. 24: 85-99.

LeNoble, M.E., W.G. Spollen and R.E. Sharp. 2004. Maintenance of shoot growth by endogenous ABA: genetic assessment of the involvement of ethylene suppression. J. Exp. Bot. 55: 237-245.

Liu, F., C.R. Jensen and M.N. Andersen. 2003. Hydraulic and chemical signals in the control of leaf expansion and stomatal conductance in soybean exposed to drought stress. Funct. Plant Biol. 30: 65-73.

Liu, F., A. Shahnazari, M.N. Andersen, S.E. Jacobsen and C.R. Jensen. 2006. Effects of deficit irrigation (DI) and partial root drying (PRD) on gas exchange, biomass partitioning, and water use efficiency in potato. Scientia Hort. 109: 113-117.

Lombardini, L., H.W. Caspari, D.C. Elfving, T.D. Auvil and J.R. McFerson. 2004. Gas exchange and water relations in 'Fuji' apple trees grown under deficit irrigation. Acta Hort. 636: 43-50.

Lötter, J.D.V., D.J. Beukes and H.W. Weber. 1985. Growth and quality of apples as affected by different irrigation treatments. J. Hort. Sci. 60: 181-192.

Loveys, B. 1984. Diurnal changes in water relations and abscisic acid in field grown *Vitis vinifera* cultivars. III. The influence of xylem derived abscisic acid on leaf gas exchange. New Phytol. 98: 563-573.

Loveys, B.R., S.P. Robinson and W.J.S. Downton. 1987. Seasonal and diurnal changes in abscisic acid and water relations of apricot leaves (*Prunus armeniaca* L.). New Phytol. 107: 15-27.

Loveys, B., J. Grant, P. Dry and M. McCarthy. 1997. Progress in the development of partial rootzone drying. Aust. GG and WM. July: 18-20.

Loveys, B., M. Stoll, P. Dry and M. McCarthy. 1998. Partial rootzone drying stimulates stress responses in grapevine to improve water use efficiency while maintaining crop yield and quality. Aust. GG and WM. Tech Issue: 108-113.

Loveys, B., P.R. Dry, B.M. Stoll and M.G. McCarthy. 2000. Using plant physiology to improve the water use efficiency of horticultural crops. Acta Hort. 537: 187-197.

Loveys, B., C. Soar, M. Stoll, M. McCarthy and P. Dry. 2002. The manipulation of grapevine leaf gas exchange through irrigation management. Aust. N.Z. Wine Ind. J. 17: 49-51.

Lovisolo, C., W. Hartung and A. Schubert. 2002. Whole-plant hydraulic conductance and root-to-shoot flow of abscisic acid are independently affected by water stress in grapevines. Funct. Plant Biol. 29: 1349-1356.

Lu, J.L., J.R. Ertl and C.M. Chen. 1992. Transcriptional regulation of nitrate reductase mRNA levels by cytokinin-abscisic acid interactions in etiolated barley leaves. Plant Physiol. 98: 1255-1260.

McCutchan, H. and K.A. Shackel. 1992. Stem-water potential as a sensitive indicator of water stress in prune trees (*Prunus domestica* L. cv. French). J. Amer. Soc. Hort. Sci. 117: 607-611.

Mills, T.M., M.H. Behboudian, P.Y. Tan and B.E. Clothier. 1994. Plant water status and fruit quality in 'Braeburn' apples. HortScience 29: 1274-1278.

Mingo, D.M., M.A. Bacon and W.J. Davies. 2003. Non-hydraulic regulation of fruit growth in tomato plants (*Lycopersicon esculentum* cv. Solairo) growing in drying soil. J. Exp. Bot. 54: 1205-1212.

Mingo, D.M., J.C. Theobald, M.A. Bacon, W.J. Davies and I.C. Dodd. 2004. Biomass allocation in tomato (*Lycopersicon esculentum*) plants grown under partial rootzone drying: enhancement of root growth. Funct. Plant Biol. 31: 971-978.

Mitchell, P.D. and D.J. Chalmers. 1982. The effect of reduced water supply on peach tree growth and yields. J. Amer. Soc. Hort. Sci. 107: 853-856.

Mpelasoka, B.S., M.H. Behboudian, J. Dixon, S.M. Neal and H.W. Caspari. 2000. Improvement of fruit quality and storage potential of 'Braeburn' apple through deficit irrigation. J. Hort. Sci and Biotech. 75: 615-621.

Mpelasoka, B.S., M.H. Behboudian and S.R. Green. 2001a. Water use, yield and fruit quality of lysimeter-grown apple trees: responses to deficit irrigation and crop load. Irrig Sci. 20: 107-113.

Mpelasoka, B.S., M.H. Behboudian and T.M. Mills. 2001b. Effects of deficit irrigation on fruit maturity and quality of 'Braeburn' apple. Scientia Hort. 90: 279-290.

Mpelasoka, B.S., M.H. Behboudian and T.M. Mills. 2001c. Water relations, photosynthesis, growth, yield and fruit size of 'Braeburn' apple: responses to deficit irrigation and crop load. J. Hort. Sci and Biotech. 76: 150-156.

Naor, A., I. Klein, D. I, Y. Gal, Z. Ben-David and B. Bravdo. 1997. The effect of irrigation and crop load on stem water potential and apple fruit size. J. Hort. Sci. 72: 765-771.

O'Connell, M.G. and I. Goodwin. 2004. Pear water relations under partial rootzone drying. Acta Hort. 664: 453-459.

Palanjian, K., L. Valenzuela, D. Neilsen, G. Neilsen and D. Eissenstat. 2005. Rapid and differential rates of root browning in apple trees under different irrigation treatments. HortScience 40: 1038.

Palmer, J.W., R. Giuliani and H.M. Adams. 1997. Effect of crop load on fruiting and leaf photosynthesis of 'Braeburn'/M.26 apple trees. Tree Phys. 17: 741-746.

Palmer, J.W., J.P. Prive and D.S. Tustin. 2003. Temperature. p. 217-236. In: D.C. Ferree and I.J. Warrington (Eds.). Apples: Botany, Production and Uses. CAB International, Oxon.

Parchomchuk, P. and M. Meheriuk. 1996. Orchard cooling with pulsed overtree irrigation to prevent solar injury and improve fruit quality of 'Jonagold' apples. HortScience 31: 802-804.

Parry, A.D., S.J. Neill and R. Horgan. 1988. Xanthoxin levels and metabolism in the wild-type and wilty mutants of tomato. Planta 173: 397-404.

Peretz, J., R.G. Evans and E.L. Proebsting. 1984. Leaf water potentials for management of high frequency irrigation on apples. Trans. ASAE. 27: 437-442.

Pierce, M. and K. Raschke. 1980. Correlation between loss of turgor and accumulation of abscisic acid in detached leaves. Planta 148: 174-182.

Poni, S., F. Bernizzoni and M. Reinotti. 2005. Whole-canopy and single-leaf gas exchange responses to partial rootzone drying in potted 'Cabernet Sauvignon' grapevines. Acta Hort. 689: 277-284.

Powell, D.B.B. 1975. Some abscisic acid relationships in apple. Riv. Ortoflorofrutt. It. 59: 424-432.

Powell, D.B.B. 1976. Some effects of water stress on the growth and development of apple trees. J. Hort. Sci. 51: 75-90.

Proebsting, E.L., S.R. Drake and R.G. Evans. 1984. Irrigation management, fruit quality, and storage life of apple. J. Amer. Soc. Hort. Sci. 109: 229-232.

Pudney S. and M.G. McCarthy. 2004. Water use efficiency of field grown Chardonnay grapevines subjected to partial rootzone drying and deficit irrigation. Acta Hort. 664: 567-573.

Robinson, T.L. and B.H. Barritt. 1990. Endogenous abscisic acid concentrations, vegetative growth, and water relations of apple seedlings following PEG-induced water stress. J. Amer. Soc. Hort. Sci. 115: 991-999.

Saab, I.N., T.H.D. Ho and R.E. Sharp. 1995. Translatable RNA populations associated with maintenance of primary root elongation and inhibition of mesocotyl elongation by abscisic acid in maize seedlings at low water potential. Plant Physiol. 109: 593-601.

Saab, I.N., R.E. Sharp, J. Pritchard and G.S. Voetberg. 1990. Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. Plant Physiol. 93: 1329-1336.

Salisbury, F.B. and C.W. Ross. 1992. Plant Physiology. Wadsworth, Belmont, CA.

Santos, T.P., C.M. Lopes, M.L. Rodrigues, C.R. Souza, J.P. Maroco, J.S. Pereira, J.R. Silva and M.M. Chaves. 2003. Partial rootzone drying: effects on growth and fruit quality of field-grown grapevines (*Vitis vinifera*). Funct. Plant Biol. 30: 663-671.

Santos, T.P., C.M. Lopes, M.L. Rodrigues, C.R. de Souza, J.M. Ricardo-Da-Silva, J.P. Maroco, J.S. Pereira and M.M. Chaves. 2005. Effects of partial root-zone drying irrigation on cluster microclimate and fruit composition of field-grown Castelao grapevines. Vitis 44: 117-125.

Sauter, A., W.J. Davies and W. Hartung. 2001. The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot. J. Exp. Bot. 52: 1991-1997.

Scholander, P.F., H.T. Hammel, E.D. Bradstreet and E.A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148: 339-346.

Schroeder, J.I., G.J. Allen, V. Hugouvieux, J.M. Kwak and D. Waner. 2001. Guard cell signal transduction. Ann. Rev. Plant Physiol. Mol. Biol. 52: 627-658.

Serageldin, I. 1999. The world water gap: world's ability to feed itself threatened by water shortage. World Water Commission, Paris. Press Release.

Shackel, K.A., M.A. Matthews and J.C. Morrison. 1987. Dynamic relation between expansion and cellular turgor in growing grape (*Vitis vinifera* L.) leaves. Plant Physiol. 84: 1166-1171.

Shackel, K.A., H. Ahmadi, W. Biasi, R. Buchner, D. Goldhamer, S. Gurusinghe, J. Hasey, D. Kester, B. Krueger, B. Lampinen, G. McGourty, M.E. Mitcham, B. Olson, K. Pelletrau, H. Phillips, D. Ramos, L. Schwankl, S. Sibbett, R. Snyder, S. Southwick, M. Stevenson, M. Thorpe, S. Weinhbaum and J. Yeager. 1997. Plant water status as an index of irrigation need in deciduous fruit trees. HortTech. 7: 23-29.

Sharp, R.E. 2002. Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. Plant, Cell and Environ. 25: 211-222.

Sharp, R.E., Y. Wu, G.S. Voetberg, I.N. Saab and M.E. LeNoble. 1994. Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potentials. J. Exp. Bot. 45: 1743-1751.

Slovik, S., W. Daeter and W. Hartung. 1995. Compartmental redistribution and long distance transport of abscisic acid (ABA) in plants as influenced by environmental changes in the rhizosphere. A biomathematical model. J. Exp. Bot. 46: 881-894.

Soar, C.J., J. Spiers, S.M. Maffei and B.R. Loveys. 2004. Gradients in stomatal conductance, xylem sap ABA and bulk leaf ABA along canes of *Vitis vinifera* cv. Shiraz: molecular and physiological studies investigating their source. Funct. Plant Biol. 31: 659-669.

Sobeih, W.Y., I.C. Dodd, M.A. Bacon, D. Grierson and W.J. Davies. 2004. Long-distance signals regulating stomatal conductance and leaf growth in tomato (*Lycopersicon esculentum*) plants subjected to partial rootzone drying. J. Exp. Bot. 55: 2353-2363.

Soejima, J., M. Watanabe, T. Moriguchi and S. Yamaki. 1990. Good correlation between enzyme-linked immunosorbent assay and gas chromatographic analysis of abscisic acid in apple organs. J. Japan. Soc. Hort. Sci. 58: 819-826.

Souza, C.R., J.P. Maroco, T. Santos, M.L. Rodrigues, C.M. Lopes, J.S. Pereira and M.M. Chaves. 2003. Partial rootzone drying: regulation of stomatal aperture and carbon assimilation in field-grown grapevines. Funct. Plant Biol. 30: 653-662.

Souza, C.R., J.P. Maroco, T.P. Santos, M.I. Rodrigues, C.M. Lopes, J.S. Pereira and M.M. Chaves. 2005. Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars. Agric., Eco. and Environ. 106: 261-274.

Stikic, R., S. Popovic, M. Srdic, D. Savic, Z. Jovanovic, L. Prokic and J. Zdravkovic. 2003. Partial root drying (PRD): a new technique for growing plants that saves water and improves the quality of fruit. Bulg. J. Plant Physiol. Special Issue: 164-171.

Stoll, M., B.R. Loveys and P. Dry. 2000. Hormonal changes induced by partial rootzone drying of irrigated grapevine. J. Exp. Bot. 51: 1627-1634.

Taiz, L. and E. Zeiger. 2000. Plant Physiology, 3rd Edition. Sinauer Ass., Sunderland, Mass.

Tal, M. and D. Imber. 1970. Abnormal stomatal behaviour and hormonal imbalance in *flacca*, a wilty mutant of tomato. II Auxin and abscisic acid-like activity. Plant Physiol. 46: 373-376.

Tan, C.S. and B.R. Buttery. 1982. The effect of soil moisture stress to various fractions of the root system on transpiration, photosynthesis, and internal water relations of peach seedlings. J. Amer. Soc. Hort. Sci. 107: 845-849.

Tardieu, F. and W.J. Davies. 1992. Stomatal response to abscisic acid is a function of current plant water status. Plant Physiol. 98: 540-545.

Tardieu, F. and W.J. Davies. 1993. Integration of hydraulic and chemical signaling in the control of stomatal conductance and water status of droughted plants. Plant, Cell and Environ. 16: 341-349.

Taylor, H.F. and R.S. Burden. 1970. Xanthoxin, a new naturally occurring plant growth inhibitor. Nature 227: 302-304.

Taylor, I.B., A. Burbidge and A.J. Thompson. 2000. Control of abscisic acid synthesis. J. Exp. Bot. 51: 1563-1574.

Taylor, I.B., R.S.T. Linforth, R.J. Al-Naieb, W.R. Bowman and B.A. Marples. 1988. The wilty tomato mutants *flacca* and *sitiens* are impaired in the oxidation of ABA-aldehyde to ABA. Plant, Cell and Environ. 11: 739-745.

Thorpe, M.R., B. Warrit and J.J. Landsberg. 1980. Responses of apple leaf stomata: a model for single leaves and a whole tree. Plant, Cell and Environ. 3: 23-27.

Toit, P.G., P.R. Dry and B.R. Loveys. 2003. A preliminary investigation on partial rootzone drying (PRD) effects on grapevine performance, nitrogen assimilation and berry composition. S. Afr. J. Enol. Vitic. 24: 43-54.

Topp, G.C., J.L. Davis and A.P. Annan. 1980. Electromagnetic determination of soil water content: Measurements in coaxial transmission lines. Water Resour. Res. 16: 574-582.

Trejo, C.L., W.J. Davies and L. del Mar P. Ruiz. 1993. Sensitivity of stomata to abscisic acid: an effect of the mesophyll. Plant Physiol. 102: 497-502.

Trewavas, A.J. and H.G. Jones. 1991. An assessment of the role of ABA in plant development, p. 169-188. In: W.J. Davies and H.G. Jones (Eds.). Abscisic Acid: physiology and biochemistry. BIOS Scientific Publishers Limited, Oxford, UK.

Turner, D.W., C.M. Menzel and D.R. Simpson. 1996. Short term drying of half the root system reduces growth but not water status or photosynthesis in leaves of passionfruit (*Passiflora sp.*). Scientia Hort. 65: 25-36.

Turner, N.C. 1979. Drought resistance and adaptation to water deficits in crop plants. p. 343-372. In: H. Mussel and R.C. Staples (Eds). Stress Physiology in Important Agricultural Crops. Wiley-Interscience, NY.

United Nations Dept of Economic and Social Affairs Population Division Web site [online], http://www.un.org/esa/population/publications/WPP2004/wpp2004.htm (2004).

Volz, R.K., F.R. Harker and S. Lang. 2003. Firmness decline in 'Gala' apple during fruit development. J. Amer. Soc. Hort. Sci. 128: 797-802.

Wahbi, S., R. Wakrim, B. Aganchich, H. Tahi and R. Serraj. 2005. Effects of partial rootzone drying (PRD) on adult olive tree (*Olea europaea*) in field conditions under arid climate. I. Physiological and agronomic responses. Agric., Eco. and Environ. 106: 289-301.

Wakrim, R., S. Wahbi, H. Tahi, B. Aganchich and R. Serraj. 2005. Comparative effects of partial root drying (PRD) and regulated deficit irrigation (RDI) on water relations and water use efficiency in common bean (*Phaseolus vulgaris* L.). Agric., Eco. and Environ. 106: 275-287.

Walker-Simmons, M., D.A. Kudrna and R.L. Warner. 1989. Reduced accumulation of ABA during water stress in a molybdenum cofactor mutant of barley. Plant Physiol. 90: 728-733.

Wan, X. and J.J. Zwiazek. 2001. Root water flow and leaf stomatal conductance in aspen (*Populus tremuloides*) seedlings treated with abscisic acid. Planta 213: 741-747.

Wang, L., H. de Kroon, G.M. Bogemann and A.J.M. Smits. 2005. Partial root drying effects on biomass production in *Brassica napus* and the significance of root responses. Plant and Soil 276: 313-326.

Wang, Z., B. Quebedeaux and G.W. Stutte. 1995. Osmotic adjustment: Effect of water stress on carbohydrates in leaves, stems and roots of apple. Aust. J. Plant Physiol. 22: 747-754.

Warrit, B., J.J. Landsberg and M.R. Thorpe. 1980. Responses of apple leaf stomata to environmental factors. Plant, Cell and Environ. 3: 13-22.

West, D.W. and D.F. Gaff. 1976. The effect of leaf water potential, leaf temperature and light intensity on leaf diffusion resistance and the transpiration of leaves of *Malus sylvestris*. Physiol. Plant. 38: 98-104.

Wilkinson, S. and W.J. Davies. 1997. Xylem sap pH increase: A drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. Plant Physiol. 113: 559-573.

Wilkinson, S. and W.J. Davies. 2002. ABA-based chemical signaling: the co-ordination of responses to stress in plants. Plant, Cell and Environ. 25: 195-210.

Wilkinson, S., J.E. Corlett, L. Oger and W.J. Davies. 1998. Effects of xylem pH on transpiration from wild-type and *flacca* tomato leaves: a vital role for abscisic acid in preventing excessive water loss even from well-watered plants. J. Plant Phys. 117: 703-709.

Yoon, T.M. and H. Richter. 1991. Stomatal conductance and leaf-water parameters of apple, pear, sweet cherry, and plum in an orchard. Gartenbauwissenschaft 56: 75-81.

Zegbe, J.A., M.H. Behboudian and B.E. Clothier. 2006. Responses of 'Petopride' processing tomato to partial rootzone drying at different phenological stages. Irrig. Sci. 24: 203-210.

Zegbe-Dominguez, J.A., M.H. Behboudian, A. Lang and B.E. Clothier. 2003. Deficit irrigation and partial rootzone drying maintain fruit dry mass and enhance fruit quality in 'Petopride' processing tomato (*Lycopersicon esculentum*, Mill.). Scientia Hort. 98: 505-510.

Zhang, S.Q. and W.J. Davies. 1989. Abscisic acid produced in dehydrating roots may enable the plant to measures the water status of the soil. Plant, Cell and Environ. 12: 73-81.

Zhang, S.Q. and W.J. Davies. 1990. Does ABA in the xylem control the rate of leaf growth in soil-dried maize and sunflower plants? J. Exp. Bot. 41: 765-772.

Zhang, S.Q. and W.H. Outlaw Jr. 2001. Abscisic acid introduced into the transpiration stream accumulates in the guard-cell apoplast and causes stomatal closure. Plant, Cell and Environ. 24: 1045-1054.

Zhang, J., U. Schurr and W.J. Davies. 1987. Control of stomatal behaviour by abscisic acid which apparently originates in the roots. J. Exp. Bot. 38: 1174-1181.