Non-hydraulic regulation of fruit growth in tomato plants (Lycopersicon esculentum cv. Solairo) growing in drying soil

Darren M. Mingo, Mark A. Bacon and William J. Davies
The Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK

Received 2 September 2002; Accepted 19 December 2002

Abstract
Tomato (Lycopersicon esculentum cv. Solairo) fruit growth, fruit mesocarp and leaf epidermal cell turgor, and fruit and leaf sub-epidermal apoplastic pH were monitored as plants were allowed to dry the soil in which they were rooted. Soil drying regimes involved splitting the root system of plants between two halves of a single pot separated by a solid impervious membrane to form a split-root system. Plants were then allowed to dry the soil in both halves of the pot (a soil-drying (SD) treatment) or water was supplied to one-half of the pot (a partial root-drying (PRD) treatment), allowing only one-half of the root system to dry the soil. A well-watered control treatment watered the soil on both halves of the pot. The rate of fruit growth was highly correlated with the soil water content of both sides of the SD treatment and the dry side of the PRD treatment. Soil drying caused a significant restriction in fruit growth rate, which was independent of any changes in the turgor of expanding fruit mesocarp cells in the PRD treatment. By supplying water to half of the root system, the turgors of mesocarp cells were maintained at values above those recorded in well-watered controls. The turgor of leaf epidermal cells exhibited a similar response. The pH of the sub-epidermal apoplastic compartment in leaves and fruit increased with soil drying. The dynamics of this increase in leaves and fruit were identical, suggesting free transport of this signal from shoot to fruit. Fruit growth rate and sub-epidermal pH within the fruit showed a strong correlation. The similarity of fruit growth response in the SD and PRD treatment, suggests that tomato plants respond to a discrete measure of soil water status and do not integrate measures to determine total soil water availability. The results of this study are not consistent with Lockhartian models of growth regulation in expanding fruit of a higher plant. A non-hydraulic, chemical-based signalling control of fruit growth in plants growing in drying soil is proposed.

Key words: ABA, chemical signalling, hydraulic regulation, pH, PRD, tomato, turgor.

Introduction
It has become clear that plants can respond to reduced soil water availability without experiencing any detectable change in shoot water relations (Gowing et al., 1990). Lockhartian models of growth regulation (Lockhart, 1965) have been shown not to hold in a variety of organs and cell types (Zhu and Boyer, 1992; Michelena and Boyer, 1982) and, although local water potential gradients may limit growth (Nonami and Boyer, 1993), demonstrations of sustained cellular turgors and significant restrictions in cell expansion (Michelena and Boyer, 1982; Termaat et al., 1985), make hydraulic explanations of such growth limitation difficult to sustain.

Several investigations have now shown that roots can sense the moisture status of the soil and respond by sending chemical signals to the shoot (Davies and Zhang, 1991; Jackson, 1993) to elicit several protective responses, including stomatal closure and a restriction in the rate of leaf expansion (Saab and Sharp, 1989; Zhang and Davies, 1989; Gowing et al., 1990), which minimize water loss. The basis of this communication and the mechanisms by which non-hydraulic regulation of leaf expansion and gas...
exchange occur, are increasingly well understood and focus on a central role for xylem-borne signals including abscisic acid (ABA) (Gowing et al., 1993), nutrients (Schurr et al., 1992), xylem sap pH (Wilkinson, 1999; Davies et al., 2002), and other plant growth regulators (Stoll et al., 2000). A pivotal role for ABA can confidently be suggested in the control of stomatal aperture and there is an increasing awareness of the sensitivity of this mechanism, which does not always require the presence of increased concentrations within the leaf to elicit stomatal closure (Wilkinson and Davies, 1997).

Similarly, evidence continues to accumulate, implicating physiological concentrations of ABA in the regulation of leaf expansion in plants growing in drying soil (Bacon et al., 1998), although other chemical regulators and interactions between them also undoubtedly play a role (Sharp et al., 2000; Sharp, 2002; Hansen and Grossmann, 2000). Surprisingly, the literature contains very few reports investigating the mechanistic regulation of fruit expansion, particularly in relation to soil water availability.

Advances in the understanding of leaf and root growth regulation in response to soil water availability will clearly inform the study of other plant organs, including fruit, and many of the mechanisms considered in these systems are likely to be important in fruit. Indeed, Thompson et al. (1998) have demonstrated that the rheological properties of the epidermis govern the rate of tomato fruit growth as they do in the expansion of root and leaf cells (Pritchard et al., 1993; Bacon et al., 1997). In vitro studies have also shown that the pH of the cell walls within the tomato fruit epidermis can modify cell wall extensibility (Thompson, 2001), with a clearly acidic optimum pH of 5.0. In observing the removal of this response by boiling epidermal strips prior to treatment, a clear similarity can be drawn between these observations and those reporting a key role for pH-sensitive cell wall expansins in regulating expansive growth in plant hypocotyls and leaves (McQueen-Mason, 1995). While enzymes such as expansins and several other cell wall regulating enzymes are known to elicit powerful controlling effects on cell expansion, only a few candidate activities have been identified in tomato fruit epidermis (Andrews et al., 2000; Fry et al., 1992), isolated and demonstrated to govern fruit growth rate (Thompson et al., 1998).

Several root-borne signals within the xylem sap have been implicated in the regulation of cell wall activities and the pH of the xylem sap has, itself, been shown to act as a powerful growth regulator via its ability to govern the distribution of ABA between internal leaf compartments (Wilkinson and Davies, 1997). While a role for these signals in regulating leaf expansion is increasingly well understood, little evidence exists to suggest a role in regulating fruit growth as water supply becomes limiting. In tomato fruit, it may be speculated, however, that signals borne within the xylem will have little effect on fruit expansion if the existence of limited xylem connections between the shoot and the tomato fruit is to be believed (Ho et al., 1987; Davies et al., 2000; Malone and Andrews, 2001). In such circumstances, it is difficult to imagine how changes in soil water status will be transmitted to growing fruit cells which are relatively isolated both chemically and hydraulically from the rest of the plant. However, previous investigations have shown that soil drying and even watering tomato plants in such a way that only part of the root system is allowed to dry (PRD – partial root drying), can limit fruit expansion rate (Davies et al., 2000) and the final size of the fruit. This investigation tests the hypothesis that such regulation is not provided by measurable changes in the turgors of those cells governing the rate of fruit expansion. In doing so the investigation provides compelling evidence that chemical signals, travelling from the root to the shoot and freely into the fruit, regulate fruit cell expansion in plants growing in drying soil.

**Materials and methods**

**Plant material and growth conditions**

Tomato seeds, *Lycopersicon esculentum*, cv Solairo (Pinetree DeRuiter seeds Ltd., Southampton, UK) were germinated in a growth cabinet in compost (Levington M3; Levington Horticulture Ltd., Ipswich, UK) under artificial lighting provided by 6×400 W halide lamps, 200 μmol m⁻² s⁻¹, on for 12 h, with a day/night temperature of 25/18 °C. Humidity was uncontrolled but was around 60–80% when the resulting seedlings were illuminated. Fourteen days after germination plants were transplanted into 100 mm diameter pots filled with compost (John Innes No. 2; J Arthur Bower’s, Lincoln, UK) and at this stage the seedlings were approximately 50 mm in height and the first set of true leaves was developing above the cotyledons. After a further 14 d the plants were transplanted into John Innes No. 2 compost in 5.0 l pots split into two compartments by a plastic dividing wall sealed into the pot. During this transplantation the roots, approximately 250–300 mm in length, were washed free of soil and split equally between the two sides of the split pot system. Plants were then placed back into the growth cabinet, under conditions defined above, and allowed to establish. Once the first truss had flowered, the plant apex was removed, as were the side shoots as they appeared, in order to generate a mature, single stemmed plant with a single fruiting truss.

The fruit used in the experiments ranged between 21–28 d post-anthesis, and between 25–40 mm in diameter.

**Soil drying experiments**

Single fruiting plants were transferred to an open-fronted controlled environment cabinet (Thompson et al., 1999) and left to acclimatize for 2 d. Lighting in the cabinet was providing by 2×240 W halide lamps delivering 200 μmol m⁻² s⁻¹, for 12 h daily. The day/night air temperature was controlled at 25/18 °C (± 1 °C).

Each pot was placed into a plastic bag to reduce direct water loss from the soil surface, but loosely fitted to provide adequate aeration of the root system. Plants were watered daily, approximately 5–6 h into the light period, after all measurement had been taken. ‘Well-watered’ plants (WW) received 400 cm² d⁻¹ of water, distributed equally between the two halves of the pot. Plants forming the soil-drying (SD) treatment had water withheld from the start of the experiment. Plants with partially drying roots (PRD) received 200
cm³ to one side of the pot, while the other side was allowed to dry for the duration of the experiment. For each plant the experimental period lasted for a total of 6 d, this included day zero for pretreatment measurements. No additional fertilization was applied to that already present in the growing medium during the experiment.

Soil moisture content within the two halves of the pots was measured daily using a Theta probe (HH2 Moisture Meter with Theta Probe type ML2X; Delta-T Devices, Cambridge, UK). Three measurements of soil moisture content were taken daily before the onset of the light period. The probe readings were calibrated to provide a measure of volumetric soil water content (VSWC).

**Cellular turgor measurements**

Fruit cell turgor (P) measurements were made using a micro-pressure probe based on the design of Murphy and Smith (1994). Fruit cell turgors were obtained by probing the mesocarp cells directly under the fruit epidermis. Turgor measurements were made 3 h into the light period. Estimates of fruit growth rate were made by measuring fruit diameter daily, using digital vernier callipers (Mitutoyo digital callipers series 500; Mitutoyo UK Ltd, Andover, UK).

**Sub-epidermal pH measurements**

Measurements of sub-epidermal apoplastic pH of fruit and abaxial leaf surface were taken daily, approximately 4 h into the light period. Using a scalpel blade, an incision of approximately 3 mm in length was made into the epidermal surface of the tissue. Using a pair of fine tweezers, the cut edge was carefully pulled away from the fruit and abaxial leaf surface to expose a section of apoplast, approximately 10–15 mm². A flat-tipped pH electrode of 3 mm diameter (PHR-146FSB Micro-combination pH electrode; Lazar Research Laboratories Inc, Los Angeles, CA, USA) was quickly placed directly onto the exposed apoplastic surface and the initial pH of the surface determined. After each individual measurement the exposed apoplastic surface was covered with a fine layer of petroleum jelly.

The growth rate of fruit was not significantly changed by measurement of cellular turgor or by epidermal pH measurements.

**Results**

**Soil-drying treatments**

During the soil-drying treatments imposed by the two watering regimes (SD and PRD), the rates of decline in soil water content in the side of the pot allowed to dry in the PRD treatment and that of the soil on both sides of the SD treatment are comparable (Fig. 1a). Initial VSWC declined from c. 35% to c.10% by the end of the experiment. Near constant VSWCs (c. 35%) were recorded on both sides of the well-watered (WW) treatment and the well-watered side of the PRD treatment.

**Fruit growth rate**

Soil drying treatments restricted rates of fruit expansion within 3 d of the start of the experiment (Fig. 1b). Initial fruit growth rates of approximately 0.5–0.7 mm d⁻¹ were reduced to barely detectable levels of expansion within 5 d (0.2 mm d⁻¹), while the rate of growth of fruit on WW plants continued at the initial rate.

The significant decline in fruit expansion rate in SD and PRD treatments limited the size of the fruit by the end of the experiment by c. 35–40%. The decline in fruit growth rate in the SD and PRD treatments was strongly correlated with the soil water content of both or the drying side of the pot, respectively (Fig. 2).

**Fruit mesocarp cell turgor**

Fruit cell P remained constant in the well-watered and PRD treatment for the entire experiment, with values of c. 0.15 MPa for a WW plant and slightly higher values of c. 0.18 MPa for a PRD plant (Fig. 1c). The fruit turgor of plants exposed to the SD treatment showed a dramatic decline from c. 0.15 MPa to 0.08 MPa between days 1 and 2. After the initial decline, P remained relatively constant at c. 0.08 MPa.

**Fruit sub-epidermal pH**

The sub-epidermal apoplastic pH of WW fruit remained significantly unchanged during the course of the experiment at a pH of c. 5.7 (Fig. 1d). A clear increase in
apoplastic pH was recorded 1 d after the onset of the SD treatment and 3 d after the onset of the PRD treatment. The pH of the apoplast rose from c. 5.7 to c. 6.1 within 1 d of the SD treatment, reaching a maximum of c. 6.4 after 5 d of the SD treatment. A delayed increase in apoplastic pH observed in the PRD treatment from 5.7 on day 2 to c. 6.4 on day 3, reached a maximum of c. 6.5 by day 4 of the experiment.

**The relationship between soil water content, fruit turgor and growth rate**

While fruit growth rate in both SD and PRD treatments showed a clear correlation with soil water content of the drying soil compartment (Fig. 2), this was not the case for fruit cell turgor (Fig. 3). Fruit cell turgors of plants given the SD treatment declined sharply (from c. 0.16 to 0.08 MPa) in response to a fall in VSWC from 30% to 25% and then remained constant at c. 0.08 MPa over a range of VSWC between 25% to 10%. Fruit cell turgor of PRD plants remained nearly constant at 0.17 MPa over a range of VSWC from 35% to 10%, a range spanning from wet to dry soil.

The disparity between fruit turgor changes in SD and PRD plants resulted in clear differences in the potential correlation between the fruit growth rates and cellular turgors measured (Fig. 4). While a potential relationship between cell turgor and growth rate was observable in SD plants, the fruit of PRD plants exhibited a range of growth rates (c. 0.6–0.2 mm d⁻¹) at near constant turgor (0.18 MPa).

**Relationship between leaf and fruit sub-epidermal pH and fruit growth rate**

The increase in fruit apoplastic pH in PRD plants was mirrored by similar increases in sub-epidermal pH within the leaves (Fig. 5). The increasing pH of the sub-epidermal apoplast was related with the decline in fruit growth rate recorded (Fig. 6), \( r^2=0.416, P<0.01 \).

**Discussion**

The results demonstrate that the restriction in tomato fruit growth observed in plants growing in drying soil is not necessarily due to changes in the turgor of those cells within the fruit that are actively expanding. While the artificial splitting of roots delivered sufficient water to the shoot to sustain favourable fruit water relations, a near
complete restriction in fruit cell expansion was observed as soil dried around some of the roots. While fruit growth rates are clearly responding to changes in soil moisture status, irrespective of the way in which plants are allowed to dry the soil, the lack of a relationship between cellular turgor and fruit growth rate in the PRD treatment provides compelling evidence that fruit growth is not always regulated by hydraulic changes in the fruit during soil drying. Although this is the first time that non-hydraulic regulation of growth has been shown in fruit, recent work has reconfirmed this on both a whole plant (Lovisolo et al., 2002) and also at the leaf level (Munns et al., 2000; Passioura and Munns, 2000). Figure 6 provides circumstantial evidence to suggest that chemical signals such as pH may have a key regulatory role in controlling fruit cell expansion during water deficit, in the absence of any change in measured turgor pressure. In doing so, this observation also raises the possibility that relationships observed between turgor and growth in this investigation and elsewhere (Matthews et al., 1984) may be correlatory and not necessarily causal.

The supply of water to one-half of the root system clearly sustained fruit turgor values close to those recorded in WW controls. The rationale for this supply of water is to sustain the water potential of the expanding cells and hence the turgor pressure required to drive expansion. It is not possible to conclude whether supply of water alone would sustain water potential. A degree of osmotic adjustment in the fruit cells of PRD plants may also account for the turgor maintenance measured. It would not, however, explain the restriction in fruit growth rate, unless precursor substrates for cell wall anabolism were being redirected to provide compatible solutes for the expanding fruit cells.

The observed similarity in the response to soil drying of fruit growth in SD and PRD plants suggests that the plants responded to a discrete measure of soil water status, rather than some integrated measure of the total soil water available. Indeed, even though PRD plants received sufficient water to sustain comparable turgors to WW controls and water was freely available within the watered half of the pot, fruit growth rates still declined at the same rate and to the same extent as those fruit of plants in the SD treatment. A fruit growth response based on some form of integrated measure of soil water content would presumably lie somewhere between the two responses observed in the WW and SD treatment. One may speculate that the perception of the lowest soil water potential around the roots, without any integration by the plant of soil water availability, may provide a plant with a sensitive, fairly conservative and rapid means by which to signal to the shoot to avoid excessive water loss as soil water content declines. This would contrast with the conclusions of Tardieu et al. (1992) who suggest that a plant ‘measures’
the access that roots have to soil water rather than soil water potential per se.

It is interesting to note that the turgors of fruit cells in the PRD treatment are marginally higher than those in the WW treatment (c. 0.04 MPa). Although no measurements were made directly in this investigation, observations in similar investigations have shown that exposure to drying soil via a PRD system will promote stomatal closure. Closure of stomata within the leaf and also potentially on the fruit (Hetherington et al., 1998) will reduce water loss resulting in this marginal increase in cellular turgor. Reduced water loss from the PRD plants will reduce the rate at which soil water is extracted, presumably explaining why soil in the whole pot of the SD treatment and half the pot of the PRD treatment dry at approximately the same rate.

The significant increase in sub-epidermal apoplastic pH in both the leaves and fruit of plants in the SD and PRD treatment and the present understanding of the role of pH in regulating cell expansion via its effects on cell wall enzyme activities and the distribution of ABA at the active site for growth regulation, provide good evidence of some form of chemical regulation of fruit expansion rate. While the range of pH values recorded lies close to those used elsewhere to elicit in vitro changes in cell wall extensibility (Thompson, 2001), the recorded values are also very similar to those in the xylem sap of tomato plants exposed to soil drying (Wilkinson et al., 1997). In this investigation, the authors measured pH values between pH 5.0 and 8.0, pH values in this range were shown to have significant effects on the access of ABA to the active sites for stomatal regulation within the leaf. Similar observations suggested that this was also the case for leaf growth regulation (Bacon et al., 1998). It is therefore tempting to suggest that the increases in sub-epidermal pH recorded in both leaves and fruit, would cause the redistribution of ABA towards the active site for growth restriction. Significant apoplastic pH increases in both the SD and PRD treatments strongly suggest that changes in water relations observed in the SD treatment are not causally related to the decline in fruit growth rate, as this was strikingly similar for the two treatments.

If the pH signal proposed elsewhere (Wilkinson and Davies, 1997) does in fact travel from root to shoot, the observed dynamics of pH increase in both leaf and fruit sub-epidermal apoplast (Fig. 5) provides a strong suggestion that there is free penetration of such signals from shoot to fruit. The hydraulic isolation of tomato fruit observed by Ho et al. (1987) and others (Davies et al., 2002; Malone and Andrews, 2001) should potentially restrict the free movement of signals from shoot to root. Ho et al. (1987) estimated that at least 90% of the total water content of a tomato fruit arrives via the phloem, with a diminishing supply being provided via the xylem. However, Ohta et al. (1997) have shown that xylem water flux into fruit varies significantly between cultivars. In addition, Davies et al. (2002) suggested that the extent of the hydraulic connection between fruit and the vegetative plant may depend on how the plant is grown. Water deficit and flooding of the soil can modify the hydraulic conductivity of the xylem connection through the pedicel and, presumably therefore, modify the penetration of chemical signals to the fruit.

Conclusions

This investigation demonstrates that restrictions in fruit growth rate in plants growing in drying soil can occur in the absence of any changes in fruit cellular turgor. It is suggested that signals borne within the xylem can travel from root-to-shoot and shoot-to-fruit to elicit a powerful regulatory effect on fruit cell expansion.

As well as providing an increase in the fundamental understanding of growth regulation under water deficit, the findings presented here also have real potential application in commercial horticulture and agriculture. The PRD technique has been adapted for use in the irrigated production of grapes, cereals, citrus, olives, and field vegetables (Dry and Loveys, 1999; Dry et al., 2001). Water is applied to different sides of the rooted crop at regular intervals to sustain the entire root mass, while allowing proportions of the roots to dry the soil. In this way, as in the experimental technique, plant water relations remain favourable. While considerable agronomic expertise is being developed, little work has been conducted to understand how fruit and leaf growth are regulated when irrigated under PRD. The investigation reported here is the first systematic analysis of this growth regulation in the context of this emerging and potentially significant agronomic technology.

One clear advantage of PRD over other forms of deficit irrigation is its ability to sustain cell turgor and prevent the shrinking and swelling of fruit that can occur when fruit turgors fluctuate during development. Rapid shrinking and swelling may cause fruit to crack and severely impair yield quality (Ohta et al., 1997). In crops where yields are maintained, it is suggested that those signals, which limit vegetative growth (often to the benefit of reproductive growth), do not penetrate into the fruit. This would appear to be the case in grape vines, in which PRD has been shown to deliver significant savings in irrigation water, quantifiable increases in grape quality and the wine produced from such grapes, without a negative effect on yield (Stoll et al., 2000). The suggestion that growth-restricting signals in such a crop do not penetrate the fruit is supported by the observation of Shackel et al. (1987) that hydraulic connections between the grape berry and plant are significantly reduced after veraison.

Several, now classic pieces of work on single-celled algae and leaf cells, stand in the literature as clear examples of data that are not consistent with the...
Lockhartian growth model (Michelena and Boyer, 1982; Zhu and Boyer, 1992; Boyer, 1993). The observations presented in this paper provide a significant contribution to this body of evidence.

Acknowledgements

We thank Dr Stuart Thompson (University of Westminster) for his tuition and useful discussions on the use and application of the pressure probe. This work was funded by the European Union INCO-MED IRRISPLIT RTD project (ICA3-CT-1999-00008).

References


Dry PR, Loveys BR. 1999. Grapevine shoot growth and stomatal conductance are reduced when part of the root system is dried. *Vitis* 38, 151–156.


