MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

Date project completed:

Research and Development

Final Project Report

(Not to be used for LINK projects)

Section 1 : Identification sheet

1. (a) MAFF Project Code	HH 1307 TPC	
(b) Project Title	Regulation of tomato fruit growth	
(c) MAFF Project Officer	Dr. Bruce Pearce	
(d) Name and address of contractor	Horticulture Research International, Wellesbourne, Warwick Postcode CV35 9EF	
(e) Contractor's Project Officer	Dr. L.C. Ho	
(f) Project start date	January 1995	Project end date: January 1998
(g) Final year costs:	**approved** expenditure	£54081
	actual expenditure	£106832
(h) Total project costs/total staff input	**approved** project	£211012
	actual project expenditure	£218036
	***approved** staff input	
	***actual** staff input	
(i) Date report sent to MAFF	21/07/98	
(j) Is there any Intellectual Property arising from this project?		NO

***staff years of direct science effort**

Section 2 : Scientific objectives / Milestones

2. Please list the scientific objectives as set out in CSG 7 (ROAME B). If necessary these can be expressed in an abbreviated form. Indicate where amendments have been agreed with the MAFF Project Officer, giving the date of amendment.

1. To define the relationship between cellular water relations (i.e. turgor and solute potential) and cell wall properties during fruit growth.
2. To assess the role of cell wall bound enzymes in relation to fruit expansion.
3. To investigate the effects of assimilate supply and plant water relations on cell expansion during fruit growth.
4. To determine how manipulation of whole-plant water relations affects fruit production and fruit growth rate.
5. Extend from CE to glasshouse in test experiments.
6. Relate seasonal variation in final fruit size to cellular water relations and cell wall properties

3 List the primary milestones for the final year.

It is the responsibility of the contractor to check fully that ALL primary milestones have been met and to provide a detailed explanation if this has not proved possible

Milestones		Target date	Milestones met?	
Number	Title		In full	on time
03/01	As for 02/1 but with manipulation of fruit temperature, fruit water status or fruit carbon availability in 3 separated experiments	March 97	Yes	Experiments completed May 97.
03/02	(1) Further quantify the effect of assimilate supply (leaf/fruit ratio, light level), plant water relations (watering, EC), and fruit temperature on the turgor in relation to fruit growth. (2) Characterise the tissue elasticity of the epidermal tissue and the possible role of peroxidase and growth regulators on the tissue strength. (3) The effect of temperature on wall bound peroxidase activity in the epidermis and on fruit growth rate in both lab and CE. (4) Characterisation of the protein of the peroxidase.	October 97	Yes	All except CE section of (3)
03/03	Write up the work for publication.	April 98	Yes	Yes

If any milestones have not been met in the final year, an explanation should be included in Section 5.

Section 3 : Declaration

4. I declare that the information I have given in this report is correct to the best of my knowledge and belief. I understand that the information contained in this form may be held on a computer system.

Signature | Date

Name: Professor I R Crute

Position in Organisation: Site Director

Section 4 : Executive Summary

A three-year collaborative project between HRI and the University of Lancaster has investigated the mechanism and regulation of tomato fruit expansion, and has developed a conceptual model of fruit growth regulation. The model has already indicated new ways in which fruit growth might be artificially regulated. Greater understanding of the intrinsic and environmental factors which regulate fruit expansion will permit growers more consistently to produce fruit of the highest quality. The rate of fruit growth is important for quality since, if it becomes excessive even transiently, there is an increased risk of disorders such as BER and surface cracking. The extent of fruit growth (i.e. the rate x duration) determines whether the fruit will make the premium size class (typically 45-55 mm diameter for round tomatoes).

A range of biochemical and water-relations approaches have been adapted from work on the mechanism of growth in stems, and applied to the growing fruit. By definition, growth of a plant organ occurs when the internal pressures tending to drive expansion exceed the restraining strength of cell walls within the tissue. Work under this project has demonstrated that these opposing forces are not distributed evenly throughout the tomato fruit; much of the restraining strength of the entire fruit is concentrated in the outermost layer, and most of the expansive forces arising from turgor pressure of cells in the pericarp is exported to this outer skin. Further investigations have consequently focused on the properties of the fruit skin.

The tomato fruit is 95% water and growth thus requires a continuous supply of water from the shoot. Work under this project has confirmed an apparent hydraulic restriction in the pathway of water flow into the fruit, which appears to change with environmental conditions (e.g. salinity) and with fruit development. The mechanism and location of this restriction is not clear, but it could have a major influence on fruit growth. Turgor pressure was found usually to be constant throughout the pericarp, or to change only slowly with fruit age and with environmental conditions. This means that both the rate and the extent of fruit growth are controlled almost entirely by the factors which determine mechanical properties of the skin.

Various techniques for measuring mechanical properties in the isolated skin were assessed, and one (the “creep” test) was found to reflect closely the properties of the fruit *in vivo*. Mechanical properties of plant tissues are thought to depend on the activities of a small number of enzymes which increase (e.g. expansin, XET) or decrease (e.g. peroxidase) the extensibility of the cell wall. Several of these enzymes were monitored during the project. Peroxidase activity in the skin was found to increase markedly at around the time fruit growth ceases, suggesting that it may play a role in determining the final size of the fruit. In particular, a number of new peroxidase isozymes were shown to appear within the cell walls, coincident with the cessation of fruit growth. These isozymes may catalyse cross-linkages within the wall which strengthen and rigidify it, and thus prevent further fruit expansion. These isozymes may provide a new opportunity for manipulating final fruit size, for example, by artificially inducing them at a specific growth stage. Further exploration of these possibilities is scheduled under a new MAFF contract (HH1323). The model of fruit growth developed here should also be applicable to comparable crops such as pepper and cucumber.

Section 5 : Scientific report

Previous work on plant stems has shown that rate of expansion of plant organs is determined by interactions between the wall properties and the turgor pressures of the constituent cells. The aims of this project were therefore to measure water relations, cell wall properties, and cell wall enzymes of the growing tomato fruit in order to analyse the mechanisms and regulation of its growth. This work has made significant advances in a number of areas.

1) Tissue Pressure.
In preliminary experiments it was noted that the epidermis of tomato fruit appeared to be under tension throughout fruit development, suggesting that "tissue pressure" (also referred to as "tissue tension") was present. In many plant organs the presence of tissue pressure can indicate that growth of the whole organ is restricted (and therefore probably regulated) mainly by one layer of tissue, usually the epidermis. Subsequently, an adaptation of previously published methods of determining tissue pressure was developed which allowed us to confirm and quantify tissue pressure within the tomato fruit (Thompson *et al.* 1998).

2) Cell wall enzymes
Following the observations described above, our investigation of those cell-wall enzymes most likely to influence mechanical properties has focused on the epidermis, and on the differences between enzyme activities in the epidermis and pericarp. Two key enzymes were examined in detail:

a). The putative wall-loosening enzyme, xyloglucan-endo-transglycosylase (XET) was found to be present at high levels in both the pericarp and epidermis of the fruit. Both epidermal and pericarp XET activities decreased as fruit growth rate declined towards maturity. An excellent correlation between the rate of fruit growth and epidermal XET activity was found (Fig. 1).

b). A peroxidase activity bound to the cell wall was also studied. This was confined to the epidermis, and it appeared only from around 40 - 45 d after anthesis, corresponding to the time at which fruit growth ceased (Fig. 2). Cell wall peroxidases are thought to be involved in stiffening of the cell wall, and may thus retard or terminate growth. It seems likely that the observed peroxidase is involved in growth termination in tomato fruit. This epidermal peroxidase has now been further characterised, as proposed in milestone 03/02 part(4), using native gel electrophoresis. Three peroxidase isozymes have been shown to appear in the wall-bound fraction of the epidermis at the time of termination of fruit growth (Fig. 3). These isozymes are absent from walls of the pericarp throughout development; they appear to provide a marker for the cessation of fruit growth. Iso-electric focusing has shown the pI of the isozymes to be around 4.6. This is similar to that of acidic peroxidases linked to cell-wall stiffening in tomato cell cultures[4]. It is evident from Fig. 3 that some of the isozymes associated with growth termination also appear in a fraction which is bound loosely to the wall (i.e. the low-salt extract). We have investigated whether the affinity of these isozymes for the cell wall alters during fruit development, and the affinity assay procedure may itself be of use in further purification and characterisation of the enzyme.

Associated work in Prof. Davies' group at Lancaster has found similar peroxidases to be associated with growth retardation and termination in grass leaves (Bacon *et al.* 1997). We are therefore able to suggest a mechanistic framework to explain the pattern of fruit growth throughout development: the rate of fruit growth is determined by epidermal XET activity until the fruit approach maturity. Growth is then terminated by the relatively sudden appearance in the epidermal cell walls of a distinctive group of peroxidase isozymes.

Manipulation of the levels of the peroxidase isozymes and of XET by transgenic or other means offers a novel approach to achieve greater control over the rate and extent of fruit growth. Fruit growth rate and size are important quality parameters for the horticultural industry. These enzymes may also control epidermal mechamical properties which are important for defects such as cracking.

3) Epidermal stress and physical properties.
Fruit turgor pressure (Fig. 4), cell sap osmotic pressure and thus fruit water potential were measured throughout development in a number of plants. The results show a steady fall in fruit turgor pressure throughout development. It is possible that this turgor decline contributes to the deceleration and final cessation of fruit growth, but estimations of the stress exerted on the epidermis (based upon fruit dimensions and architecture) suggest that this is not a primary factor.

[Continued on 5 additional sheets].

Experiments using a 'creep' extensiometer were employed to investigate the material properties of fruit epidermis (milestone 03/02). No consistent relationship was found between the growth rate of the fruit, and the elasticity or plasticity of the skin. However, the rate of prolonged slow stretching (creep) correlated well with the rate of growth. The creep rate of epidermal strips from fruit which had ceased to grow was consistently lower than that from growing fruit, provided that the load per strip was > 30g. This behaviour is consistent with the observation that a minimum turgor is required for growth in most plant organs *in vivo*. The threshold weight also appears consistent with the observed threshold turgor required for growth of tomato fruit, although it is notoriously difficult to compare the effects of linear and multidimensional stresses in plant tissues.
Cell wall acidification is thought to mediate regulation of growth of many plant organs ("acid growth"), and bathing epidermal strips in buffers of lower pH was found to increase the creep rate, suggesting that regulation of wall pH may also be a component of growth regulation in tomato fruit. The creep rate was also increased in strips bathed in ascorbate solution. Since ascorbate is an inhibitor of many peroxidases, this supports our hypothesis that peroxidases are involved in cell wall stiffening.

4) Environmental effects on fruit turgor and growth.
The effects of a number of environmental factors on fruit growth and turgor pressure have been assessed.

a). Fruit turgor and growth were observed during the light and dark periods of a diurnal cycle whilst the aerial parts of the plant were maintained at a constant temperature of 18 °C. No effect on growth or fruit turgor was observed (Fig 5) supporting the suggestion of Pearce, Grange and Hardwick[3] that the diurnal variations of growth rate they observed could arise largely from changes in temperature.

b). Fruit turgor and growth rate has been measured while raising the temperature from 19°C to 29°C (revised milestone 03/01). Such treatment caused fruit turgor to decrease rapidly by 0.02 MPa. Although this decrease is small, it represents a significant proportion of the total fruit turgor during later development; turgor may decline to 0.1 MPa by the time growth ceases. The rate of fruit growth increased at higher temperatures but this effect was smaller than typically observed in other plant organs. The Q_{10} is for growth rate is often about 2 in plant tissues, but in tomato fruit Q_{10} was 1.8 between 15 °C and 20 °C, and it decreased progressively towards higher temperatures (1.15 from 18 °C to 28 °C; 1.0 from 26 °C to 31 °C; see Fig. 6). These changes correspond with progressively greater reductions in fruit turgor pressure at higher temperatures. It is also reported that the duration of fruit expansion in tomato is shorter at higher temperature[1]. Taken together, these factors explain why final fruit size is reduced at higher temperatures. A slight turgor increase was observed in the leaves after the temperature was increased from 18°C to 28°C, which probably reflects stomatal closure, potentially reducing the rate of carbon assimilation.

c). We have verified earlier reports[2] that the tomato fruit has restricted hydraulic connections to the shoot. During droughting of rooted side-shoots, the fruit water potential and turgor were unaffected until the leaf water potential fell below that of the fruit (Fig. 7). Interestingly, in plants grown from seed in special enclosed pots designed to allow pressurisation of the roots, the hydraulic isolation of the fruit appeared virtually complete in root pressurisation experiments although leaf water relations were affected in the expected manner. Such an experiment is shown in Fig 8. In other experiments, up to 0.29 MPa was applied to the roots with no effect on fruit growth rate or turgor pressure. This isolation was also apparent during soil drying episodes, with fruit growth continuing in such plants even after the foliage had been completely wilted for two days. Interestingly an increased (though still limited) hydraulic connection between the fruit and the remainder of the plant could be induced in these plants by a mild soil drying episode early in fruit development. Limited observations suggest that such fruit were also less prone to BER. We have not established whether the hydraulic conductivity is increased as a direct result of soil drying or was caused by some other factor associated with this treatment such as the reduction in plant size, or the reduction in oxygen availability to the roots. It is possible that further investigation along these lines would identify new approaches to regulation of the balance of xylem and phloem transport, perhaps leading to increased control over fruit properties and quality in horticulture.

Experiments investigating longer-term effects of elevated temperatures in controlled environment (intended to fulfil part (3) of 03/02) were aborted because of a major failure of CE equipment, and because a high proportion of the fruit had BER. Preparations are underway to repeat these experiments using an NFT system to ensure more consistent supplies of water and nutrients.

Key aspects of these findings have been published in the form of scientific papers, listed below.

References:

1. De Koning A.N.M. (1994) Development and dry matter distribution in glasshouse tomata. PhD thesis, Wageningen Agriculture University.
2. Ehret D.L. and Ho LC (1986) J. Expt. Bot. 37, 1294-1302.
3. Pearce B.D., Grange R.I. and Hardwick K. (1993) J. Hort. Sci. 68: 1-11.
4. Schnabelrauch *et al.* (1996) The Plant Journal 9, 477-489.

Publications:

D.S. Thompson, W.J. Davies & L.C. Ho. (1998) Tomato fruit growth regulation by epidermal cell wall enzymes. *Plant, Cell and Environment* (in press).

W.J. Davies, D.S. Thompson & J.E. Taylor. (1998) Manipulation of growth of horticultural crops under environmental stress. Proceedings HRI Conference on Genetic and Environmental Manipulation of Horticultural Crops, 1997 (ed. K. Cockshull), CAB (in press).

D.S. Thompson, P.W. Smith, W.J. Davies & L.C. Ho. (1998) Interactions between environment, fruit water relations and fruit growth*Acta Horticulturae* (in press).

M.A. Bacon, D.S. Thompson & W.J. Davies (1997) Can cell wall peroxidase activity explain the leaf growth response of *Lolium temulentum* L. during drought ? *Journal of Experimental Botany* 48: 2075-2085.

Presentations were also made at four international conferences.

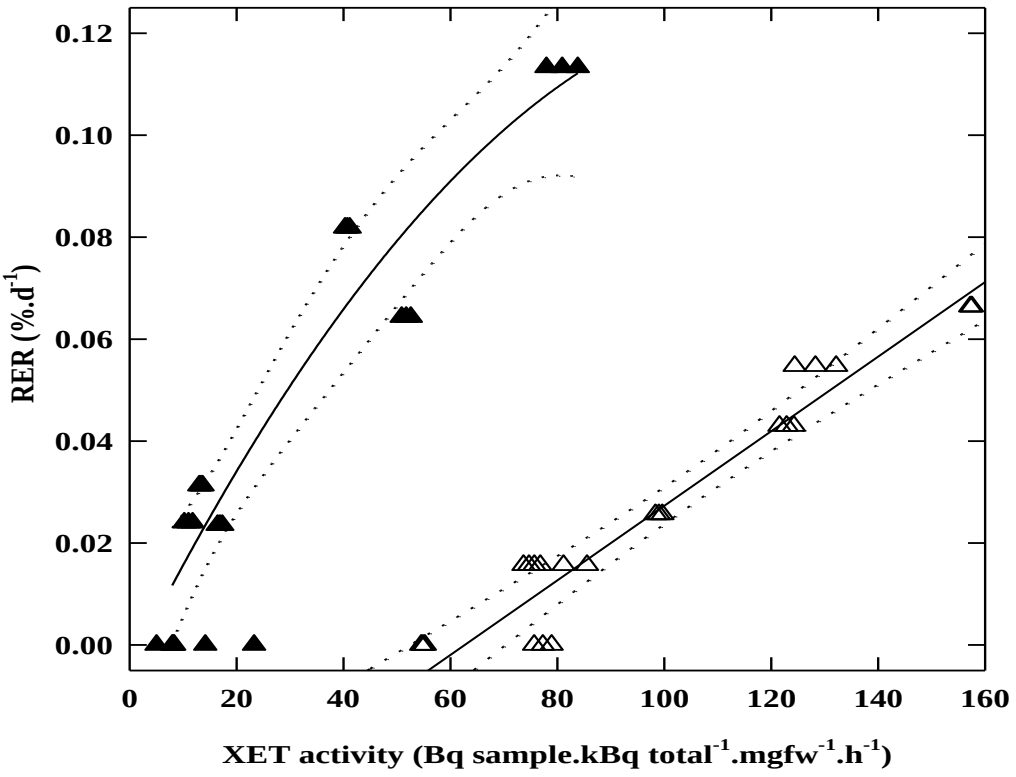

Fig 1. The relationship between the fruit growth (RER =relative rate of growth in surface area), and RER and XET activities in the epidermis (∆) and pericarp (▲). Duplicate measurements are shown. The solid lines are fitted by the method of least squares, first order for the epidermal data, and second order for the pericarp. The dotted lines represent 95% confidence limits.

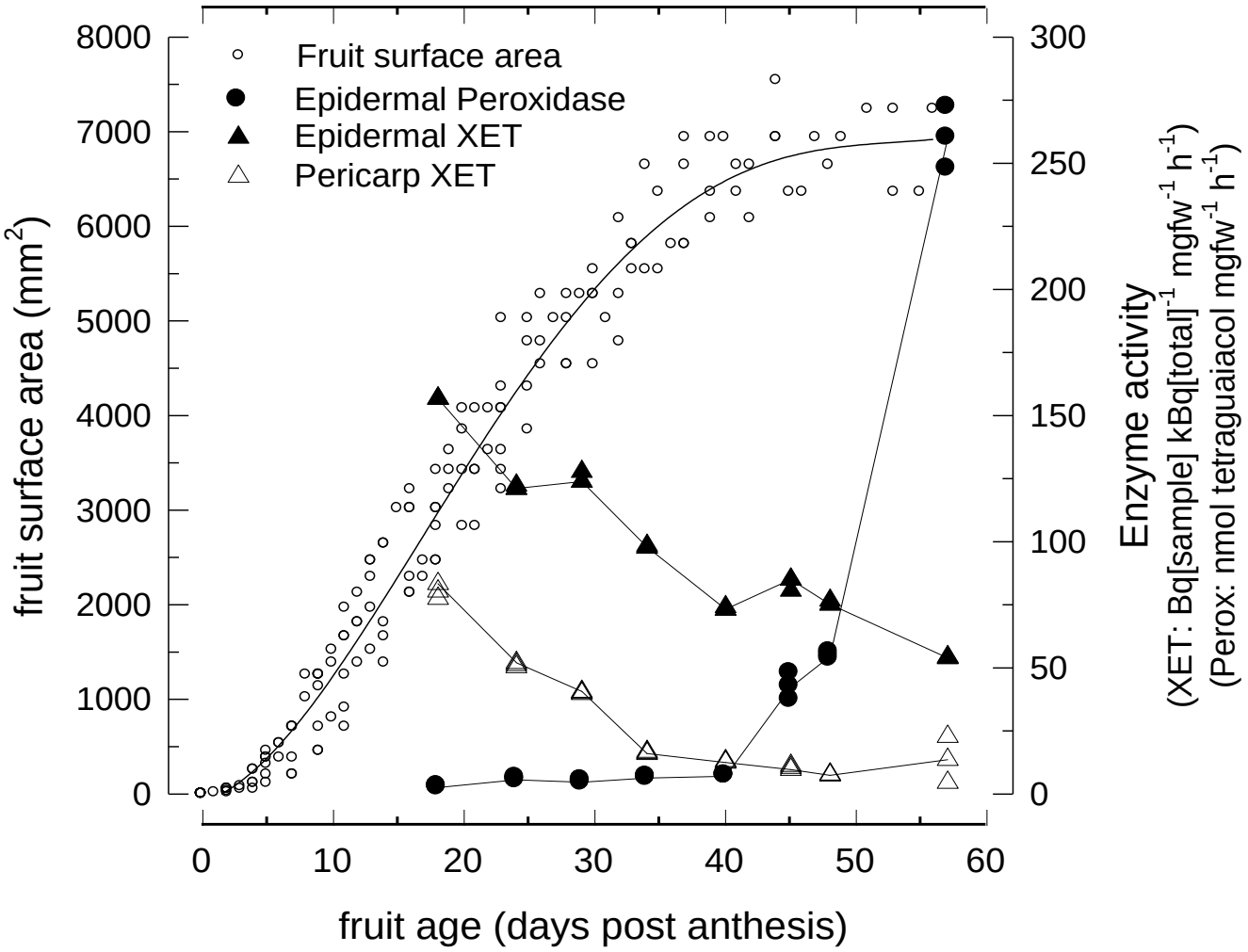

Fig. 2. Correlation between cessation of fruit growth (here shown as surface area) and the increase in epidermal peroxidase activity. Activity of XET in the pericarp and epidermis is also shown for comparison.

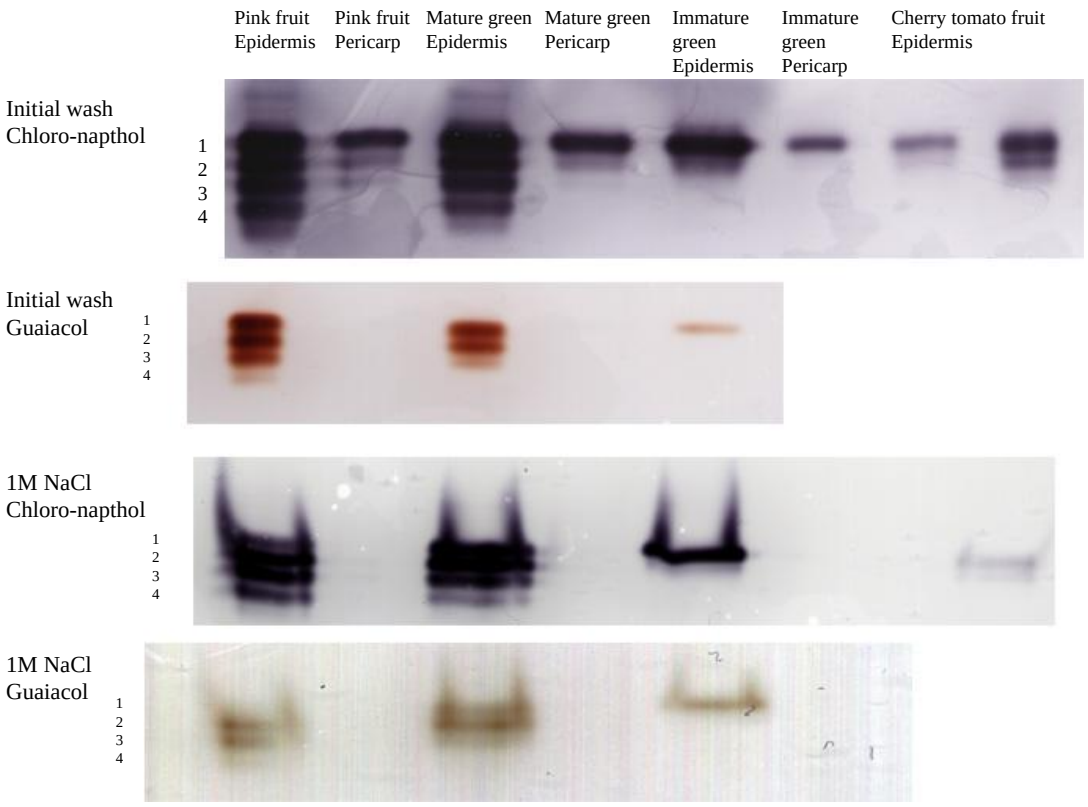

Fig 3. Gel electrophoresis of soluble and wall-bound peroxidase isozymes from fruit of various developmental stages and types. Soluble ("initial") and wall-associated ("1M NaCl") fractions are

shown separately. Specificity for two substrates (chloronaphthol & guaiacol) is also compared.

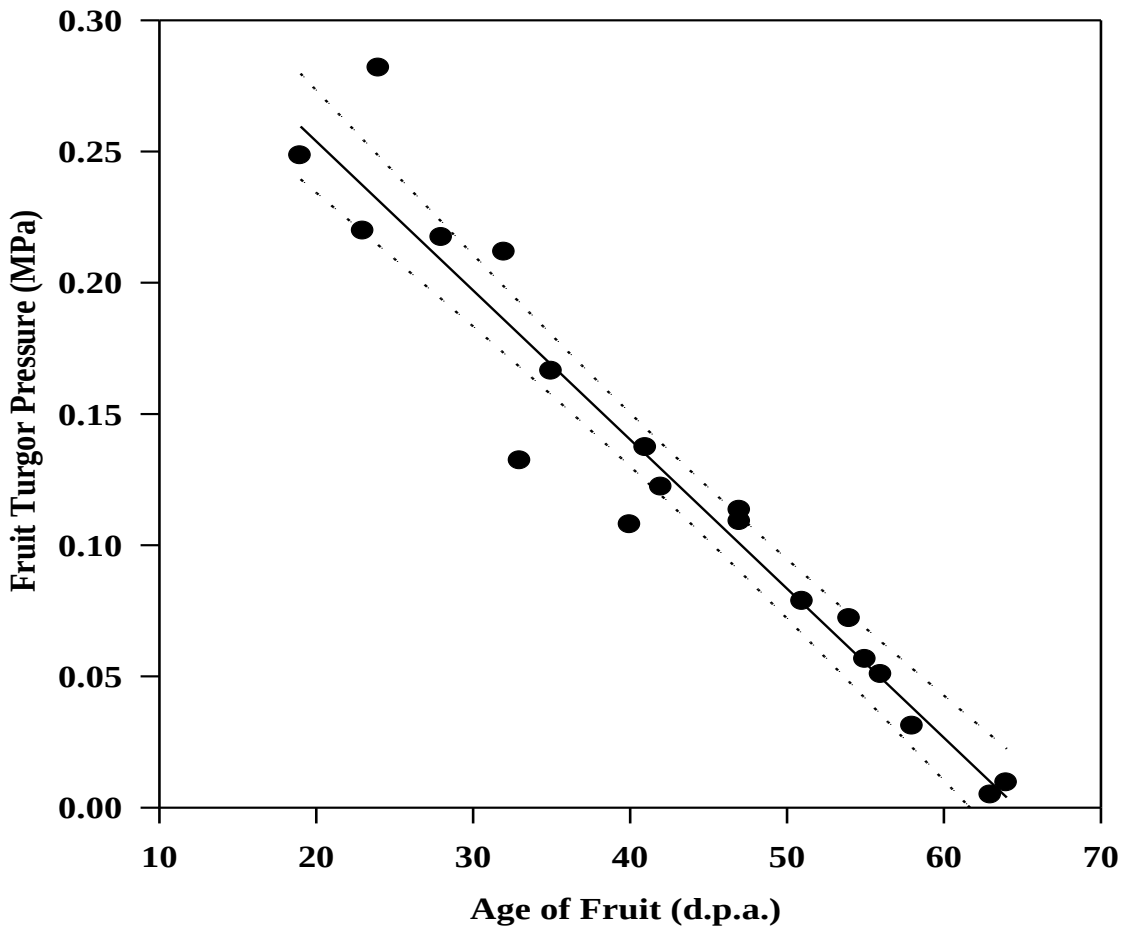

Fig 4. Effect of fruit age on pericarp cell turgor pressure.

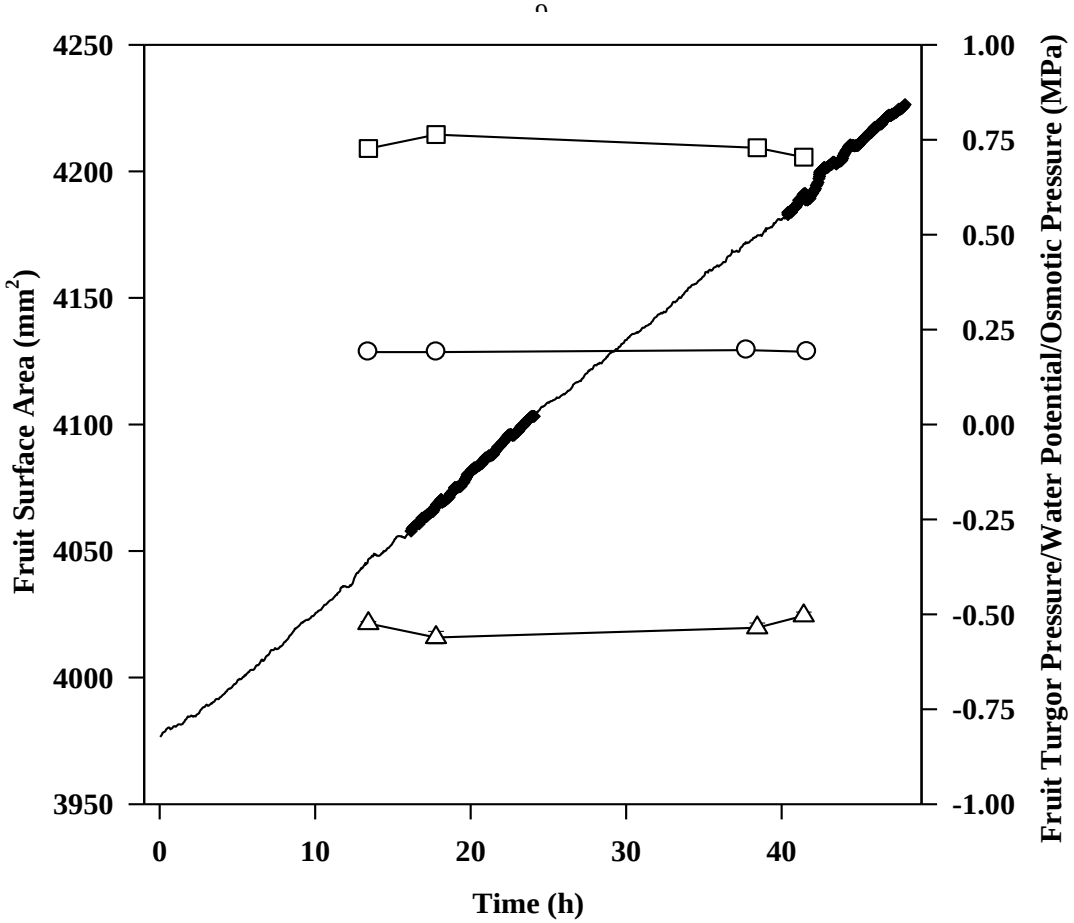

Fig 5. The effect of diurnal changes in light intensity on fruit turgor pressure (○), fruit osmotic pressure (□) and fruit water potential (△). Cumulative fruit size is shown (diagonal line) with dark periods shown in heavy type.

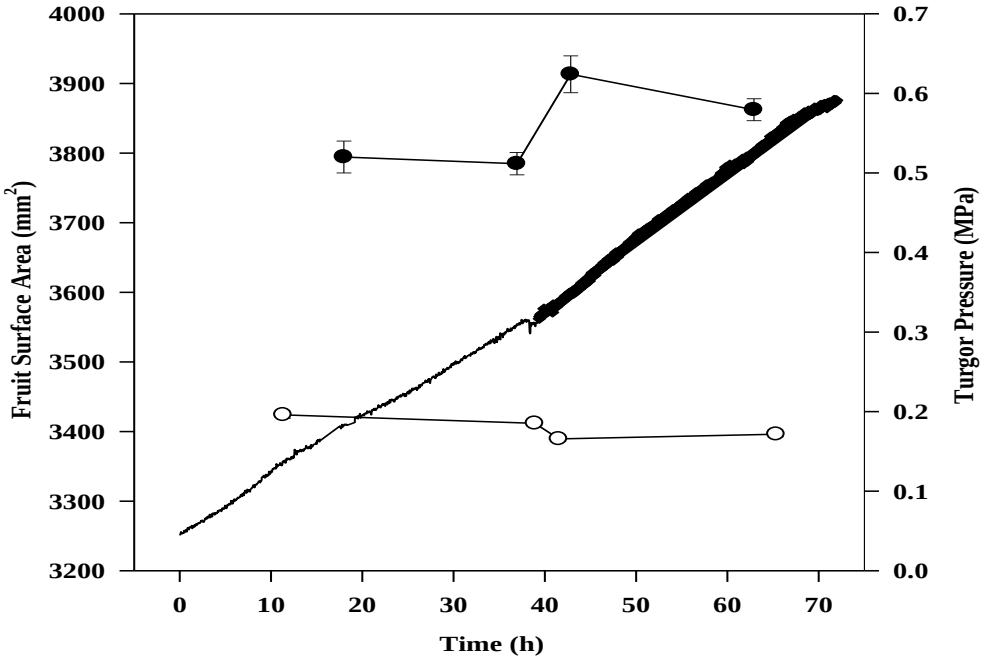

Fig 6. Fruit growth and leaf mid-rib and fruit turgor pressures (leaf mid-rib-●, fruit-○; ± s.e.). The thin line represents cumulative fruit size at 18 °C and the thick line at 28 °C.

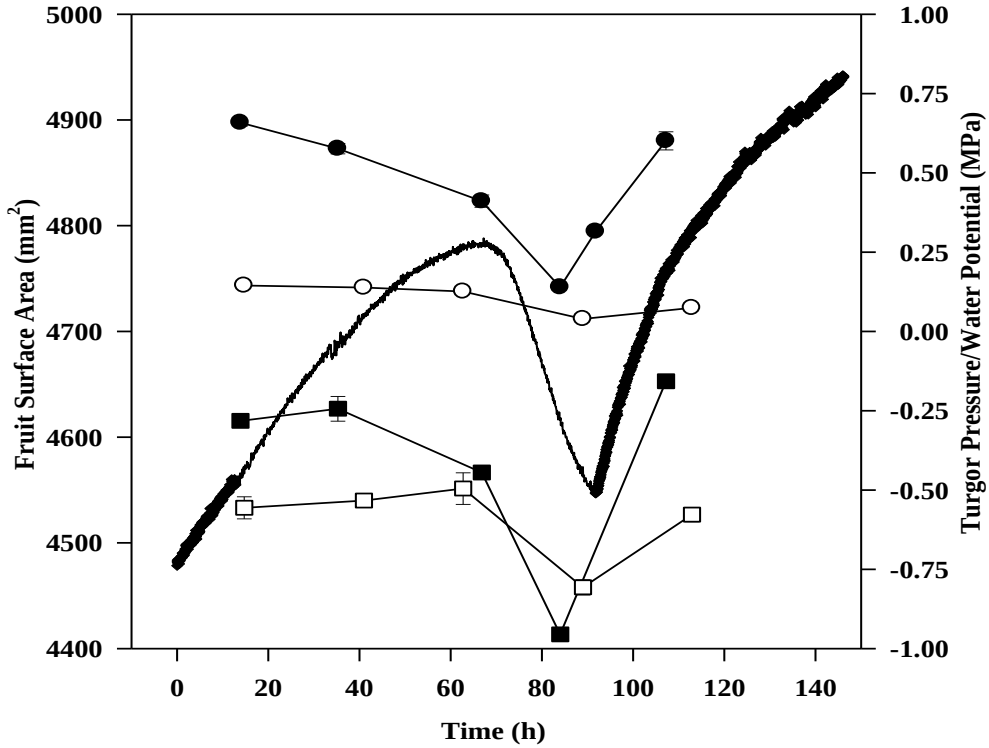

Fig 7. Fruit growth, fruit turgor pressure and water potential (turgor pressure - ○, water potential - □; ± s.e.), and leaf mid-rib turgor pressure and water potential (turgor pressure - ●, water potential - ■; ± s.e.) during a soil drying episode. The line represents cumulative fruit size. The line is thin when water was witheld and becomes thick again when the plant was rewatered.

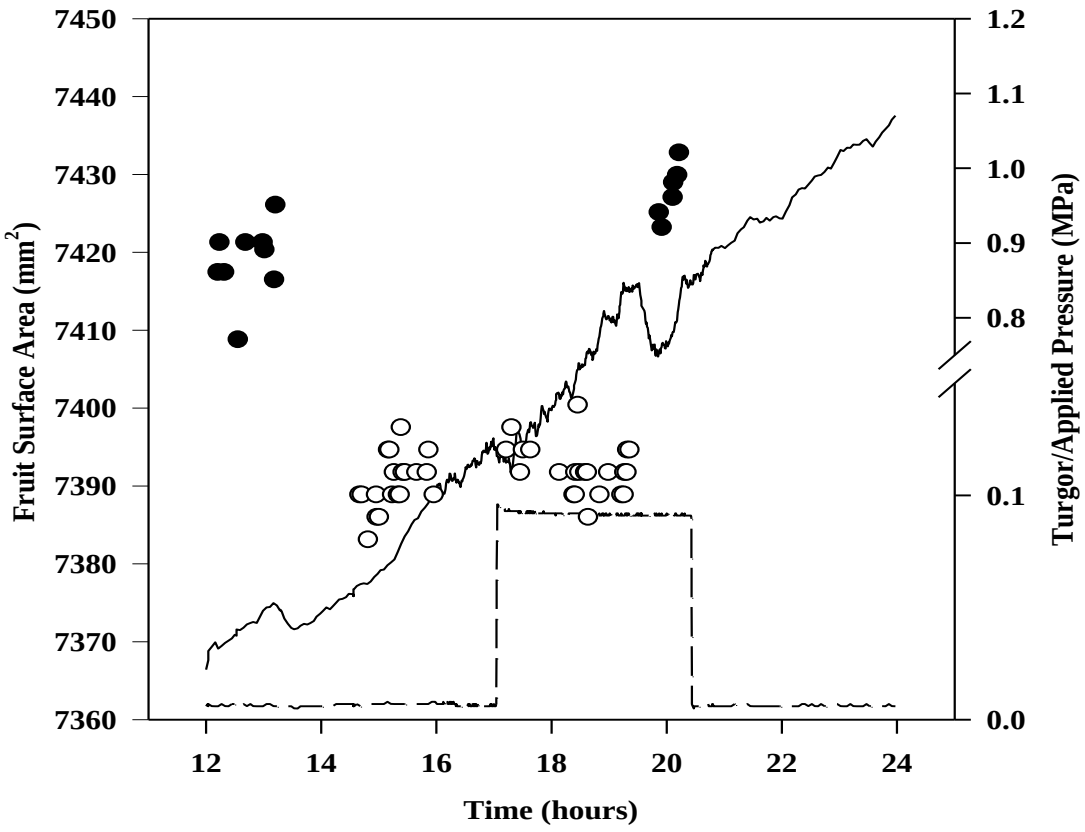

Fig 8. Fruit growth and leaf mid-rib and fruit turgor pressures (leaf mid-rib-●, fruit-○; ± s.e.) during application of pressure to the plant's roots. The solid line represents cumulative fruit size and the dotted line the pressure applied to the roots.