Project title: The optimisation of Summer Fruit Quality in Tomato

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Authentication

I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

Signature……

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Executive Summary

Introduction

Common summer fruit quality defects of tomatoes grown under glasshouse conditions in the UK include *uneven ripening*, *dark patches* and *fruit softness*. However the precise environmental conditions that caused these defects were unknown. Anecdotal evidence suggested that the most severe incidences of fruit quality defects occurred during periods of high temperature. The overall aim of the project was to quantify the critical limits of key environmental factor(s) that lead to summer fruit quality defects. The project was carried out at HRI Efford and Wellesbourne over a three-year period from 1998 to 2000 and addressed five key hypotheses:

- *That temperature has a direct effect on fruit quality*
- *That temperature can affect quality at various stages of fruit development*
- *That K and Ca accumulation in the fruit play a key role in the incidence of these defects*
- *That changes in water relations in the fruit under high temperature have an important role in mediating fruit quality defects*
- *That the root environment has an important role in regulating fruit nutrient accumulation*

The role of environmental factors in fruit quality defects

The main experiments were carried out annually with a large-scale glasshouse grown crop at HRI Efford. To explore the role of temperature in causing fruit quality defects, short term 'pulses' of high temperature were used. These took the form of 3 and 7-day pulses, within an eight or four-week cycle. Whilst the crop was exposed to high temperature for a short period, the eight-week cycle allowed fruits ranging from anthesis to mature green at the time of exposure to be monitored through to picking. Fruit quality was continuously monitored throughout this period to detect responses to high temperature. This approach revealed that the incidence of *uneven ripening*, *dark patches* and *softness* peaked in the week immediately following the heat pulse event. The heat pulse therefore primarily affected quality between the mature green and colour stage 4/5 phase of fruit ripening. The longer 7-day, compared with the 3-day pulse, exacerbated the incidence of the key defects.

The project also addressed the importance of the magnitude of the heat pulse and employed three levels of elevated temperature plus the control over a three day period (i.e. mean temperatures of, 20.4; control, 22.2, 24.2, and 25.9°C). Linear relationships were successfully formulated for increasing mean 3-day pulse temperature and the incidence or severity of key quality defects. For example, fruit *firmness* was decreased by 0.07 N mm^{-1} and *uneven ripening* increased at the rate of 2.7 units of score per °C rise in the pulse mean temperature.

To further investigate the role of high temperature at the mature green phase of fruit development in triggering fruit quality defects, attached and detached fruit studies were carried out. Fruits were either allowed to develop to maturity attached to plants raised in a controlled environment (CE) or detached from plants grown under glasshouse conditions at the mature green stage of ripening. Ripening occurred for both sources of fruit under CE conditions at set temperatures between 18.0 and 31.6°C, at constant levels of light and vapour pressure deficit (VPD). The detached fruit behaved in a similar fashion to attached fruits exposed to similar temperatures under glasshouse conditions. However attached fruit on plants grown under CE constant temperature conditions proved unsuitable for use in predicting the likely impact of high temperature events on fruit quality defects at the crop level. Nevertheless, these data strongly suggest that fruit quality defects were primarily linked to temperature and not VPD or solar radiation. Furthermore, covariate regression analysis for fruit quality defects and glasshouse environmental variables suggested that a role for VPD in the incidence of the key fruit defects could not be substantiated.

The roles of nutrition, water and fruit structure

Fruits with heat induced defects did not exhibit significant changes in cell structure or water relations, and were not depleted in K or Ca, sugars or the major acid, citrate. However, affected fruits did exhibit reduced malic acid concentrations in response to increasing temperature suggesting that this acid acted as a carbon substrate for enhanced respiration during the mature green and colour stage 4/5 phase of ripening.

K and Ca accumulation in the fruit invariably exhibited a seasonal decline. This corresponded with a decrease in fruit juice acidity and *firmness* and an increase in the incidence of *uneven ripening*. Nutritional amendments, root-zone warming and new root treatments all proved to be ineffective in reversing this trend of decreasing accumulation of K and Ca within the fruits, and associated loss of quality towards the end of the season. K and Ca accumulation may be primarily modulated by reduced demand in the ageing shoot, rather than by factors that may restrict the uptake of K and Ca by the roots.

Verification of critical limits

Uneven colouring of the fruit that occurred under elevated pulse temperatures may have been due to localised inhibition of ripening. Direct measurements of fruit pericarp temperature in the glasshouse in the summer revealed values greater than 30.0°C, which is of significance as ripening is inhibited above 30.0°C. Improving the supply of water and nutrients to the shoot during the heat pulse clearly had little impact on the incidence of defects or fruit water relations. The elevated **temperature** of a pulse event appeared to lead to fruit quality defects by primarily affecting the biochemistry of ripening between the mature green and colour stage 4/5. It was found that both the magnitude and duration of a pulse event are therefore important factors in assessing the likely

impact of high temperature on the incidence of fruit quality defects. *Uneven ripening* showed a substantial response to high temperature heat pulses and this was identified as the major defect caused by high temperature and was thus used in the process of 'verification of critical limits'. For *uneven ripening*, a pulse temperature of 25.0°C lasting for seven days would cause substantial commercial damage while a pulse temperature of 26.0°C lasting for three days would cause similar or even greater commercial damage. However to more fully interpret the critical threshold of temperature in a commercial situation, an unacceptable 10 % loss in Class I fruit would require a pulse duration of 3 days at a temperature of 23.0°C.

Summary

The findings of this investigation conclusively demonstrate that key summer fruit quality defects are caused principally by high **temperature** events during the late maturation phase of ripening (between mature green and colour stage 4/5). Both an increasing magnitude and duration of a heat pulse exacerbated the level of defects. Linear relationships between selected defects and heat pulse temperature have been successfully formulated and can be used to predict the anticipated impact of mean temperature over a 3-day period in the range 20.0-26.0°C, on marketable fruit quality. A critical limit of temperature, above which unacceptable commercial losses will be incurred due to key summer fruit quality defects, has been identified as 23.0°C over 3-days.

Practical section for growers

Background

Progress in glasshouse design and crop management in the UK has led to high yielding tomato crops. However, although increasing yield is a key objective to maintain profitability, improving fruit quality is essential for growers to sustain their share in a competitive market. The ability to produce a high quality fruit that is appealing to both the eye and palate is becoming crucial for UK growers because of increasing competition from North African and Mediterranean regions. Considerable advances have been made in understanding the physiology and biochemistry that underlie defects such as blossom end-rot but the events that lead to other defects such as *uneven ripening* and *dark patches* remain largely unknown.

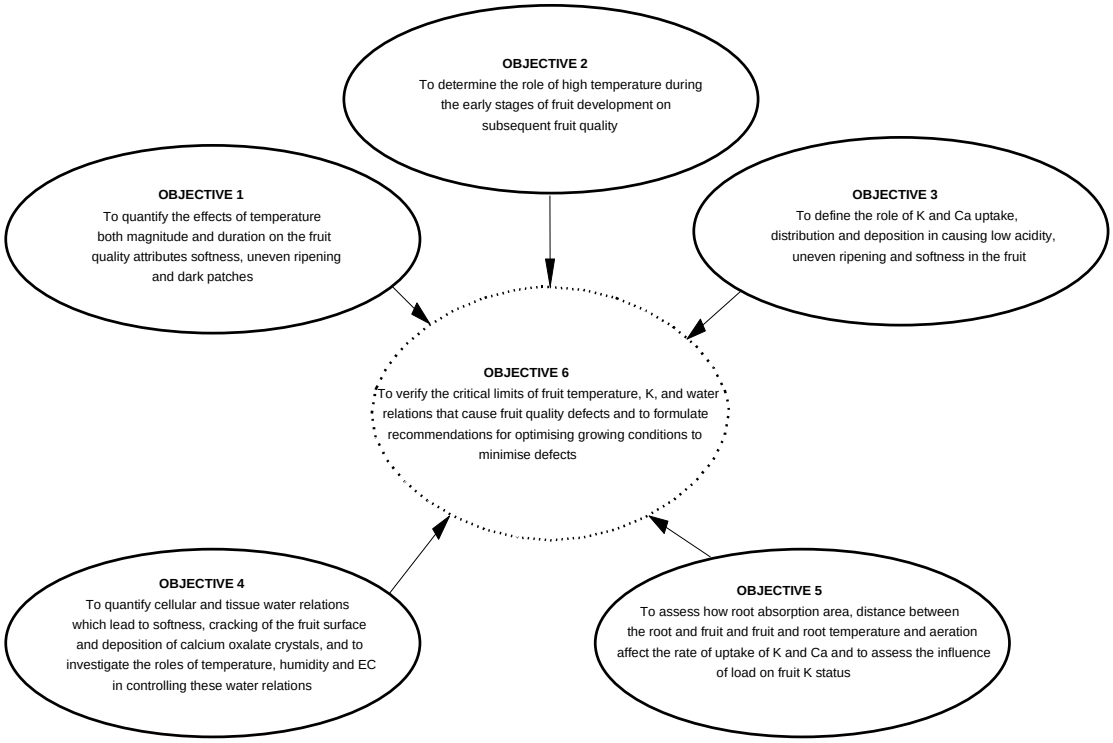
During periods of hot weather venting at present is the primary means with which to cool the glasshouse and provide airflow to meet the blueprint temperatures. This process allows CO_2 to escape and therefore deplete the concentration within the glasshouse. Growers may therefore restrict venting to attain the high CO_2 concentrations required for high yielding tomato production in the UK. Venting therefore provides only approximate control for glasshouse cooling and the resulting temperatures in the UK glasshouses during the summer can peak above the blueprint. These peaks or pulses of temperature are of concern to growers as anecdotal evidence suggests that they frequently, but not always, coincide with *soft* or *uneven ripening* fruit. There is little knowledge of which environmental factor(s) are important in causing such fruit quality defects. It is also unknown at which stage of development the fruits are most susceptible to *softening* or *uneven ripening* or the mechanisms involved in mediating these defects in quality.

The overall objective of the project was to quantify the critical limits of key environmental factor(s) that lead to summer fruit quality defects.

The work in this HORTLINK programme was formulated to explore five hypotheses formulated as potential causes of the summer fruit quality defects:

- *That temperature has a direct effect on fruit quality*
- *That temperature can affect quality at various stages of fruit development*
- *That K and Ca accumulation in the fruit play a key role in the incidence of these defects*
- *That changes in water relations in the fruit under high temperature have an important role in mediating fruit quality defects*
- *That the root environment has an important role in regulating fruit nutrient accumulation*

These hypotheses were addressed through five associated research Objectives which fitted together as depicted in the following diagram:



A key feature of the experimentation was the use of heat pulses applied to glasshouse compartments for three or seven-day periods at intervals of four or eight weeks and at a range of elevated temperature. This was done to explore the effects of both the magnitude and duration of the heat pulse on tomato summer fruit quality. These experiments were carried out on crops grown under near-commercial conditions during the periods April to October 1998, 1999 and 2000 at HRI Efford. Sub-treatments were applied within the main treatments to satisfy the other Objectives, while further small-scale experiments were carried out in controlled environments at HRI Wellesbourne and Efford. The findings were used to quantify the critical limits of the environmental factors involved (Objective 6).

Summary of Results

- Periodic temperature pulses as short as 3 days increased the incidence of *uneven ripening* and *dark patches* and decreased fruit *skin strength* and pericarp *firmness*. (Objective 1)

- The summer fruit quality defects *uneven ripening*, *softness* and *dark patches* were principally caused by high temperature during the mature green and colour stage 4/5 of ripening. All other stages of fruit development were apparently unaffected by these temperature pulses. (Objective 2)

- Mean temperature during a pulse event over 3 days was used to construct linear relationships for temperature and key fruit quality defects. Therefore whilst critical conditions that precipitate quality problems have been identified within the limitations of available experimental conditions decreasing fruit quality is related to a gradual increase in temperature above 20.0 °C. For example, fruit *firmness* decreased at a rate of 0.07 N mm^{-1}; and *uneven ripening* increased at a rate of 2.7 units of score per °C rise in mean pulse temperature, spanning the mean pulse temperature range of 20.0 to 26.0 °C. (Objective 1)

- High temperature pulses are likely to reduce overall consumer acceptability, although this may not always affect % Class I yield based on common European Standards of assessment. However a critical mean threshold temperature and duration were identified as 23 °C over 3 days to produce a 10 % loss in Class I fruit. (Objective 1 and 6)

- Decreases in *skin strength* due to the heat pulse are further exacerbated by truss pruning because the reduced numbers of fruits in a truss exhibit a higher incidence of *netting* (skin surface fine cracking). (Objectives 1 and 5)

- Malate concentration in the fruit juice declined at a rate of 0.025 mg ml^{-1} per °C rise in mean pulse temperature. Some loss of *malic acid* can be expected in the *uneven ripening* fruit, but *citric acid* and the sugars *glucose*, *fructose* and *sucrose* are likely to be unaffected by the heat pulse. A heat pulse event should not markedly affect 'taste' in recently picked fruits. (Objective 1)

- The fruit quality defects did not appear to result from changes in the structure of the fruit, the accumulation of K and Ca or altered water relations within the fruit. (Objective 3)

- An increase in yield, due to accelerated ripening should be expected during the first week following a heat pulse event. There will be a compensatory decrease in yield mainly in the second week but also extending into the third week. After four weeks the yields should have returned to normal expected levels. (Objective 1)

- Altering the water availability to the crop by modulating EC from 1.8 to 4.8 mS to solar radiation in the range 500 to 50 W m^{-2}, has no beneficial effect over constant EC (2.8 mS) for improving fruit quality attributes. The use of applied EC as low as 1.8 mS does not appear to result in any increases in *uneven ripening*. (Objective 4)

- There is a seasonal decline in K and Ca concentrations in the fruit. This trend is not altered by root-zone temperature, high supply of K, a new root system or fruit number. (Objectives 3 and 5)

Conclusions

- Temperature is by far the principle factor determining *uneven ripening* and *softness* and appears to affect the biochemistry of ripening in fruit just entering the ripening sequence.

- Apart from potential cultivar differences, there would seem no evidence to implicate a role as yet for other environmental factors such as humidity, pipe temperature and root-zone temperature in the incidence of summer fruit quality defects.

- Rates of incidence of summer fruit quality defects as related to temperature and critical thresholds of heat pulse variables have been identified within the limitations of available experimental conditions.

- The specific impacts of peaks of temperature or average temperature for periods of less than 3 days might be further investigated to account for varying commercial experiences.

Action points for growers

- The linear relationships formulated for temperature on fruit defects can be used to predict anticipated impacts on marketable fruit quality. These predictions can be used to justify intensification of temperature control measures or to inform marketing of likely quality issues, caused by high temperature events.

- The critical mean threshold temperature and duration were identified as 23.0 °C over 3 days that produced a 10 % loss in class I fruit. Daytime temperatures that exceed 30.0°C can inhibit fruit ripening.

- Growers should give full priority to reducing temperature when such periods occur. Increased venting and reduced pipe heating are clear targets while the maintenance of high CO_2 levels should be considered of secondary importance during such temperature excursions.

- Other ways of attempting to control fruit defects including, EC, root temperature, nutrient feed K and Ca concentrations and distance between the roots and fruit appear to be ineffective under high temperature conditions.

- It is not recommended that the supply of K and Ca is altered or the root environment is modified to combat fruit defects in the late season. This is because the demand for K, Ca and water may be primarily modulated in the ageing shoot rather than by factors that restrict the uptake of K and Ca by the roots.

Practical and Financial Anticipated Benefits

The project will benefit the industry's technology base by developing strategies for the prevention of summer fruit quality defects. New approaches adopted to minimise *softness*, *uneven ripening* and other summer quality defects will reduce the proportion of fruit in lower quality grades without incurring yield penalties. Furthermore, heat pulses accelerate ripening and cause fluctuations in yield. By avoiding high temperature events that cause week-to-week fluctuations in yield, growers will be better able to effectively schedule labour inputs and marketing. This will enable more consistent and efficient production of high quality British tomatoes. The relationships formulated between increasing temperature and the incidence of fruit defects found in the current project should enable growers to predict and anticipate fruit quality on a week-to-week basis.

The wholesale value of the UK heated tomato crop is £70 m. Due to summer quality defects, growers estimate that 2 % of production goes to waste at a loss to the industry of £2.1m. Improvements in the quality of British tomato fruit to the extent that losses through downgrading to Class II and to waste became negligible, would represent a saving to the industry of over £3m.

Progress against Objectives and Milestones

Objective and Milestone	Progress
Objective 1 **To quantify the effects of temperature, both magnitude and duration, on the fruit quality attributes, softness, uneven ripening and dark patches**	
Milestone **1.1** High and low continuous temperature regimes and heat-pulse treatments imposed to assess impact on fruit yield and quality.	Completed year 1
1.2 Heat pulse treatments of varying duration imposed to explore effects on fruit yield and quality.	Completed year 2
1.3 Correlative study of fruit quality with environmental conditions on commercial nurseries.	Completed years 1, 2 & 3
1.4 Establish range of temperature experienced in fruit at different heights within the canopy.	Completed years 1 & 2
1.5 Explore structural changes within the fruit, which are associated with softness and dark patches.	Completed year 2
1.6 Quantify the impact of air temperature *per se* on fruit quality with plant and fruit at the same temperature and with individual trusses at different temperatures.	Completed years 2 & 3

Objective and Milestone	Progress
Objective 2 **To determine the role of high temperature during the early stages of fruit development on subsequent fruit quality**	
Milestone **2.1** Quantify the effects of quality responses to heat pulse treatments	Completed years 1& 2
2.2 Assess the impact of localised heating of individual trusses at different stages of fruit growth.	Completed year 2
Objective 3 **To define the role of K and Ca uptake, distribution and deposition in causing low acidity, uneven ripening and softness in the fruit.**	
3.1 Assess the impact of low and high K supply on fruit quality.	Completed year 1
3.2 Explore the impact of air temperature, K supply and new roots on fruit acidity and blotchy ripening	Completed years 1 & 2
3.3 Investigate the seasonal decline in K status and acidity and its relationship with NADP-malic enzyme	Completed years 1, 2 & 3

Objective and Milestone	Progress
Objective 4 **To quantify cellular and tissue water relations which lead to softness, cracking of the fruit surface, and deposition of calcium oxalate crystals, and to investigate the roles of temperature, humidity and EC in controlling these water relations**	
Milestone **4.1** Quantify the effects of manipulating feed EC (osmotic potential) according to the demand for water by the plant and its interaction with atmospheric heat pulse events.	Completed year 2
4.2 Determine the effects of root temperature and its interaction with atmospheric heat pulse on fruit water relations.	Completed year 2
Objective 5 **To assess how root absorption area, distance between the root and fruit and root temperature and aeration affect the rate of uptake of K and Ca and to assess the influence of load on fruit K status.**	
5.1 Quantify the uptake of K and Ca by young tomato plants as influenced by root-zone temperature in laboratory studies.	Completed year 1
5.2 Undertake a literature review on the uptake of K and Ca as influenced by root-zone temperature and aeration.	Completed year 2

Objective and Milestone	Progress
Milestone **5.3** Quantify the effectiveness of manipulating distance between fruits and roots on the uptake of K and Ca into fruit by the formation of new roots.	Completed year 2
5.4 Assess the impact of root warming on fruit K and Ca status and quality.	Completed year 2
5.5 Quantify the effect of fruit load on K status in the fruit.	Completed year 3
Objective 6 **To verify the critical limits of fruit temperature, K, and water relations that cause fruit quality defects and to formulate recommendations for optimising growing conditions to minimise defects.**	
Milestone **6.1** Verification of the critical levels of temperature, K and water relations undertaken and recommendations for the Industry formulated.	Completed year 3

Science Section

Introduction

Under summer conditions in the UK, fruit quality defects such as *softness*, *uneven ripening* and *dark patches* can periodically occur. Anecdotal evidence suggests that this can happen during bouts of elevated glasshouse temperature. Additionally, this loss of quality can be associated with a time when fruit acidity falls, particularly in the late summer and early autumn and appears to coincide with reduced uptake of K (Voogt, 1993). The lowering of quality is a serious issue for growers as produce may be downgraded or rejected from supermarkets and thus generates a loss of confidence in the product.

The experimentation was designed to identify the environmental factors and processes that trigger fruit *softness* and *uneven ripening* defects and the seasonal decline in K content and acidity in fruit juice. The research addressed 5 key hypotheses:

- *That temperature has a direct effect on fruit quality*
- *That temperature can affect quality at various stages of fruit development*
- *That K and Ca accumulation in the fruit play a key role in the incidence of these defects*
- *That changes in water relations in the fruit under high temperature have an important role in mediating fruit quality defects*
- *That the root environment has an important role in regulating fruit nutrient accumulation*

It was not known whether discolouration and softening defects can be triggered by elevated air temperature and, if so, how these defects are affected by the magnitude and duration of a high temperature pulse event. The development stage at which fruits are sensitive to temperature i.e. during early development or during ripening was not known. Previous work had shown that high temperatures may cause localised inhibition of lycopene synthesis in the exposed part of the fruit (Tomes, 1963), leading to 'blotchy' colouration. Low-acidity detected in the fruits may be the consequence of low malate metabolism or low citrate production due to high loss of malic acid through faster respiration at elevated fruit temperature (Knee & Finger, 1992). Thus, whilst plausible causal mechanisms existed, the timing and impact of high air temperature on the fruits of a large-scale crop had yet to be quantified.

Potassium deficiency has frequently been implicated in physiological ripening defects such as *blotchy ripening* (Hobson *et al.,* 1977). However, whether the defect can be reduced by increased supply of K seems unlikely (HH1316/PC122). Increased Ca supply may also influence fruit quality defects such as *gold-spot*. Recent findings from the project HH1316/PC122

have shown that high day time vent set temperature (25.0°C) as well as increased Ca supply in the transpiration stream can increase the incidence of *gold-spot*. *Gold-spot* may therefore be influenced by a combination of factors including Ca supply from the roots and by fruit temperature, which may alter the relative rate of Ca deposition as crystals of calcium oxalate in the fruit. Furthermore, the softening may be caused by the loss of Ca from the cell wall. Therefore, the effect of high temperature on fruit Ca required investigation. It was thus important to gain an understanding of the potential role of these two nutrients in fruit quality defects under high temperatures.

The discolouration of fruits, particularly in the areas that appeared to have failed to ripen, may actually be due to cell damage. The dull appearance of parts of the fruit surface may also coincide with the presence of fine cracks in the cuticle or epidermis. These could be caused by direct effects of low humidity on the structure of the cuticle (Luque *et al.,* 1995; Luque & Heredia, 1994), by fluctuations in the fruit water potential, or by excess tissue pressure on the epidermis (Thompson *et al.,* 1998). Fruit *softness* may also involve water relations where the decrease in '*firmness*' may be triggered, in part, by a loss of cell turgor. Changes in fruit water relations under high temperature events may be pivotal in our understanding of the processes involved in discolouration and fruit *softness*. However, alterations in microscopic cell structure within the epidermis under high temperature have not been studied. Microscopic analysis of the pericarp exhibiting localised *uneven ripening*, *dark patches* and '*softness*' will give us some insight into possible processes at the cellular level that affect fruit structure.

Whilst fruit quality defects may be triggered by conditions in the aerial environment which in turn may directly or indirectly influence nutrition and water relations within the fruit, there may also be some influence of the root environment. The uptake of K by fruiting tomato plants is known to decline in the summer (Voogt, 1993) perhaps as a result of lower root absorption capacity associated with root ageing, poor aeration or high root temperature. Low K content of the leaves is also known to correlate with *blotchy ripening* (Adams *et al.,* 1978). The K content of the juice is also lower in *blotchy* fruit (Hobson *et al.,*1977) and this coincides with lower juice acidity (Davies & Winsor, 1967; Winsor & Adams, 1976).

The conditions that lead to fruit defects and reduced fruit juice acidity are complex and are likely to involve both root and shoot environments. An improved supply of water and nutrients to the shoot and fruits may offset the impact of high temperature experienced in the summer months that may lead to *fruit softness* and ripening defects. The work in this HORTLINK project was formulated to explore the five hypotheses outlined above and was targeted at understanding the causes of defects in fruit quality. Ultimately the aim was to quantify the critical limits of the environmental factor(s) involved (Objective 6).

The results reported in the following sections relate to all the key findings observed over the three years of the study. Greater detail on some of the findings can be found in the two interim reports for 1998 and 1999.

Materials and Methods

Glasshouse facility

Eight glasshouse compartments (8.0 x 9.6 m; 76.8 m^2) comprising the south half of the M-Block facility at HRI-Efford were used for the main environmental treatments of the three summer fruit quality experiments in 1998, 1999 and 2000. Each compartment had independent computer control and single aspirated screen measurements of wet and dry bulb temperatures were made at the top of the crop canopy (Mulholland *et al.,* 2000). There were four experimental rockwool beds (14 m^2), containing plants with independent nutrient feeds in each compartment that were used as sub-plot treatments. Single rows of plants were used as guard rows along the East side and West side within each compartment. Due to the requirements of layering, there were no guard plants on the ends of the individual rows.

All plants received solutions containing the following levels of nutrients unless specifically altered to meet the requirements of individual experiments (mM): K 10, NO_3-N 11, Mg 3, P 1; and (µM) NH_4-N 250, Fe 36, Mn 9, B 37, Zn 15, Cu 2 and Mo 1. Solution pH was maintained at between 5 and 6 by adjustment with nitric acid and EC was 2.8 mS.

Plant material and crop management

Tomato plants cv Solairo and Espero were sown by a commercial propagator (Table 1; Almodington Nurseries, UK) and blocked on into small rockwool cubes (Grodan DM65, 10 x 10 x 6.6 cm, Grodan, Denmark). Plants in small rockwool cubes were transferred to the experimental glasshouse compartments (Table 1) and placed on standard rockwool slabs (Grodan Talent, 120 x 15 x 7.5 cm, Grodan, Denmark). Solairo and Espero were used in 1998, but in subsequent experiments during 1999 and 2000 a single cultivar, Solairo, was utilised. Each experimental bed comprised 6 rockwool slabs. On each slab were placed 2 rockwool cubes each containing 2 tomato plants. Thus the initial plant population was 24 plants 14 m^{-2} or 1.7 plants m^{-2}. Side shoots were routinely removed from the plants except on two occasions; firstly when one shoot was left in on alternate plants and later when a shoot was allowed to develop on the remaining plants (Table 1). Thus the plant population was eventually increased to 3.4 m^{-2} to match increasing light levels. De-leafing was carried out to the level of the lowest unpicked truss. The timing and duration of experimental treatments are shown in Table 1 for each of the three years. During the winter / spring period the glasshouse atmosphere was enriched to target concentrations of 1000 µmol mol^{-1} CO_2. During the experiments atmospheric CO_2 concentration was maintained at 500 µmol mol^{-1} to accommodate increased venting in the summer months.

Table 1. Timetable for crop management and experimental treatments 1997-2000.

Task	Year		
	1997-98	1998-99	1999-00
Sowing	11 Nov 97	11 Nov 98	14 Dec 99
Delivery	1 Dec 97	3 Dec 98	11 Jan 00
Slab contact	7 Jan 98	7 Jan 99	2 Feb 00
I^{st} sideshoot	21 Jan 98	27 Jan 99	31 Jan 00
2^{nd} sideshoot	18 Feb 98	1 Mar 99	21 Feb 00
Compartment Treatments	6 April 98	26 Apr 99	14 Apr 00
Sub-plot treatments	2 April 98	19 Apr 99	17 Apr 00
Treatments finished	21 Sep 98	4 Oct 99	18 Sep 00

Experimental treatments

Overview of whole compartment manipulation of glasshouse temperature

Whole compartment treatments involved the manipulation of temperature and the impact of temperature on selected fruit quality defects. During 1998 the effect of high temperature was explored as a continuous high temperature regime, and as a heat pulse regime. The pulse was applied using elevated heating and set points day and night (Table 2) for 7 days (168 h) and the crop monitored for the following 7 weeks. This allowed all fruits exposed to the heat pulse to develop and be harvested before the next pulse was applied. The pulse method was applied in the second year (1999) to explore the impact of the two durations, 72 and 168 h, of the pulse event on fruit quality. To apply each heat pulse within the same 'pulse week', the 168 h pulse ran from Monday to Monday and the 72 h pulse from Friday to Monday. Lag '0' in 1999 therefore corresponds to the pulse week and Lag 1, to the assessment week following the pulse. Lag 1 in 1999 and 2000 are similar in that they are the first quality assessments following the high temperature pulse treatment. To complete the heat pulse experimentation, the impact of the magnitude of 3-day (72 h) pulse events was examined in the final year, 2000, with three different day/night heating and vent set point regimes plus the blueprint control (Table 2).

1998 Experiments

The 1998 experiment compared a temperature pulse applied for seven days once every eight weeks with a control treatment grown under normal environmental conditions (Appendix 1, Fig. 36). In addition, a constant high temperature treatment was maintained in two compartments throughout the summer period (Table 2). There were four pulse treatment compartments and these were divided into 2 sets of 2 pulse treatments, pulse A and Pulse B, for staggered application of the heat pulse. The first application of Pulse A was in week 15, whereas the first application of pulse Pulse B was in week 19.

Subsequent pulses were then applied at eight-week intervals in each heat pulse compartment. This staggered application time meant that replicated heat pulses could be applied in every month to ensure that heat pulse effects could be sampled in every month. The actual application of heat pulses to individual compartments for 1998 is shown in the left-hand panel of Table 3.

The four sub-treatments were factorial combinations of two varieties, Solairo or Espero, and two levels of K, 10 and 4 µM applied to the crop. The K treatments were intended to address the importance of K supply as a factor that affects the decline of K accumulation in fruits during the long season production cycle.

Table 2. Heating set points and ventilation set points for control and pulse treatments, for all three years.

Treatment	Heating set point Day / night	Ventilation set point Day / night
1998		
Control	20/16.0 °C	21/17.0 °C
Continuous high	20/16.0 °C	25/21.0 °C
Pulse (7 day)	25/21.5 °C	27/23.0 °C
1999		
Control	19/15.5 °C	20/16.5 °C
Pulse (7 and 3 day)	25/21.5 °C	27/23.5 °C
2000		
Control	18/15.0 °C	19/16.0 °C
Pulse (3 day)	21/18.0 °C	22/19.0 °C
Pulse (3 day)	24/21.0 °C	25/22.0 °C
Pulse (3 day)	27/24.0 °C	28/25.0 °C

1999 Experiments

In 1999 high temperature pulses were again applied once every eight weeks. A heat pulse that lasted for three days was compared with the previously implemented 7-day regime. To achieve maximum power for estimating heat pulse effects, all eight experimental compartments were used for four replicates of the two pulse treatments. Each pulse treatment had four staggered times of application so heat pulses were applied in every two weeks of the season giving a detailed sampling of seasonal conditions and of the stage of development of the crop. The actual allocation of heat pulse treatments to compartments is shown in the centre panel of Table 3. As all eight compartments were committed to the heat pulse treatments, four untreated control plots from the MAFF root physiology experiment on the North side of the experimental facility were used as a control treatment (Appendix 1; Fig. 37). Both the MAFF experiment and the LINK experiment had the same variety (Solairo) and cultural operations so that their standard control regimes were directly comparable. Previous experience has shown that all16 compartments in the M-Block facility are highly comparable and this was the justification for using the MAFF experiment to provide the control for the LINK experiment.

The four sub-treatments were the four combinations of two root-zone temperature treatments and two EC levels. A temperature of 25.0°C was maintained continuously by flat heating panels that were positioned below the rockwool slabs (Heatwave Panel, Hotbox International, Lymington, Hampshire, UK). A constant EC and solar radiation modulated (SRM) EC were applied in the nutrient feed. The SRM feed EC was achieved by modulating applied EC between 1.8 and 4.8 mS, which corresponded with the light range 50 to 500 wm^{-2}. The ranges of EC and light were selected so that the average applied EC over the course of the experiment was similar to the control (2.8 mS). EC in the runoff from the rockwool slabs was monitored continuously by using a LVZ EC drain unit (LVZ Automation Ltd., Bognor Regis, UK).

To explore the effect of new roots on K uptake and accumulation, an adventitious root system was initiated from the stem during July 1999, near to the newly developing trusses on sections of stem that had recently been layered. This treatment was carried out as a sub-treatment within the MAFF root physiology commissioned project (HH1320SPC), using the cultivar Solairo (Appendix 1, Fig. 37). Positioning the stem section between two tightly bound moist, small rockwool cubes caused a new root system to develop. After 14 days there were sufficient roots produced to make slab contact with the existing rockwool slab and allow the growth and development of the 'new roots'.

2000 Experiments

The main Efford 2000 experiment compared three different achieved heat mean pulse temperatures, 22.2^{o}C, 24.2°C and 25.9^{o}C using a 3-day pulse period (Table 4; Appendix 1, Fig. 38), plus a standard control (20.4°C). From the results of the 1999 experiment, it was apparent that the effect of a 3-day heat pulse was sufficient to cause a significant increase in the incidence of the key fruit quality defects within the first 4-week period following a pulse event with little effect in later weeks. These data justified the application of a heat pulse treatment at a four-week instead of an eight-week interval. Every developing fruit would experience two heat pulses but from the 1999 results it could be assumed that only the second pulse would affect fruit quality. The advantage of using a four-week interval was that the number of heat-pulses per compartment could be doubled and therefore the expected amount of information per compartment could also be doubled. There were two compartments for each temperature and as each pulse treatment had two staggered times of application there was a heat pulse application in every two weeks of the season, as in 1999. The remaining two compartments were used as control compartments with a normal growing regime. The allocation of the main treatments to compartments is shown in the third panel of Table 3. Fruit load was manipulated by decreasing the number of fruits to five per truss, to address whether reduction in fruit load could lead to improvements in the K concentration and quality of fruits.

High temperature effects on attached and detached fruit

Attached fruit

Tomato plants cv. Liberto were grown under four continuous aerial temperatures at 14.0, 18.0, 22.0 and 26.0°C at constant VPD (0.6 kPa) in a controlled environment facility (Weiss) at HRI Wellesbourne. Ten randomly selected ripe fruit (colour stage 4 / 5) were harvested from each treatment on five dates throughout the experimental period, providing a total of fifty fruits to be assessed (200 in total). Once fruits were picked, they were packaged and dispatched to HRI Efford for assessments of quality, which included:

- Visual defects including, *uneven ripening*, *gold spot*, *gold marbling*, *netting* and *blossom end-rot.*
- The diameter of fruits and fruit *skin strength* (*load* at penetration (N) and *firmness* at penetration (N mm^{-1}) were measured.
- The fruit were split where one half was dried (80.0°C for 72 h) for mineral analysis and the other frozen / thawed and fruit juice extracted for determination of pH and Brix.

Individual truss heating experiments were attempted under CE conditions but did not provide sufficient fruit due to limited fruit set under the applied high temperatures used (constant 26.0°C).

Detached fruit

Twenty mature green fruits of cv Espero were removed from a separate B-Block glasshouse at HRI Efford in 2000. The crop was grown under similar cultural and environmental conditions to Solairo in the summer fruit quality HORTLINK M-Block controls. The sets of twenty detached fruits were immediately placed in shelf life rooms at HRI Efford with achieved mean temperatures over a 3-day exposure period of 20.2, 22.9, 26.8 and 31.6°C; VPD and light levels were set to similar levels for all four temperature treatments. The fruits were then assessed for *firmness* and *uneven ripening*.

Data analysis

All experimental data from each designed experiment was analysed by analysis of variance by linear regression analysis, using Genstat5. For a detailed representation of the experimental layouts in each year see Appendix 1 (Figs 36-38).

Plant and environment monitoring

Fruit temperature and 'within-canopy' measurements of VPD (1999)

Measurements of temperature (copper constantan thermocouples) and VPD were recorded for selected heat pulse events throughout the experimental period. Fruit temperature was measured by 1 cm long 1mm diameter copper constantan thermocouples, inserted at a 60° angle through the skin and into

the fruit pericarp at the shoulder. To minimise short-term fluctuations in heating of the fruit due to solar radiation or 'sun flecks', the thermocouple was situated on the 'north side' of the growing fruit, thus providing a reliable integrated estimate of pericarp temperature. Thermocouples were placed in immature green fruit (proximal plus one fruit position) two trusses above a truss with a proximal fruit at the breaker plus one ripening stage, throughout the experimental period for three fruits of two heat pulse treatments and one control. Data was logged every 30 s using a Campbell CR10X data logger and AM416 multiplexer (Campbell Scientific, Shepshed, UK) from which 30 min means were calculated and stored. Concurrent measurements of VPD and air temperatures were made at the same level of the fruits containing thermocouples using 'stand alone' measurement and logging units (Tinytalk, RS, UK).

Solar radiation

Solar radiation was measured by a Kipp solarimeter positioned on the roof of the M-Block glasshouse complex. Within the glasshouse incident radiation was measured using one m long tube solarimeters (Mulholland *et al.,* 1998) at two locations, one within the top section of the glasshouse roof structure and the other at the level of the fruits being monitored for temperature. Tube solarimeters were calibrated against a Kipp solarimeter before and after the experiment. Data was logged every 30 s using a Campbell CR10X data logger and AM416 multiplexer (Campbell Scientific, Shepshed, UK), from which hourly means were calculated and stored.

Temperature within the root-zone

Temperature in the root-zone was measured with copper constantan thermocouples. All four subplots in each of two compartments within the eight-compartment experiment were monitored in 1999. Two thermocouples were placed mid way along each of the two slabs' length 1 m apart at half of the slab depth.

All measurements of root-zone temperature were recorded at 30 s intervals and stored as 30 min means using a Campbell CR10X datalogger and AM416 relay multiplexer (Campbell Scientific, Shepshed, UK).

Sapflow

Sapflow in intact plants was measured using a heat balance method which is described in detail elsewhere (Steinberg *et al.,* 1989). The installation and use of the gauges followed the recommendations of the manufacturer (Dynamax, Texas, USA). The outputs from the gauges were monitored every 15 s, and stored as 30 min means (Campbell CR10X datalogger and AM416 relay multiplexer; Campbell Scientific, Shepshed, UK), for subsequent computation of sapflow rates.

Table 3. Glasshouse environmental regimes applied to the eight glasshouse compartments in each of the three fruit quality experiments in 1998, 1999 and 2000.

Week	1998 Experiment Compartment Number								1999 Experiment Compartment Number								2000 Experiment Compartment Number							
	9	10	11	12	13	14	15	16	9	10	11	12	13	14	15	16	9	10	11	12	13	14	15	16
15		H	P	C	P	C		H									28	22	C	25	C			
16		H		C		C		H											C		C			
17		H		C		C		H	7	3									C		C	28	25	22
18		H		C		C		H											C		C			
19	P	H		C		C	P	H							3	7	28	22	C	25	C			
20		H		C		C		H											C		C			
21		H		C		C		H			7	3							C		C	28	25	22
22		H		C		C		H											C		C			
23		H	P	C	P	C		H					3	7			28	22	C	25	C			
24		H		C		C		H											C		C			
25		H		C		C		H	7	3									C		C	28	25	22
26		H		C		C		H											C		C			
27	P	H		C		C	P	H							3	7	28	22	C	25	C			
28		H		C		C		H											C		C			
29		H		C		C		H			7	3							C		C	28	25	22
30		H		C		C		H											C		C			
31		H	P	C	P	C		H					3	7			28	22	C	25	C			
32		H		C		C		H											C		C			
33		H		C		C		H	7	3									C		C	28	25	22
34		H		C		C		H											C		C			
35	P	H		C		C	P	H							3	7	28	22	C	25	C			
36		H		C		C		H											C		C			
37		H		C		C		H			7	3							C		C	28	25	22
38		H		C		C		H											C		C			
39		H		C		C		H					3	7					C		C			
40		H		C		C		H											C		C			
	Heat pulse treatment interval: 8 weeks P = Seven day heat pulse H = Continuous high temp. C = Standard control Blank is untreated								Heat pulse treatment interval: 8 weeks 7 = Seven day heat pulse 3 = Three day heat pulse Blank is untreated								Heat pulse treatment interval: 4 weeks 22= Three day pulse; day vent set 22.0^{0}C 25= Three day pulse; day vent set 25.0^{0}C 28= Three day pulse; day vent set 28.0^{0}C C = Standard control Blank is untreated							

SRM applied EC

In this treatment the applied EC was modulated on the basis of light intensity. The Priva computer calculated a set point EC of between 4.8mS and 1.8mS over the light range 50 Wm^{-2} to 500 Wm^{-2} (measured by Kipp solarimeter positioned on the M-Block glasshouse roof). Thus at high light intensities a low EC was calculated and at low light intensity a high EC was calculated.

However, there was a considerable buffering effect from the irrigation water that was already resident in the mixing tank and the header lines and dripper lines of the irrigation system. It was calculated that the ratio between applied volume per irrigation round and total volume resident in the system was approximately 1:10. Thus the changes in EC would take approximately 10 irrigation rounds before having a noticeable effect at the dripper. In terms of achieved applied EC, instead of attaining a modulated effect in response to

instantaneous light on an hourly basis the treatment gave a response to the average light received over a period of hours previously. Routine samples of applied and run off solution were taken three to four times per week and EC measured continuously using a drain unit connected to the Priva computer system.

Assessment of fruit quality and yield

Fruit quality assessment framework

A random sample of eight, size D (47-57 mm in diameter) fruits picked at colour stage 4/5 was drawn from the complete harvest of each plot on each Monday throughout the heat pulse experiment. The eight fruits from each plot were then assessed individually for ripening defects using a five point scoring system for each defect separately.

The five scores were 1A, 1B, 1C, II and Waste where 1A indicated no defect, 1B and 1C indicated slight and moderate defect, respectively, within Class I, Score II indicated sufficient defect to downgrade to Class II and Waste indicated sufficient defect to downgrade to waste (Appendix 2). For analysis, the defect measurements on the individual fruit in a sample were pooled using a weighted combination of the defect scores with weights chosen to reflect the severity of the defect. In this report, 1A fruit is given a weight of zero, 1B fruit is given a weight of one, 1C fruit is given a weight of two, Class II fruit is given a weight of three and Waste fruit is given a weight of four.

The overall weighted score was then scaled to cover the range 0 to 100. Thus a single 1A fruit would have a weighted score of zero, 1B a weighted score of 25, 1C a weighted score of 50, a Class II fruit a weighted score of 75 and a waste fruit a weighted score of 100. The combined weighted score of the eight fruits in a sample therefore gave an average weighted defect score in the range 0 to 100 (**Eq. 1**).

Eq. 1 Weighted score =(1B+1C*2+II*3+waste*4)*100/N (where N=32 assuming eight-fruit samples)

Tissue sampling for visual defects, firmness, mineral, sugar and acid content

Eight fruits selected randomly from the total yield for each subplot were used for visual assessments of fruit quality (Appendix 2). These assessments were carried out on the Monday of each week of the experimental period, and yield and size grade-out was recorded on three occasions during the week (Monday, Wednesday and Friday).

Load (N) which gives an estimate of skin strength and *firmness* (N mm^{-1}), which is related primarily to the *firmness* of the pericarp were measured on eight fruit using a materials testing system (Model LRX, Lloyd Instruments, Hants, UK). This consisted of a 5 mm diameter round-ended probe travelling

at a constant velocity (0.17 mm s^{-1}) into whole tomato fruit. These same eight fruit were split in half with a sharp blade, one half dried at 80.0 °C in a fan assisted oven for 48 h and the other placed in a freezer and stored at minus 20.0°C. The dried fruits were then ground (< 1 mm), re-dried at 80.0 °C oven for 5 h prior to Kjeldahl digest. The digest was made up to a 50 cm^3 sample volume, K was analysed by flame emission spectrometry and Ca by inductively coupled plasma atomic emission spectrometry (ICP). The frozen fruit tissue was defrosted at room temperature the extract filtered and the juice collected. 1.2 ml of the extract was sub-sampled and placed in 1.5 ml eppendorf microtubes and stored at minus 20.0 °C prior to analysis for sugar and acid content.

Determination of sugars and acids

Brix and pH values were determined for the bulk fruit juice immediately after collection using a standard refractometer and pH meter respectively. Three sugars *glucose*, *fructose*, *sucrose* and two acids *citrate* and *malate* were determined from the subsampled frozen fruit juice extracts, using a modified enzyme-linked colorimetric assay. Samples for analysis were diluted 1:100 in distiled H_2O. The assay was run so that all three sugars could be determined sequentially on a single aliquot of diluted juice, in a single assay well; the acids were run independently.

Water relations

Fruit water relations were assessed by measuring fruit juice osmotic potential. A pericarp strength bioassay, was used to estimate fruit turgor from osmotic pressure (Thompson *et al.,* 1998). Osmotic potential was measured on 100 µl of fruit juice expressed from sections of pericarp using a freezing point osmometer (MOD 200, Camlab Ltd., Cambridge, UK). Fruit was prepared for the tomato strength bioassay by removing 5 mm thick equatorial slices from four fruits selected for fruit quality assessments as described above. The locules, columella, radial pericarp wall and placenta were removed to leave a hoop comprising the outer pericarp wall and epidermis. Tension in the epidermis (turgor as influenced by osmotic pressure) was then tested by floating the hoop in incubation solutions of PEG 8000 (Sigma, UK). A range of solutions was used with the following osmotic pressures; 0.34, 0.47, 0.51, 0.72, 0.86, 0.91, 1.16 MPa, and hoops floated on each solution for 18 h. Hoops were then cut and the degree of opening of the hoops was measured after 30 s. The turgor as influenced by osmotic pressure at which opening was zero was extrapolated by linear regression. These measurements were made once, in the week immediately before and after a pulse event. The hoop bioassay therefore provided information on the tissue pressure properties of the epidermis and pericarp.

Assessment of tomato fruit structure

Cryo scanning electron microscopy (CryoSEM) was used to examine morphological differences in cells and tissues between normal and affected areas of tomato fruits exhibiting *uneven ripening* and *gold-spot*. Tomato fruits

were examined on four occasions during 1999. From each fruit several 25 mm long tissue wedges were removed to allow imaging of the cuticle and up to 5 mm depth into the fruit. In most cases comparisons were made between normal and affected tissue within the slice i.e. an unripe region adjacent to a ripe one. They were mounted onto a Cryo stub using carbon conducting cement, partially air-dried and then frozen in liquid Nitrogen and maintained on cold stages at around minus 180.0°C to allow imaging in the high vacuum of the SEM without collapse and distortion of the cells. A gold coating, routine for SEM, was applied to the samples prior to viewing. With this technique cell contents at the cut surface were largely lost but differences such as cell size, size distribution, uniformity and also cell wall and cuticle thickness are measurable. Another feature that was measurable was the distribution and depth below the cuticle of the vascular tissue / bundles.

Root-zone temperature and nutrient uptake and accumulation in model plants

Plants of cv Solairo were grown under controlled environment conditions (20.0/20.0 ^{o}C day/night temperature, 14 h photo- and thermoperiod and 400 μmol m^{-2} s^{-1} PAR, VPD 0.5 kPa. Twenty-one days after emergence, seedlings of uniform size were transferred to 0.5 L LDPE pots containing 480 ml of continuously aerated 0.2 strength Hewitts nutrient solution. Target temperatures were achieved gradually over a 2-3 h period to produce constant root-zone treatments of 10.0, 20.0, 30.0 and 40.0°C respectively.

Approximately 24 h prior to destructive harvesting nutrient solution was changed (sub-samples taken and analysed for nutrient feed mineral content) and nutrient uptake was calculated by depletion of individual elements from the solution and water uptake by gravimetric determination. At harvest once the plant had been removed a 100 ml nutrient solution sub-sample was removed and analysed for pH, EC, K, NO_3, Ca, Mg, P, Fe, Zn, Mn, Cu, B, Na, Cl and P. To estimate evaporative water losses sampled pots were refilled with nutrient solution and a wooden dowel inserted into the foam support for each root-zone temperature treatment and weighed at the beginning and end of a 24 h period (encompassing light and dark periods).

Four independent water baths were used for the four temperature treatments. Within each water bath, 12 plants were arranged on a 4 x 3 rectangular grid pattern. Plants were sampled at 5, 12 and 15 days after the imposition of root-zone temperature treatments (DAIT) using an augmented Latin square sampling scheme i.e. a 3 x 3 Latin square with one repeated row. Thus, four plants were sampled from each bath on each of three occasions.

Data were analysed by analysis of variance using Genstat5. The temperature treatments were unreplicated as only four water baths were available for the experiment, therefore all estimates of standard errors of the difference and significant levels for treatment effects are based on plant-to-plant variability within water baths. However, water bath conditions were highly regulated and it is unlikely that bath-to-bath variability was sufficiently large to affect the reliability of the statistical analysis.

The response to root-zone temperature was modelled by a second degree polynomial ($y = \mu + \alpha x + \beta x^2$) fitted to treatment means for individual harvests. The polynomial response curves were used to estimate the temperature that gave a response to maximum by solving the equation; $0 = \alpha + \beta 2x_{max}$ for x_{max}. Back substitution of x_{max} into the polynomial response function for y gave the maximum response y_{max}.

Results

Objective 1:
To quantify the effects of temperature, both magnitude and duration, on the fruit quality attributes, softness, blotchy ripening and dark patches.

Temperature treatments

Microclimate within the compartments

The crop was exposed to a range of temperature treatments, which varied in magnitude and duration (Table 4). The mean temperatures achieved within short-term heat pulse events of two durations (72 and 168 h) in 1998 (72 h; 24.7°C) and 1999 (72 and 168h; 25.0°C) were broadly similar. In 2000, the crop was exposed to different magnitudes of temperature and mean temperatures achieved within the pulses were 1.8, 3.8 and 5.5°C above the control mean temperature for the equivalent 72 h period (Table 4 and Fig. 1). Replicated detailed measurements of fruit temperature demonstrated that mean daily fruit temperatures were up to 0.6°C higher than the corresponding air temperature. However, for the purposes of identifying responses to heat pulse events, these differences were sufficiently small to justify the use of compartment temperatures as the temperature variable in subsequent analyses.

Temperature differences due to the heat pulse events were controlled treatment effects but changes in the VPD were consequential on the temperature treatments and were not directly controlled. Observed VPD changed in response to the temperature treatments (Table 5) and cannot be clearly distinguished from the effects of temperature. However, the effects of VPD and temperature were not completely confounded. For example, humidity increased under elevated temperatures in 1998 and 1999 but in 2000, the highest temperature treatment (+5.5°C) produced a less humid environment than the other heat pulse and control treatments (Table 5). Thus, as VPD was not a simple direct function of temperature, there was some opportunity for studying the effects of humidity on fruit quality independent of the effects of temperature. However, the observed range of mean VPD during continuous high temperature or pulse events was small (0.5-0.7 kPa) and would not normally be considered deleterious to vegetative growth or fruit quality during either winter (Fussell *et al.,* 1992) or summer (PC30/30a) conditions. Therefore, in this study, temperature rather than humidity must be regarded as the main ‘driving’ variable for the observed fruit quality effects.

Table 4. Mean temperatures (°C) within the control heat pulse or continuous high temperature treatments. Refer to Table 2 for details of heating and venting set points during the day and night time periods.

Year	Heat pulse Control	High-Continuous	72 h pulse	168 h pulse
1998	21.3	23.6		24.7
1999	21.3		25.0	25.0
2000	20.4		22.2 24.2 25.9	

Table 5. Mean VPD (kPa) within control, heat pulse or continuous high temperature treatments. Refer to Table 2 for details of heating and venting set points during the day and night time periods.

Year	Heat pulse Control	High-Continuous	72 h pulse	168 h pulse
1998	0.6	0.5		0.5
1999	0.6		0.5	0.5
2000	0.6		0.5 0.5 0.7	

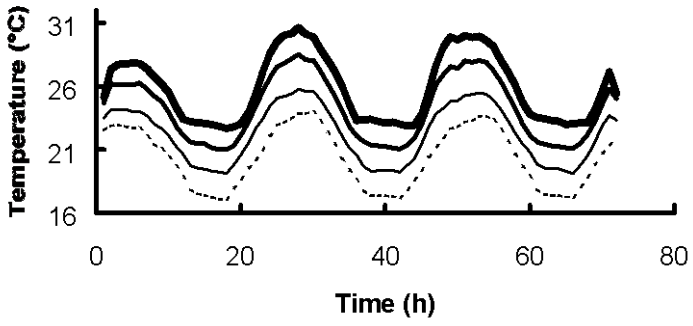


Figure 1. Mean seasonal heat pulse trends for 2000. Pulses were applied for 72 h and mean pulse temperatures were 20.4 (dashed line) 22.2, 24.2 and 25.9°C represented by increasing line thickness.

Quantifying fruit quality attributes

An important distinction arose during the 1998 experimentation regarding the categorisation of 'blotchy fruit'. These were not strictly blotchy but were classified as *unevenly ripened* fruit (Plate 1 and Appendix 2). True blotchy fruit are structurally different from normally ripening fruits (Hobson & Davies, 1976). However, for fruits exhibiting *uneven ripening*, we could find no structural differences for the normally ripened and unripe green areas (Plate 1).

To relate the weighted score used in the analysis of variance to the expected percentage Class I (A+B+C) commercial grade out, the percentage Class I fruit was plotted graphically against the estimated weighted score for each fruit sample. Calibration graphs are shown for *uneven ripening*, *dark patches*, *gold marbling*, *gold-spot* and *netting* (Fig. 2a,b,c,d,e), and are intended to show the expected percentage grade out corresponding to the weighted scores achieved within the experiment. As the observed percentages are based on only eight fruits they can fall only within the nine discrete categories 100%, 87.5%, 75%, 62.5%, 50%, 37.5%, 25%, 12.5% or 0%. Ordinary regression analysis was not appropriate for this type of percentage data therefore calibration curves were fitted using a logistic regression analysis assuming a binomial distribution for the percentage Class I fruit within each sample. The fitted relationship may provide a useful aid to estimating the likely amount of downgrading due to any particular weighted score value. Figures 2a, 2b, 2c, 2d and 2e can be used to predict the expected percentage Class I fruit for any given weighted score defect assuming that the fruit was downgraded according to that particular defect. For example, Figure 2a shows that a weighted score of 30 due to *uneven ripening* corresponds to about 97% Class I indicating that any set of conditions that gives a weighted *uneven ripening* score of 30 will cause a downgrading from 100% to about 97% due to that defect.

Crosses mark the actual observed percentage Class I fruit for each sample of eight fruit relative to the weighted defect score. The scatter of the actual observed values about a fitted line is an indication of the reliability of that line as a predictor of fruit quality. Figure 2a appears reasonably reliable, Figures 2b,c,d and e are less reliable but provide some useful information.

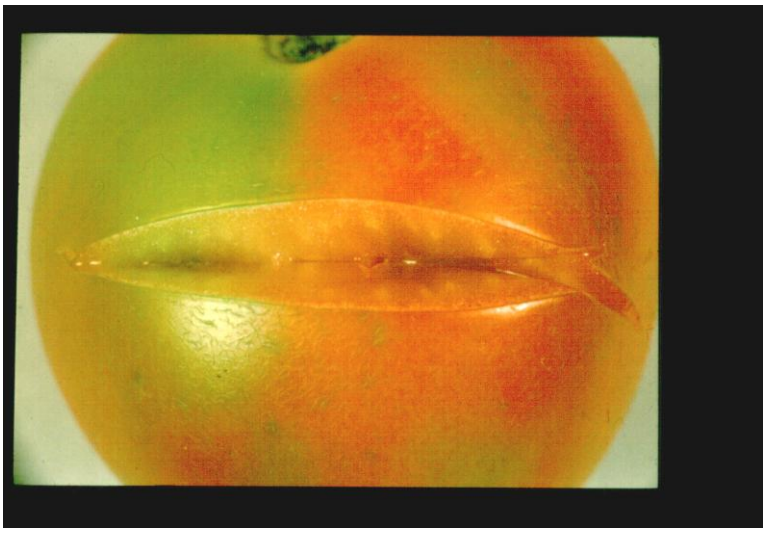
Further calibration graphs showing the relationship between percentage Class (A+B) fruit and the weighted scores was undertaken based on information supplied by VHB that Grade C fruit can become commercial Class 2 if present in sufficient numbers. For example, more than 4 grade C fruits in a box downgrades the whole box. Therefore the percentage of fruit better than Class C may give a clearer indicator of real commercial damage. The graphical plots of Class (A+B) fruits may give a fuller understanding of fruit quality criteria in a commercial situation (Fig. 3).

Softness, uneven ripening and dark patches

Continuous high and high temperature pulses

Experiments carried out in 1998 established that fruit softness and visual defects were increased at all times in response to continuous high temperature. When the achieved glasshouse mean temperature was raised by 3.3°C above the control during a heat pulse event the incidence *of uneven ripening* and *dark patches* was significantly increased, whilst fruit *firmness* was decreased (data not shown). These defects in fruit quality appeared in the first fruit pick and quality assessment immediately following a pulse event, or 'Lag week 1' (data not shown, but dealt with in detail in the following two sections).

a)



b))

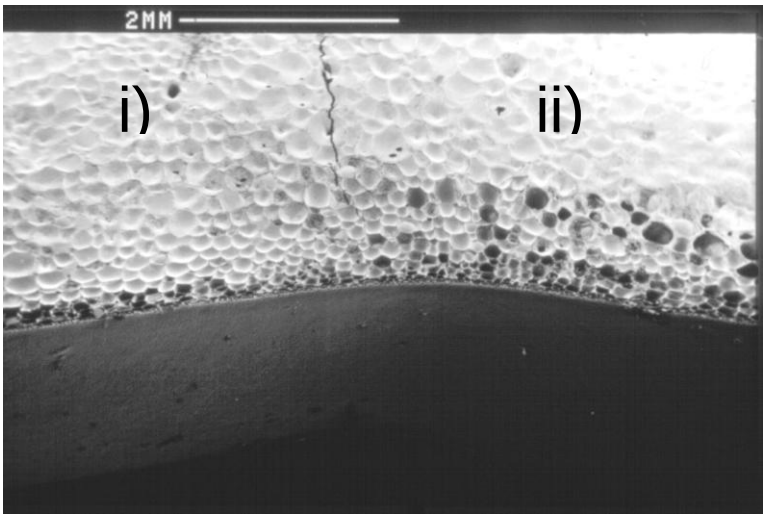


Plate 1. Photograph of a) typical **unevenly ripening** fruit and b) SEM of section through i) green and ii) ripened areas of an **unevenly ripening** fruit. Note no detectable difference in cell size in either section and the crack although it conveniently delimits the two areas arose artificially during sample preparation.

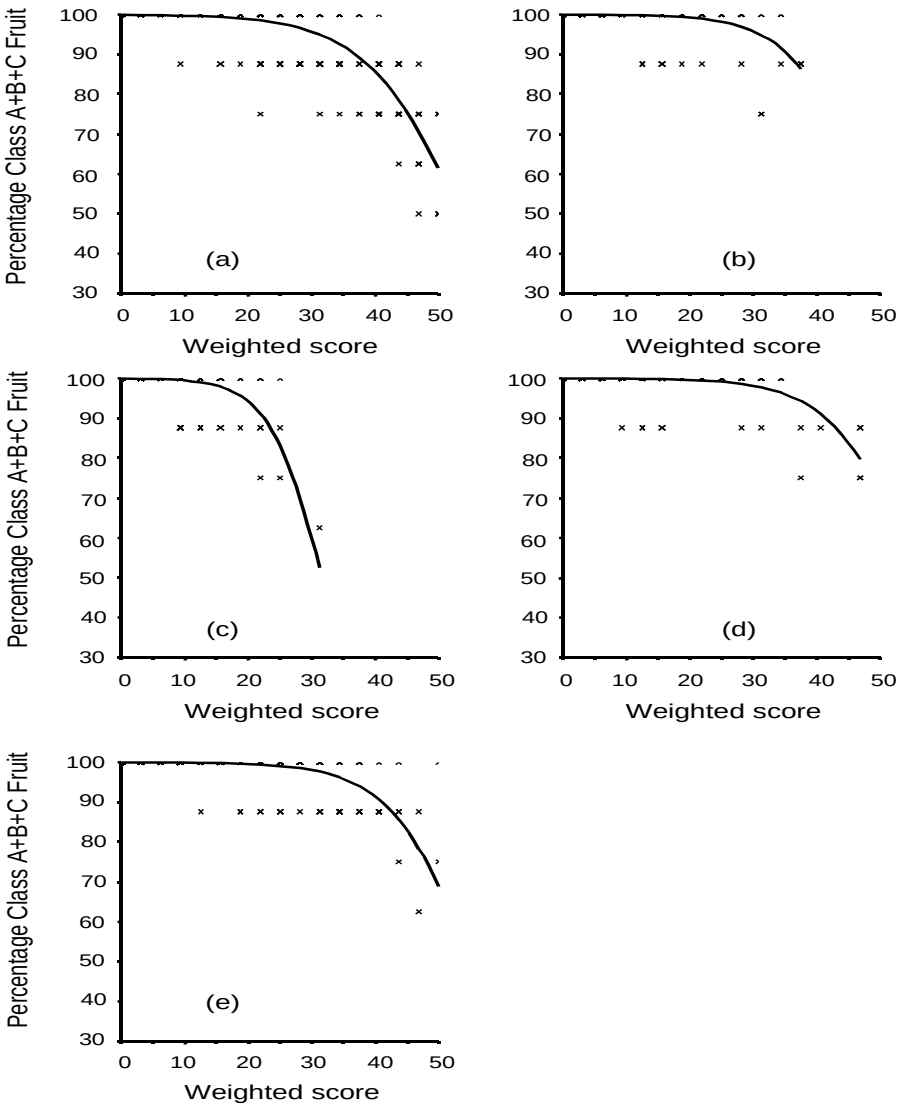


Figure 2. The relationships between percentage Class I grade-out (Class 1 A+B+C) and weighted score for a) **uneven ripening** b) **netting** c) **dark patches** d) **gold-spot** and e) **gold marbling**. The fitted curves were constructed from back transformed logistic regression values.

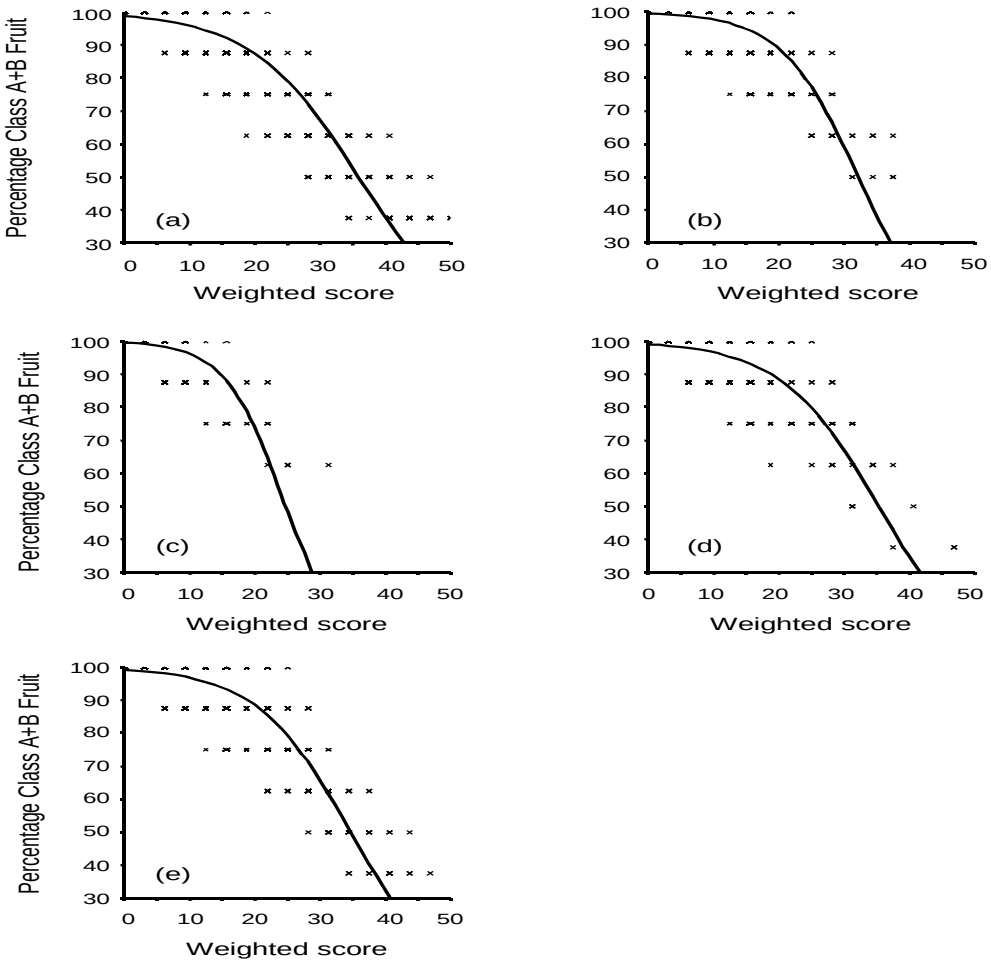


Figure 3. The relationships between percentage Class I grade-out (Class 1 A+B) and weighted score for a) **uneven ripening** b) **netting** c) **dark patches** d) **gold-spot** and e) **gold marbling**. The fitted curves were constructed from back transformed logistic regression values.

Effect of pulse duration

Pulses were applied in 1998 in eight-week cycles for pairs of replicate compartments. The eight-week cycle was introduced to cover the entire growth period from anthesis to fruit pick. Although this experiment gave some preliminary information that a week long (7-day) heat pulse could decrease fruit quality, the results of the first year's experimentation at Efford needed to be extended to explore the relationship between the duration of the heat pulse event and the incidence of key defects. To address this question, during 1999 pulses were applied as either 3 or 7-day events. Fruit was picked routinely for yield assessment during this period. Both the 7-day and the 3-day temperature pulses were timed to finish on the same day and fruit was sampled immediately following the termination of a heat pulse. The Lag 1 record is the first harvest immediately following a pulse (Monday) and the subsequent Lag records followed on subsequent Mondays through to the end

of each eight-week cycle. This sampling routine was repeated throughout the 24 weeks of the heat pulse experiment. In the 1999 series of experiments, Lag week 8 was re-labelled Lag week 0 and has been shown graphically immediately prior to Lag week 1. Due to the cyclic imposition of heat pulse treatments Lag 0 is synonymous with Lag week 8 and follows immediately after Lag week 7.

(1) Skin strength (load) and pericarp firmness

For these experiments, *fruit load and firmness,* which, respectively, give an estimate of skin and pericarp strength were markedly decreased in the week following the pulse event ($P<0.001$; Lag 1; Fig. 4a, b); there was only weak evidence that greater pulse duration exacerbated these effects.

(2) Uneven ripening, dark patches and yield

The incidence of *uneven ripening* and *dark patches* was increased in response to the heat pulse ($P<0.001$; Fig. 5a, b). However, the duration of the pulse appeared to have some effect on the incidence of these two defects, whereby *uneven ripening* exhibited a significant increase at the 7 compared with the 3-day pulse at Lag 1 ($P<0.05$; Fig. 5a). In addition to an increased incidence in these important quality defects, yield was increased for fruits at colour stage 4/5 in the Lag 1 period ($P<0.001$; Fig. 6; Table 6).

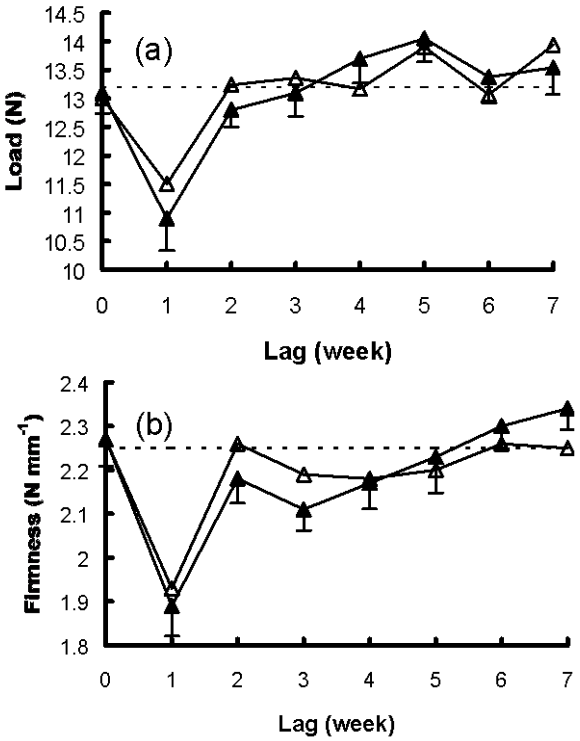


Figure 4. The effect on a) **load** and b) **firmness** of 7 (▲) and 3 d (Δ) heat pulse treatments. The dashed horizontal line represents the average north side M-Block control treatment and the vertical lines are standard errors of the difference (SED) with 6 degrees of freedom (d.f.).

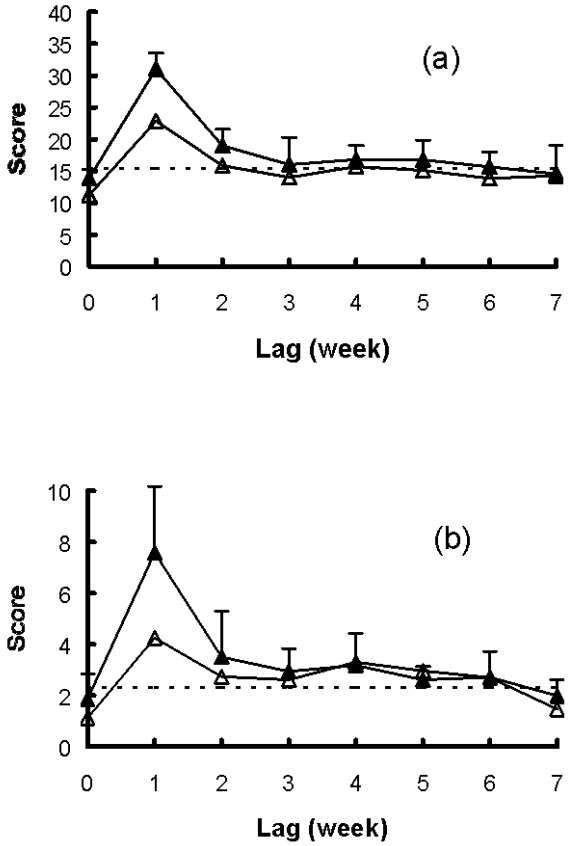


Figure 5. The effect on a) **uneven ripening** and b) **dark patches** of 7 (▲) and 3 d (Δ) heat pulse treatments. The dashed horizontal line represents the average north side M-Block control treatment and the vertical lines SED with 6 d.f.

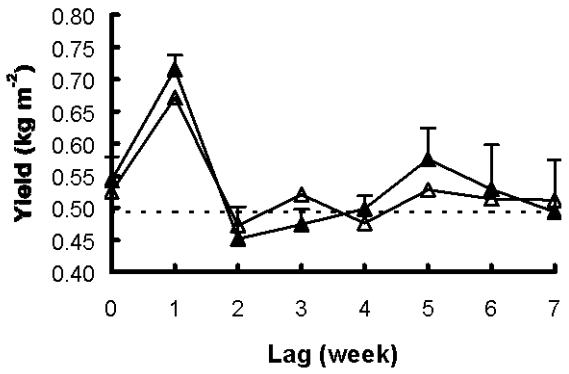


Figure 6. The effect on mean individual pick **fruit yield** during each Lag week of 7 (▲) and 3 d (Δ) heat pulse treatments. The dashed horizontal line represents the average north side M-Block control treatment and the vertical lines are SED with 6 d.f.

Table 6. The effects of 7-day and 3-day temperature pulse treatments on percentage Class I grade-out and total yield in kg m^{-2} for the eight Lag weeks following the end of a heat pulse event in the 1999 LINK experiment. The control means were from the four control plots of the 1999 MAFF experiment on the north side of M-Block and each control mean covers the same set of harvests as the corresponding Lag treatment mean.

Lag Week	Harvest Day	**Percentage Class I**			**Yield kg m^{-2}**		
		7-day	**3-day**	**Control**	**7-day**	**3-day**	**Control**
Lag 0	Mon	94.1	94.6	94.3	.631	.634	.547
	Wed	94.0	94.7	93.8	.429	.457	.433
	Fri	93.3	95.5	94.3	.559	.483	.422
Lag 1	Mon	93.5	95.0	94.9	.998	.756	.599
	Wed	93.5	94.0	94.5	.506	.554	.362
	Fri	95.6	94.8	95.2	.629	.704	.626
Lag 2	Mon	95.9	95.6	95.9	.575	.607	.725
	Wed	95.0	95.7	93.8	.399	.425	.425
	Fri	95.7	95.3	96.2	.428	.446	.531
Lag 3	Mon	96.7	95.8	95.2	.610	.686	.785
	Wed	95.0	95.3	95.5	.378	.408	.428
	Fri	94.3	94.7	95.6	.483	.534	.474
Lag 4	Mon	95.8	95.2	96.4	.643	.646	.581
	Wed	94.4	96.3	93.9	.464	.448	.313
	Fri	95.3	95.2	97.7	.511	.491	.454
Lag 5	Mon	96.3	96.0	96.0	.782	.736	.627
	Wed	95.1	95.5	94.7	.427	.427	.347
	Fri	95.4	95.6	94.4	.637	.568	.448
Lag 6	Mon	95.8	95.9	96.9	.740	.745	.485
	Wed	95.8	96.3	95.6	.491	.480	.425
	Fri	95.3	96.6	93.6	.538	.522	.347
Lag 7	Mon	96.1	96.8	96.3	.723	.738	.609
	Wed	95.9	96.4	94.6	.389	.388	.382
	Fri	94.6	96.0	95.6	.539	.565	.481

(3) Heat pulse effects on percentage Class I fruit and yield

The Lag 0 corresponds to the temperature pulse week lasting from Monday to Sunday inclusive for the 7-day pulse and Friday to Sunday inclusive for the 3-day pulse. This means that fruit harvested on the Wednesday and Friday of Lag 0 of the 7-day pulse treatment would have had some exposure to a heat-pulse immediately before harvest whereas the 3-day heat pulse treatment fruit would have had no such exposure. Table 6 shows a loss of quality on the Friday of Lag 0 for the 7-day pulse treatment of 1 % which is comparable with the loss of quality for this treatment on the following Monday (1.4%). The fruit harvested on the Friday would have experienced about a 4-day heat pulse and this result is consistent with the temperature pulse effects having an almost immediate effect on fruit quality. The 3-day pulse produced a slight reduction in percentage Class I fruit on the Wednesday and Friday of Lag 1. The effects of the temperature pulse treatments on yields was associated with a substantial yield increment on the Monday and a smaller increase on the Wednesday immediately following a 7-day heat pulse. There was also a small increase in yield of harvested fruit on the Friday of lag week zero for the 7-day pulse treatment but of a much smaller magnitude than the yield increment on

the following Monday. However, there appeared to be a subsequent compensatory decrease in yield for Lag 2 and Lag 3 compared with the control. The 3-day heat pulse had a smaller overall effect on yields compared with the 7-day heat pulse and the yield increments occurred throughout the Monday, Wednesday and Friday of Lag 1 rather than being concentrated mainly on the Monday. However, the overall pattern of yield effects due to the heat pulses was broadly similar for both treatments (Fig. 6).

The relationship between the length of the temperature pulse and the percentage *uneven ripening* in Class (A+B+C) and in Class (A+B) is shown in Figures 7 and 8, respectively, for the eight fruit samples taken on each of the eight Mondays following a pulse. These graphs were fitted to the data by assuming a binomial classification of the fruit in each sample and by fitting an estimated response curve for percentage acceptable fruit against pulse duration using the Genstat GLM package. It is apparent that the grading standard used for the eight-fruit samples was much more severe than that used for the harvest yields. Whereas reductions of up to 1.4 % were found in the percentage Class I grade out (Table 6), reductions of almost 10 % were found in Lag week 1 for a 7-day pulse (Fig. 7a). The Lag 1 plots in Figures 7 and 8 show clearly the effects of pulse duration on percentage loss of quality and the smooth trends through the 0-day, 3-day and 7-day periods show the progressive effects of lengthening pulse period on the incidence of *uneven ripening*. The plots for the subsequent Lag periods show no evidence of temperature trend effects and confirm that the impact of temperature on *uneven ripening* is immediate and disappears after at most one week following a temperature pulse event. Similar plots for all other defects in 1999 are presented in Appendix (3).

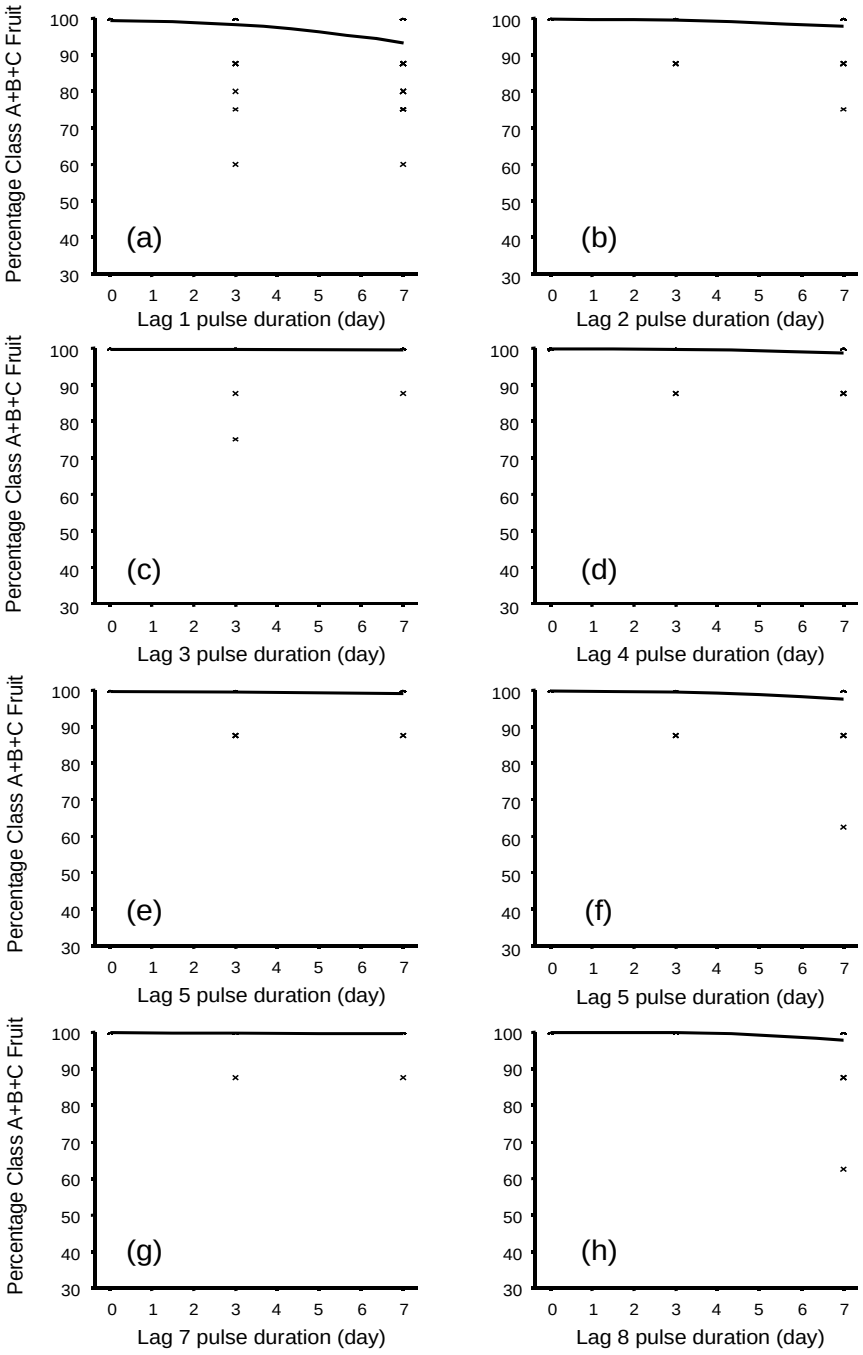


Figure 7. The relationship between % Class I fruit (Class A+B+C) for **uneven ripening** and pulse duration in a-h, for Lags 1-8 respectively.

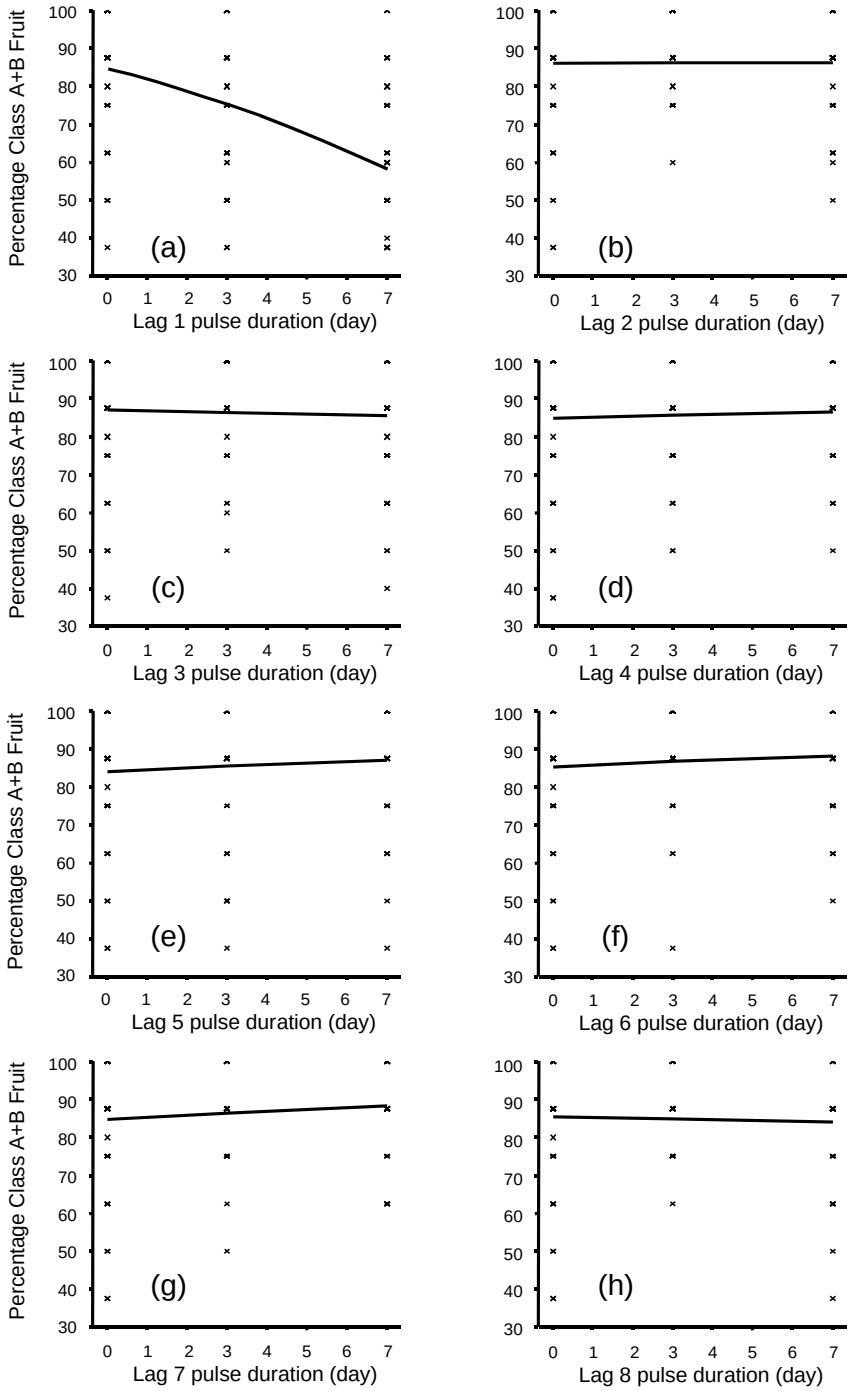


Figure 8. The relationship between % Class I fruit (Class A+B) for **uneven ripening** and pulse duration in a-h, for Lags 1-8 respectively.

Effect of magnitude of the pulse

(1) Skin strength (load) and pericarp firmness

In 2000 load and firmness were markedly decreased in response to increasing mean pulse temperature (Figs 9 and 10). The introduction of fruit pruning treatments, by restricting the numbers of fruits per truss to 5 compared with around 8 in the non-pruned control, appeared to decrease *skin strength* in Lag week 1 ($P<0.01$; Fig. 9a,b). However, pruning had little effect on fruit pericarp *firmness* (Fig. 10a,b).

The effects in Lag 1 appeared to respond in a linear fashion to increasing temperature. Additional linear regression analysis for Lag 1 demonstrated a significant relationship between increasing mean pulse temperature and decreasing *fruit load and firmness* ($P<0.001$; Fig. 11a). The difference in *fruit load* between the pruned and non-pruned treatments was reflected in the different regression lines ($P<0.001$; Fig. 11a), with the pruned fruit showing a lower *load* at low temperature but with a flatter response to increasing temperature compared with the non-pruned fruit. At 26.0°C the two regression lines for load converged. This suggests that pruning had a greater effect on *skin strength* or *load* at mean pulse temperatures below 26.0 °C. As with *load*, *firmness* exhibited a linear decrease with increasing temperature ($P<0.001$; Fig. 11b). However, in contrast to *load*, the relationships for pruned and non-pruned fruit were similar (Fig. 11b).

(2) Uneven ripening

Uneven ripening was increased in response to temperature in Lag 1 of the pulse (Fig. 12a, b) as had been observed in 1999 (Figs 5a,7a,8a). The incidence of this defect exhibited a linear response to increasing temperature ($P<0.001$; Fig. 13). Whilst the differences between the pruned and non-pruned fruit for *uneven ripening* were difficult to assess visually using Figures. 12a, b, a linear regression showed clear differences between the two sub treatments ($P<0.05$; Fig. 13). The regression lines exhibited a similar pattern to the *load* regression lines (Fig. 11a) with the regression lines converging at a high temperature around 26.0°C. Below that temperature there appeared to be a lower incidence of *uneven ripening* in the pruned compared with the non-pruned fruit ($P<0.05$; Fig. 13).

(3) Dark patches

Dark patches also exhibited an increase in incidence with elevated temperature mainly in Lag 1 ($P=0.05$; Fig. 14a, b). Linear regression analysis showed that this relationship was similar for the pruned and non-pruned plants and that increasing incidence in Lag 1 was linearly related to temperature ($P<0.01$; Fig. 15).

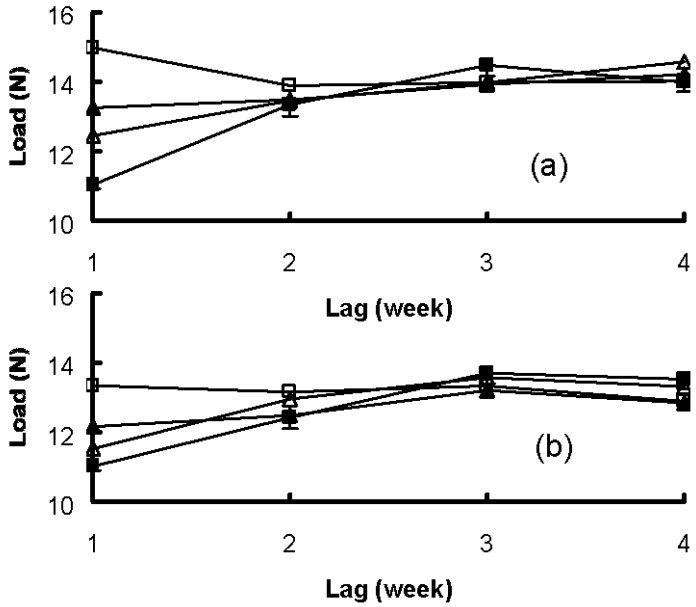


Figure 9. The effect on fruit **skin strength** or **load** of three heat pulse regimes (19/16 °C day/night ventilation set point; (□), 22/19 (▲), 25/22 (Δ) and 28/25 °C (■) in a) non-pruned and b) pruned treatments. Vertical lines are SED with 3 d.f.

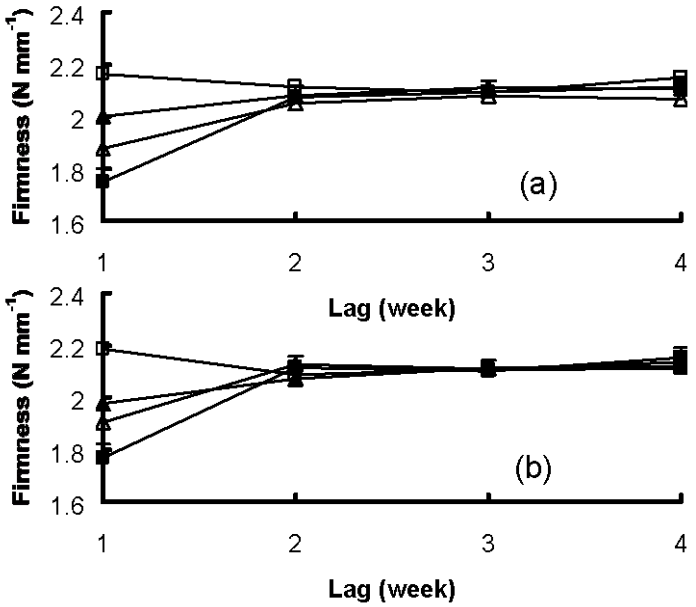


Figure 10. The effect on fruit **firmness** of three heat pulse regimes (19/16 °C day/night ventilation set point; (□), 22/19 (▲), 25/22 (Δ) and 28/25 °C (■) in a) non-pruned and b) pruned treatments. Vertical lines are SED with 3 d.f.

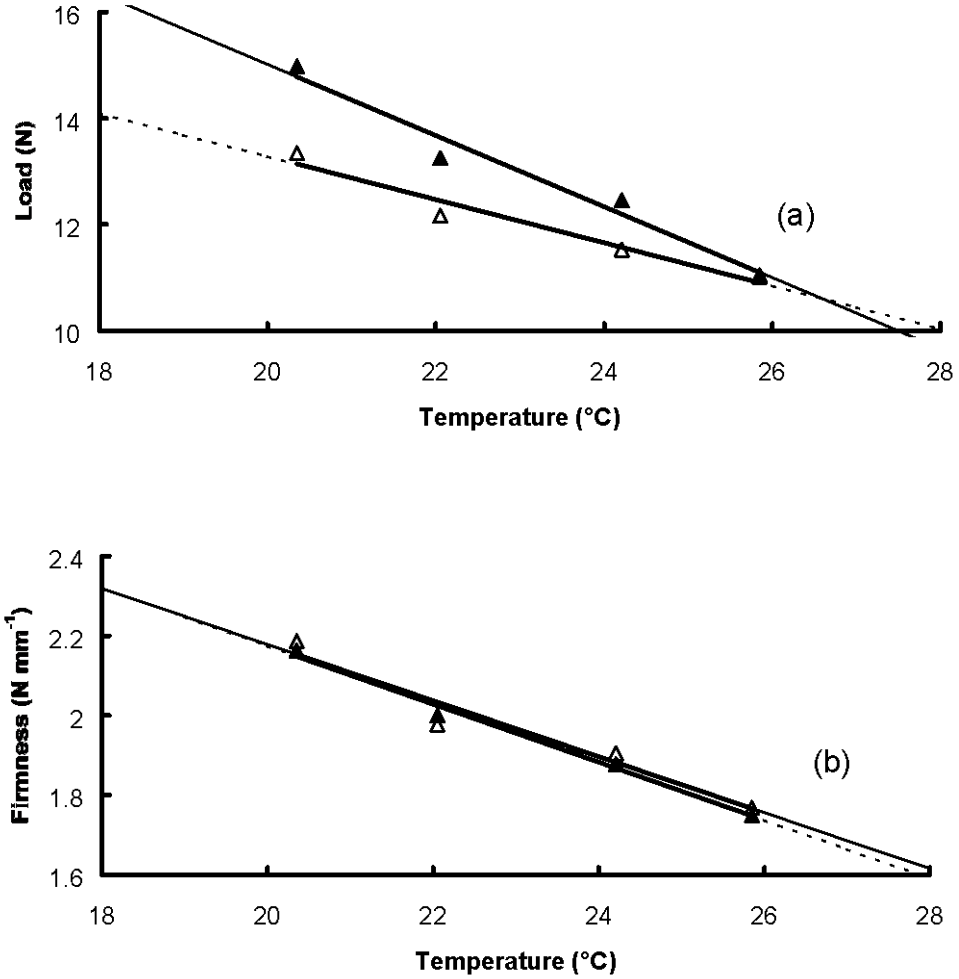


Figure 11. Relationship between heat pulse temperature in Lag week 1and a) **load** (skin strength) and b) **firmness** in non-pruned (▲) and pruned (Δ) plants. Regression analysis produced the following relationships for load, non-pruned=-0.670(T)+28.419 ($P<0.001$; $R^2=0.95$) and pruned=-0.406(T)+21.399 ($P<0.001$; $R^2=0.99$) and firmness non pruned=-0.073(T)+3.634 ($P<0.001$; $R^2=0.99$) and pruned=-0.070(T)+3.586 ($P<0.001$; $R^2=0.95$), with 3 d.f.

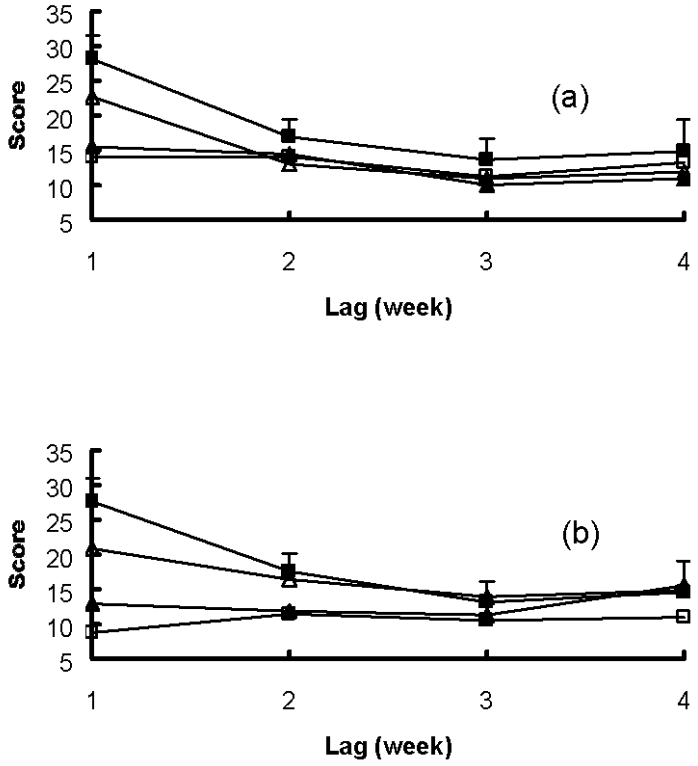


Figure 12. The effect on **uneven ripening** of three heat pulse regimes (19/16 °C day/night ventilation set point; (□), 22/19 (▲), 25/22 (Δ) and 28/25 °C (■) in a) non-pruned and b) pruned treatments. Vertical lines are SED with 3 d.f.

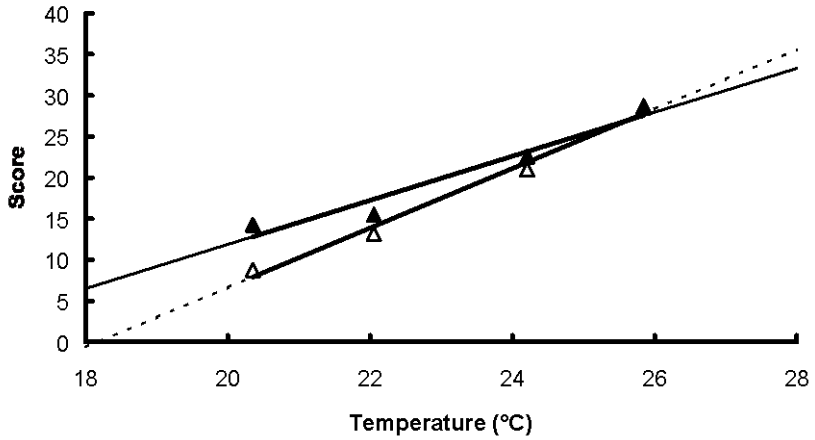


Figure 13. Relationship between heat pulse temperature in Lag week 1 and the incidence of **uneven ripening** for non-pruned (▲) and pruned (Δ) plants. Regression analysis produced the following relationships for non-pruned=2.680(T)-41.728 ($P<0.001$; $R^2=0.99$) and pruned=3.620(T)-65.776 ($P<0.001$; $R^2=0.95$), with 3 d.f.

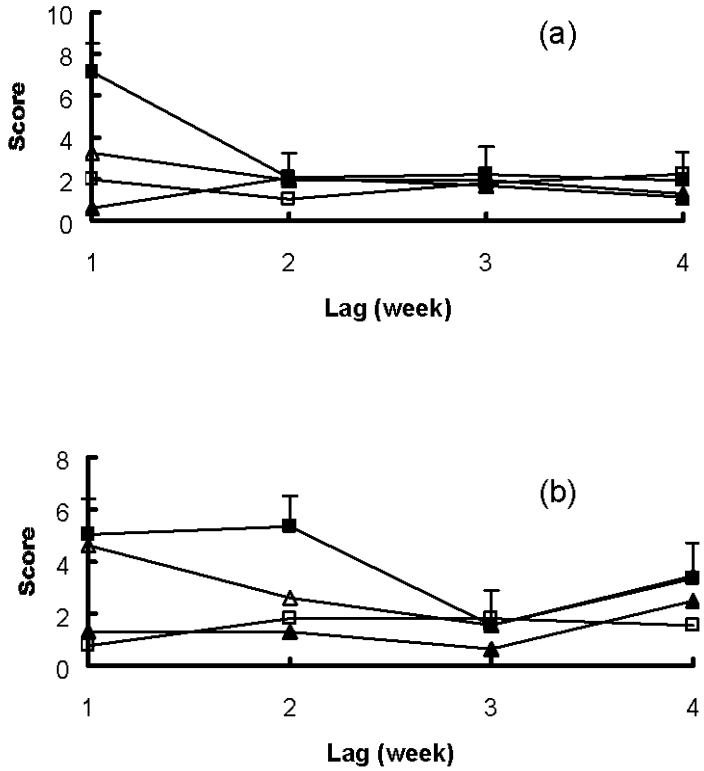


Figure 14. The effect on **dark patches** of three heat pulse regimes (19/16 °C day/night ventilation set point; (□), 22/19 (▲), 25/22 (Δ) and 28/25 °C (■) in a) non-pruned and b) pruned treatments. Vertical lines are SED with 3 d.f.

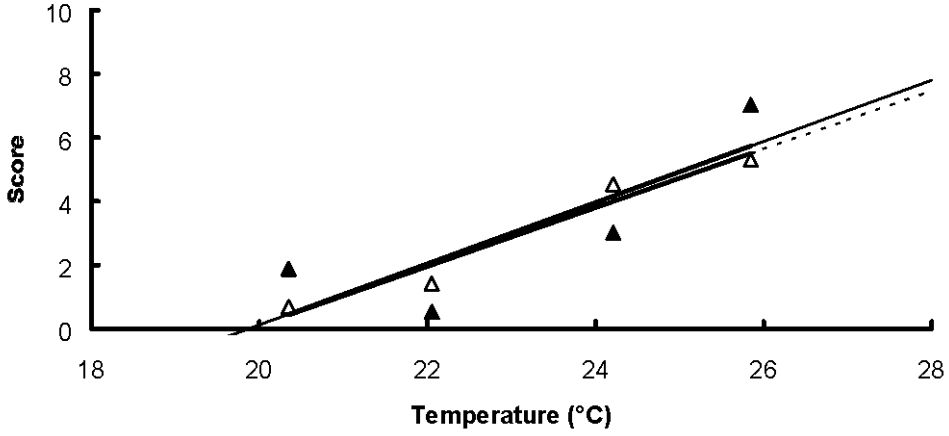


Figure 15. Relationship between heat pulse temperature in Lag week 1 and **dark patches** in non-pruned (▲) and pruned (Δ) plants. Regression analysis produced the following relationships for non-pruned=0.960(T)-19.080 ($P<0.01$; $R^2=0.68$) and pruned=0.920(T)+18.286 ($P<0.001$; $R^2=0.98$) with 3 d.f.

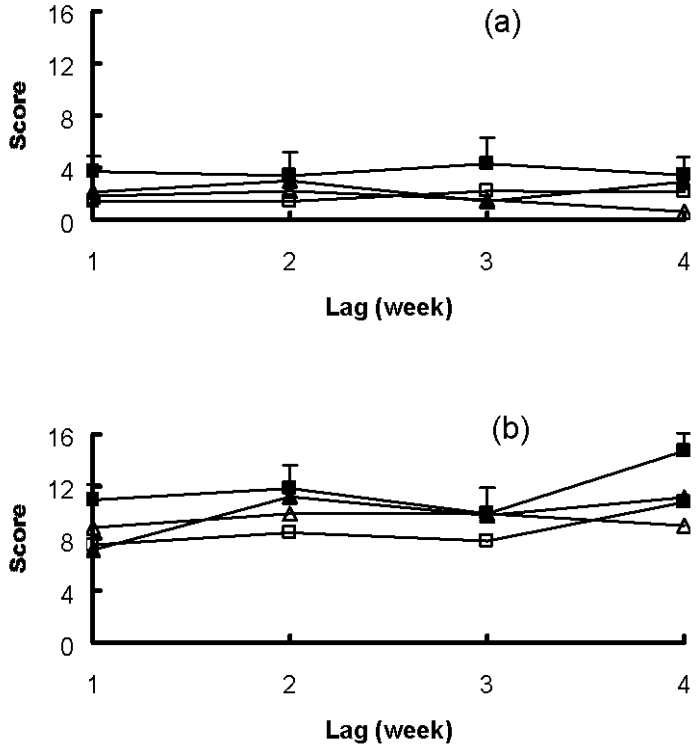


Figure 16. The effect on **netting** of three heat pulse regimes (19/16 °C day/night ventilation set point; (□), 22/19 (▲), 25/22 (Δ) and 28/25 °C (■) in a) non-pruned and b) pruned treatments. Vertical lines are SED with 3 d.f.

(4) Netting

Netting was markedly and consistently higher in the pruned fruit compared with the non-pruned control ($P<0.001$; Fig. 16). As *netting* is due to disruption of the skin surface, this may have been related to decreased skin strength in the pruned fruits (cf Figs. 9a, b and 11a).

(5) Heat pulse and pruning effects on yield and percentage Class I fruit

Table 7 shows the effects of the 3-day heat pulse temperature treatments on the non-pruned fruit treatment in 2000 and the general pattern of heat pulse effects on both quality and yield is very comparable with the results observed from the corresponding 3-day pulse treatments in 1999. The effects on percentage Class I were slight but there was evidence of a loss of quality on the Wednesday of Lag 1 with a loss of about 1% in Class I yield due to the highest pulse temperature treatment compared with the control treatment. The effect of temperature on yield was very clear with a steady increase in yield increment due to heat pulse-temperature increments on the Wednesday and the Friday of Lag 1. There was little evidence of heat-pulse effects on yields on the Monday immediately following a heat pulse event. There were corresponding yield decreases due to increasing heat pulse temperature effects in Lags 2 and 3 indicating compensatory losses of yield (Table 7).

Table 7. The effect of a range of 3-day heat pulse treatments on percentage Class I grade-out and total yield in kg m^{-2} for the four Lag weeks following the end of a heat pulse event in the 2000 LINK experiment for **non-pruned** fruit.

Lag week	Harvest Day	**Percentage Class I**				**Yield in kg m^{-2}**			
		20.4°C	22.2°C	24.2°C	25.9°C	20.4°C	22.2°C	24.2°C	25.9°C
Lag 1	Mon	96.5	96.3	96.6	96.4	.669	.687	.708	.667
	Wed	97.8	97.0	97.1	96.8	.405	.492	.590	.614
	Fri	97.1	97.9	96.3	96.7	.476	.573	.656	.654
Lag 2	Mon	97.2	96.7	96.6	96.6	.736	.693	.660	.606
	Wed	97.0	97.4	96.3	97.4	.511	.525	.455	.424
	Fri	96.5	97.7	98.9	96.6	.531	.537	.556	.516
Lag 3	Mon	97.3	97.4	97.6	97.3	.681	.742	.697	.601
	Wed	97.1	97.4	97.3	97.4	.455	.429	.394	.381
	Fri	96.9	97.1	96.9	96.8	.515	.497	.448	.408
Lag 4	Mon	97.4	96.9	96.0	97.4	.719	.661	.644	.626
	Wed	96.4	96.0	95.6	96.2	.500	.538	.537	.510
	Fri	96.7	96.7	97.5	96.6	.530	.567	.540	.555

Table 8 shows the effects of a 3-day heat pulse on the pruned fruit treatment and shows a pattern of heat pulse effects on both quality and yield that is broadly similar to the pattern of effects on the non-pruned fruits. However, the effects of the temperature pulse on the yield increments of the pruned fruits during Lag 1 appear more marked than the effects on the non-pruned fruits. There is clear evidence of yield increments due to the heat pulse effects on the Monday of Lag 1 and the increases on the Wednesday of Lag 1 appear larger than the increments for the corresponding non-pruned fruit, after allowing for the lower overall yields of the pruned fruit. Further experimental investigation of temperature pulse effects on the distribution of fruit between the various Class I size grades for the pruned and the non-pruned fruits could be of value. It was also noticeable that percentage Class 1 fruits were relatively lower for the pruned compared with the non-pruned fruit (Tables 7 and 8). This may have been due to increased *netting* in the pruned compared with the non-pruned fruits at high temperature (Fig. 16b).

Table 8. The effects of a range of 3-day heat pulse treatments on percentage Class I grade-out and total yield in kg m^{-2} for the four Lag weeks following the end of a heat pulse event in the 2000 LINK experiment for **pruned** fruit.

Lag week	Harvest Day	**Percentage Class I**				**Yield in kg m^{-2}**			
		20.4°C	22.2°C	24.2°C	25.9°C	20.4°C	22.2°C	24.2°C	25.9°C
Lag 1	Mon	96.4	95.4	95.8	94.7	.610	.591	.653	.658
	Wed	97.5	96.6	97.6	96.8	.390	.466	.550	.624
	Fri	95.8	96.7	95.9	95.9	.428	.474	.553	.563
Lag 2	Mon	96.3	95.8	95.6	95.1	.614	.600	.620	.580
	Wed	95.1	96.1	96.6	95.9	.445	.416	.391	.405
	Fri	96.2	97.1	96.7	96.4	.479	.431	.442	.409
Lag 3	Mon	95.7	95.9	95.3	96.1	.621	.618	.636	.518
	Wed	97.1	97.3	95.6	96.5	.412	.379	.378	.356
	Fri	97.5	95.5	95.1	96.4	.520	.373	.361	.343
Lag 4	Mon	97.2	95.9	95.9	95.3	.673	.545	.558	.573
	Wed	97.3	95.2	96.4	96.2	.432	.452	.513	.479
	Fri	97.3	95.3	96.7	97.0	.471	.477	.483	.489

Figures 17 and 18 show the percentage Class I grade-out (A+B+C) and the percentage (A+B) grade-out, respectively, for the temperature treatment effects on the sampled fruit defects for each of the four Lag weeks following a temperature pulse event. Comparison of the observed percentage Class I grade-out of the Lag 1 Monday yields (Tables 7 and 8) against the estimated percentage Class I grade-out of the fruit samples (Fig. 17), show clear inconsistencies in the standard used to assess fruit quality. The loss of percentage Class I fruit due to uneven ripening in 2000 was estimated to be about 10% (Fig. 17) at the highest temperature pulse treatment. For percentage Class (A+B) fruit (Fig. 18) the effects of temperature was even more pronounced. However, the actual amount of downgraded harvested fruit on the Monday following a heat pulse event was less than 2 % (Tables 7 and 8). Taken together, the evidence from the 1999 and 2000 experiments, suggests that the grade-out standard for the plot yields may have been less stringent than the standard for the sampled fruit visual defect analysis.

The limited evidence from the harvest grade-out suggests that loss of percentage Class I fruit during the Lag 1 week of a 3-day heat pulse was greater on the Wednesday and possibly on the Friday than on the Monday compared with the 7-day pulse (Table 6). This suggests that the Monday fruit sample may have missed the peak of the fruit defects caused by the 3-day heat pulse and, in retrospect, fruit should have been sampled on each harvest day throughout the week following a heat-pulse event. The 7-day pulse defects were greater than the 3-day pulse defects (Figs 7 and 8), but it was impossible to determine whether the extra incidence of defects was due entirely to the extra heat exposure or was due partly to defects that developed over a longer time period than three days (Figs 7 and 8). However it is clear from the evidence of the later Lag data periods in a heat pulse cycle that all the defects occurred substantially within the first week after exposure to a heat pulse event (Figs 7,8,17,18). Plots for all other sample defect data collected in 2000 are presented in Appendix 4.

Overall, these results support the interpretation that temperature pulses affect the quality of fruit near the picking stage, that the effects are observed almost immediately and peak within three or four days of a short temperature pulse application and that the effects substantially disappear within about a week. Temperature pulse effects in the short term on yield follow a similar pattern compared with fruit quality defects showing an increment within three or four days following a short temperature pulse application. However there is a compensatory loss of yield in the second and third weeks following a pulse event.

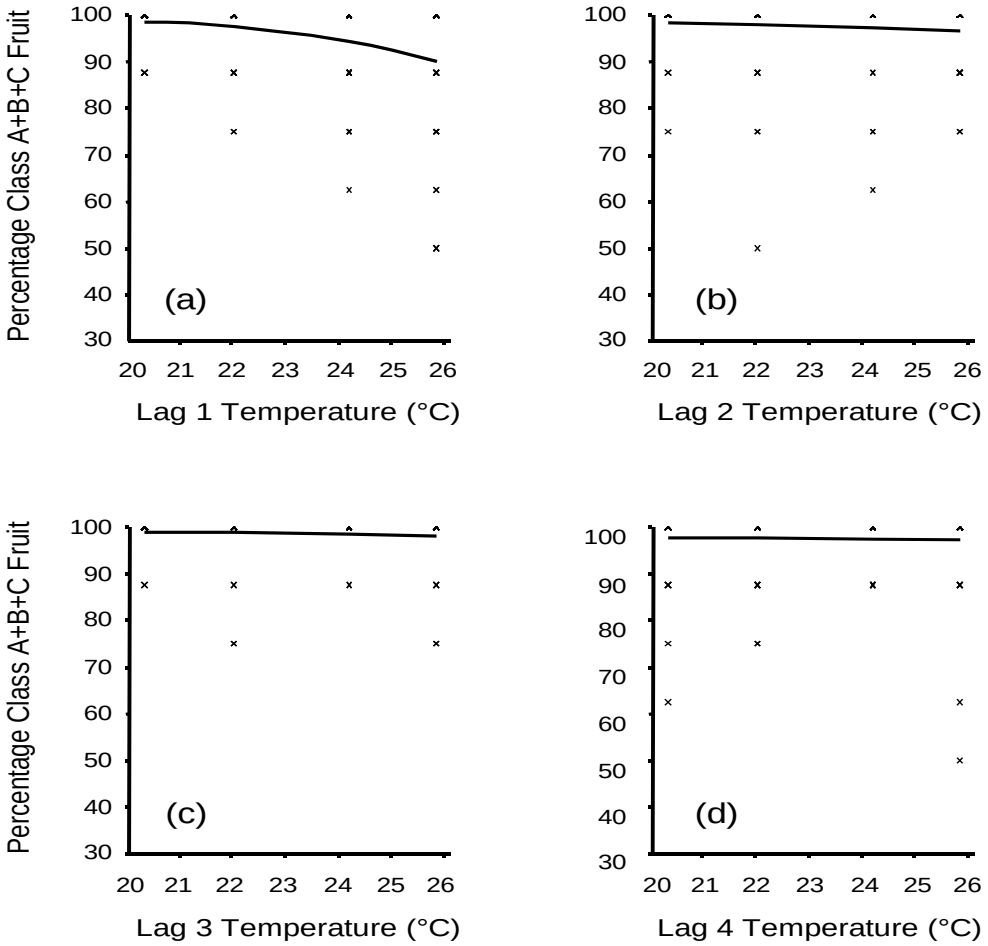


Figure 17. The relationship between % Class I fruit (Class A+B+C) for **uneven ripening** and mean pulse temperature in a-d, Lags 1-4 respectively.

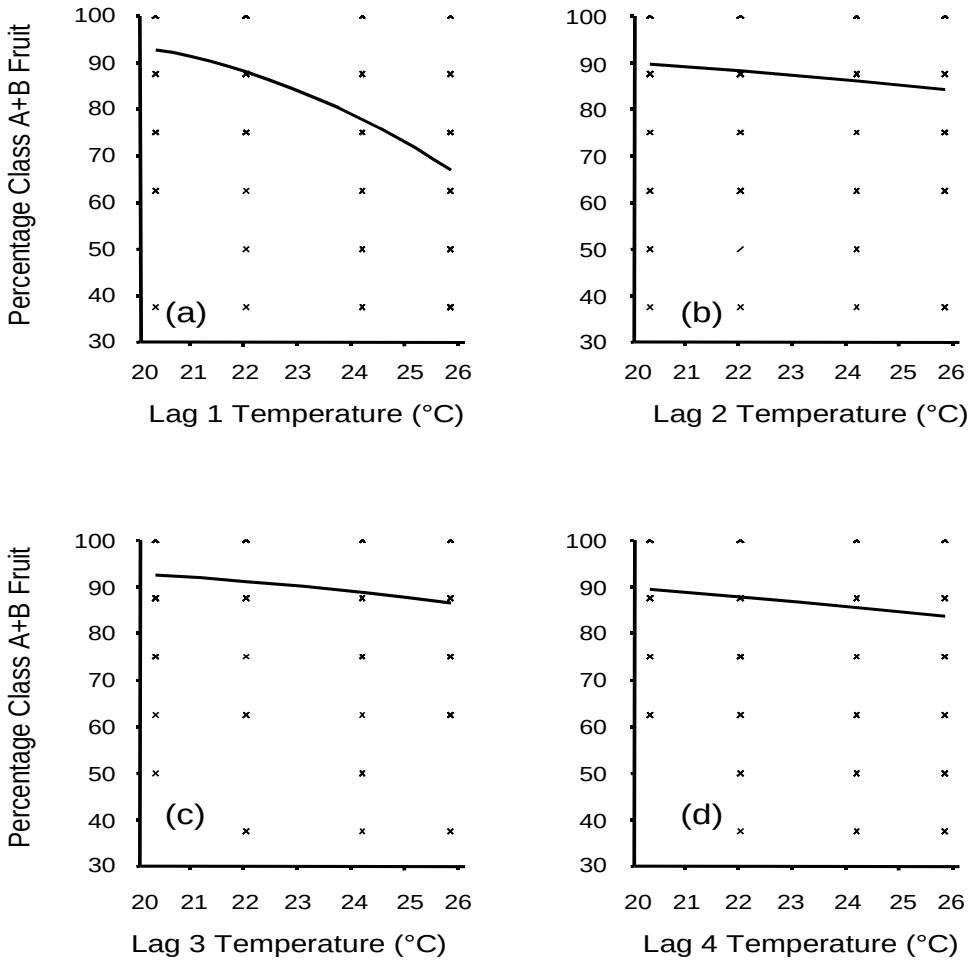


Figure 18. The relationship between % Class I fruit (Class A+B) for **uneven ripening** and mean pulse temperature in a-d, Lags 1-4 respectively.

(6) The relative effects of pipe and air temperature and humidity on fruit quality defects

Temperature was the controlled environmental variable in all the experiments but as other concomitant variables change in response to a heat pulse it was considered appropriate to explore the impact of certain other environmental factors on fruit quality. In addition to air temperature, pipe temperature and humidity were recorded during the heat pulse events and these variables have been analysed as covariates that may have affected fruit quality independently of the set temperature levels.

The effects of the covariates for the linear trend effects of pipe-temperature and air-temperature at Lag 1 after fitting a linear trend for evenly-spaced temperature levels are shown in Tables 9 and 10 respectively. The purpose of these models was to test for any additional effects of pipe-temperature or air-temperature on the measured quality variables after eliminating the effects of the evenly-spaced temperature levels. However, the correlation coefficients between the evenly-spaced temperature levels and the pipe-temperature and air-temperature variables were, respectively, 0.992 and 0.998 and these correlations show that pipe-temperature and air-temperature effects cannot be separated from the effects of the intended temperature levels. Tables 9 and 10 are therefore of little value for exploring the effects of pipe-temperature and air-temperature separately from the effects of the evenly-spaced temperature treatments.

Table 11 shows the linear effects of relative humidity on fruit quality at Lag 1 after allowing for the effects of the set temperature levels. The correlation between relative humidity and the evenly-spaced temperature levels was 0.602 and this shows that although relative humidity was strongly correlated with temperature, it was not fully explained by temperature. Table 11 may therefore provide some useful information on relative humidity effects after allowing for linear temperature effects. Due to the limited number of degrees of freedom available for estimating residual error in the compartment stratum, the error variance is poorly determined and none of the variance ratios for relative humidity in Table 11 achieve the nominal 5% F-level of 7.71. Nevertheless, the effects of relative humidity do approach the 5% significance level for *gold marbling* and *netting* and the regression coefficients of 0.59 (SE=0.224) for *gold marbling* and –0.249 (SE=0.096) for *netting* respectively do provide some weak evidence that high humidity increased *gold marbling* and reduced *netting*. **Substantially similar effects were found when humidity was expressed as VPD.** As this project was not designed to study the impact of humidity *per se*, these results are best regarded as hypotheses that need to be tested by further experimentation. No firm conclusions can be drawn from this study on the effects of humidity on fruit quality.

Table 9. Analysis of Lag 1 mean square effects due to **pipe-temperature** after allowing for the linear trend effect of evenly spaced temperature levels.

		Defect									
		Blotchy		Gold spot		Gold Marbling		Netting		Dark patches	
Source	df	MS	VR	MS	VR	MS	VR	MS	VR	MS	VR
Blocks	1	1452.6		446.1		1585.5		61.2		26.1	
Temps	1	7759.9	47.1	472.0	13.8	334.0	16.7	272.3	24.2	854.3	16.2
Pipetemp	1	39.0	0.2	81.7	2.4	353.2	17.6	33.3	2.9	1.0	0.0
Resid	4	164.7		34.2		20.1		11.3		52.7	

Table 10. Analysis of Lag 1 mean square effects due to **air temperature** after allowing for the linear trend effect of evenly spaced temperature levels.

		Defect									
		Blotchy		Gold spot		Gold Marbling		Netting		Dark patches	
Source	df	MS	VR	MS	VR	MS	VR	MS	VR	MS	VR
Blocks	1	1452.6		446.1		1585.5		61.2		26.1	
Temps	1	7759.9	46.7	472.0	8.8	334.0	3.6	272.3	23.5	854.3	16.3
Airtemp	1	32.5	0.2	3.4	0.1	57.0	0.6	31.9	2.7	2.7	0.1
Resid	4	166.3		53.7		94.1		11.6		52.3	

Table 11. Analysis of Lag 1 mean square effects due to **relative humidity** after allowing for the linear trend effect of evenly spaced temperature levels.

		Defect									
		Blotchy		Gold spot		Gold Marbling		Netting		Dark patches	
Source	df	MS	VR	MS	VR	MS	VR	MS	VR	MS	VR
Blocks	1	1452.6		446.1		1585.5		61.2		26.1	
Temps	1	7759.9	64.4	472.0	8.9	334.0	8.4	272.3	37.2	854.3	33.4
RH	1	216.1	1.8	5.9	0.1	274.8	6.9	49.1	6.7	109.5	4.3
Resid	4	120.4		53.1		39.6		7.3		25.6	

(7) Heat pulse effects on fruit chemical composition

Glucose and *fructose* concentrations showed no consistent response to the heat pulse (Figs 19 and 20). However for *glucose*, concentrations increased during Lag weeks 1-4, and were more apparent in the non-pruned compared with the pruned fruit. These increases may have been due to an overriding seasonal effect that was related to increasing light levels during the summer period. This effect was not observed for *fructose*. Pruning, however, appeared to have an effect on sugar content where the overall concentrations for *glucose* and *fructose* were increased in the pruned fruits compared with the non-pruned controls ($P<0.05$; Figs 19 and 20).

Fruit juice *citrate* concentration was unaffected by the heat pulse in both the pruned and the non-pruned treatments (Fig. 21). However, there was a decline in citrate concentration in the Lag weeks 3 and 4 in the pruned compared with the non-pruned treatments ($P<0.01$; Fig. 21). This appeared to coincide with a seasonal decline in citrate concentration rather than a specific effect of the heat pulse. In contrast, *malate* was significantly reduced

in response to increasing mean pulse temperature (P<0.001; Fig. 22a,b). *Malate* concentrations were consistently higher in the non-pruned compared with the pruned fruits (P<0.001; Fig. 22a, b, 23). Linear relationships with increasing temperature were found in the Lag 1 phase of the pulse cycle (P<0.001; Fig. 23), whereby malate concentrations were reduced at a rate of 0.025 and 0.018 mg ml^{-1} for non-pruned and pruned fruit respectively per °C rise in pulse mean temperature.

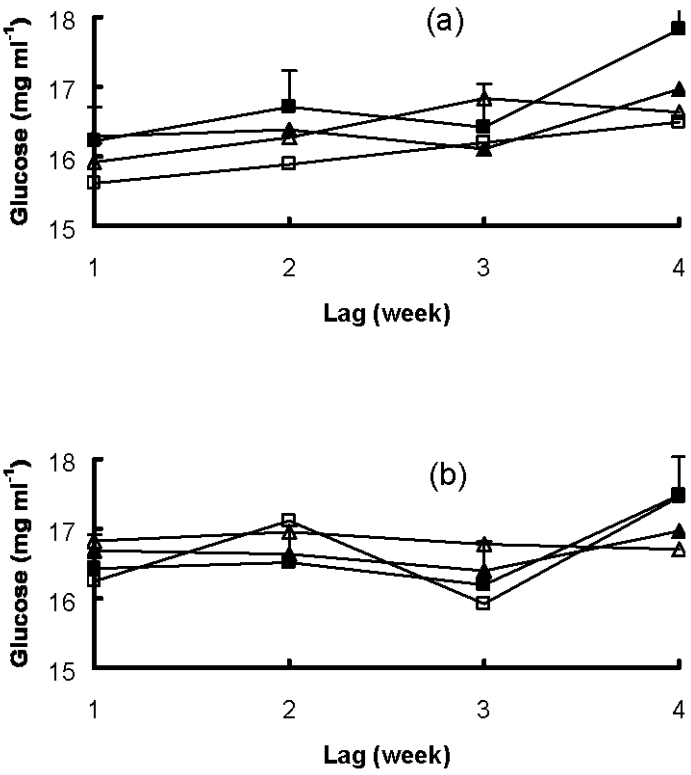


Figure 19. The effect on the **glucose** concentration of fruit juice of three heat pulse regimes (19/16 °C day/night ventilation set point; (□), 22/19 (▲), 25/22 (Δ) and 28/25 °C (■) in a) non-pruned and b) pruned treatments. Vertical lines are SED with 3 d.f.

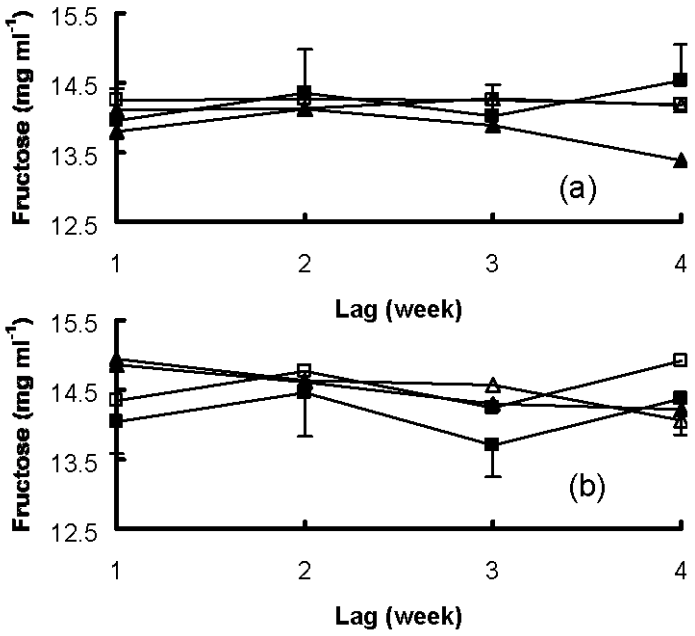


Figure 20. The effect on the **fructose** concentration of fruit juice of three heat pulse regimes (19/16 °C day/night ventilation set point; (□), 22/19 (▲), 25/22 (Δ) and 28/25 °C (■) in a) non-pruned and b) pruned treatments. Vertical lines are SED with 3 d.f.

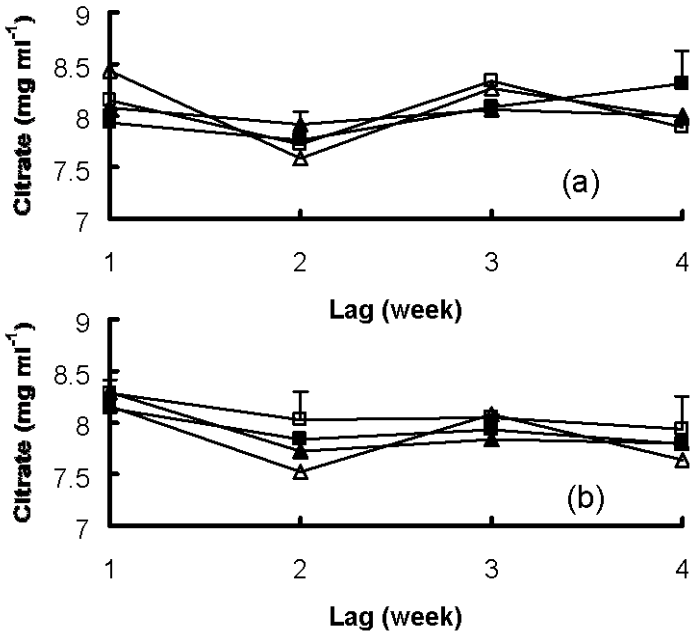


Figure 21. The effect on the **citrate** concentration of fruit juice of three heat pulse regimes (19/16 °C day/night ventilation set point; (□), 22/19 (▲), 25/22 (Δ) and 28/25 °C (■) in a) non-pruned and b) pruned treatments. Vertical lines are SED with 3 d.f.

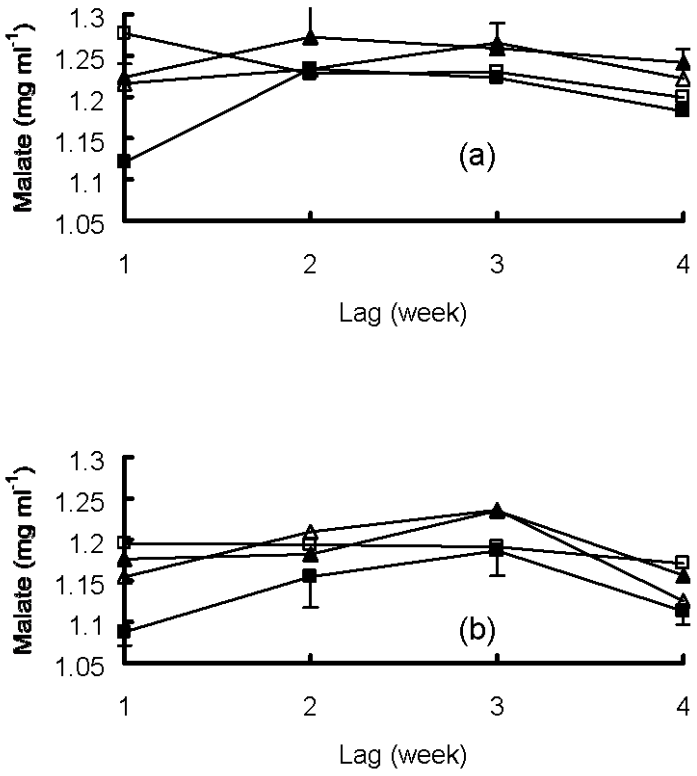


Figure 22. The effect on the **malate** concentration of fruit juice of three heat pulse regimes (19/16 °C day/night ventilation set point; (□), 22/19 (▲), 25/22 (Δ) and 28/25 °C (■) in a) non-pruned and b) pruned treatments. Vertical lines are SED with 3 d.f.

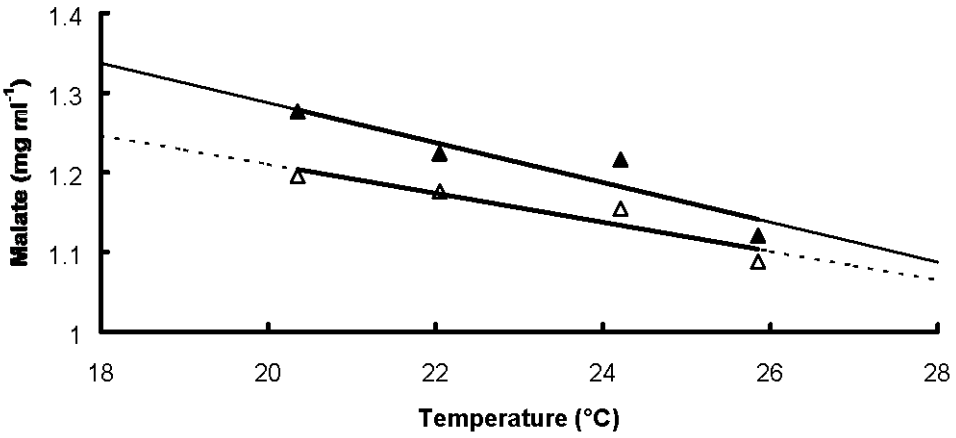


Figure 23. Relationship between heat pulse temperature in Lag week 1 and fruit juice **malate** concentrations in non-pruned (▲) and pruned (Δ) plants. Regression analysis produced the following relationships for non-pruned=-0.025(T)+1.7885 ($P<0.001$; $R^2=0.86$) and pruned=-0.018(T)+1.575 ($P<0.001$; $R^2=0.88$) with 3 d.f.

Objective 2:
To determine the role of high temperature during the early stages of fruit development on subsequent fruit quality

The experimentation in 1998 and 1999 was designed to explore the effect of the heat pulse on fruit development from near ripe fruit through to those in the early stages of growth. Thus an eight-week cycle encompassed the entire growth cycle of individual fruits of tomato under near commercial glasshouse conditions.

All the fruits were examined as they came to harvest, but no deleterious effect on quality for the key defects was observed in young fruit that was exposed to high temperatures during the heat pulse event. These fruit did not appear to suffer the same damaging effects on quality observed for near mature fruit at the time of the pulse. For example, it was only in Lag week 1 after the pulse event, that any effect on *uneven ripening* was observed and subsequent Lag weeks of the pulse showed no effect (Fig. 5a). Similarly fruit *load* and *firmness* (Fig. 4a, b) and *dark patches* (Fig. 5b) were only significantly altered in Lag week 1 of the pulse. However in the 1998 and 1999 experiments increases in Lag 7 of the eight-week pulse cycle were observed for *gold marbling* (data not shown). However, the incidences of such defects late in the pulse cycle, although consistent, were of considerably less importance than the incidence of defects in the Lag 1 immediately following the pulse event and did not merit further detailed examination. Therefore we can conclude that key quality defects are not induced by high temperature in the early stages of fruit development.

Objective 3:
To define the role of K and Ca uptake, distribution and deposition in causing low acidity, blotchy ripening and softness in the fruit

Fruit Ca and K in ripe fruit were largely unaffected by the heat pulse regimes at any stage of fruit development (Figs 24 and 25). However, both K and Ca exhibited a seasonal decline in concentration in mature tomato fruit (Fig. 26).

Associated with the seasonal decline in these key minerals, there was a four-fold increase in *uneven ripening* (Fig. 27a) and a 12% decrease in *fruit firmness* i.e. the fruit became softer (Fig. 27b) over this 20 week period.

A strong relationship was found between declining K and increase in pH as the season progressed (Fig. 27c). In the 20 weeks from May to September, the K concentration was reduced at a rate of 0.095% per week resulting in a reduction from 5 to 3.1% (i.e. 38% reduction). Similarly the Ca concentration within the fruit was reduced at a rate of 0.003% per week resulting in a reduction of 0.14 to 0.08% (i.e. 43% reduction).

Although the heat pulse increased the incidence of *uneven ripening* and *softness*, neither K nor Ca concentrations in the ripe fruit were affected by the heat pulse treatment. However, the seasonal decline in K and Ca coincided with increased *uneven ripening* and *softness*. Therefore the role of K and Ca in these key defects induced by a high temperature heat pulse event remains unclear.

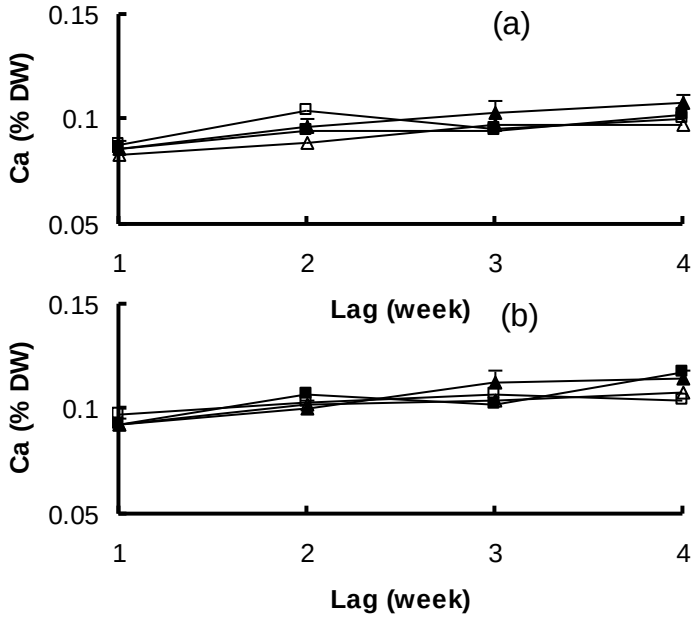


Figure 24. The effect on fruit **Ca** concentration of three heat pulse regimes (19/16 °C day/night ventilation set point; (□), 22/19 (▲), 25/22 (Δ) and 28/25 °C (■) in a) non-pruned and b) pruned treatments. Vertical lines are SED with 3 d.f.

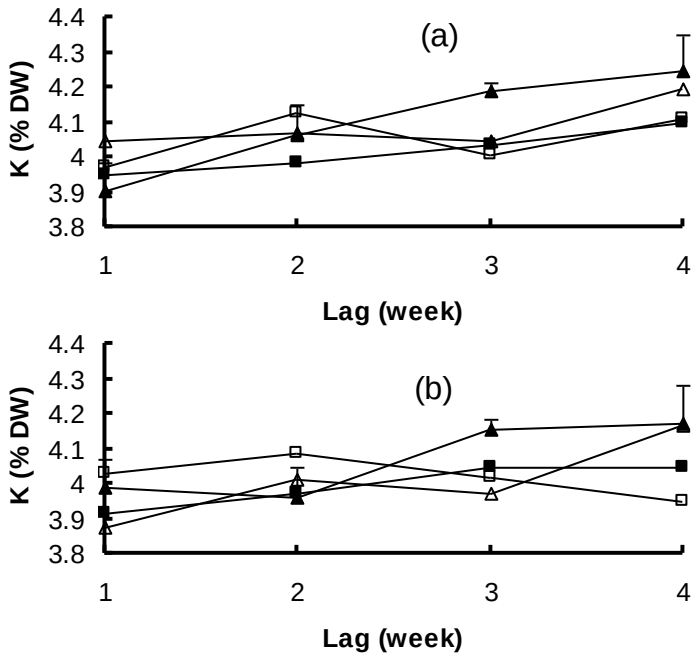


Figure 25. The effect on fruit **K** concentration of three heat pulse regimes (19/16 °C day/night ventilation set point; (□), 22/19 (▲), 25/22 (Δ) and 28/25 °C (■) in a) non-pruned and b) pruned treatments. Vertical lines are SED with 3 d.f.

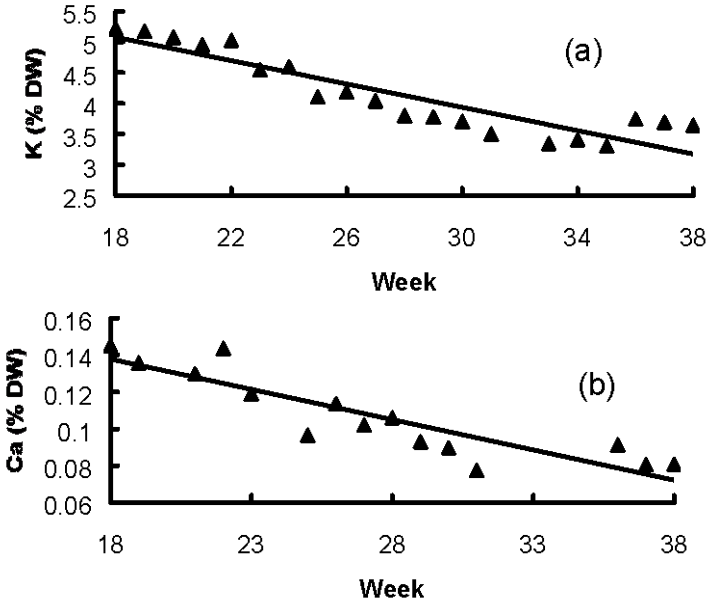


Figure 26. The decline in a) **K** and b) **Ca** over the 2000 season in unpruned fruit. The regression analysis produced the following relationships for K=-0.095(x)+6.783 ($P<0.001$; $R^2=0.85$) and Ca=-0.003(x)+0.196 ($P<0.001$; $R^2=0.82$).

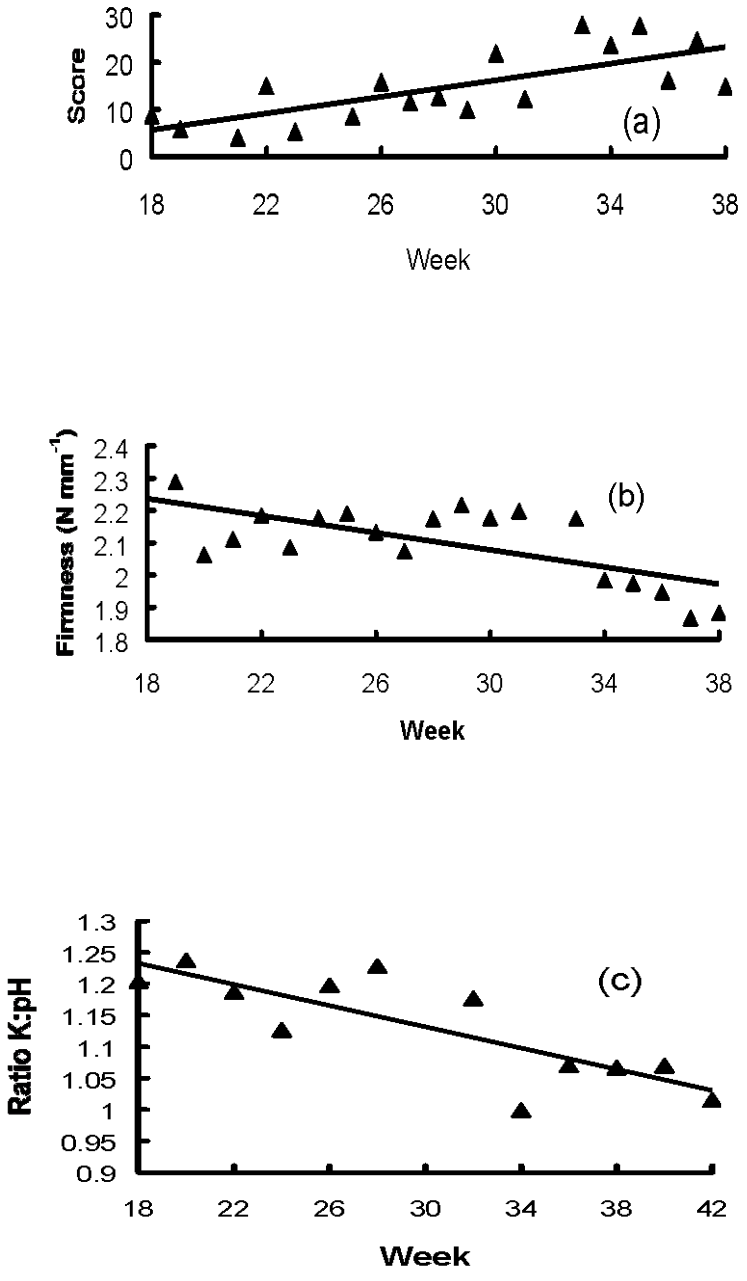


Figure 27. The increase in the incidence of a) **uneven ripening** and the decrease in b) **fruit firmness** in 2000 and c) the fruit juice **K/pH ratio** over the 1999 season. The regression analysis produced the following relationships **for uneven ripening**=0.878(x)+10.129 ($P<0.01$; $R^2=0.58$), **firmness**=-0.013(x)+2.486 ($P<0.01$; $R^2=0.45$) and the ratio of **K/pH**=-0.0084(x)+1.385($P<0.001$; $R^2=0.67$).

Objective 4:
To quantify cellular and tissue water relations which lead to softness, cracking of the fruit surface and deposition of calcium oxalate crystals and to investigate the role of temperature, humidity and EC in controlling these water relations

Water supply, yield and quality

The modulated EC system in use at Efford responded to high light by reducing EC (Figs 28 and 29). This response was not instantaneous for reasons outlined in the Materials and Methods. However, the technique demonstrated that an EC regime in the range 1.8 to 4.8 had little effect on yield or fruit quality (data not shown).

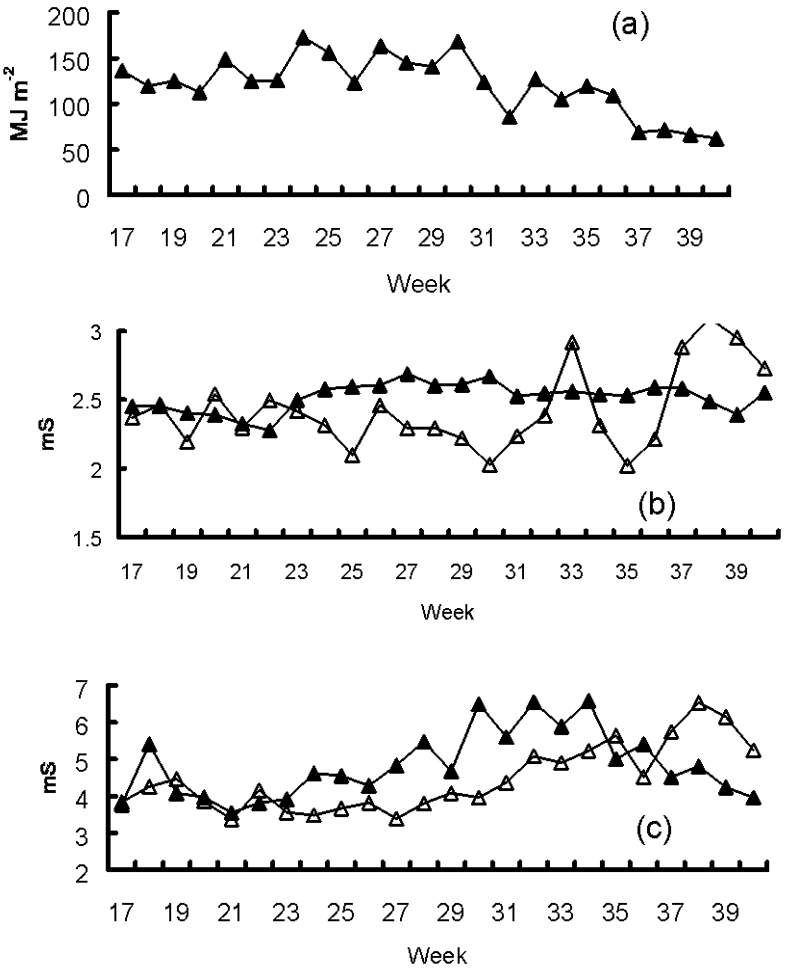


Figure 28. Environmental conditions for a) short wave solar radiation b) EC in the applied nutrient feed and c) EC in the runoff or drain during 1999, for constant (▲) and modulated (Δ) EC.

The seasonal modulated EC plots showed that EC was higher in the modulated EC treatments at the start of the season (low light), lower in mid summer (high light) and higher at the end of the season, due again to low

light. There was an expectation that modulating EC during high light periods would reduce the availability of K to the fruits and thus increase *uneven ripening*. However, there was no significant effect of modulated EC compared with constant EC on the concentration of K within the fruit or on the incidence of *uneven ripening*. There was also no significant effect of the SRM EC treatment on firmness implying that attempted manipulation of turgor pressure within the fruit via altering water flux within the plant was ineffective in preventing *softness* during a heat pulse episode.

There was also a lack of effect of EC on the incidence of *uneven ripening* or *gold-spot*. However, clear evidence of the deposition of calcium oxalate crystals in individual cells of the pericarp, was found in the fruit with *gold-spot* (Plate 2). But the incidence of *gold-spot* correlated poorly with fruit firmness in any of the years (data not shown).

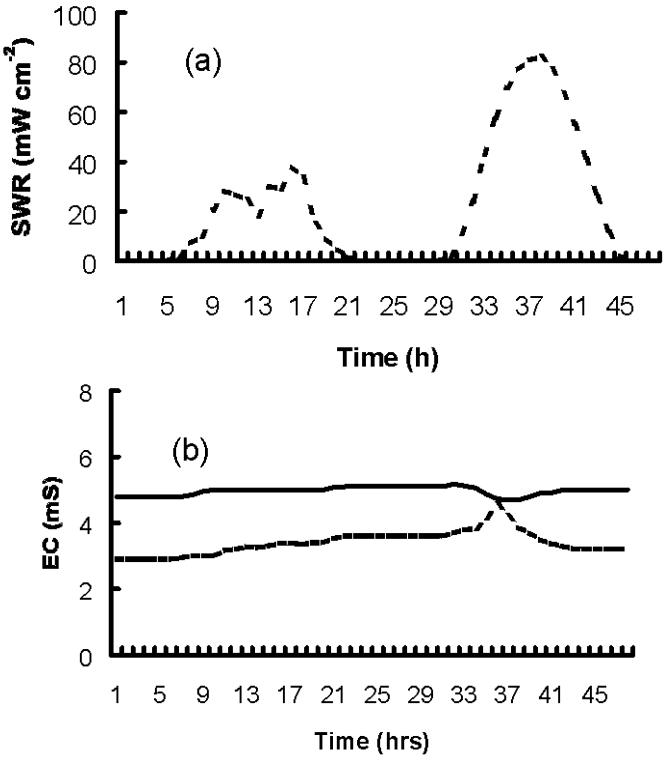
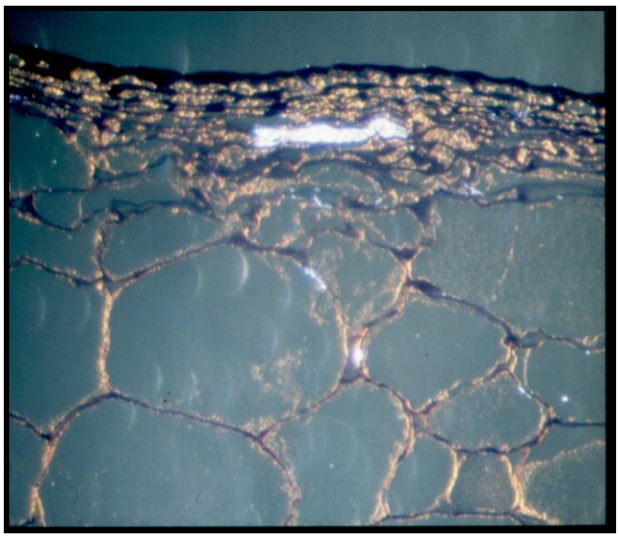


Figure 29. Time course of a) short-wave radiation (SWR) and b) EC in the runoff of constant (solid line) and modulated EC treatments (dashed line) on 22-23 July 1999.

(a)



(b)

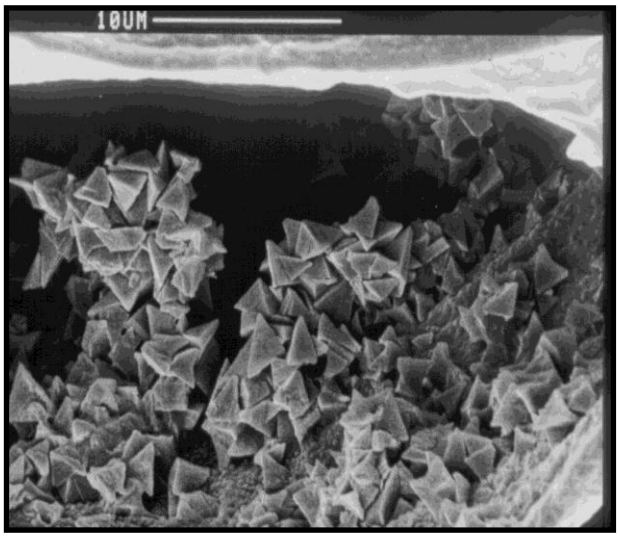


Plate 2. An image of a) **gold-spot** ‘cell’ full of pyramidal Calcium oxalate crystals and b) the same cell under scanning electron microspcopy.

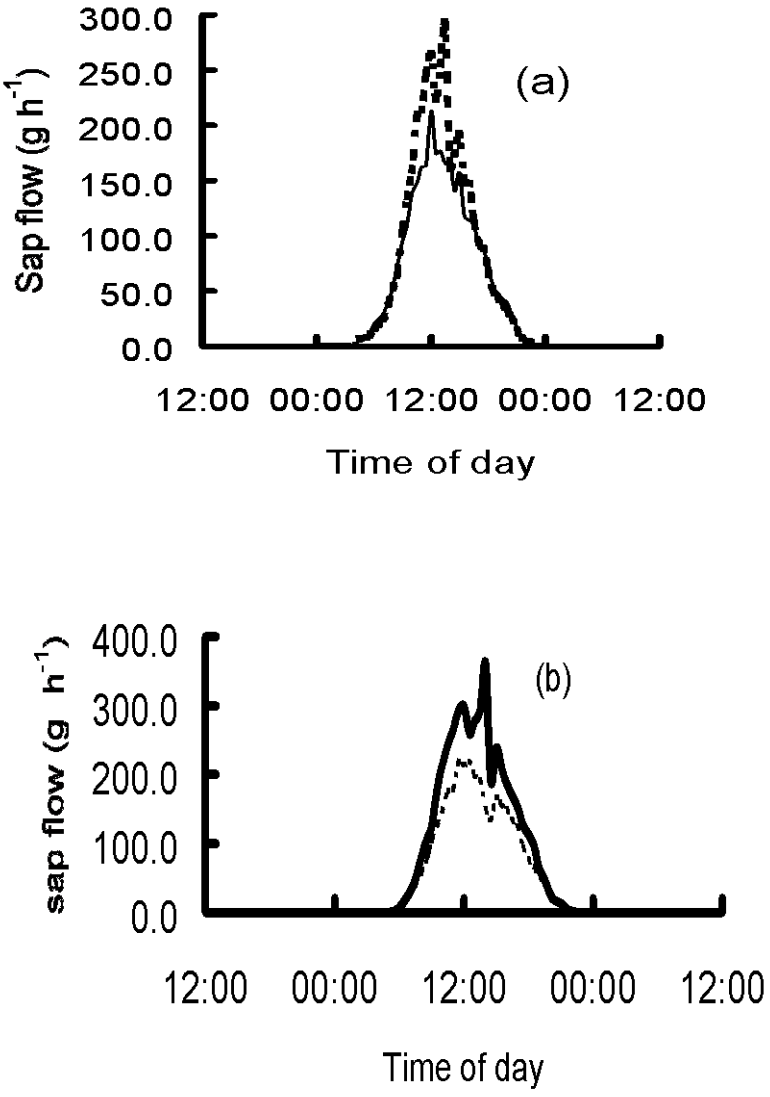


Figure 30. The effect of a) constant (thin solid line) and modulated EC (thick dashed line) for a diurnal course of sapflow and b) elevated RZW (thick line) versus control on sap flow. Note increased sap flow in both the modulated EC and RZW treatment.

Water flux within whole plants

The EC regimes imposed clearly had an impact on the transpirational flux within intact plants. A typical diurnal course of sapflow is shown in (Fig. 30a), where plants growing at constant EC show a lower water flux than modulated EC plants. At midday when the differences in sapflow were greatest, plants growing under modulated EC would be subjected to a lower EC under high

light conditions compared with constant EC conditions. Root-zone warming (RZW) had the effect of increasing sapflow (Fig. 30b), but had little effect on the mineral content of the fruits (data not shown). Whilst water flux was clearly affected by nutrient feed EC or RZW, this appeared to have little effect on pericarp strength. This is a measurement related to tissue turgor pressure and is equivalent to the osmotic pressure required to inhibit opening of the hoops (zero opening) as extrapolated from the intercept of the regression line with the *x* axis (Fig. 31).

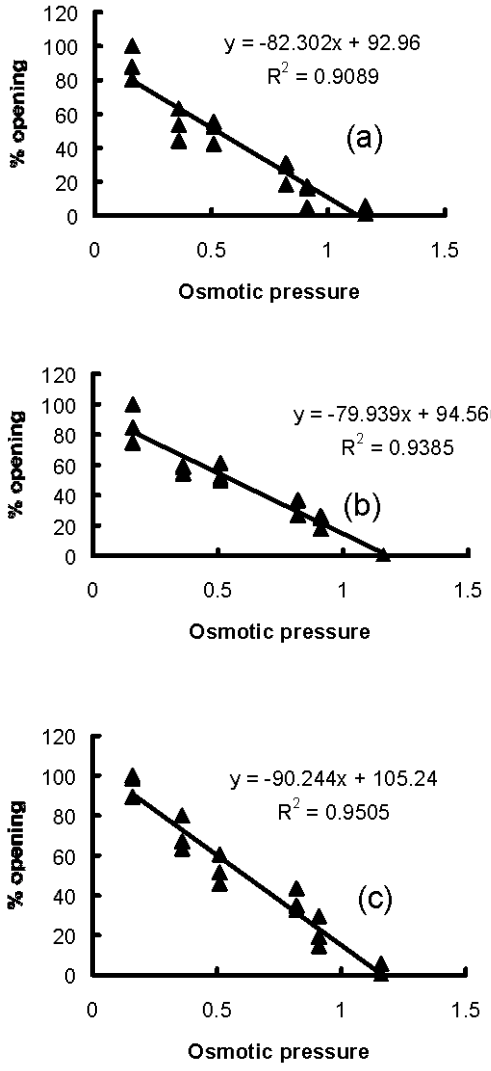


Figure 31. The effect of heat pulse and EC on pericarp strength, a measure of turgor as affected by osmotic pressure in the bathing solution, in a) the control b) constant EC after a 7-day pulse and c) modulated EC after a 7-day pulse. The point at which there was zero hoop opening was 1.13, 1.18 and 1.17 MPa for graphs a,b and c respectively. The linear regression equations and R^2 values are shown on each graphical plot

The tissue pressure within the fruits was therefore not affected by heat pulse or modulated EC. The lack of impact of varying water flux through the plant on fruit water relations may be a function of the leaves modulating the control of water flux in the shoot. Therefore large fluctuations in transpiration may have a minimal impact on the water / solute relations of the fruits as most of the shoot water is moving to and being lost by the leaves through stomata.

However, this differential effect of high and low EC was not observed under heat pulse conditions, where the sapflow was suppressed regardless of nutrient feed EC (data not shown). It could be argued that plants subjected to elevated air temperatures within a pulse would exhibit a higher water loss compared with control plants. However, the decrease in transpirational flux may have been influenced by the increase in humidity for the majority of achieved mean heat pulse temperatures (Table 5). Furthermore, whilst in 1998 there was some evidence to suggest that the heat pulse event could reduce the osmotic potential of the fruit juice (data not shown), more detailed analysis of pericarp strength in 1999 suggested that there was little effect on fruit turgor pressure (Fig. 31). This is important, as turgor is a key factor in determining *fruit firmness* (Seymour and Gross, 1996), and indicates that something other than water availability was promoting *softening* in fruits subjected to a heat pulse.

The differences in sap flow observed under the various treatments appeared to have no detectable effect on the water relations of ripe fruit using the tomato strength bioassay technique. There was also little impact on nutrient accumulation with the fruits through manipulating the EC and temperature of the rooting medium. The heat pulse also appeared to have little effect on altering water and nutrient fluxes to the fruit. Previous work by other researchers has suggested that the fruit are relatively hydraulically isolated from water fluxes within the xylem compared with the leaves (Davies *et al.,* 2000). Our data appears to concur with these findings.
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Objective 5:
To assess how root absorption area, distance between the root and fruit and root temperature and aeration affect the rate of uptake of K and Ca and to assess the influence of load on fruit K status.

Root zone temp and nutrient uptake in model plants

The uptake of water, potassium, calcium and nitrate was increased with increasing root-zone temperature ($P < 0.001$) up to an optimum (T_{opt}). The order of uptake for key nutrients was $K > NO_3 > Ca$ at T_{opt}. Enhancement of water and nutrient uptake transport at T_{opt} coincided with increased leaf area and root growth (data not shown). T_{opt} occurred between 23.6 and 24.7 °C for nutrient uptake and plant growth in cv Solairo (Table 12).

The data presented are of short term studies of root-zone temperature effects on ion uptake in young plants. These data should be interpreted with caution, when considering ion uptake in long-term experiments where temperature effects on root growth, root morphology and root:shoot ratio can become prominent factors in the regulation of ion uptake (Cumbus and Nye, 1982). However, root-zone warming at between 22.0 and 27.0°C in NFT culture for crops with a high fruit load had been shown to improve yield and fruit quality, particularly in conjunction with low air temperatures at night (Hurd and Graves, 1985; Maher, 1980). In this instance, short-term studies appear to confirm that efficiency of water and nutrient capture is optimised at 25.0°C. However, root-zone warming to 25.0°C in large-scale crop experiments during the summer months had little impact on the uptake of K and Ca compared with control plants (data not shown).

New roots

To address how root absorption area and distance between the roots and fruits affected K and Ca uptake, new roots were initiated from existing layered stems. Fruit yield was actually improved compared with control plants with no extra roots. This may in part have been due to increased survival of plants because of apparent decreased susceptibility to *botrytis* in extra rooted plants. K and Ca accumulation in the leaves and fruit was unaffected by the growth of new roots (data not shown) suggesting that their accumulation would not be increased by a new root system produced closer to the target organs.

Manipulation of growth to alter assimilate partitioning to favour root growth over shoot growth may not be a useful way to increase uptake. With this scenario demand for K and Ca may actually diminish because of reduced shoot growth; therefore the increased uptake capacity provided by enhanced root growth becomes ineffective.

Table 12. The impact of root-zone temperature on the influx of potassium, nitrate and calcium. The rate of nutrient influx for harvests 1, 2 and 3. DATI denotes days after treatment imposition, X and Y_{max} indicate the optimum levels of root-zone temperature (T_{opt}) and water uptake rates respectively.

Variable	DATI	Potassium	Nitrate	Calcium
Slope (15°C) ($\mu mol\ g^{-1}\ FW\ ^{\circ}C^{-1}\ h^{-1}$)	5	0.72	0.26	0.16
	12	1.43	0.56	0.33
	15	1.49	0.76	0.49
X_{max} (°C)	5	23.60	23.90	23.60
	12	24.10	24.00	23.90
	15	24.30	24.40	24.70
Y_{max} ($\mu mol\ g^{-1}\ FW\ h^{-1}$)	5	9.36	3.59	2.00
	12	20.20	7.51	4.27
	15	21.70	10.08	5.46

Fruit load

Fruit load was manipulated by reducing the numbers of fruit per truss from typically eight to five fruits. Whilst treatments began in April it was not until June that significant decreases in yield were observed. Prior to this, changes in fruit size distribution were observed where a greater proportion of larger fruits were produced compared with the non-pruned control treatment ($P<0.001$; data not shown). Yield reduction in the pruned compared with the non-pruned treatment became larger towards the end of the experiment (September). These significant yield losses may have been in part due to the presence of significant levels of blossom end rot (BER; data not shown). However, whilst BER is associated with Ca deficiency to the fruits, fruit Ca concentration in healthy ripe fruit was actually increased compared with the non-pruned controls particularly later in the production cycle ($P < 0.001$; Fig. 32a). Despite reducing fruit number and thus load, no improvement in K concentration in the fruit was observed (Fig. 32b).

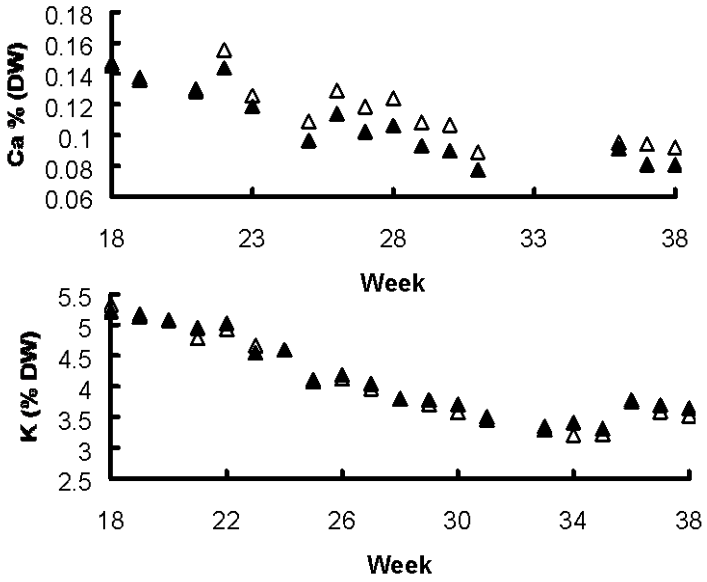


Figure 32. The effect of fruit load on fruit a) **Ca** and b) **K** concentration for non-pruned (▲) and pruned (Δ) fruits during the year 2000 experiment.

Objective 6:
To verify the critical limits of fruit temperature, K, and water relations that cause fruit quality defects and to formulate recommendations for optimising growing conditions to minimise defects.

It became clear as the experiment progressed that although nutrient feed and manipulation of physical characteristics of the roots and rooting medium had some effect on the growth and nutrient accumulation in the fruits, pulses of high **temperature** had an overriding effect on the key fruit quality defects. These defects were induced late in the fruit maturation process between mature green and colour stage 4/5 of ripening. At this stage, no appreciable role for water relations or K accumulation within the fruit could be detected. High temperatures may have primarily affected the biochemistry of the later stages of fruit ripening. In addition to the analysis of temperature heat pulse effects, the effects of other factors including pipe-temperature and humidity expressed as either RH or VPD on fruit quality were analysed by covariate analysis. As pipe-temperature was almost totally confounded with heat pulse effects, it was impossible to separate the effects of heat pulse and pipe-temperature in this study. However RH and VPD showed only a moderate correlation with heat pulse effects. After fitting the heat pulse effects, there was sufficient variability in the observed humidity to provide a weak test for humidity effects on fruit quality. No significant impact of humidity on fruit quality was detected. Further work would however be required to explore the potential role of e.g. VPD separately and in combination with temperature on fruit quality defects.

Mean fruit temperature was similar to air temperature therefore *air temperature* was considered to be *the driving variable* for the range of observed summer fruit *quality defects*.

Fruit quality throughout the project was assessed both by comparison of the *observed* percentage Class I grade-out of the plot yields and by the quality grading of eight-fruit samples collected on each Monday during the experimental period. However, there were significant discrepancies between the results of the two methods. Taking the experiment as a whole, this appears to suggest that the standard used to assess fruit quality in the sampled fruit was very different from that used for the plot yields. For future work the two methods of quality assessment need to be more closely aligned. However, for the present project, the use of the *estimated* percentage Class I grade-out from the sampled fruit appears to give the best understanding of treatment effects on fruit quality in a commercial situation. For this reason, only the assessments of the individual defect scores and the estimated percentage Class I grade-out derived from the sampled fruit scoring system were used for validating critical temperature limits for summer fruit quality defects.

Using a benchmark relationship for pulse length (duration), mean pulse temperature (magnitude) and estimated level of fruit quality defect or % Class I fruit (Fig. 33) the process of ‘verification of critical limits’ was developed.

In 2000, year 3 of the experimental programme, four levels of pulse temperature was achieved and linear relationships were formulated between temperature and each defect. These relationships allowed calculation of the likely impacts of temperature in the range 20.0-26.0°C for the individual summer fruit quality defects examined.

Glasshouse verification from 1999 and 2000

The relationships between pulse duration in 1999 and pulse magnitude in 2000 was compared for each key defect and each Lag week following a heat pulse but significant fruit quality defects were found only in the week immediately following a pulse. Therefore only the Lag week 1 data has been presented in the analysis developed here.

Uneven ripening showed a substantial response to high temperature heat pulses and this disorder has been identified as a major defect caused by high temperature effects. Figures 33a and b show the effects of pulse duration on percentage Class I (A+B+C) and Class I (A+B) fruit, respectively, for the harvest immediately following a heat pulse and Figures 33c and 33d show the corresponding effects for pulse temperature. The temperature used for the pulse treatments in 1999 was 25°C therefore the 3-day pulse effect in 1999 should have been comparable with the estimated 25°C 3-day pulse effect in 2000. Comparison of Figure 33a with 33c at the comparable pulse length and temperature shows a somewhat greater percentage of Class I (A+B+C) fruit in 1999 than in 2000 (approx. 97% versus 93%). However, comparison of Figure 33b with 33d shows excellent agreement with approximately 75% Class I (A+B) fruit in both years. This shows that the results of the two trials were reasonably consistent over the two years of experimentation.

The effects of the 7-day pulse on *uneven ripening* immediately following the end of the pulse was greater than the effects of the 3-day pulse (see Fig. 5a) but some caution is needed in the interpretation of this result. The fruit sample records were taken on the Monday immediately following the end of a pulse and any defect requiring more than three days to develop would not have been detected in the Monday sample for the 3-day pulse treatment. However, comparison of the commercial yield grade-out for the 7-day and the 3-day pulse effects in Table 6 supports the interpretation that, overall, the 7-day effect on fruit quality was more severe than the 3-day effect. Although there is some evidence that the effect of the 3-day pulse on quality was greater for the Wednesday harvest than for the Monday harvest, the 7-day effect was greater than the 3-day effect on both the Monday and the Wednesday. The 3-day effect appears slightly greater than the 7-day effect on the Friday following the heat pulse but even if real, this would be insufficient to alter this interpretation.

Although Figure 33 a, b suggests that the effects of a temperature pulse is approximately linearly related to pulse length, there are insufficient time points on the graph to be fully confident that the linear relationship will hold for very short pulse lengths. Nevertheless, there is evidence that the level of fruit quality defects increased almost linearly with both the magnitude and duration of the heat pulse (Fig. 33). For *uneven ripening*, a pulse temperature of

25.0°C lasting for seven days would cause substantial commercial damage while a pulse temperature of 26.0°C lasting for three days would cause similar or even greater commercial damage (Fig. 33a,b).

Both the magnitude and the duration of the temperature pulse therefore had a deleterious impact on fruit quality. An approximate 10% loss of quality due to the 7-day pulse in 1999 and a similar 10% loss due to the 26.0°C 3-day pulse was observed in 2000 (Fig. 33a,c; Class I A+B+C). The temperature pulse level in 1999 was 25.0°C therefore the 3-day effect in 1999 should be comparable with the 25.0°C effect in 2000. In fact, the graphs in Figure 33 suggest that the 2000 effect (Fig. 33c) was somewhat stronger than the 1999 effect (Fig. 33a) but, overall, the results from the two years appear reasonably consistent. Certainly, the amount of loss in the proportion of Class I fruit due to the extreme treatments would be commercially significant in both years. However to more fully interpret the impact of temperature in a commercial situation Figures 33 b and d (Class I A+B) can be used to extrapolate the critical limits of duration and magnitude of the heat pulse. For example, a commercially unacceptable 10 % loss in fruit quality would require a pulse duration of 3 days (Fig. 33b) at a temperature of 23.0°C (Fig. 33d).

Linear regressions constructed from year three of the project data were used to provide a benchmark relationship for temperature effects on the individual fruit quality defects. Data points from previous years including large-scale glasshouse work at Efford and other experiments were superimposed on these regressions to test the robustness of the various relationships for selected summer fruit quality defects (Fig. 34).

Uneven ripening exhibited a good relationship with temperature between years (Fig. 34a). *Dark patches* were inherently variable, more so than any other fruit quality defect but fruits clearly had more *dark patches* at high temperature compared with controls (Fig. 34b). *Load* also provided a robust relationship between different experiments in which the decrease in skin strength was linearly related to increasing temperature (Fig. 34c). Fruit *firmness* measurements appeared to show a good relationship with temperature within years but *firmness* was higher in 1999 at all temperatures compared with 2000 (Fig. 34d).

Controlled environment detached and attached fruit

Controlled environment studies allowed examination of the impact of temperature *per se* on selected fruit quality defects. Data are presented for *uneven ripening* and *firmness* (Fig. 34a,d) and attached fruits from plants grown at constant temperatures exhibit considerable scatter from the relationship formulated from glasshouse studies. Fruits detached from glasshouse grown crops and then placed in controlled environments at constant temperatures and allowed to ripen exhibited a closer relationship with the fitted line compared with the attached fruit CE grown plants. Thus it appears that robust predictions of the likely impacts on *uneven ripening* and reduced *firmness* can be made from measured air temperature alone, whether on detached or attached fruits at the time of the high temperature heat pulse

event provided they originate from large-scale crop, glasshouse environments. However relatively low light levels in CE compared with glasshouse environments and unnatural constant temperatures as used in the whole plant attached fruit studies may be unsuitable for predicting the likely impact of high temperatures on summer fruit quality defects.

Grower holdings

Uneven ripening and *dark patches* showed good agreement for two of the growers AVN and Cantelo with the linear temperature relationship formulated from Efford 2000 glasshouse experimental data (Fig. 35a,b). The VHB results did not fit the linear temperature relationship and why they should be so different to the other data remains unclear. The relationship for fruit *load* and *firmness* was much more variable (Fig. 35c,d). *Firmness* appears to be sensitive to changes in temperature, and therefore considerations such as the conditions under transport, time between pick and assessment may be critical to the production of accurate and meaningful data.

The relationships developed for temperature and defect level appear to be sufficiently robust to be used by growers to achieve standards that are acceptable to their customers. Further testing of these relationships with more growers would be desirable and for measurements such as *firmness*, greater consideration needs to be given to the methods used to collect the data. Cross calibration of the *firmness* testing apparatus used by growers has been carried out within this project to produce experimentally acceptable standard values of fruit physical attributes. However, conditions in transport for independent assessments at HRI Efford on fruit from growers and time after the fruits being detached from the plant and time of sampling need to be carefully considered. In this way more standard data sets can be generated particularly for important variables such as fruit *firmness*.

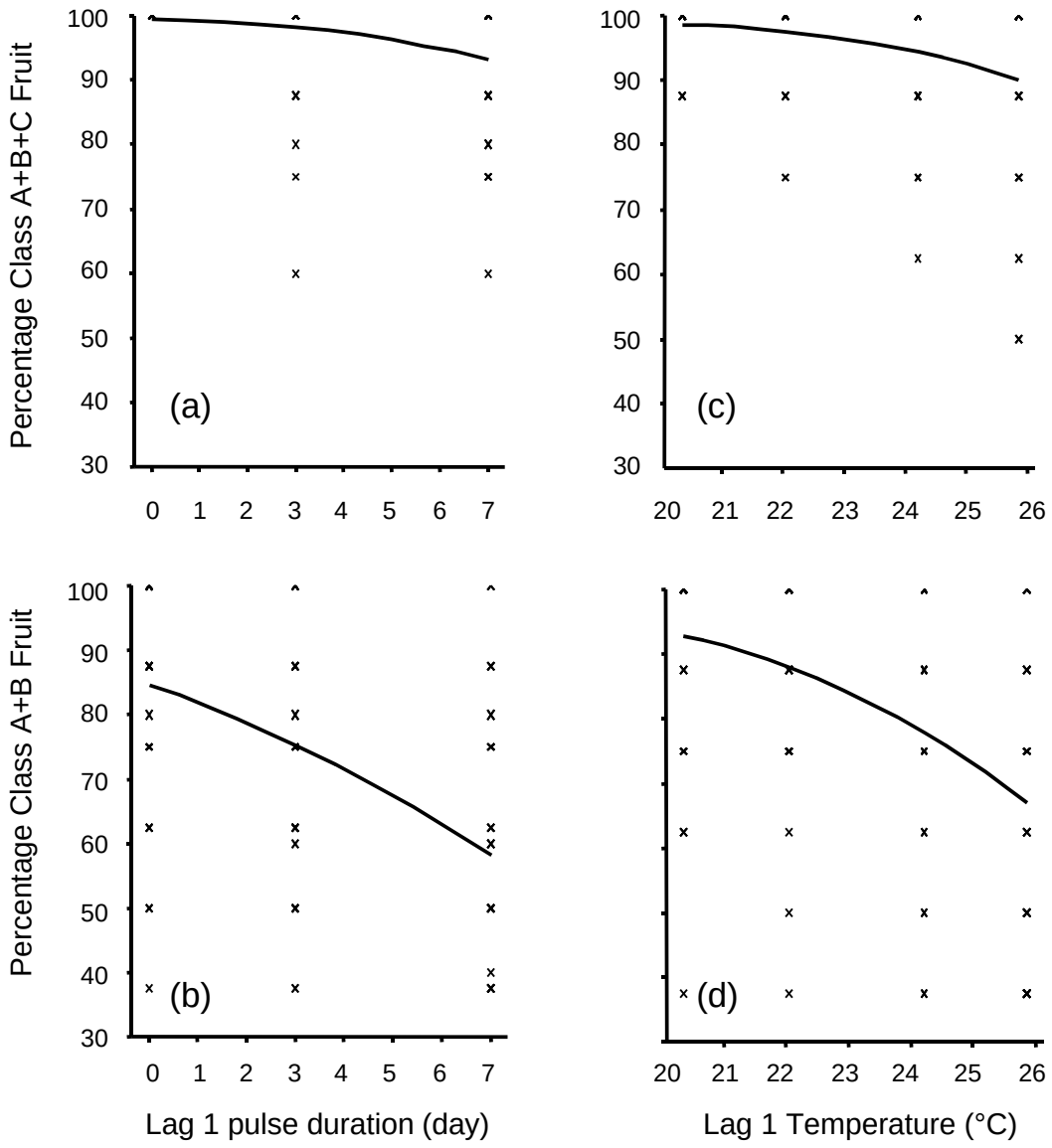
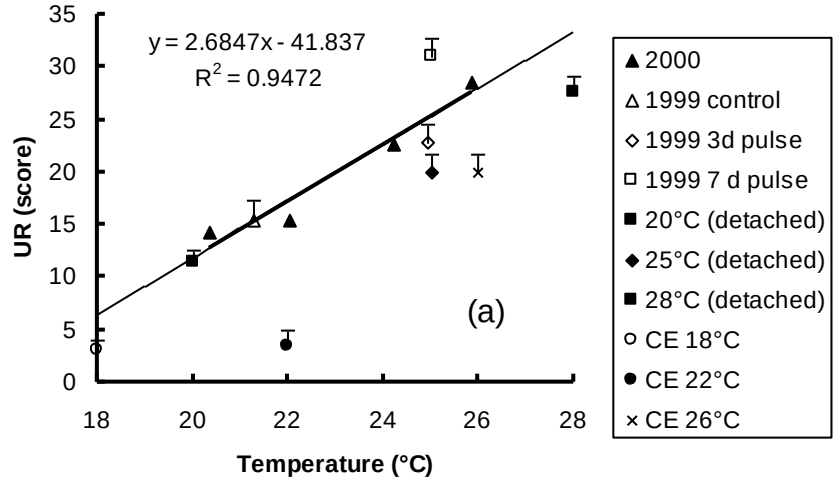
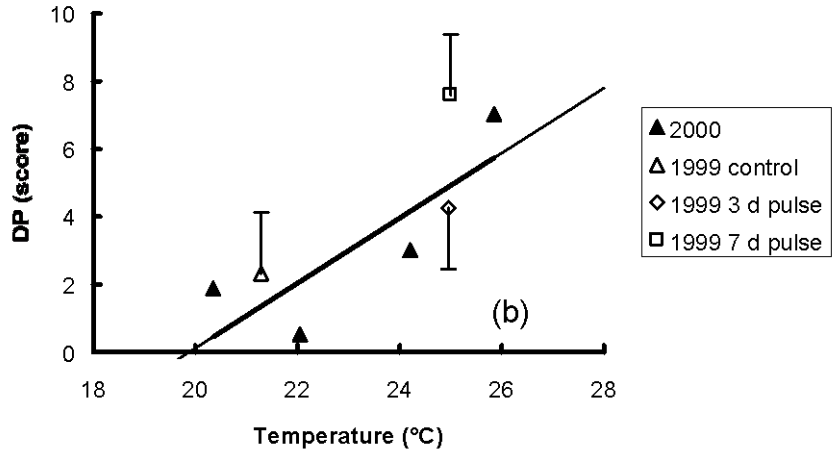


Figure 33. The relationship between the estimated % Class I fruit for **uneven ripening** (Class A+B+C; a,c) and (Class A+B; b,d) in Lag 1 of the cycle for heat pulse a,b) **duration** (1999) and b,d) **mean temperature** (2000) for a 3-day pulse (magnitude).





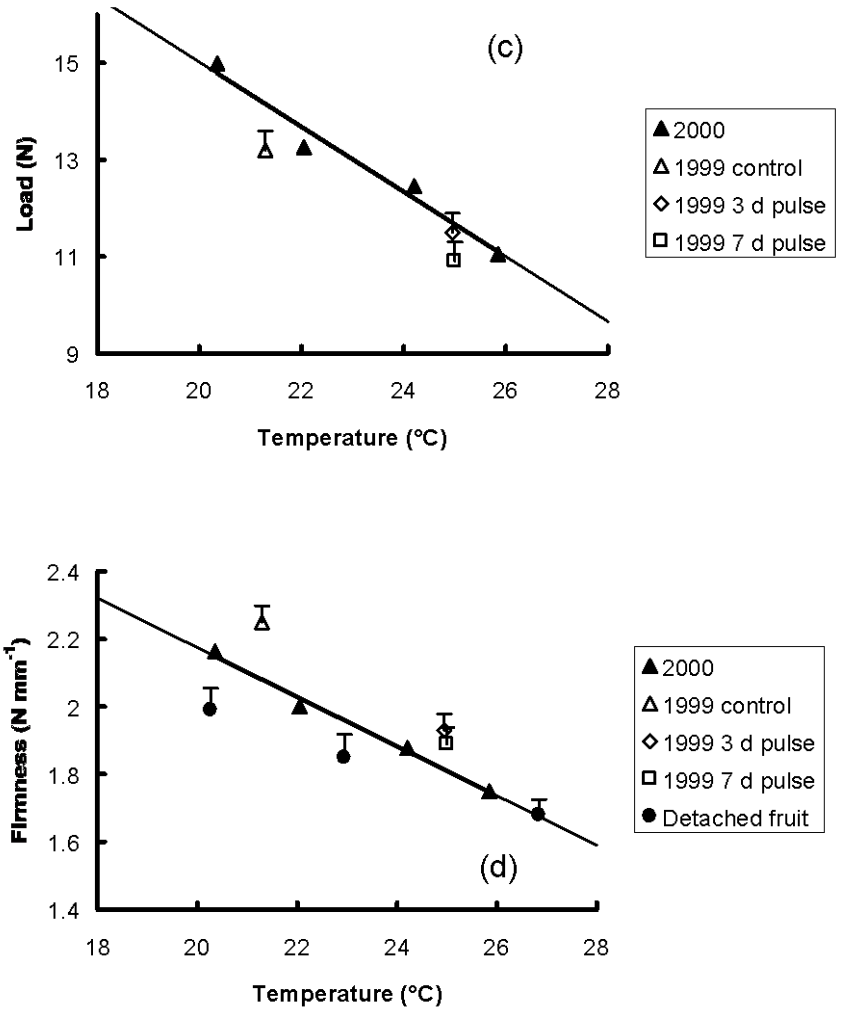
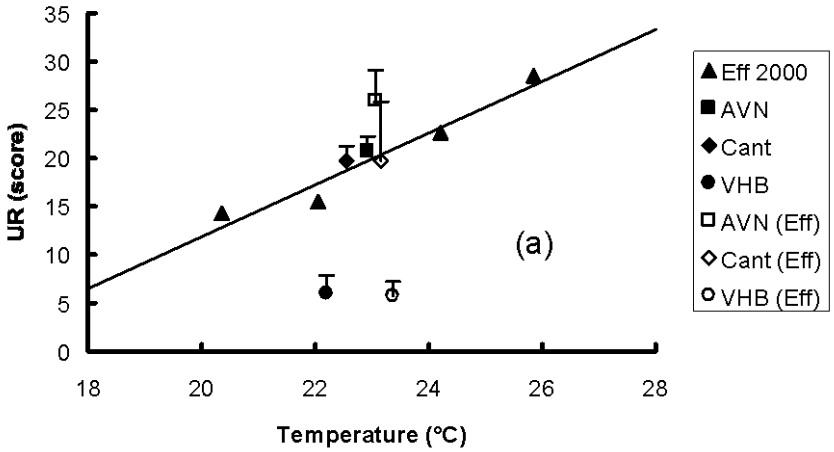
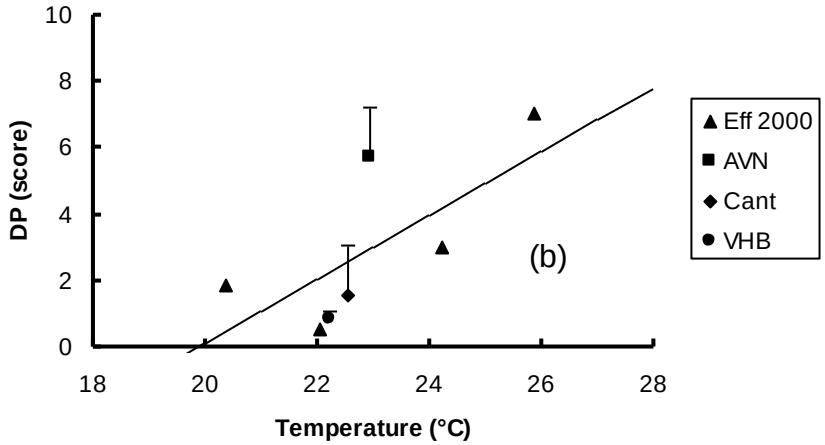
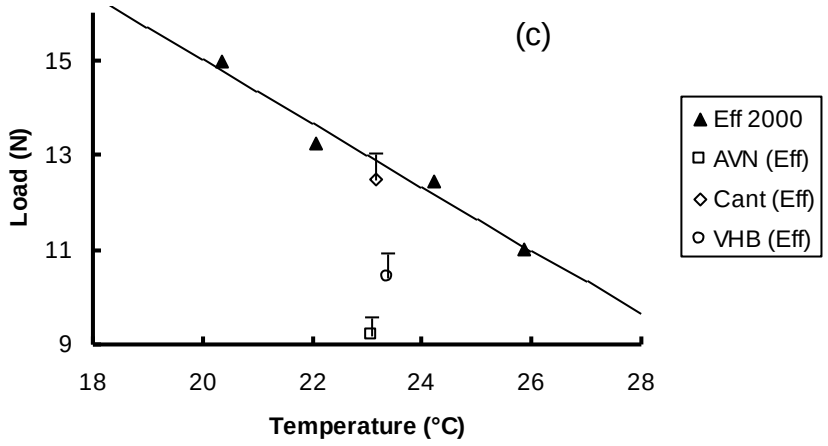


Figure 34. Relationship between heat pulse temperature in Lag week 1 for the magnitude 2000 experiment in non-pruned plants for a) **uneven ripening** b) **dark patches** c) **load** and d) **firmness**. Regression relationships and goodness of fit are as detailed within Objective 1. Various data are plotted graphically with a single standard error from previous 1999 glasshouse experiments and in particular for **uneven ripening**, detached fruit exposed to constant mean temperatures under controlled environment conditions at HRI Efford. In a) data from the growers and HRI Efford are plotted for separate assessments of **uneven ripening** and temperature measurements. Efford assessments were plotted against measurements of temperature made by Tinytalk temperature measurement and dataloggers positioned within the canopy rather than the use of growers' measurement of screen temperature.







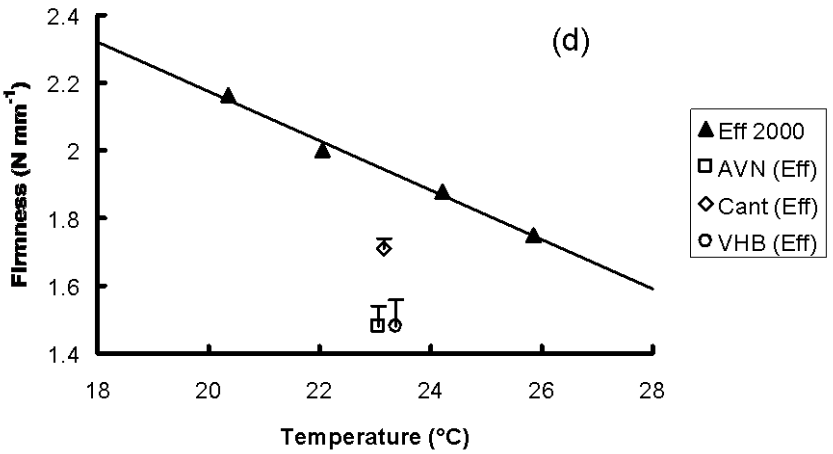


Figure 35. Relationship between heat pulse temperature in Lag week 1 for the magnitude 2000 experiment in non-pruned plants for a) **uneven ripening** b) **dark patches** c) **load** and d) **firmness**. Regression relationships and goodness of fit are as detailed within Objective 1. Various data are plotted graphically with a single standard error from growers holding along with the mean temperature for 3 days prior to pick. Fruits from the same pick were assessed separately at both Efford and by the growers.

Discussion of Results

Glasshouse experiments were designed to expose long season tomato crops to periodic high temperature pulse events during the summer months. These experiments demonstrated that temperature is a key environmental factor and that after only three days of high temperature conditions fruit quality defects such as *uneven ripening*, *dark patches* and *softness* are triggered. In *uneven ripening* and fruits with *dark patches* there were no detectable changes in the cell structure of the pericarp, nor were the water relations perturbed. Initially *unevenly ripened* fruit were thought to be 'blotchy', but lack of structural changes strongly suggested that these fruit were not exhibiting true blotch (Hobson *et al.,* 1977). In addition blotchy ripened fruit exhibit significant reductions in sugar and acid concentrations and may even be firmer than unaffected fruits, due to the green unripened areas (Hobson and Davies, 1976). Therefore an important distinction was made after the first year of the project where, apparently blotchy fruit were re-classified as *uneven ripening* fruit. These fruits were correctly categorised and assessed in years two and three of the project as *uneven ripening* fruit.

The impact of the heat pulse on fruit quality was confined to a structurally sound and normally developing fruit, in the late maturation phase of fruit ripening between mature green and colour stage 4/5. Exposing the crop to pulses of either 3 or 7-day duration in an eight-week cycle elucidated this. The eight-week cycle encompassed the entire fruit growth cycle of a typical fruit cycle from anthesis to pick at colour stage 4/5. Only those fruits that were picked immediately after a pulse event exhibited significant levels of key defects. Fruits picked over the following seven weeks, had been at an earlier stage of development at the time of the pulse and these fruits failed to exhibit any loss in visual or physical attributes. Importantly, and contrary to some of the projects original hypotheses, there was no reduction in K, Ca, sugars or the primary acid, citrate in affected fruits.

The findings from the current study suggest that both the magnitude and duration of the heat pulse can increase the incidence of fruit quality defects. For *uneven ripening*, a pulse temperature of 25.0°C lasting for seven days would cause substantial commercial damage while a pulse temperature of 26.0°C lasting for three days would cause similar or even greater commercial damage (Fig. 33a,c; Class I A+B+C). However to more fully interpret the critical threshold of temperature in a commercial situation, an unacceptable 10 % loss in Class I fruit would require a pulse duration of 3 days (Fig. 33b) at a temperature of 23.0°C (Fig. 33d; Class I A+B).

Linear regressions constructed from year three of the project data were used to provide a benchmark relationship for temperature effects on the individual fruit quality defects within the mean pulse temperature range of 20.0 to 26.0°C. This effect appeared to be controlled solely by air temperature, as no manipulation of nutrition, plant water balance, or fruit load appeared to ameliorate the likely incidence of the key defects. Similarly, solar radiation and atmospheric humidity appeared to play no direct role in the incidence of

the defects. Although humidity was increased, partially through reduced venting to achieve the pulse temperatures, the levels were not within the range that can cause loss of *firmness* under glasshouse summer conditions (Fussell *et al.,* 1992; PC30/30a).

Within the heat pulse, maximum temperatures within the treatment range peaked at over 30.0°C through parts of the day for both fruit and air temperature. As fruit and air temperature were closely aligned, air temperature was sufficient for the prediction of the incidence of the key fruit defects. Temperatures over 30.0°C may be of significance as numerous post harvest studies have demonstrated that fruit ripening is inhibited at and above 30.0°C (Cheng, 1988; Mitcham and McDonald, 1992). In detached fruit studies within the controlled shelf life facility at Efford in 2000, *uneven ripening* fruits were found at constant temperatures below 30.0°C but at 30.0°C ripening was inhibited. During ripening, temperature influences the rate of pigment synthesis, and thus uneven pigmentation can be caused by a localised temperature effect on a fruit (Koskitalo and Ormrod, 1972). Thus a combination of accelerated and inhibited ripening in a single *uneven ripening* fruit may have the overall effect of reducing *fruit firmness*. This effect appears to be exacerbated for 3-day mean pulse temperatures of between 20.0 to 26.0°C.

At the onset of ripening an increase in respiration, called the climacteric peak, occurs which then subsequently declines slowly. The carbon sources for respiration include carbohydrates. For example, the metabolism of malic acid increases during tomato fruit ripening (Jeffery *et al.,* 1984). Indeed, whilst malate was present at approximately a seven-fold lower concentration compared with citrate in mature fruits, it did exhibit a linear reduction in concentration in response to increasing temperature. In addition, the malate concentration was greater in the pruned compared with the non-pruned control treatment. However, the rate of loss of malate due to increasing temperature was similar for both treatments. This indicates that fruits exposed to high temperatures had high rates of respiration, which may be involved in the loss of quality. However, unravelling the exact sequence of events that leads to a multi-factor, reduction in quality, is clearly complex.

Polygalacturonase (PG) is an important softening enzyme in tomato. PG is absent in green fruit and accumulates in large quantities during ripening (Tucker and Grierson, 1982). As ripening continues, solubilization of the cell wall becomes more extensive. Increased temperature during a pulse and the decreases in fruit *firmness* may be critically related to the activity of PG. Indeed, mutants of tomato that are deficient in PG synthesis show very little softening (Grierson and Kader, 1986). However, PG alone is insufficient to induce softening and the process appears to involve a cocktail of closely related enzymes, including PG, and is an area that requires continuing fundamental and basic research (Seymour and Gross, 1996). Research on these enzymes in the late maturation phase of fruit ripening in response to high temperature may be key to understanding the process of fruit softening. Comparison of lycopene, carotenoids and PG activity in *uneven ripening* fruit

may be a useful next step in being able to quantify the impact of high temperature effects on visual appearance.

The current investigation has demonstrated that K and Ca concentrations in mature fruit at colour stage (4/5) invariably exhibited a seasonal decline (Fig. 26), which corresponded with a decrease in fruit juice acidity (Fig. 27c). Nutritional amendments, root-zone warming and new root treatments all proved to be ineffective in reversing this trend of decreasing accumulation within the fruits, and associated loss of quality. Fruits tended to become *softer* and to have higher incidences of *uneven ripening* towards the end of the summer, or early autumn period. However, although manipulation of the root-zone did not yield any positive benefit in reversing this decline in fruit quality, it could be that the demand for K, Ca and water is actually reduced in the shoot. Therefore K and Ca accumulation may be primarily modulated in the ageing shoot rather than in the roots. It is possible that alteration to the shoot environment, such a supplementary lighting could go some way to reverse this temporal decline in fruit quality, through the increased accumulation of sugars acids and nutrients in the fruit.

Conclusions

- The summer fruit quality defects *uneven ripening*, *softness* and *dark patches* were principally caused by high **temperature** during the mature green and colour stage 4/5 of ripening.

- Both the *magnitude* and *duration* of the heat pulse event are important factors when assessing the likely impact on the incidence of fruit quality defects. For *uneven ripening*, a pulse temperature of 25.0°C lasting for seven days would cause substantial commercial damage while a pulse temperature of 26.0°C lasting for three days would cause similar or even greater commercial damage. However to more fully interpret the critical threshold of temperature in a commercial situation, an unacceptable 10 % loss in Class I fruit would require a pulse duration of 3 days at a temperature of 23.0°C.

- Highly significant linear regression relationships were formulated from the temperature magnitude experiments in 2000, and the key fruit quality defects *uneven ripening*, *dark patches* and *fruit softness*.

- The linear relationships derived from experimental glasshouse trials were used to compare the goodness of fit with data collated from grower holdings. Good agreements were found with mean temperature and the incidence of *uneven ripening* and *dark patches* for two out of three grower holdings tested. Some considerable discrepancy was found for *fruit firmness* parameters. These measurements were made solely at Efford and therefore issues of the exact time of pick, time from pick and conditions of transport from e.g. grower holdings need to be carefully considered in the measurement and collection of such data

- *Malic acid* although present in small amounts compared with *citric acid* concentration was linearly reduced with increasing temperature, indicating that *malate* can act as a carbon source for increased rates of fruit respiration. The concentrations of *citric acid* and the sugars *glucose*, *fructose* and *sucrose* were not affected indicating that taste would not be greatly affected by increasing pulse temperature.

- The heat pulse primarily affected the late maturation phase of fruit development; some increases in *gold-spot* and *gold marbling* were detected seven weeks into the pulse cycle but were considered economically unimportant.

- *Netting* is associated with low skin strength (*load*) and is enhanced by both high temperature and high fruit growth rate (pruned fruit). However *softness* is influenced solely by high temperature.

- The defects did not appear to result from changes in the structure of the fruit, the accumulation of K and Ca or altered water relations within the fruit.

- Modulating the water availability to the crop in the EC range 1.8-4.0 mS had no beneficial effect over constant EC (2.8 mS) in reducing the incidence of fruit quality defects.

- Amendments to the root environment did not appear to offset the seasonal decline in K and Ca accumulation in the fruit and suggests that the demand for K, Ca and water may be primarily modulated in the ageing shoot rather than in the roots

Technology transfer and further work

Publications

- One scientific paper will be prepared for publication in an international scientific journal
- An article will be submitted to the popular press such as The Grower
- An article will prepared for the HDC News
- An HDC technical paper will be prepared and presented through the Tomato Growers Association

Presentations and demonstrations

- A presentation will be given to the TGA/HDC/MAFF tomato conference in 2001

Exploitation plans

- Further work is required to validate the linear relationships formulated between the key fruit quality defects and temperature.
- Further experimentation should be attempted to elucidate whether it was the average temperature over a pulse period or whether 'spikes' of elevated temperature over shorter durations (i.e. less than 3 days) are of equal or greater significance in increasing the incidence of summer fruit quality defects.
- The work has elucidated that it is only the late phase of fruit maturation is affected by periodic high temperature pulse events. An in depth study into the effects of high temperature of the biochemistry of late fruit ripening will provide greater insight into the mechanisms controlling fruit quality under such conditions.
- We have no information as to how the heat pulse events impact on subsequent post harvest quality attributes in particular, firmness taste and flavour prior to delivery to the customer and how acceptable these fruits are to the consumer.
- These experiments have provided a large amount of information on temperature pulse effects on fruit ripening in a commercially grown tomato crop. The yield data shows an immediate increase following a pulse followed by a compensatory dip in the following weeks. In 1999 the data shows the effects of a 7-day or a 3-day heat pulse on yields during the following eight weeks whereas in 2000 the data shows the effects of a 3-day pulse applied at three different mean pulse temperatures on yields during the following four weeks. The pulses were applied twice as frequently in 2000 as in 1999 and this should give information on the

cumulative effects of temperature on accelerated ripening. A statistical model analysis of the fruit yield data could provide quantitative predictions of temperature pulse effects on yield in commercial scale crops. In addition Class I fruit was fully graded throughout the season and a statistical model analysis will also show any effects on the fruit size distribution due to the temperature pulse effects on ripening. This model will address the weekly variation in fruit yield and proportion of Class I grade-out.

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Appendix 1

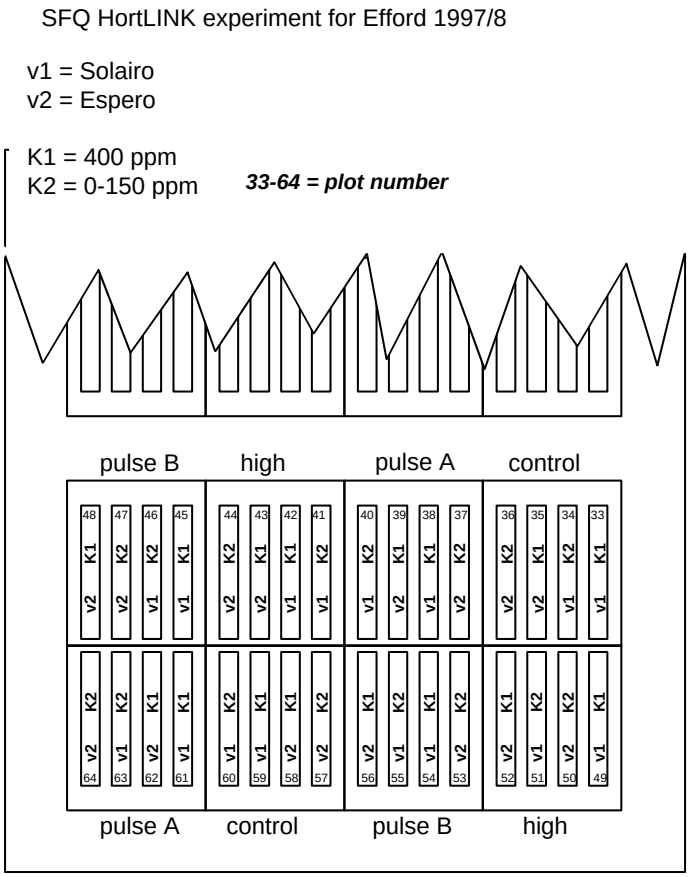
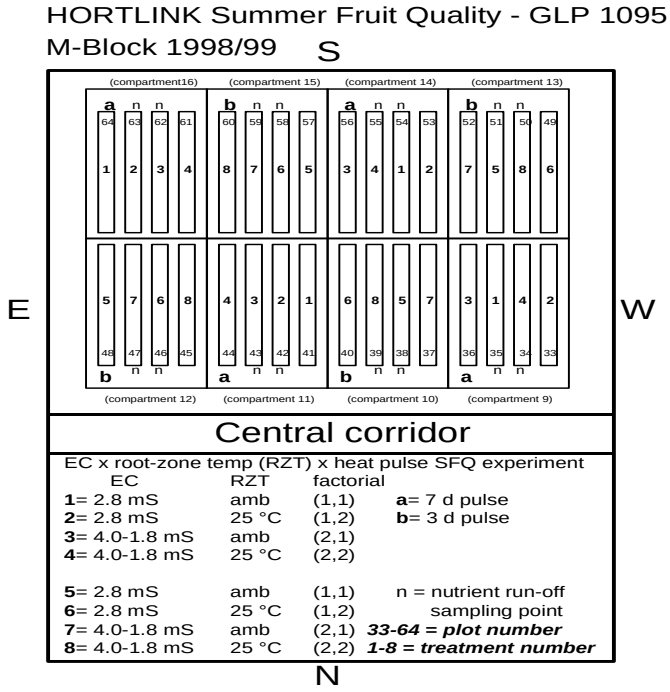


Figure 36. Year 1 (1998) experimental layout. There were three elevated temperature treatments plus the blueprint control. Sub-plot treatments comprised two genotypes (Solairo and Espero) and two levels of K.



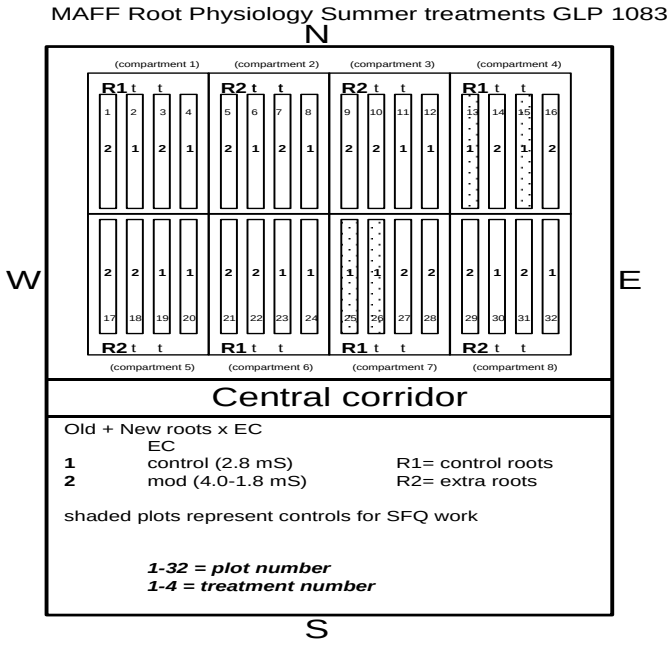


Figure 37. Year 2, (1999) experimental layout. The top diagram illustrates the experimental treatments in the south side M-Block at Efford, which encompassed two heat pulse treatments of varying duration, 3 and 7 days and four subplot treatments comprising modulated and constant EC and two root-zone temperatures, ambient and a constant elevated 25 °C. Four plots (13,15, 25 and 26) in the bottom diagram that formed part of the North side Root Physiology experiment were used as ambient EC controls for the Summer Fruit Quality experiment.

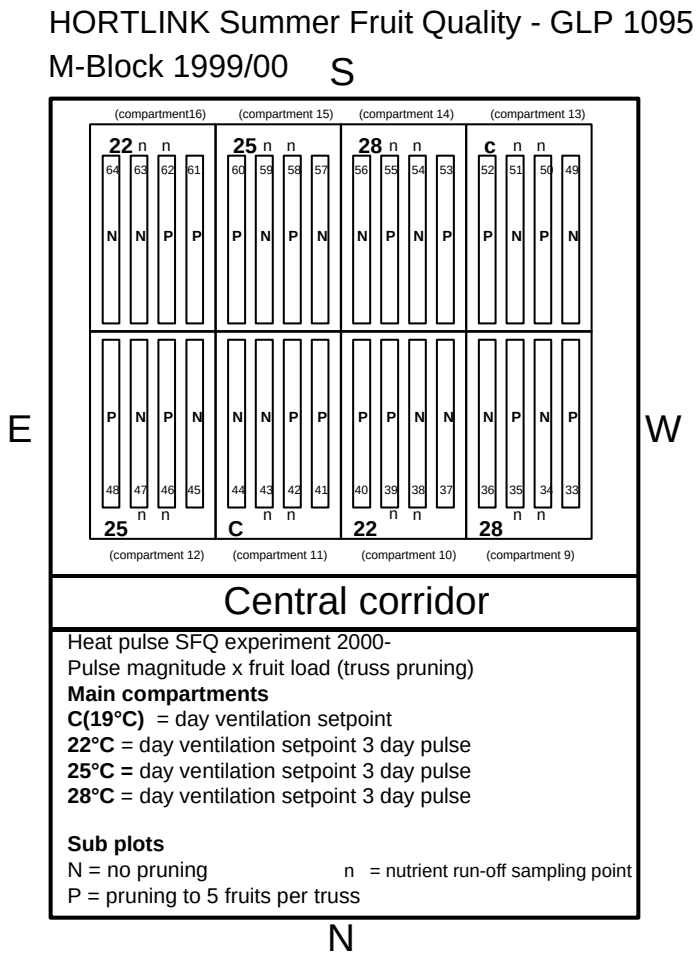


Figure 38. Year 3, (2000) experimental layout. Three heat pulse treatments were applied with an ambient blueprint control for three days with two sub-plot treatments, pruned with five fruits per truss and a non-pruned control.

Appendix 2
Defect recording system

The scoring system for defects is based on the EC common standards for quality of round tomatoes:

1A	Absent or virtually absent	Class I
1B	Present at a low levels	Class I
1C	Noticeable but still acceptable for	Class I
2	Present at acceptable level for	Class II
3	Unacceptable level	Waste

Acceptable levels of common defects

Defect	1B	1C	2	3
Blotchy Ripening	1-3 blotches < 5mm diam.	4-6 blotches any one 6 - 10mm	> 6 blotches or any one 11-30mm	Any one blotch 30mm diam.
Dark Patches (like bruises)	1-3 blotches < 5mm diam.	4-6 blotches any one 6 - 10mm	> 6 blotches or any one 11-30mm	Any one blotch > 30mm diam.
Uneven Ripening	1 ATB Col Stage difference	> 1 ATB Col Stage difference	-	-
Gooseberry Veining	<30% surface area	>30% surface area		
Softness	Moderately firm	Slightly soft	Soft (tender)	Very soft
Gold Spot	< 100 spots/cm² or < 10mm radius around calyx	> 100 spots/cm² and 11-20mm radius around calyx	> 100 spots/cm² and > 20mm radius around calyx	-
Gold Marbling (flecking)	1-20mm diam.	21-40mm diam.	>40mm diam.	-
Net Cracking (russetting)	wide net or < 1cm² close net	1 - 2 cm² close net	< 50% close net	> 50% close net
Concentric Cracking	Total length of all cracks < 5mm	Total length of all cracks 6 - 10 mm	Total length of all cracks 11 - 30mm	Total length of all cracks > 30mm
Blossom-End Rot	-	-	-	Any

To provide a general score to represent the level of a defect averaged across treatments the following weightings were used:
Score = ((0*1a)+(1*1b)+(2*1c)+(3*II)+(4*waste))*100/sample number

Appendix 3
1999 estimated response curves for percentage acceptable fruits against pulse duration

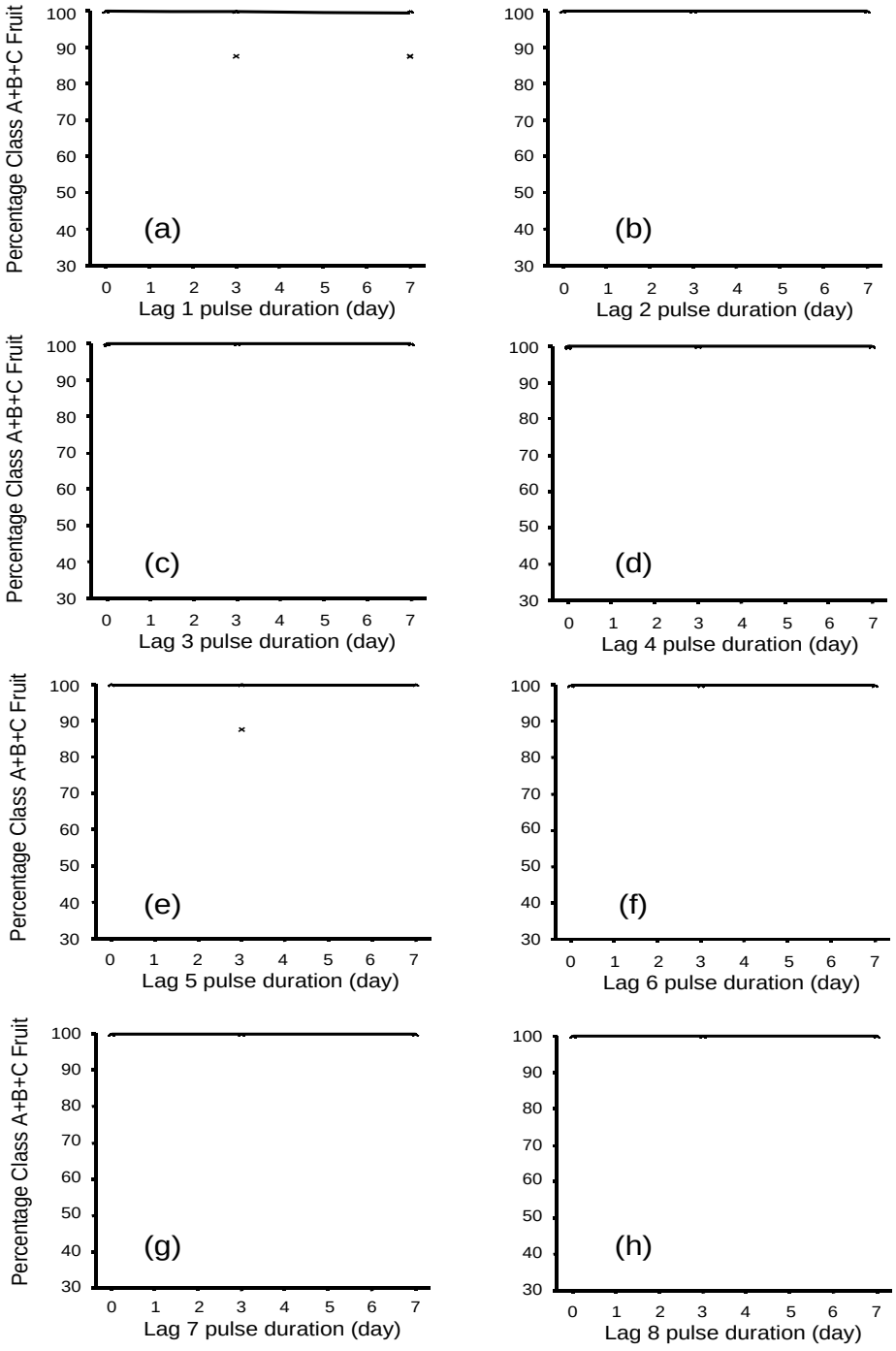


Figure 39. The relationship between % Class I fruit (Class A+B+C) for **netting** and pulse duration in a-h, for Lags 1-8 respectively.

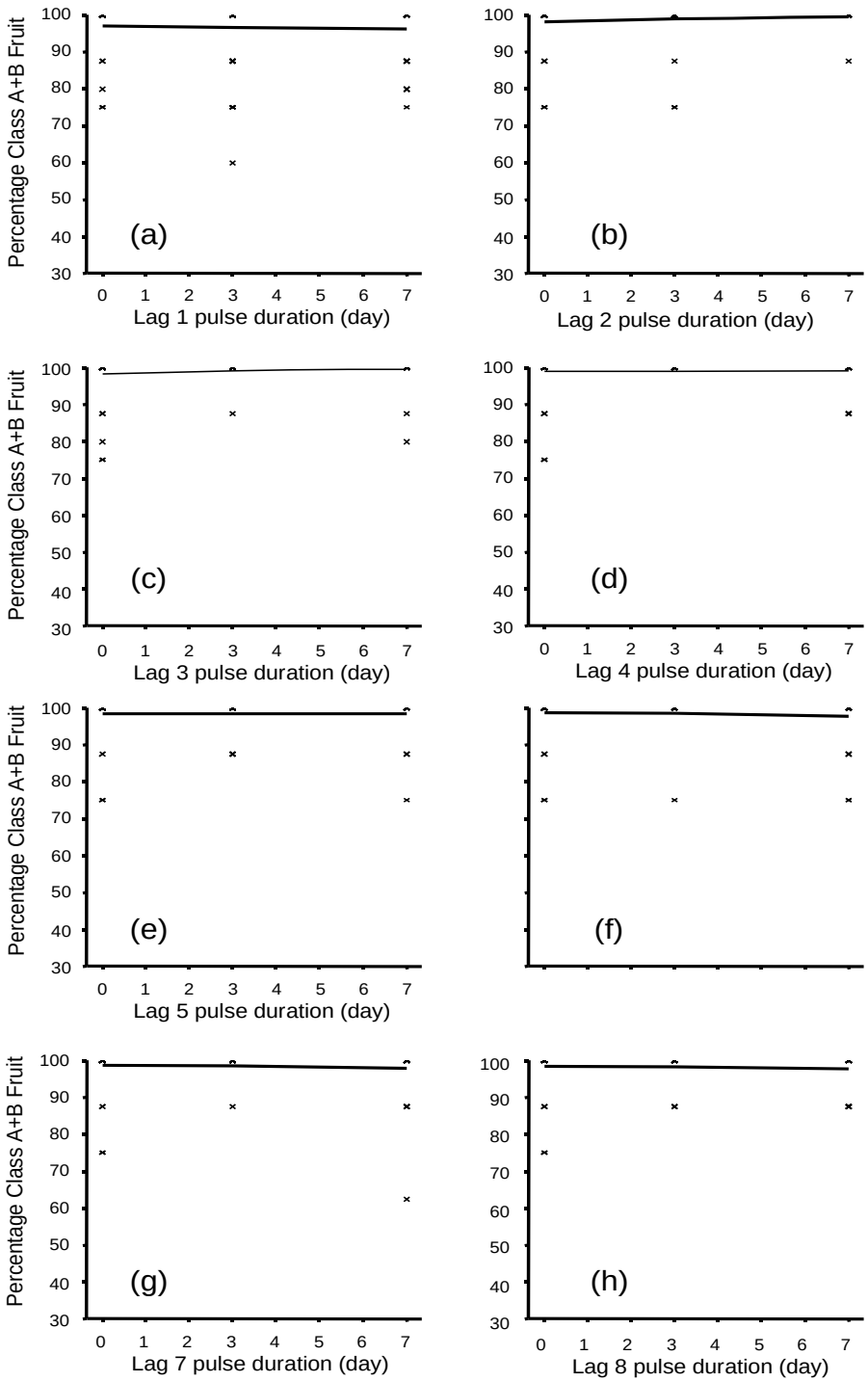


Figure 40. The relationship between % Class I fruit (Class A+B) for **netting** and pulse duration in a-h, for Lags 1-8 respectively.

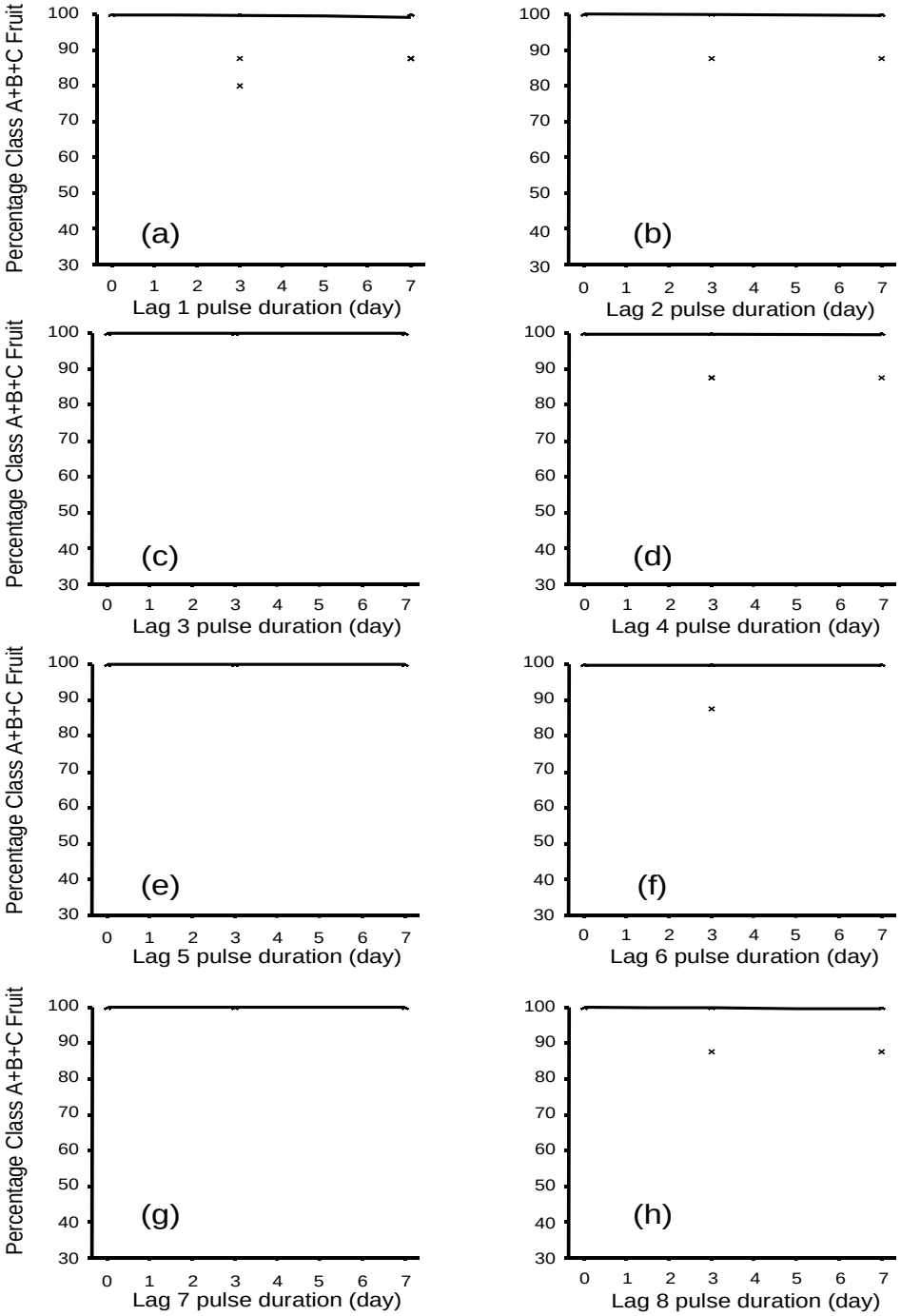


Figure 41. The relationship between % Class I fruit (Class A+B+C) for **dark patches** and pulse duration in a-h, for Lags 1-8 respectively.

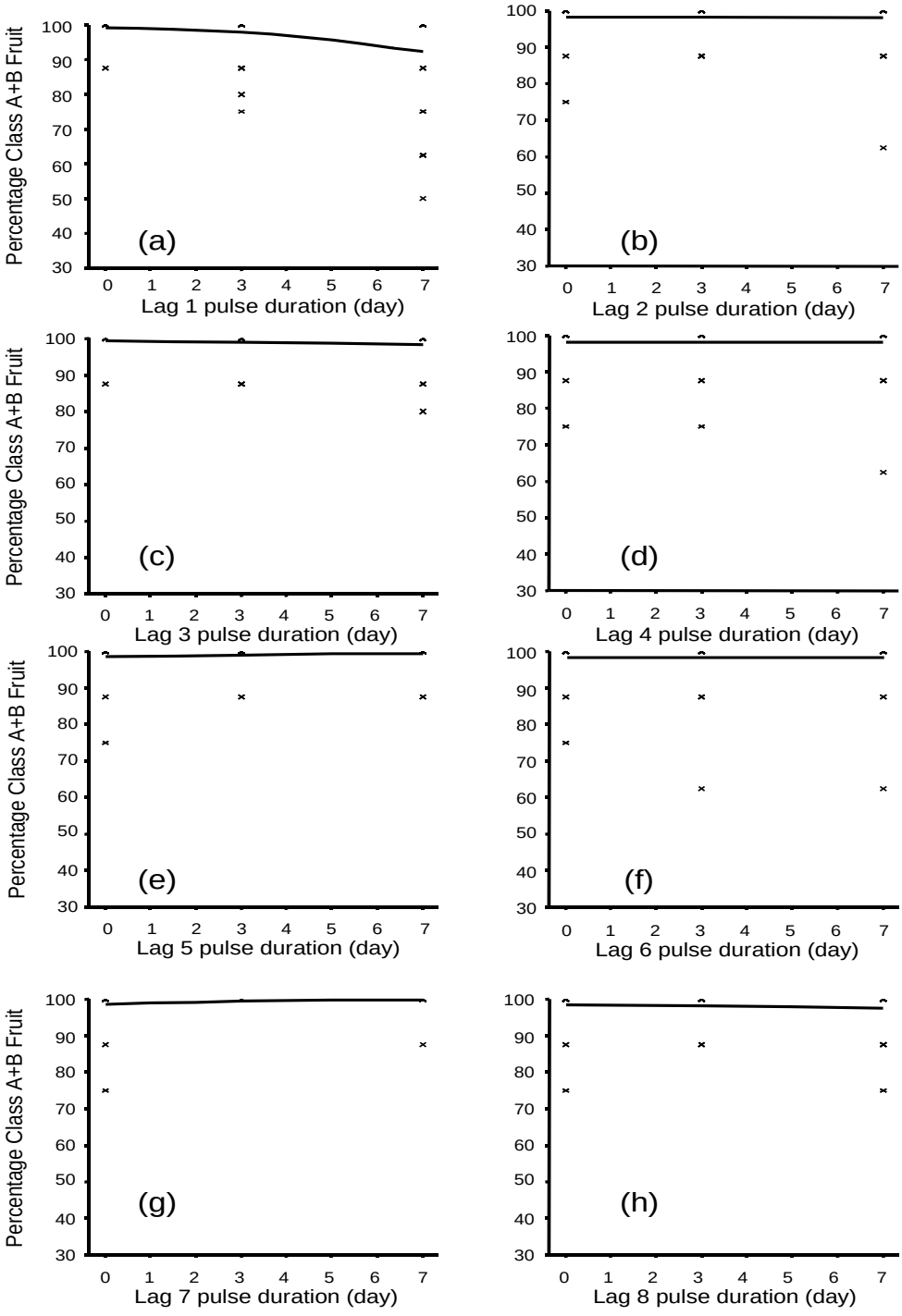


Figure 42. The relationship between % Class I fruit (Class A+B) for **dark patches** and pulse duration in a-h, for Lags 1-8 respectively.

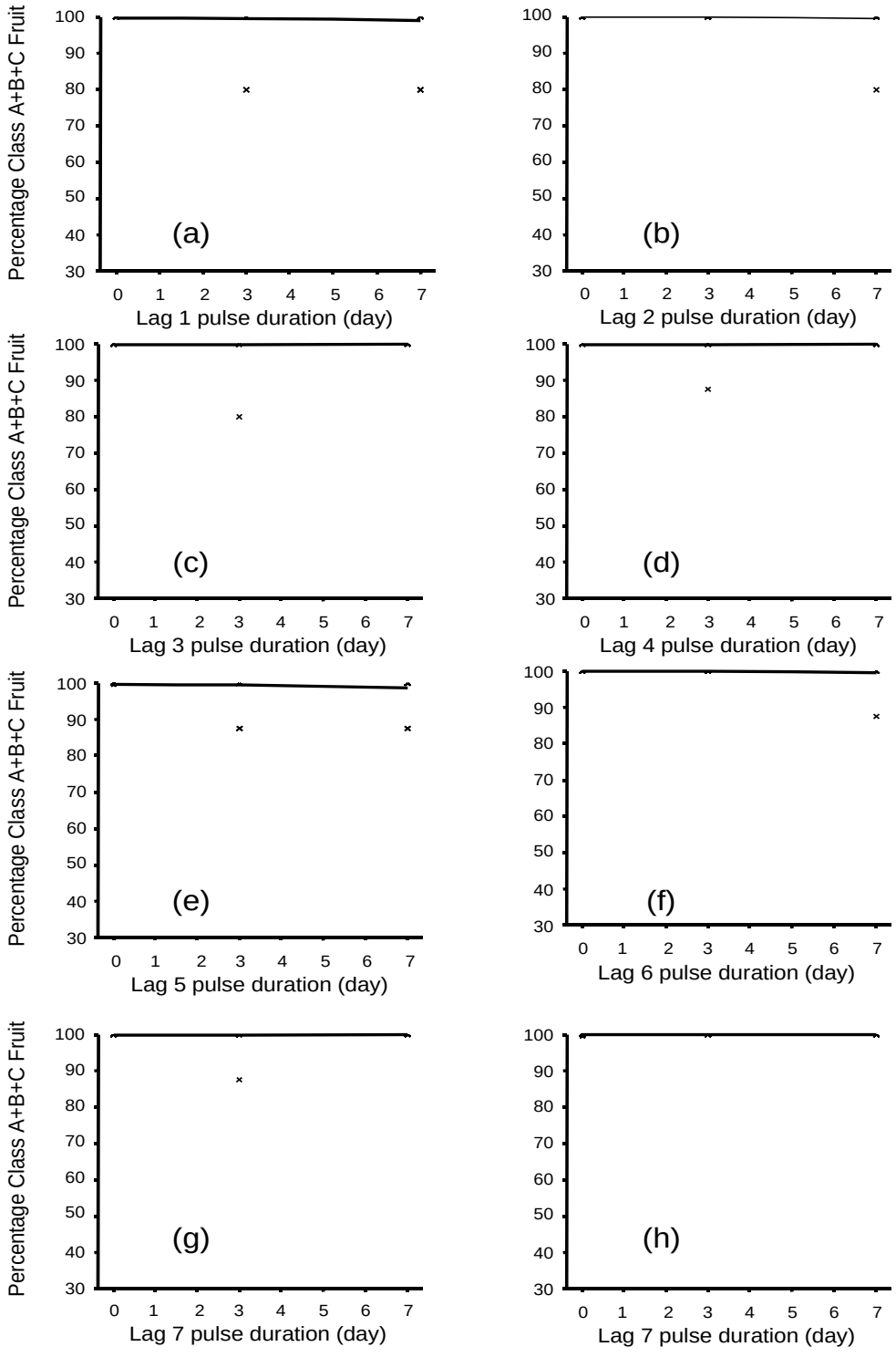


Figure 43. The relationship between % Class I fruit (Class A+B+C) for **gold-spot** and pulse duration in a-h, for Lags 1-8 respectively.

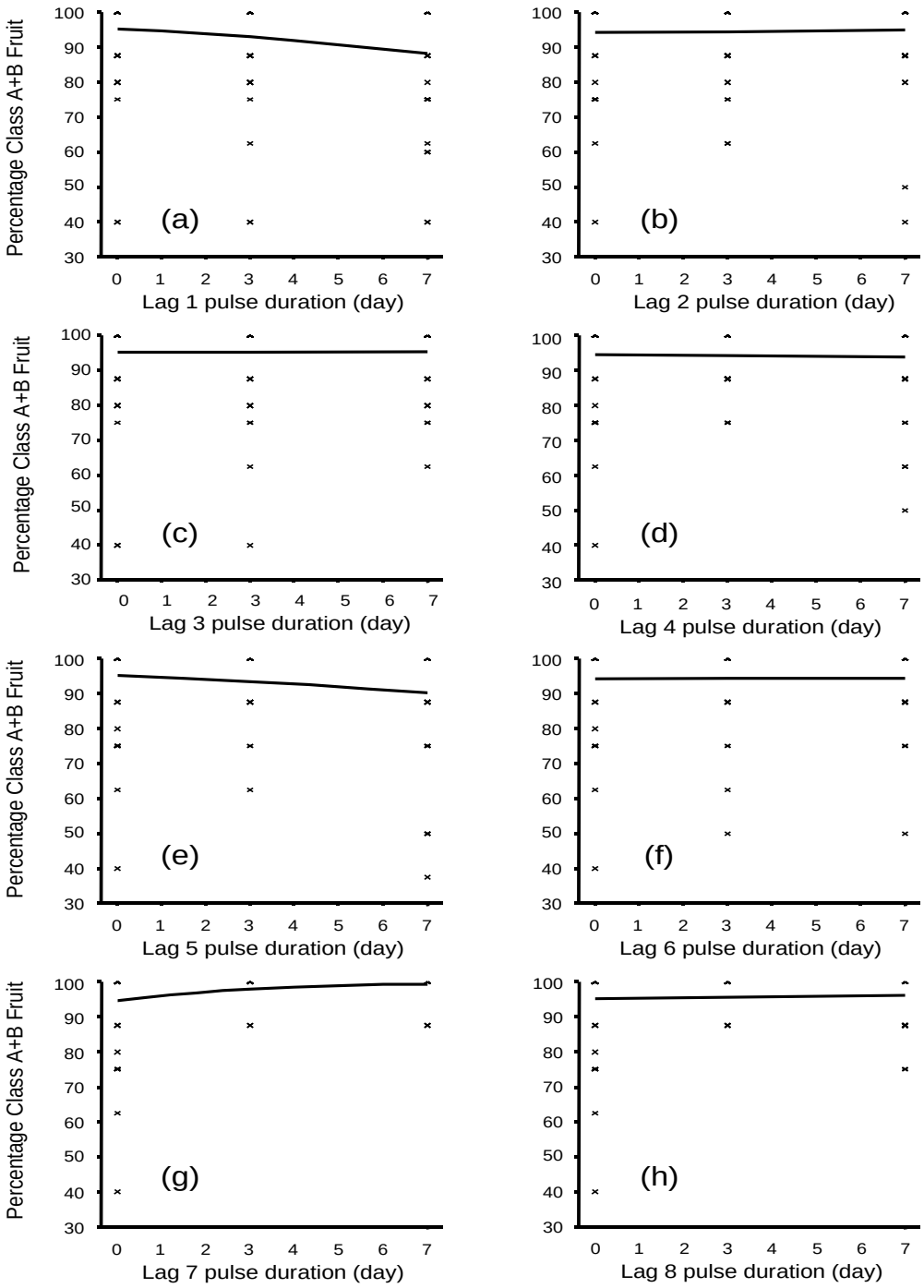


Figure 44. The relationship between % Class I fruit (Class A+B) for **gold-spot** and pulse duration in a-h, for Lags 1-8 respectively.

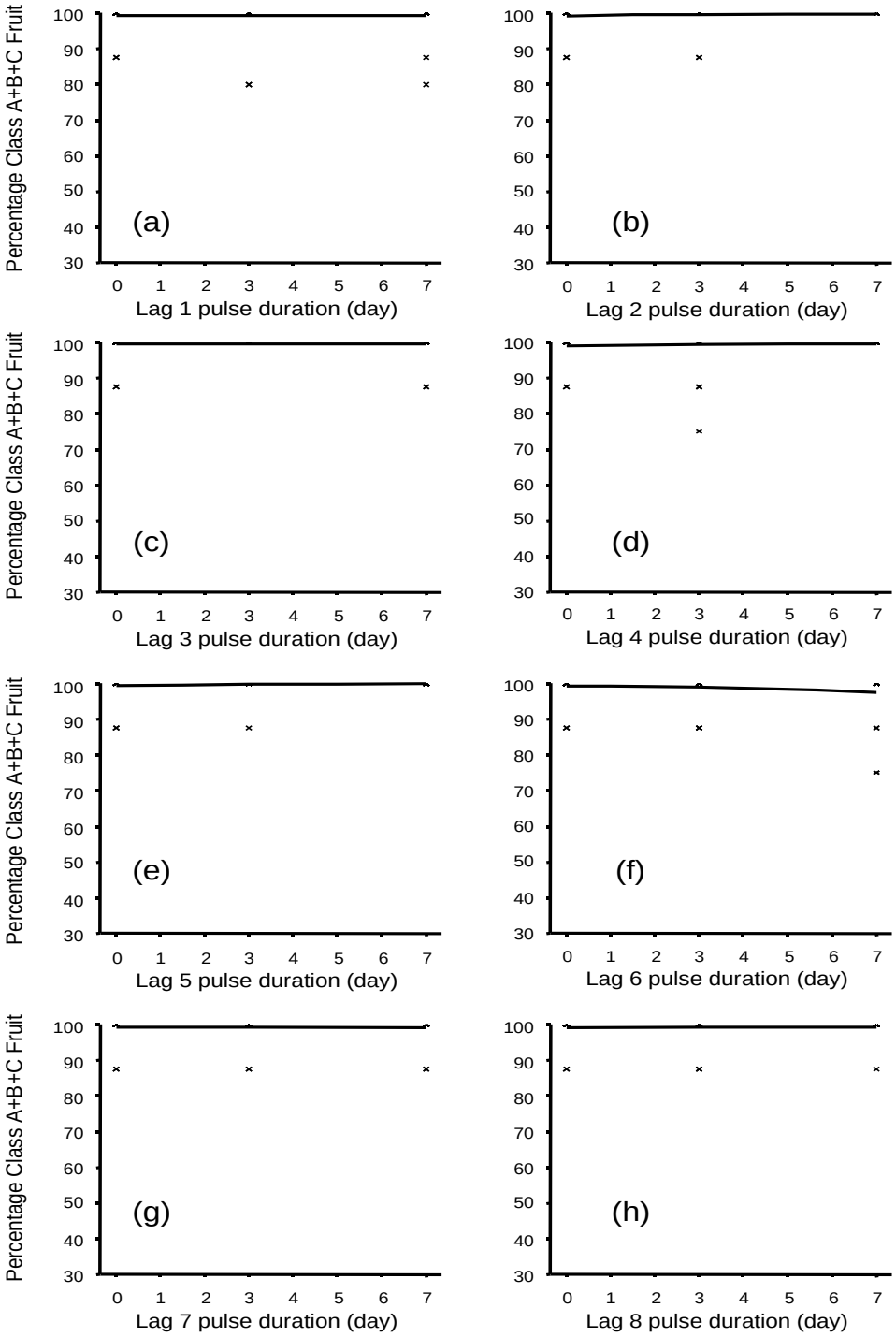


Figure 45. The relationship between % Class I fruit (Class A+B+C) for **gold marbling** and pulse duration in a-h, for Lags 1-8 respectively.

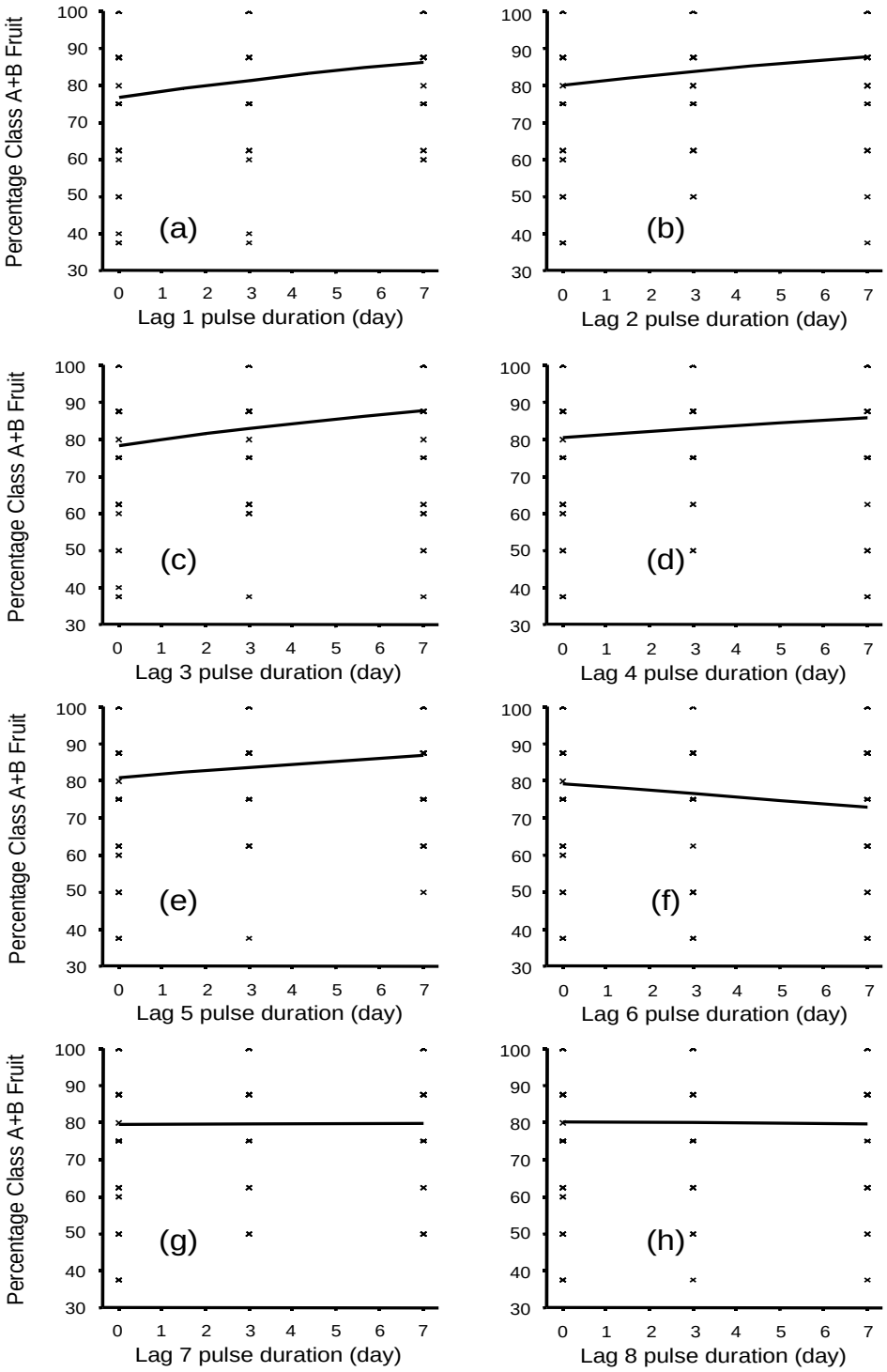


Figure 46. The relationship between % Class I fruit (Class A+B) for **gold marbling** and pulse duration in a-h, for Lags 1-8 respectively.

Appendix 4
2000 estimated response curves for percentage acceptable fruits against measured pulse temperature

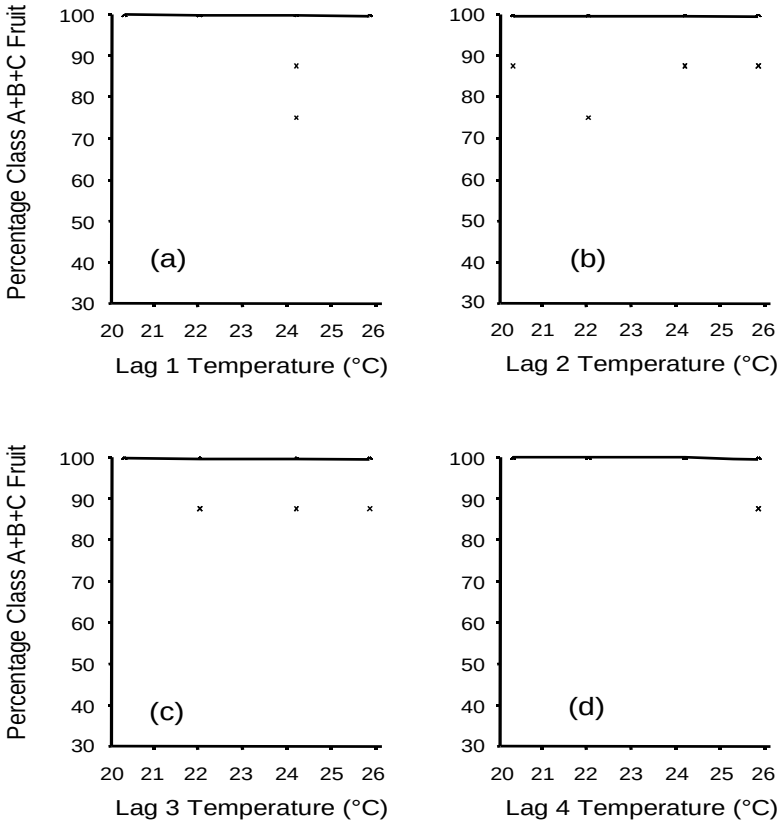


Figure 47. The relationship between % Class I fruit (Class A+B+C) for **netting** and mean pulse temperature in a-d, Lags 1-4 respectively.

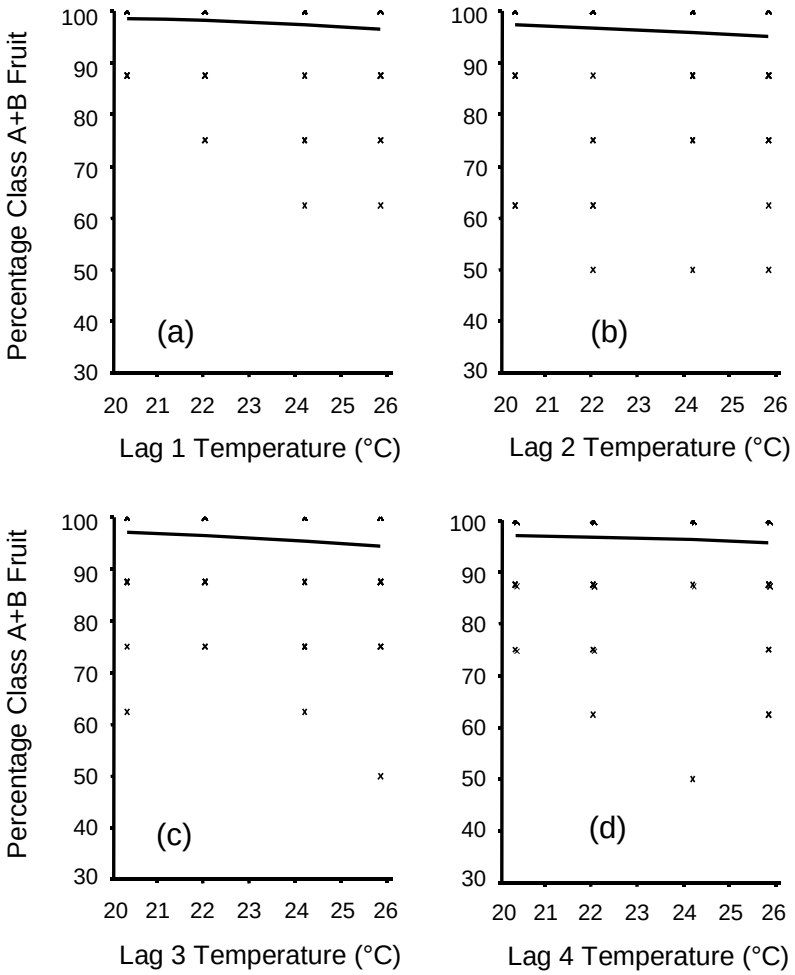


Figure 48. The relationship between % Class I fruit (Class A+B) for **netting** and mean pulse temperature in a-d, Lags 1-4 respectively.

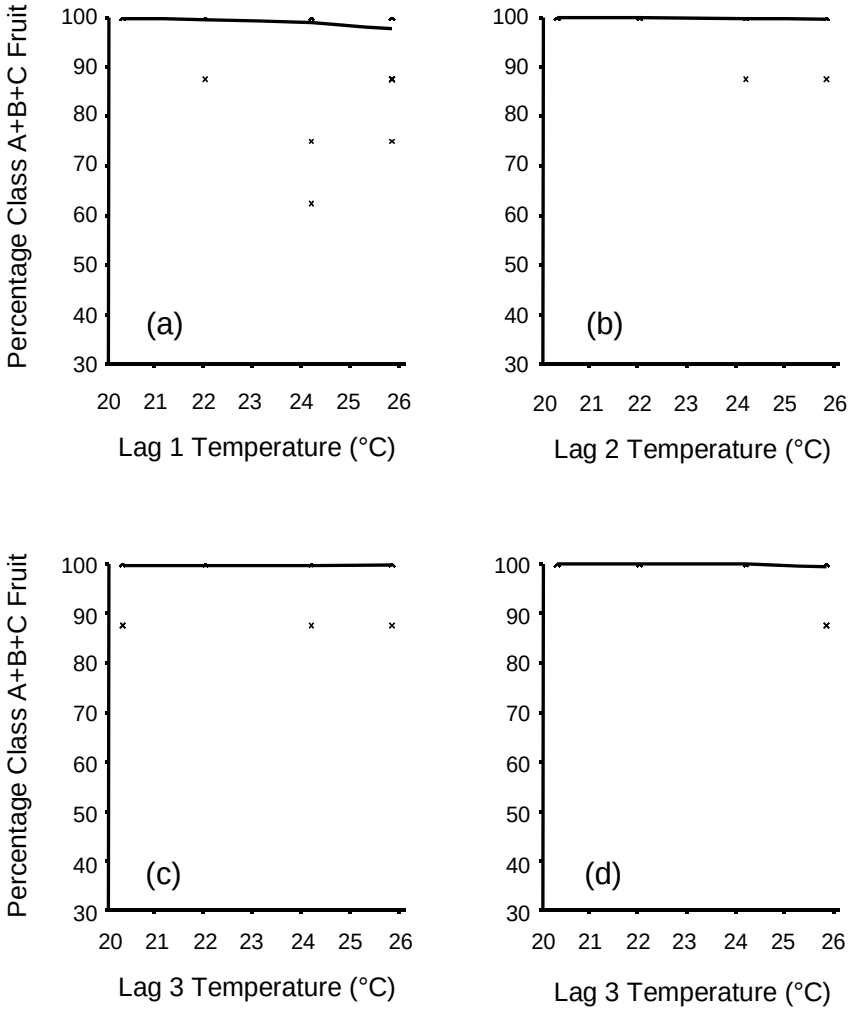


Figure 49. The relationship between % Class I fruit (Class A+B+C) for **dark patches** and mean pulse temperature in a-d, Lags 1-4 respectively.

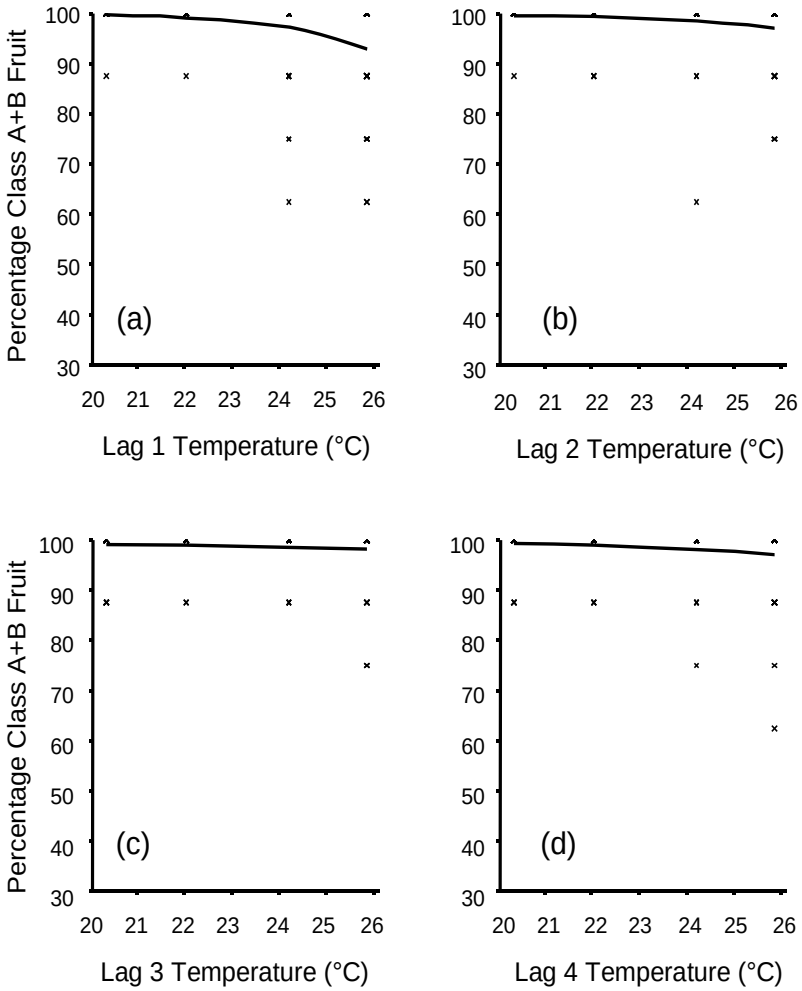


Figure 50. The relationship between % Class I fruit (Class A+B) for **dark patches** and mean pulse temperature in a-d, Lags 1-4 respectively.

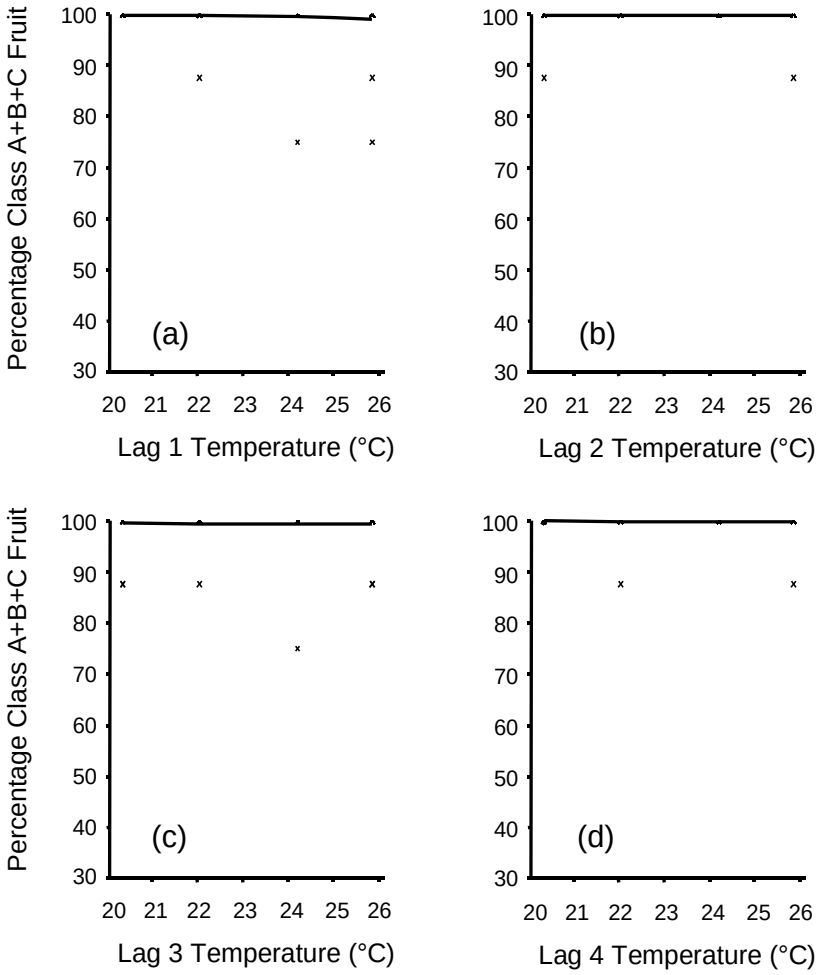


Figure 51. The relationship between % Class I fruit (Class A+B+C) for **gold-spot** and mean pulse temperature in a-d, Lags 1-4 respectively.

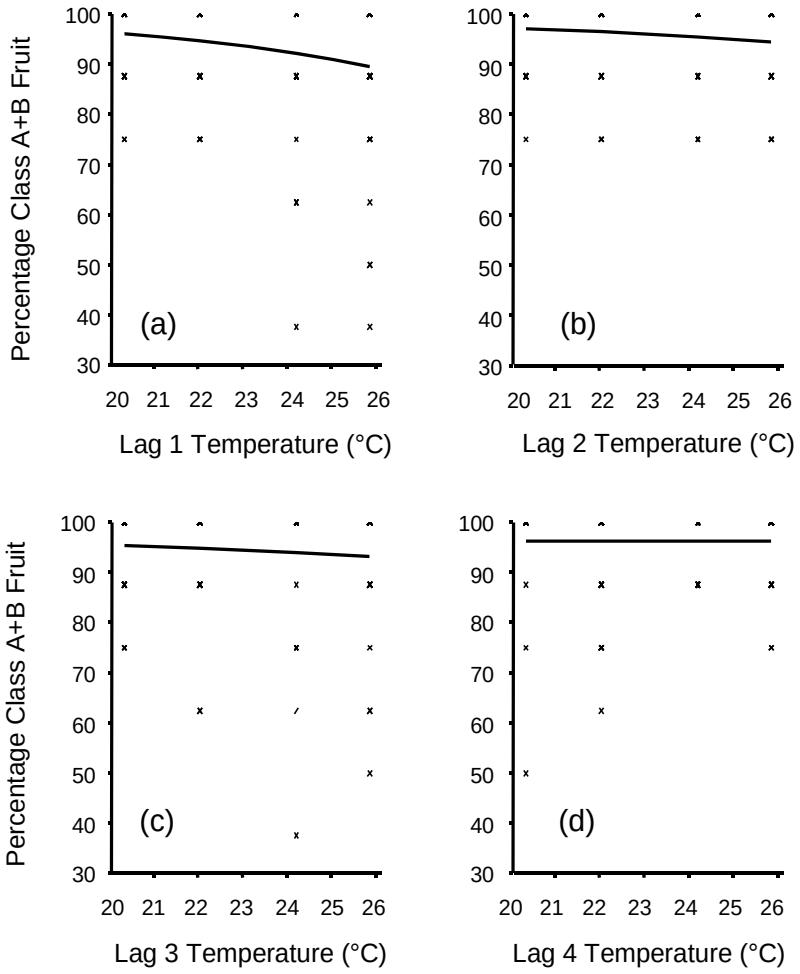


Figure 52. The relationship between % Class I fruit (Class A+B) for **gold-spot** and mean pulse temperature in a-d, Lags 1-4 respectively.

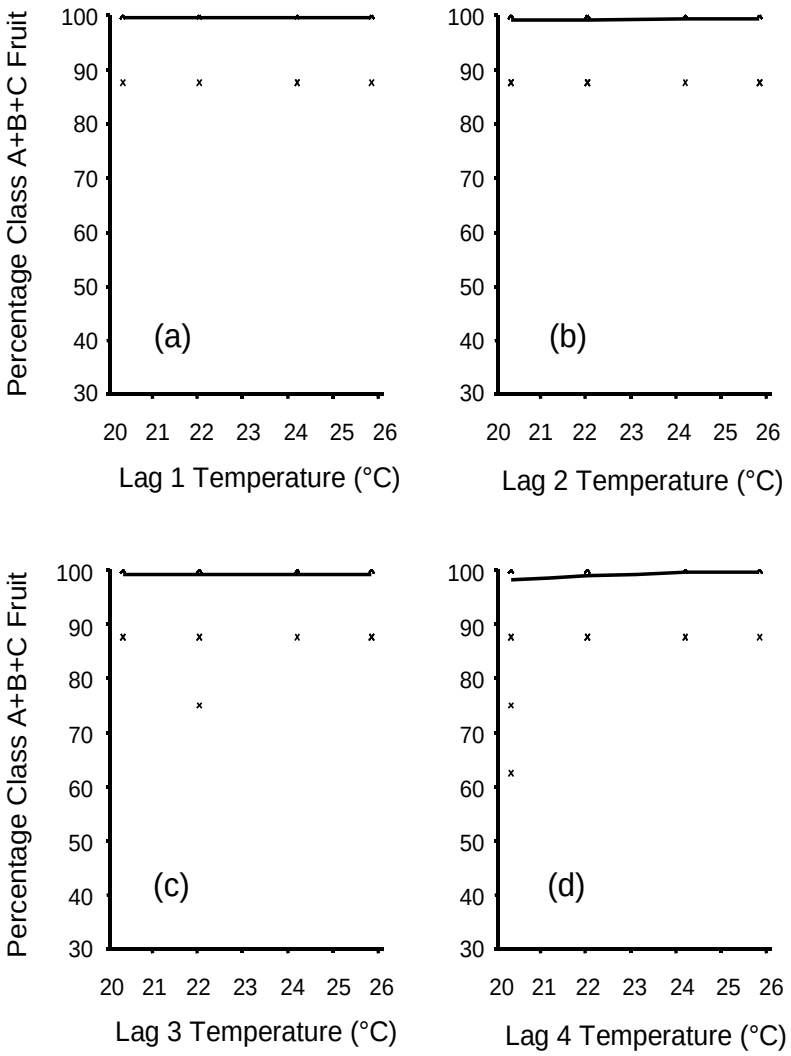


Figure 53. The relationship between % Class I fruit (Class A+B+C) for **gold marbling** and mean pulse temperature in a-d, Lags 1-4 respectively.

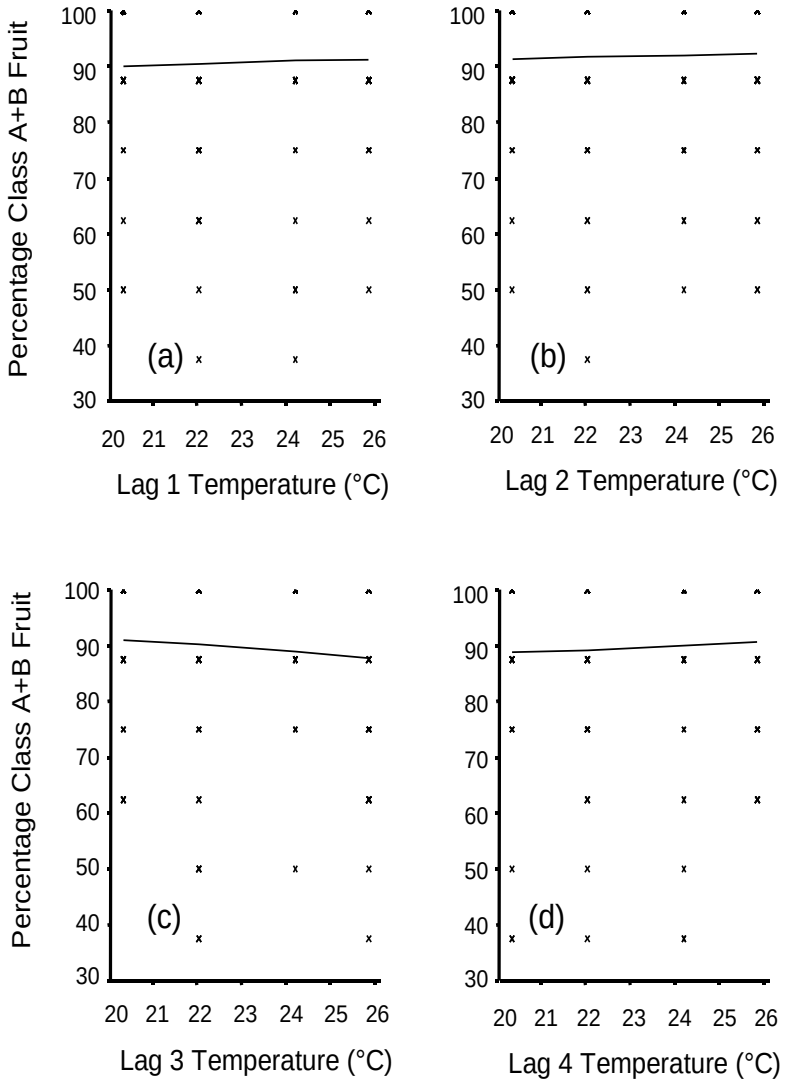


Figure 54. The relationship between % Class I fruit (Class A+B) for **gold marbling** and mean pulse temperature in a-d, Lags 1-4 respectively.