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AUTHENTICATION

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

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Grower Summary

Headline

- Forced air circulation and careful choice of polythene film may be able to ameliorate thermodormancy in everbearing strawberries.

Background and expected deliverables

Thermodormancy triggers in everbearing strawberry were investigated in detail for the first time in the DEFRA HortLINK project 215, completed March 2004 at the University of Reading (Angenendt and Battey, 2003; Wagstaffe and Battey, 2004, 2006a & b).

Heat induced cropping troughs, or thermodormancy, can reduce commercial everbearing strawberry yields by 30% (Grower, Week 34 2003). The 2004 and 2006 seasons were particularly affected by thermodormancy, possibly due to higher than average night-time temperatures and/or high relative humidity levels.

The UK soft fruit industry has increased the production of everbearing strawberry varieties to exploit the lucrative out-of-season market. Significant advances in breeding have provided varieties with improved fruit quality, making everbearer production for multiple retailers feasible, but the extended growing season makes the crop susceptible to thermodormancy. This topic is growing in importance within the context of climate change and protected cropping systems.

Over the three years of this project, physiological triggers for thermodormancy were studied as well as interactive effects of variety choice, vapour pressure deficit, osmotic potential (EC of the nutrient solution) and crop load. Further practical methods of ameliorating the severity of thermodormancy were investigated. These included plastic film greenhouse cladding materials, both with and without internal shading as well as forced air circulation. The establishment of the cause and nature of thermodormancy triggers and their alleviation may enable crop husbandry methods to be adapted.

Summary of the project and main conclusions

Four experiments were conducted. The first of these investigated the interaction of high temperature with other parameters of the growing environment. The second looked at the impact of reducing crop load during periods of high temperature. The third determined the effect of high temperatures on pollen performance. The fourth investigated the potential of heat control films.

1) ***Interaction of high temperature with other parameters of the growing environment***

Experiments were designed to investigate the possibility that manipulating either the EC of the feeding solution or levels of relative humidity may ameliorate the negative impact of high temperature. For this purpose, plant growth, flowering, cropping and stress status were monitored. In a separate experiment transpiration was assessed by monitoring stomatal conductance.

EC of the feeding solution

- Three feed-levels (Low, Normal and High) were applied to varieties Everest, Flamenco and Diamante grown in a semi-commercial pipe-and pot system within a glasshouse.
- The three feed-level treatments applied during high temperature treatment in July had no significant effect on the flowering response.
- Yield patterns were however affected. A trend was observed for higher average weekly yield in the low-feed treatment in both 'Flamenco' and 'Diamante' and a higher total yield in 'Everest' in the high-feed treatment.
- The response to feed-levels was therefore variety dependent.
- A possible explanation for the lack of any clear cut response may be a possible capacity to compensate for the change in salt level in the feed. This suggests a need to use a wider range of electrical conductivities (salt levels) in the feed solution to establish a physiological response.

Varying levels of relative humidity

- By varying relative humidity levels with two temperature treatments (22^{o}C – previously established as an optimum growth temparture for 'Everest'; 26^{o}C – previously shown to result in thermodormancy in 'Everest') five vapour pressure treatments were applied to 'Everest' plants grown in controlled environment cabinets.
- 'Everest' plants at 22^{o}C were found to have significantly higher yields than those at 26^{o}C irrespective of vapour pressure deficit treatments.
- A trend could be observed, however, of increased yields in August when plants were exposed to high vapour pressure deficits at both 22^{o}C and 26^{o}C for most of July.

Transpiration factors

- Differences in daily transpiration patterns were measured by monitoring stomatal conductance in 'Everest' and 'Diamante' grown in glasshouse compartments at three temperatures (14°C, 22°C and 26°C).
- Transpiration levels were highest at 22°C in both 'Everest' and 'Diamante', which was in agreement with optimum yield production in 'Everest' only. In comparison, 'Diamante' produced its highest yields at 14°C.
- These variety differences again demonstrated the relevance of genetic background.

2) Reducing crop load

- The potentially ameliorating effect on a thermodormancy response of a reduction in crop load (truss removal) during periods of high temperature was investigated for 'Everest' and 'Diamante' grown in temperature controlled glasshouse compartments.
- Reducing crop load was not beneficial in ameliorating the thermodormancy response in either variety.
- Truss removal therefore did not improve cropping patterns of treated plants in comparison to non-treated plants.

3) Pollen performance

Several experiments were conducted in the second and third year on pollen quality and successful pollination in 'Everest' and 'Diamante' plants grown in controlled environment cabinets.

High temperature effects on pollen quality

- Significant effects of different day/night temperature regimes on pollen quality and successful pollination were observed.
- Pollen germination rate, pollen viability and pollen tube growth, were significantly reduced in both varieties in response to high temperatures.
- In the second year of this project, pollen germination declined to zero in both strawberry varieties following high day/night temperature application.
- In the third year of this project similar results were found: pollen germination declined to zero in 'Everest' and to 6.5% in 'Diamante' following a high temperature episode.

- This also indicates that pollen performance in strawberry is variety dependent.
- These reduced rates of pollen germination in response to high temperature exposure are in agreement with other recently published research.

Pollen quality effects on cropping

- Subsequent low cropping levels and poor fruit set observed in the current study can be attributed to a reduction in pollen performance.
- Differences between varieties were found: cropping dips following exposure to 30ºC/19ºC were more pronounced in 'Everest' than in 'Diamante'.

Recovery of optimum pollen performance

- Both varieties exhibited a strong capacity to recover pollen germination rates after the termination of high temperature events.
- This implies that, provided the high temperature event is not prolonged, no permanent damage to the plant's pollen system is caused. This may also be due to the perpetual flowering habit of these varieties. Thus, if flower buds develop when the plant is not under thermal stress, then the flowers produce viable pollen.
- The recovery of pollen performance on return to lower temperature conditions appeared to be variety dependent.
- In 'Everest' most flowers that opened within 5 days after the termination of the high temperature treatment completed fruit set in experiments conducted in the second year of this project. This implies a strong capacity of 'Everest' plants to recover flower quality. However, in 'Diamante' flower quality improved more slowly.

Ameliorating the high temperature effect

- Lower night temperatures appeared to have an ameliorating effect on pollen performance.
- The pollen germination rates did not decline to zero in either variety when high day temperatures were combined with low night temperatures (for example, 26°C/11°C day/night).
- This finding is in agreement with previous work at the University of Reading on 'Everest' where cool night temperatures had an ameliorating effect on the severity of the thermodormancy response.

4) Heat control films & forced air circulation

The potential of heat control films to reduce the impact of high temperature on everbearer strawberry production was investigated in the third year of this research. A suite of 10 tunnels specifically designed to study plastic films were clad with 4 experimental films and an additional treatment with forced air circulation applied. Varieties 'Everest' and 'Albion' were used.

The five treatments were as follows:

a) A standard clear horticultural film (UVI/EVA) with no heat control properties and with forced air circulation in the tunnel (fitted fans)
b) A standard clear horticultural film (UVI/EVA) with no heat control properties and without forced air circulation in the tunnel (no fans) = CONTROL
c) A light diffusing film with known heat control properties (Luminance THB)
d) A modified Luminance THB heat control film with 25% shade
e) A modified Luminance THB heat control film with 33% shade

- Relatively cool outside temperatures over the summer of 2008 resulted in no significant thermodormancy induction in any treatment.
- This was confirmed by the flower-to-fruit conversion data.
- However, some flower abortion was observed in 'Albion' in August but the levels were not representative of a typical thermodormancy response.
- Plants growing under the shaded films produced lower flower numbers.
- In 'Everest' cropping patterns were significantly lower during August under the two diffusive films with internal shading but through September average plant weekly yield under these films was significantly higher. This observation has implications for late season fruit production in this variety.
- In 'Albion' differences in cropping patterns between the film treatments were not apparent. Differences in flowering and cropping and plant growth between the clear films with and without forced air circulation were also not so apparent.
- Both internal shading incorporated into the plastic film and forced air circulation reduced the temperature inside the tunnels compared to the control.
- Mean air temperature in the tunnels with forced air circulation was lower than the tunnels covered with the shading plastic films, suggesting that both

approaches could be beneficial in reducing thermodormancy in the event of high outside temperature conditions.

In conclusion, the reduction in pollen performance appears to be the main contributing factor to flower abortion and low fruit set and thus, it forms a significant part of the thermodormancy response. In addition, reduced flower numbers were observed following high temperature exposure in 'Everest and 'Diamante', indicating a reduction in flower initiation and/or emergence.

The key role of pollen performance within the thermodormancy response implies that the use of crop husbandry techniques that aid in temperature reduction, such as ventilation and potentially heat control cladding materials, may be of value in reducing thermodormancy in everbearing strawberry.

Financial benefits

A deeper understanding of the key processes regulating thermodormancy and its prevention will enable an increased production of everbearing strawberries to extend the UK strawberry season. The resulting improvement in continuous cropping will enhance customer confidence in everbearing varieties, thus increasing sales.

Action points for growers

This project has been directed towards dissecting the components of the physiological basis of thermodormancy on the one hand and on the other hand towards assessing practical methods to alleviate its severity on a commercial scale.

- Temperature alone seemed to be the main trigger of thermodormancy.

- Any steps that can be taken to enhance pollen performance during and after high temperature events are likely to be of major significance in ameliorating thermodormancy.

- Plastic film cladding materials with light diffusion properties and internal shading positively affected yield levels in September in one of the two varieties tested. This has important implications for late season fruit production for the current variety.

- Forced air circulation could be an effective greenhouse cooling strategy as well as shading. The combination of forced air circulation and shading for maximum

temperature control in case of high outside temperatures is suggested for commercial protected everbearing strawberry production.

Science Section

1. Introduction

The use of everbearing strawberry varieties has significantly increased the UK soft fruit industry by extending the lucrative out-of-season market. However, the yields of everbearing strawberry varieties can be detrimentally reduced following spells of high temperature, as often experienced between June and September. During such periods the plants appear dormant due to a lack of flowers and fruit. Consequently this phenomenon has been referred to as 'thermodormancy' by commercial growers (Angenendt and Battey, 2004). Although this term is a misnomer (Wagstaffe and Battey, 2006b) as the physiological basis for this response is not related to dormancy per se, we continue to use this term throughout this study when referring to reduced flower and fruit numbers as a result of high temperature exposure.

Although everbearing strawberries have been found to give greater production volumes per year, previously their fruit characteristics were deemed inferior to those of Junebearers (Hancock, 1999). Due to significant advances in breeding (Simpson *et al*., 1997), varieties with improved fruit quality have recently made everbearer production for multiple retailers feasible. Moreover, tunnel-type greenhouse technology has increased the production, quality and season-length for a range of soft fruit crops and is now used for at least 80% of the UK strawberry crops. Therefore, growers now predominantly use a combination of the traditional Junebearing strawberries with everbearing varieties in staggered production systems under semi-permanent tunnel structures, supporting cropping from May to October and beyond.

Against this background of season extension heat-induced cropping troughs are a significant problem. They have major cost implications for the UK strawberry industry, as commercial everbearing strawberry yield reductions of 30% have been experienced (Grower, Week 34 2003). In 2006, for example, approx. 30% of the everbearing strawberry production was lost in the period between late July and late August. Such events are compelling evidence of a need to further understand the phenomenon of thermodormancy and to explore possible methods for its amelioration by the development of novel heat control and other strategies.

Thermodormancy triggers in everbearing strawberry were investigated in detail for the first time in the DEFRA HortLINK project 215, completed in March 2004 at the University of Reading (Wagstaffe and Battey (2004, 2006 a b). In this four-year research project detailed transfer treatments from a commercial 'pipe and pot' system into temperature controlled glasshouses and controlled environment growth cabinets were used. Key findings included the establishment of 26^{o}C as capable of triggering thermodormancy in 'Everest' from an

exposure of 5 days, with an increase in severity following longer periods of exposure. This significant cropping trough could, however, be prevented by a cool night-temperature (13°C). In a reverse 13°Cday / 26°C night treatment it was shown that thermodormancy in 'Everest' is strongly related to night-temperature.

These findings were broadly in agreement with Japanese research in this field, where high numbers of flower buds were found to abort when plants of everbearing varieties were exposed to high temperatures during the summer period (Kumakura and Shishido, 1995). Furthermore, an average air temperature of 26°C in August was found to be capable of preventing the plants from producing flower buds and significant dips in flowering were observed (Taimatsu *et al*., 1991). The initiation of flowers was also found to be reduced by high temperature events (Oda and Yanagi, 1993; Yanagi and Oda, 1990, 1992 and 1993). In a more recent study, flowering was found to be accelerated when plants were not exposed to high day/night temperatures during the summer (Kumakura *et al*., 2005).

These observations confirm that everbearing strawberries are very heat sensitive during flowering and reproductive growth which continues for several months in these perpetual fruiting crops. Knowledge of regional climate and microclimate, as well as of crop husbandry practices, is therefore of importance in these types of study. Moreover, the developmental stages at which high temperature causes detrimental physiological effects must be identified in order to elucidate the physiological basis of thermodormancy.

Crucial stages in floral development in any crop are pollination and fertilization as a good yield usually depends upon good pollination, successful fertilization of the flower and satisfactory fruit set. The production of viable pollen, successful pollination and pollen tube growth are often critical for the processes of flowering and fruiting. In general, the production of viable pollen within the flower starts with the successful meiotic phase within the anther; then pollen development follows, starting with the formation of pollen mother cells and the induction of meiosis and ending with the formation of the pollen grains (Dickinson, 1987; Stanley and Linskens, 1974). After the production of pollen, and provided that successful pollination has taken place, the pollen must germinate and fertilize the ovules of the flower. During its growth the pollen tube encounters a complex and unstable environment, starting with the capture of the pollen grain by the stigma, hydration, the induction of germination, the growth of the tube through the style and finally the entry of the tube into the embryo sac and gamete discharge (Heslop-Harrison, 1987). During these stages the tube interacts with the stigma surface materials, the underlying cells, the secretions in the transmitting tissue and finally with the female gametophyte making the process of fertilization naturally complex. Additional external stresses, such as high temperatures, would be expected to have a confounding effect on the successful fertilization of the flowers.

In strawberry, pollen viability has been shown to be reduced at high temperatures in certain short-day strawberry varieties, but plants were also found to be capable of producing heat tolerant pollen (Ledesma and Sugiyama, 2005; Leech *et al*., 2002), even in temperatures above 25°C (Voyatzis and Paraskevopoulou-Parousi, 2002). Similar results have been found in tomato (Pressman *et al*., 2002; Song *et al*., 1999), in which the response of pollen to heat treatments can be genotype dependent (Abdul-Baki and Stommel, 1995). In rice, the exposure of spikelets of several genotypes to short episodes of high temperature caused sterility (Jagadish *et al*., 2007). In groundnut, high temperatures caused a decrease in pollen viability which resulted in yield reduction due to inhibited floral development (Kakani *et al*., 2002; Vara Prasad *et al*., 1999 and 2001). The same was reported for pepper (Mercado *et al*., 1997). High temperature can also have negative effects on the receptiveness of the stigma in peach (Hedhly *et al*., 2005).

Consequently, the effect of high temperature on pollen viability and pollen germination after pollination and the successful growth of the tube were considered key processes for the understanding of thermodormancy. As a result, their investigation was incorporated in a change to the original proposal in years 2 and 3 of the current study.

Crop load of most fruiting plants affects growth and development; according to various studies, fruit thinning can significantly enhance yield and quality of the product on crops like pecan, sweet orange and pepper (Smith *et al*., 1993; Syvertsen *et al*., 2003; Fukumoto *et al*., 2004 respectively). Particularly in orange trees, an increased crop load was found to affect nitrogen and carbohydrate concentration within the plant as well as net assimilation of CO_2, stomatal conductance and the size of the leaves (Syvertsen *et al*., 2003). Moreover, high crop load effects have been shown to interact with high temperature stress in Japanese plum varieties causing significant decreases in yield potential (Naor *et al*., 2004). In addition, a high flower load has been found to affect subsequent crop load and consequently yield and quality of fruit in European plums (Meland, 2007). In an attempt to ameliorate the severity of thermodormancy, a systematic approach to crop thinning in a high temperature environment was therefore investigated as part of this study.

Aims of this research

The project aimed to define the physiological basis of thermodormancy in everbearing strawberries and to establish the potential to reduce its severity on a field scale. The establishment of the cause and nature of thermodormancy triggers was considered key to enable crop husbandry methods to be adapted in order to aid season extension of everbearing strawberry in the UK.

The sensitivity of everbearing strawberries to temperature is variety specific. It was therefore important for the choice of varieties to reflect a wide genetic diversity. The four varieties chosen for the current study were 'Everest', 'Flamenco', 'Diamante' and 'Albion'. 'Everest' was raised from a cross between the varieties 'Irvine' and 'Evita' and previous thermodormancy work of relevance to this project was conducted in this variety (HortLink 215). 'Flamenco', was also bred in the UK and is a cross between 'Evita' and 'EMR 77' (Meiosis, 2006). Varieties 'Diamante' and 'Albion' are day-neutral varieties that have been developed by the University of California strawberry breeding program (UC Davis Strawberry Breeding Program) (Baruzzi *et al*., 2006). 'Albion' is the newer release of the two and is expected to offer an alternative for strawberry growers to the industry standards 'Everest' and 'Diamante' in the commercial UK everbearer market (Hargreaves Plants, 2009). It can be argued that the genetic basis of any of the commercial everbearers is small and that threshold temperature sensitivity, for example, may be similar to that previously established for 'Everest'; the differences in cropping patterns, however, imply that a range of responses will be found to thermodormancy triggers. On a commercial scale even small differences are multiplied to have a significant impact on yield.

SEASON 1

In the first year, factors that could potentially interact with high temperature events to trigger thermodormancy were studied. The interaction of changes in relative humidity with high-temperature events were investigated by applying various vapor pressure deficits (VPD) to plants grown in controlled environment cabinets. In a further experiment, the impact of changes in osmotic potential of the feed applied during high temperature exposure was studied in a glasshouse experiment within a semi-commercial pipe and pot system. In addition to standard flowering and cropping data, key observations included the timing of the onset of flower abortion, levels of chlorophyll fluorescence and stomatal conductance.

SEASON 2

Using controlled environment cabinets, the effects of high day and night temperatures were investigated both on the quality and performance of pollen (pollen viability and germination), and the occurrence of floral abortion (pre- or post-anthesis). The potentially ameliorating effect of crop load reduction (truss removal) on the severity of the thermodormancy response was investigated in a separate glasshouse experiment.

SEASON 3

In the final season, further studies on pollen performance (pollen viability and germination) both *in vitro* and *in vivo* were conducted using controlled environment cabinets. In a further experiment, a suite of 10 polytunnels were used to investigate the potential of novel heat control films (tunnel cladding materials) with varying degrees of internal shading for temperature reduction.

2. Materials and Methods

2.1 Crop husbandry methods

In most experiments in this project plants were potted of 2l or 3l pots using standard strawberry peat compost (Bulrush Horticulture Ltd. UK) which enabled easy transfer of the plants between different glasshouse locations for treatment application when necessary. A commercial, soft fruit liquid fertilizer mix was used throughout (Avoncrop Ltd, UK, 3-1-6 plus micronutrients). A stock solution of the liquid fertilizer was diluted with mains water to give the desired EC (1.4ms). The acidity of the liquid feed was kept between pH5.8 and 6.2 by addition of dilute nitric acid. Drip irrigation was used throughout (flow rate $2l.h^{-1}.dripper^{-1}$; Field Ltd. UK) via automated irrigation pumps (5/6 irrigations per day).

2.2 Glasshouse facilities

Glasshouse experiments were performed in the inner six or a linear array of eight temperature-controlled glasshouse compartments (3.7m x 7m) set to provide minimum temperatures of 4, 10, 14 18, 22 and 26^{o}C, with ventilation at temperatures 4^{o}C above these set points (Fig. 1). Plants were grown under natural daylengths. Mean diurnal temperatures were calculated from data recorded on a datalogger (DT500, Delta Electronics, UK) using aspirated Type K thermocouples (30-s scans, logged every 5 minutes)

Figure 1: Temperature-controlled glasshouse compartments.

2.3 Controlled environment facilities

Controlled environment growth chambers (cabinets) (Saxcils – Saxton Ltd. UK) were used in experiments where accurate control of day/night temperatures and relative humidity was required (Fig. 2). Temperature was measured in the cabinets (every two minutes) via Type K thermocouple probes and recorded as 2 hour means with data loggers (DT500, Delta

Electronics, UK). The light source in the cabinets consisted of 53x58 watt, warm white fluorescent tubes (F58W, Sylvania, UK) and 30x15 watt tungsten bulbs (Crompton Lamps, UK) in each cabinet. The photon flux density (PFD) of the light within the cabinets was 350 $\mu mol.m^{-2}.s^{-1}$. The Red/Far Red light ratio, measured with a 660/730 nm sensor (Skye Instruments Ltd, UK), was on average 6. The vertical air flow in the cabinets was from bottom to top and was on average, 0.52 $m^{-1}.s^{-1}$. The carbon dioxide concentration in the cabinets was kept at an ambient level.

Figure 2: Controlled environment cabinets.

2.4 Flowering, cropping and growth measurements

Flowering and cropping were measured at weekly intervals throughout each experiment. Flower number, fruit number and fruit fresh weight (g) per plant were recorded. Flowers were tagged weekly to monitor the duration of fruiting and to record flower-to-fruit conversion rates.

At the end of each experiment a destructive harvest was conducted to measure vegetative growth; either on all experimental plants or on a randomly selected sample of experimental plants. Growth measurements included leaf number and area, crown number and inflorescence number and the fresh and dry weights of these components. These data were used to include total plant fresh and dry weight, harvest index (HI) (total yield $plant^{-1}$ / total yield $plant^{-1}$ + total plant fresh weight), leaf weight ratio (LWR) (leaf dry weight / total plant dry weight), leaf area ratio (LAR) (leaf area / total plant dry weight) and specific leaf area (SLA) (leaf area / leaf dry weight).

2.5 Physiological measurements

Physiological responses of plants to the treatments applied included chlorophyll fluorescence and stomatal conductance.

Stomatal conductance was measured using a dynamic diffusion, non-ventilated porometer (AP4, Delta-T Devices, UK) on one newly expanded leaf per plant. The AP4 porometer measures stomatal conductance by comparing the precise rate of humidification within a small cuvette to readings obtained with a perforated calibration plate.

Chlorophyll fluorescence was used to estimate plant stress by measuring photosynthetic efficiency as light re-emitted as chlorophyll fluorescence. (Maxwell and Johnson, 2000; DeEll and Toivonen, 2003; Papageorgiou and Govindjee 2004) using a chlorophyll fluorescence meter (Hansatech Ltd, UK). Minimal fluorescence (F_0) of a light-adapted leaf that was darkened for a period of 20-25 minutes was used as a measure of the stability of the light harvesting complex. Maximum fluorescence (F_m) during a saturating light flash was measured and the difference between the two parameters is (F_v) was calculated ((F_v) = (F_m)-(F_0)). The ratio Fv/Fm ratio is a measure of the maximum quantum yield of PSII, and was used as an index for the extent of stress. The performance index (PI) of PSII which is a measure of the photosynthetic efficiency of the leaf based on the energy absorbed by the leaf's reaction centers was also measured. Chlorophyll fluorescence measurements were carried out on one newly expanded leaf per plant.

2.6 Statistical analysis methods

Flowering, cropping and growth data from each experiment were converted into an electronic format using Microsoft Excel spreadsheet software (Microsoft Corp. USA) to enable further calculation and analysis. Physiological measurements were electronically downloaded from the instruments. Analyses of Variance (ANOVAs) were used to determine the level of significance of treatment effects using Genstat 8.1 from VSN international and Minitab 15 (Minitab Inc.). Comparisons of means were made using least significant differences (LSD) either at the 5% or 1% level. In all experiments, individual plants were used as the experimental unit. Graphs were drawn with the use of Microsoft Excel graphs facilities.

2.7 Pests and diseases

Biological control methods were used in the current project where possible and the chemical applications were always kept to the minimum effective level**.** The School of Biological Sciences at the University of Reading implements standard procedures and health and safety protocols for pests and diseases control.

2.8 Materials and Methods relating to specific experiments.

2.8.1 **The interaction of high temperature with other environmental parameters on flowering, cropping and plant growth and development in everbearing strawberry varieties.**

Experiment 1

This experiment was conducted to establish the effect of different EC levels in the feeding solution (which create different osmotic potentials) on a thermodormancy response in three everbearing strawberry varieties.

Tray plants of cv. Everest and bare rooted plants of 'Flamenco' and 'Diamante' were planted into peat compost in 2-litre pots on 12 April 2006 and placed into a 'pipe and pot' system in a heated glasshouse compartment (Fig. 3). Further details of plant husbandry are given in section 2.1. Pest and diseases control methods used are given in section 2.7.

High temperature (>26^{o}C day and night) was applied to all plants in late July for 18 days concurrently to the application of three different feed levels: low (EC 1.0mS), normal (EC 1.4mS) and high (EC 2.0mS). This resulted in 9 treatments: 3 EC levels x 3 varieties. Replicate plants were placed in a completely randomised design.

Figure 3: The 'pipe and pot' system.

Following the 18-day application of high temperature and 3 EC levels, the set-point temperature of the compartment was reduced to ambient (through venting) and the EC level returned to the standard 1.4mS.

Weekly flower counts (flower no / plant) and cropping measurements (fruit number and fruit weight / plant) were taken to determine the flowering and cropping patterns over the season (late May to early October 2006).

Weekly stomatal conductance and chlorophyll fluorescence measurements were carried out as described in section 2.5.

Minimum and maximum daily temperatures recorded in the compartment are presented in Figure 4.

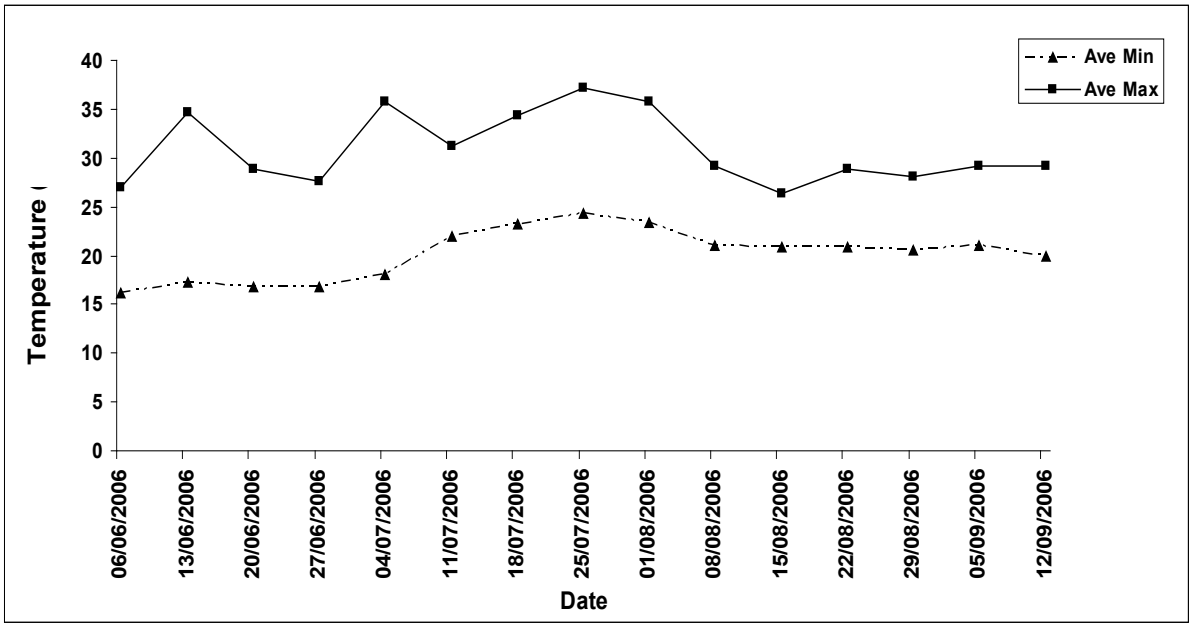


Figure 4: Average weekly min and max temperature (ºC) in the glasshouse compartment across the season

Experiment 2

This experiment investigated the interaction between relative humidity and temperature (and therefore vapour pressure deficit) in determining the severity of the thermodormancy response.

'Everest' plants were established as in experiment 1 and then transferred into controlled environment cabinets between 05/07/06 and 28/07/06. Five cabinets were used to provide two temperatures (22ºC & 26ºC) with two and three vapour pressure deficit levels respectively (Table 1). Each cabinet contained 12 plants (Fig. 2). The day-length was kept at 16 hours light and 8 hours dark in all treatments. Following treatment application in July, plants were transferred into their previous position in the glasshouse compartment containing the 'pipe and pot' system (Fig. 3).

Table 1: Treatment details of experiment 2.

Treatment no	Temperature (constant D/N) (°C)	RH (%)	VPD
1	**26**	**80**	**0.67**
2	**26**	**55**	**1.51**
3	**26**	**45**	**1.85**
4	**22**	**75**	**0.66**
5	**22**	**40**	**1.58**

Flowering and cropping patterns were determined as described in section 2.1.

Chlorophyll fluorescence measurements (Section 2.5) were taken at the beginning, middle and end of the transfer period as well as once weekly until the end of the season.

Additionally, stomatal conductance measurements (Section 2.5) were taken at certain times during the season.

2.8.2 Effects of different temperature conditions on the stomatal opening patterns, flowering and cropping of two everbearing strawberry varieties.

Plants were cropped at three temperatures, in this small-scale experiment, to investigate differences in daily transpiration patterns.

Plants were established as described for experiment 1 and then transferred into three different factorial glasshouse compartments at set point temperatures of 14°C, 22°C and 26°C at the start of the cropping (late June). Six plants per variety were placed into each compartment (Fig. 1).

Flowering and cropping patterns were determined as described in Section 2.1.

Daily stomatal conductance patterns (Section 2.5) were described by hourly porometer measurements (9am – 6pm) on three different dates, and therefore under different light levels.

Chlorophyll fluorescence measurements (Section 2.5) were taken at certain times during the season.

The experiment was carried out on two occasions. In the first, the everbearing varieties used were ‘Everest’ and ‘Flamenco’. Forty eight plants of each variety were used; these were tray plants of ‘Everest’ and bare rooted, cold stored plants of ‘Flamenco’ (supplied by Hargreaves Plants Ltd., UK). The plants were planted on 12th April 2006 and placed in a ‘pipe and pot’, semi-hydroponic system in a heated glasshouse compartment at the School of Biological Sciences in Reading. Crop husbandry methods used are described in Section 2.1.

On the second occasion, 60 plants of the everbearing variety ‘Everest’ were planted 12/04/2006 in the same heated greenhouse compartment as the first experiment at a set point temperature of 22°C. During a 23 day period in mid July (05/07 – 28/07/2006) plants were transferred into controlled environment cabinets for treatment application. The controlled environment cabinets are described in Section 2.3.

2.8.3 The potentially ameliorating effect of crop load reduction during periods of high temperature on the severity of the thermodormancy response in two everbearing strawberry varieties.

Plant material

Varieties ‘Everest’ and ‘Diamante’ were used. Tray plants of ‘Everest’ and bare rooted, cold stored plants of ‘Diamante’ were planted in 2-litre pots at 07/05/07. Plants were initially grown in a glasshouse compartment (Figure 5) at a set point temperature of 22°C (ambient RH). Half of the plants were later transferred to another compartment for temperature treatment application. The crop husbandry techniques and materials used are described in Section 2.1.

Experimental design and treatment application

Treatment application took place for 20 days from 13/08/2007 when plants were exposed to either 22°C or 28°C. At the same time, crop load treatments were applied with three levels of truss removal: 0%, 25% and 50%. The trusses were removed when the plants were at full crop load.

There were two temperature treatments x three truss removal treatments x two varieties (thus 12 treatments in total). There were six replicate plants per treatment resulting in a total of 72 plants (36 per variety). The statistical design was an incomplete randomised block design. The treatment details are summarised in Table 2.

Figure 5: The temperature controlled glasshouse compartments.

Table 2: Treatment details for crop load experiment.

Temperature compartment	Variety	% truss removal
22°C	Everest	0
		25
		50
	Diamante	0
		25
		50
28°C	Everest	0
		25
		50
	Diamante	0
		25
		50

Flowering and cropping measurements

Weekly flowering and cropping measurements were conducted in the same way as in the first experiment. In addition, flower buds were recorded and labelled; they were recorded again at fully open flower (fully open petals), and then, for a third time when they had turned into fully ripened fruit (full colour development). Labelling took place twice a week throughout the season. The bud-to-flower conversion rates and their subsequent flower-to-fruit

conversion rates were calculated for every plant; the time needed to transit between the stages of development was also calculated. These observations were of importance when comparing optimum to high growth temperatures in the two varieties. Therefore, these measurements were taken only on plants with an unaltered crop load.

2.8.4 **Effects of high temperature on pollen performance of the everbearing strawberry varieties 'Everest' and 'Diamante'.**

These experiments investigated the effects of different day/night temperature regimes on pollen performance in terms of pollen viability, *in vitro* germination capacity and pollen tube growth. This was related to subsequent flowering and cropping patterns in two everbearing strawberry varieties. Two experimental replications were carried out during a seven-month period in 2007 in the first instance and then a further experiment with slightly modified design conducted again in 2008.

Plant material

The everbearing varieties used were 'Everest' and 'Diamante'. Twenty four tray plants of 'Everest' and 24 bare rooted, cold stored plants of 'Diamante' (supplied by Hargreaves Plants Ltd., UK) were planted in 2 litre pots on 5 May 2007 using standard strawberry peat compost (Bulrush Horticulture Ltd). In the following year, 24 tray plants of 'Everest' and 24 bare rooted, cold stored plants of 'Diamante' (Hargreaves Plants Ltd., UK) were planted in a similar way on 14 May 2008. Further details of crop husbandry are given in section 2.1.

Experimental design and treatments

Controlled environment growth chambers (Saxcils – Saxton Ltd, UK; see Section 2.3) were used to control day and night temperature. Crop husbandry details are described in Section 2.1. The plants remained in the cabinets for the entire duration of the experiment, from 12/06/2007 – 31/08/2007. The high-temperature treatments were applied for 19 days in July 2007. The temperature regimes consisted of an optimum treatment (control) (22°C day temperature / 15°C night temperature, 16 h light / 8 h dark); this regime averaged 19.5°C and was chosen based on ambient on-site temperature data from previous years. The application of this temperature regime was continuous and acted as the control. The second treatment was 26°C day / 11°C night (16 h light / 8 h dark) with an average temperature of 19.5ºC but a larger day/night temperature differential and was used to assess the effect of a cool night temperature. The third temperature regime was 30°C day / 19°C night in the first experiment and 30°C day / 20°C night in the second (16 h light / 8 h dark) and was used to provide the high temperature treatment (average 26.5°C), which is above the threshold-temperature for inducing thermodormancy (Wagstaffe and Battey, 2006a and b). All cabinets

were kept at the same temperature as the Control before and after high-temperature treatment application.

For the 2008 experiment the plants again remained in the cabinets for the duration of the experiment. The high-temperature treatment was applied for 30 days, in total from 18/07/2008 until 18/08/2008. Six plants of each variety were placed in each cabinet (temperature regime). The temperature regimes consisted of the same optimum temperature (control – continuous application) as for the 2007 experiments and a second regime which provided the high temperature treatment: 30°C day / 20°C night (16 h light / 8 h dark) (average 26.6°C) in the first instance (20 days); this was increased to 35°C/20°C D/N (average 29.7°C) for a further 10 days in order to provide an extreme temperature regime. All cabinets were kept at the same temperature as the control before and after treatment application.

Flowering and cropping patterns

Weekly flowering and cropping measurements were taken throughout the duration of the experiments. Details are given in section 2.1.

Pollen measurements

Pollen viability, pollen germination capacity and pollen tube growth were determined *in vitro* on a weekly basis throughout the duration of the experiments. To measure pollen viability, acetocarmine staining solution (1%) was used (Ledesma and Sugiyama, 2005). Pollen was collected from plants in a Petri dish and taken to the laboratory where it was placed onto normal microscope slides, mixed with the stain and assessed after a period of approx. 5-10 minutes at ambient room temperature. Acetocarmine is a vital stain and it has been used extensively in a large number of species for assessing viability of pollen (Mercado *et al*., 1997; Singh *et al*., 2004; John and Prabhakara, 2005; Zebrowska, 1995). The number of stained against non-stained grains were recorded under a light microscope. For the germination test, the 'sitting drop culture method' was used (Shivanna and Rangaswamy, 1992). The germination medium used is well established (Brewbaker and Kwack, 1963) and has been found suitable for more than 86 species (Shivanna and Rangaswamy, 1992; Feder, 1990; Jayaprakash and Sarla, 2001; Honsho *et al*., 2007; Wheeler and McComb, 2006; Youmbi *et al*., 2004). This consisted of 100 mg/l sucrose, 100 mg/l boric acid, 300 mg/l calcium nitrate, 200 mg/l magnesium sulphate and 100 mg/l potassium nitrate.

Several incubation times were tested: 1, 2, 4, 6, 8 and 12 hours at a temperature of 24±2°C. An increase in germination incidence and tube length was observed for a period up to 4 hours incubation and no significant changes were observed after that period. Thus it was concluded that the cultures should be incubated for 4-6 h at a temperature of 24±2°C, in agreement with Ledesma and Sugiyama (2005) and Kakani *et al*. (2002). After incubation the cultures were fixed using 30% ethanol (Shivanna and Rangaswamy, 1992) and

germination incidence and tube growth were evaluated. When the pollen tube of a grain was equal or longer than the diameter of the grain, it was considered to have germinated (Ledesma and Sugiyama, 2005; Kakani *et al*., 2002; Shivanna and Rangaswamy, 1992). In order to minimise experimental errors, pollen was collected from all of the plants and most of the flowers within a particular treatment plot and mixed into a single Petri dish, then, from that dish, 4 or 6 replicate slides / germination cultures were made. For each of these replicates the numbers of stained against non-stained, or germinated against non-germinated, pollen grains were recorded from 6-10 random microscopic fields under a 100x magnification. The number of stained or germinated grains was calculated as a percentage of the total number of counted grains. This was done for all replicates of a treatment plot resulting in a mean percentage for each plot for each date. The total number of grains counted for each calculation was around 400 – 600 grains irrespective of the size of the population within the slide/culture.

The length of the pollen tubes was measured with a micrometer slide (Graticules Ltd. UK) and a Graticule eyepiece under a high-powered microscope (140x magnification). Mean tube length per treatment per date was determined using the same counting and calculation method as for the germination assessment. The level of anther dehiscence *in situ* was also recorded in terms of how many plants had dehisced anthers per treatment plot. In addition, the deterioration of the macroscopic appearance of flowers during high temperature application was recorded photographically.

Other measurements

In the 2008 experiment, chlorophyll *a* fluorescence measurements were taken on the plants during as well as after treatment application. In addition, the plants were destructively harvested in the end of the experiment to measure vegetative growth as described in Section 2.4.

Statistical analysis

The measurements on pollen were conducted weekly, which enabled analysis of variance to determine the level of significance of the temperature treatment effects on pollen viability and germination capacity. The experiment was treated as a completely randomised design. The germination results were tested against the binomial distribution (Mead *et al*., 2003) in order to determine if there was a significant population effect (Shivanna and Rangaswamy, 1992). The flowering and cropping data were analysed as described in Section 2.6.

2.8.5 Evaluation of the potential of different heat control films (tunnel cladding materials) to ameliorate high temperature induced reductions in flowering and cropping of the everbearing strawberry varieties 'Everest' and 'Albion'.

Plant material, crop husbandry and site details

The experiment took place in 10 tunnels specifically designed for studying plastic films at the School of Biological Sciences Field Unit at Shinfield, Reading. Each tunnel measured 3mX2.7mX6.8m (W x H x L) (Figure 6). The strawberry varieties used were 'Everest' (and 'Albion'. One hundred and twenty tray plants of 'Everest' and 'Albion' (supplied by Hargreaves Plants Ltd., UK) were planted in the end of May 2008 into 1m long peat-filled Growbags (Bulrush Horticulture Ltd. UK) (4 plants per bag) and the bags were placed on supports approximately 80cm high (Figure 6). Details concerning feeding and irrigation system are described in section 2.1.

Figure 6: The spectral films suite and the experimental set-up.

Experimental design and treatments

Twelve plants from each variety were arranged in two separate rows with guard plants at the end of each row. Each tunnel was covered in a different film treatment.

Five films were examined:

a) A standard clear horticultural film (UVI/EVA) with no heat control properties and with artificial air circulation in the tunnel (fitted fans)

b) A standard clear horticultural film (UVI/EVA) with no heat control properties and without artificial air circulation in the tunnel (no fans)

c) A light diffusing film with known heat control properties (Luminance THB)

d) A modified Luminance THB heat control film with added 25% shade

e) A modified Luminance THB heat control film with 33% shade

All films provided by the British Polythene Industries Agri., UK.

Measurements

Environmental measurements:

Outside and inside-tunnel temperature was continuously recorded throughout using T-type thermocouples. Tube solarimeters (Szeicz *et al*., 1964) were used for measuring the total solar radiation inside each tunnel. The temperature and light data from each tunnel were recorded every 30s and half-hourly averages were stored in a data logger (DT500, Delta Electronics, UK).

Other measurements:

Flowering and cropping measurements were conducted weekly as described in section 2.4. The spectral transmission of the films were measured at the beginning and end of the experiment using a M300EA monochromator, Benthams Instruments Ltd., UK. The plants were destructively harvested in the end of the experiment to measure vegetative growth.

3. Results

3.1 The interaction of high temperature with other environmental parameters on flowering, cropping and plant growth and development in everbearing strawberry varieties.

Experiment 1

In this experiment, the effects of EC on yield were studied. In all varieties two flowering peaks were observed during the experimental period (fig. 7) separated by a period of limited flowering following the high temperature event. The largest decline in flowering was observed in variety 'Everest'. Less flowering occurred in variety 'Diamante' throughout the experimental period.

Whilst there was a significant effect of variety on flowering (P<0.05), there was no effect of feed treatment on flowering apart from some effect in variety 'Diamante' (P<0.05). Variety 'Everest' gave the highest mean flower number plant^{-1}week^{-1} followed by 'Flamenco', whilst 'Diamante' gave the lowest numbers of flowers plant^{-1}week^{-1} ('Everest' = 13 flowers/plant/week > 'Flamenco' = 10 flowers/plant/week > 'Diamante' = 7 flowers / plant / week, LSD=1.58, P<0.05).

The cropping patterns over the season showed one minor followed by a major cropping peak (Fig. 8). These were separated by a decline in cropping during August, following the high temperature episode. This was consistent across all varieties. As with flowering, cropping differed significantly between varieties with the highest overall yield in 'Everest' followed by 'Flamenco' and Diamante showed the lowest yield (Table 3). However, cropping during the dip in yield which followed the high temperature episode was similar in all varieties (53.2 grams/plant/week for 'Everest', 49.3 for 'Flamenco' and 42.3 for 'Diamante', LSD=5.05, P<0.05). In contrast with flowering, feed had a significant effect on yield in 'Flamenco' and 'Diamante' (P<0.05) where the low feed treated plants showed higher yield for both varieties.

Table 3: Average weekly yield expressed as grams of fruit plant^{-1} week^{-1} for the low, normal and high feed treatments for 'Everest', 'Flamenco' and 'Diamante'.

Variety	Feed		
	Low	**Normal**	**High**
Everest	52.8g	51.8g	54.9g
Flamenco	55.6g	46.8g	45.7g
Diamante	49.1g	39.5g	38.4g

LSD = 5.05 (P<0.05)

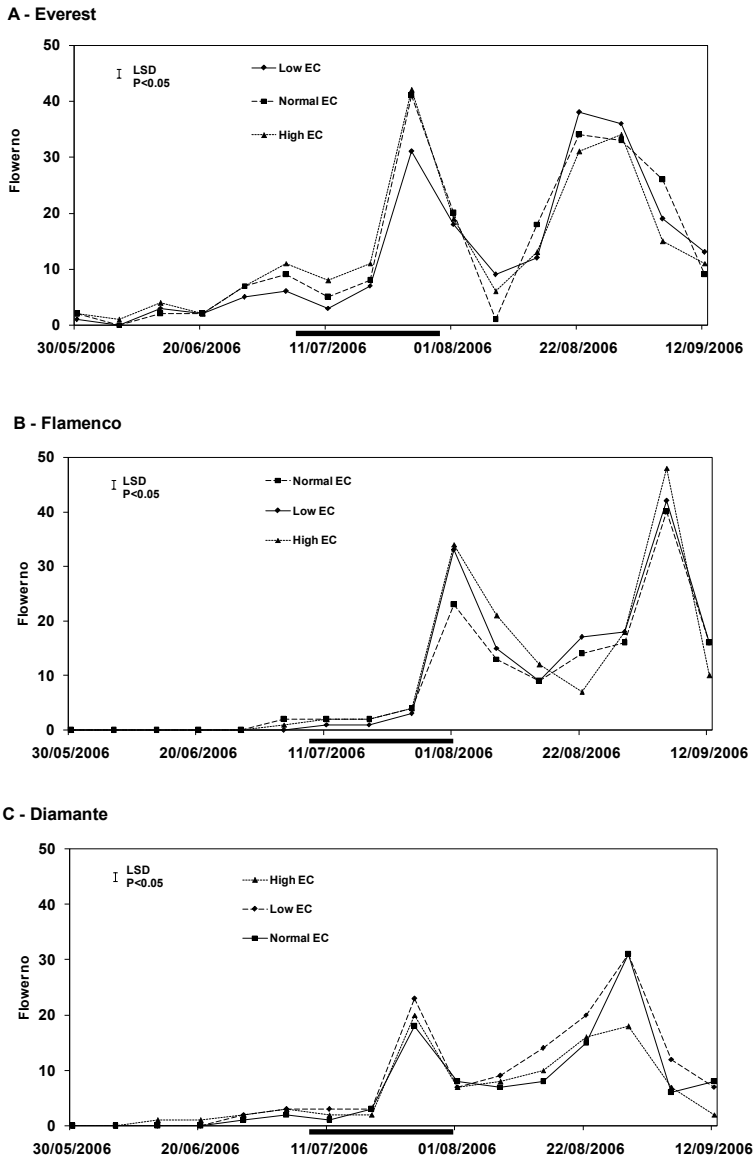


Figure 7: Flowering (expressed as mean weekly flower numbers per plant against date) across the season for variety (A, Everest; B, Flamenco; C, Diamante) grown under 3 EC treatments (low, normal and high EC). The Least Significant Difference (LSD) of the means for the interaction of EC*variety is 1.58 (*P*<0.05). The thick lines on the x axis indicate the EC and temperature treatment application period.

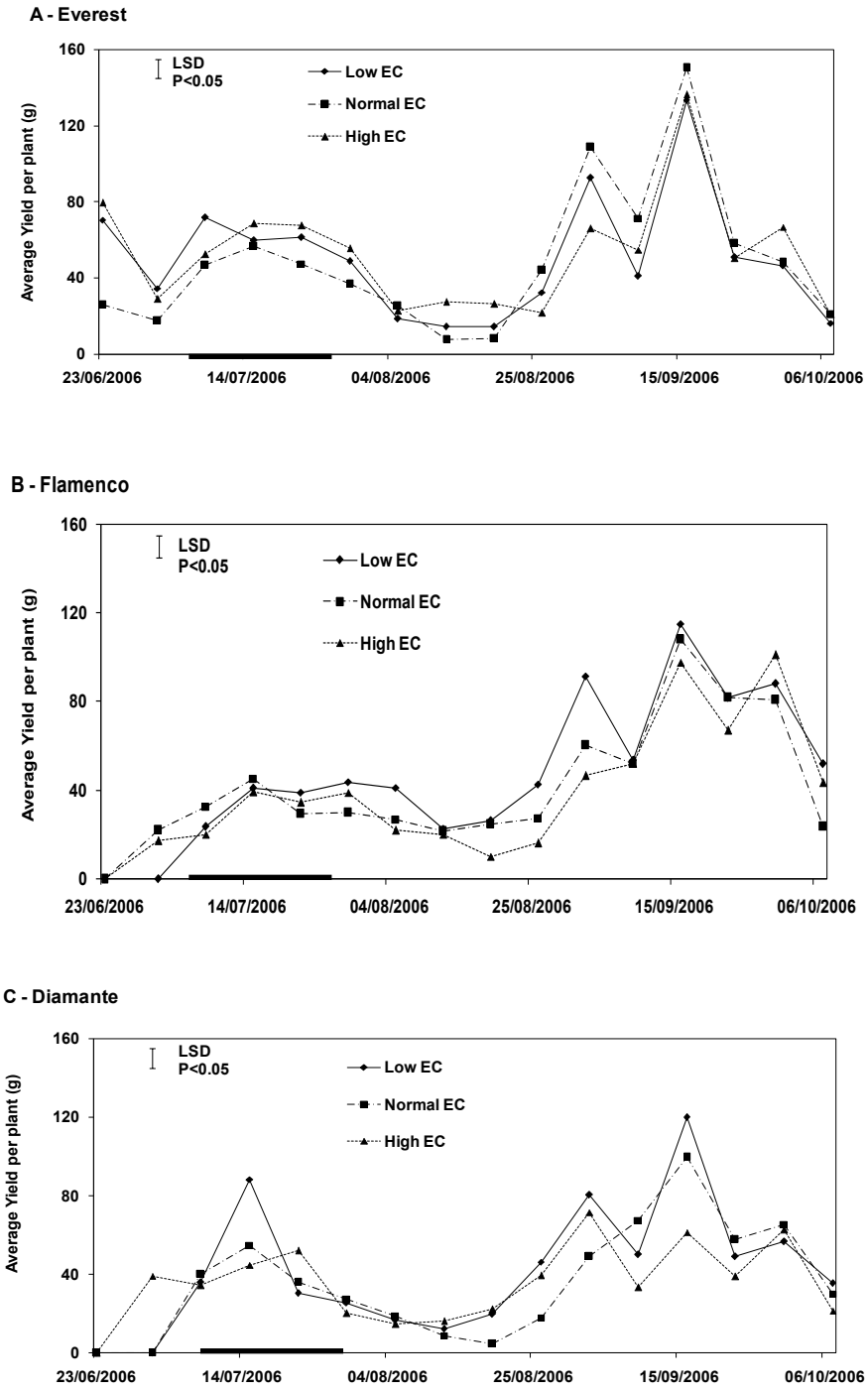


Figure 8: Cropping patterns across the season (expressed as mean weekly fruit fresh weight (g) per plant for the 3 varieties (A, Everest; B, Flamenco; C, Dimante) treatments (low, normal and high). The LSD of the means for the interaction of the factors (EC*variety) is 5.05 (*P*<0.05). The thick lines on the x axis indicate the EC and temperature treatment application period.

The average time from flowering to fully ripened fruit did not differ significantly between varieties and between feed treatments. In late July, where there was a peak in flowering in all varieties at the end of the period of high temperature, the proportion of those flowers that developed into fully ripened, marketable fruits was very low in all varieties (Fig. 9).

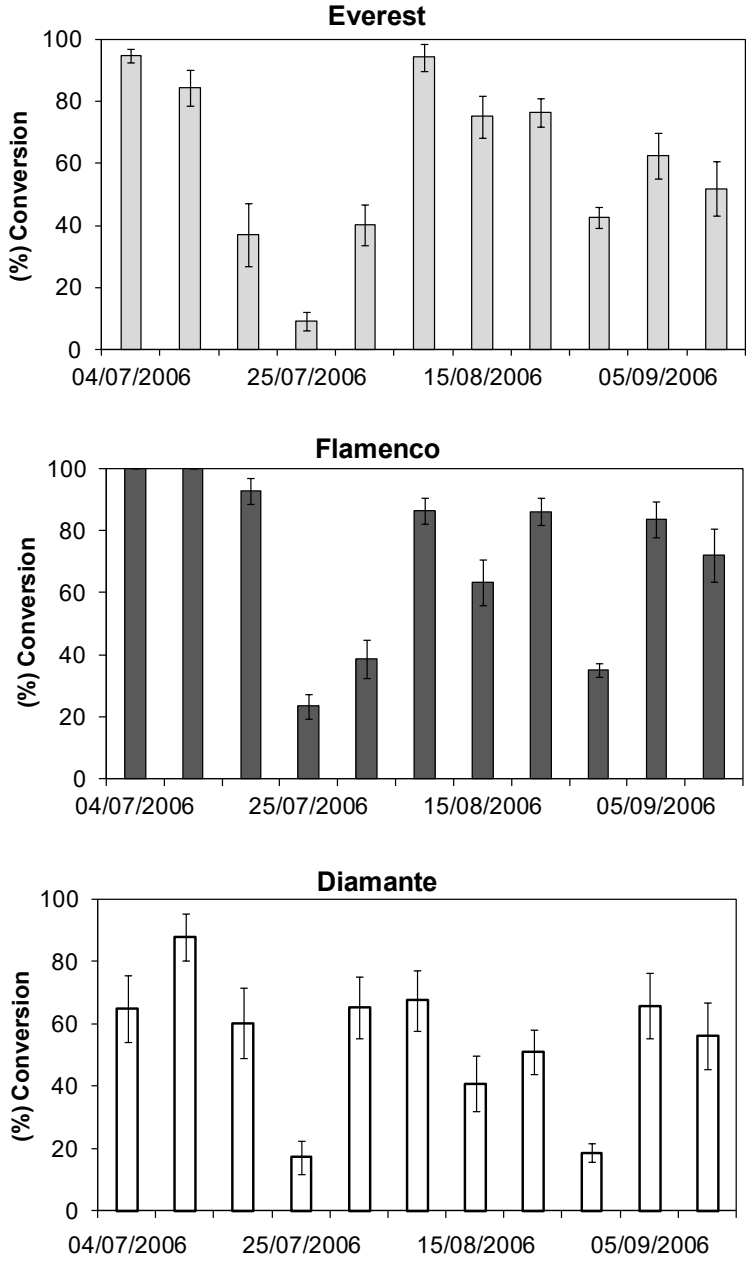


Figure 9: Average weekly flower-to-fruit conversion rates (%) for 'Everest', 'Flamenco' and 'Diamante' during the part of the season when the major flowering peaks occurred. The vertical bars show the ±Standard Errors of the means. The LSD for the means is 22.09 (P<0.05).

NB: Since the feed treatment did not have a significant effect on flowering, the conversion rates have been calculated at the variety level. The average time required for a fully bloomed flower to become a fully ripened fruit was 25 days on average, and was not significantly different between varieties or feed treatments. The dates on the graphs are flowering dates.

Total yield per plant differed significantly between varieties (P<0.05) but not between EC treatments within each variety (Fig. 10).

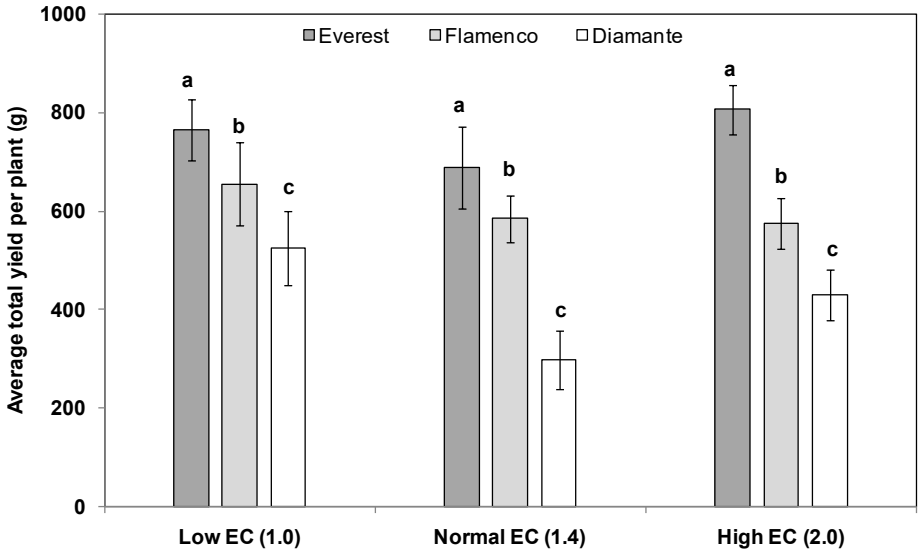


Figure 10: Mean total yield per plant for 'Everest', 'Flamenco' and 'Diamante' for the 3 EC treatments. The vertical bars are standard errors of the means. Columns marked by different letters are significantly different from each other within each EC treatment (P<0.05).

Proportions of unmarketable fruit (recorded after high temperature treatment) were high in late July – early August in all varieties but were lower for most of the rest of the season (Fig. 11). However, a high proportion of unmarketable fruits were observed in 'Everest' and 'Flamenco' during late September – early October.

Experiment 2

'Everest' plants were transferred from a glasshouse compartment into controlled environment cabinets between 05/07/06 -28/07/06. Five cabinets were used to provide two temperatures (22ºC & 26ºC) with two and three vapour pressure deficit levels respectively, to investigate the interaction between relative humidity and temperature in determining the severity of the thermodormancy response.

Contrasting vapour pressure deficit in conjunction with normal and high temperatures, did not significantly affect flowering in 'Everest'. There was a trend of lower flower numbers in plants growing at 1.5vpd, but this was not significant (P<0.05). Temperature also did not have a significant effect as plants in both temperatures flowered almost identically (Fig. 12).

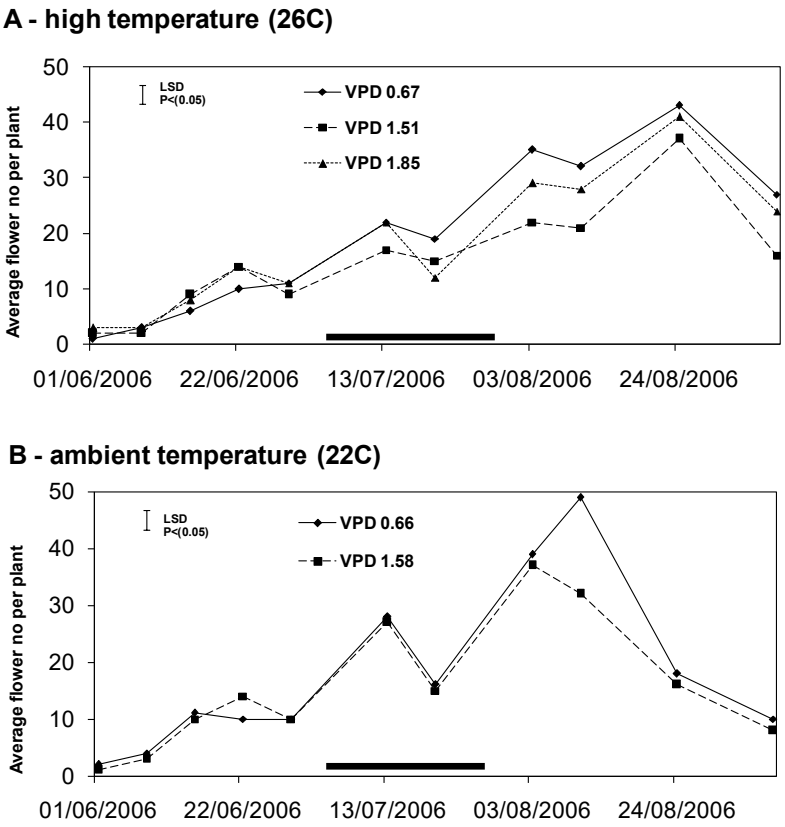


Figure 12: Mean flower number per plant plotted against date for the 5 VPD treatments and the 2 temperature treatments. The period of treatment application is indicated by the thick lines on the x axis. The LSD of the means for the interaction of the factors is 3.44 (P<0.05).

Plants at 22ºC had significantly higher yields than those at 26ºC irrespective of the vapour pressure deficit (VPD) treatment (P <0.003). In comparison, the different VPD treatments within each temperature did not significantly affect fruit production (Fig. 13). This analysis was based on the entire season; however, a trend could be found for higher yields in the highest VPD treatments in both the 26ºC and the 22ºC plants throughout August (Fig. 13).

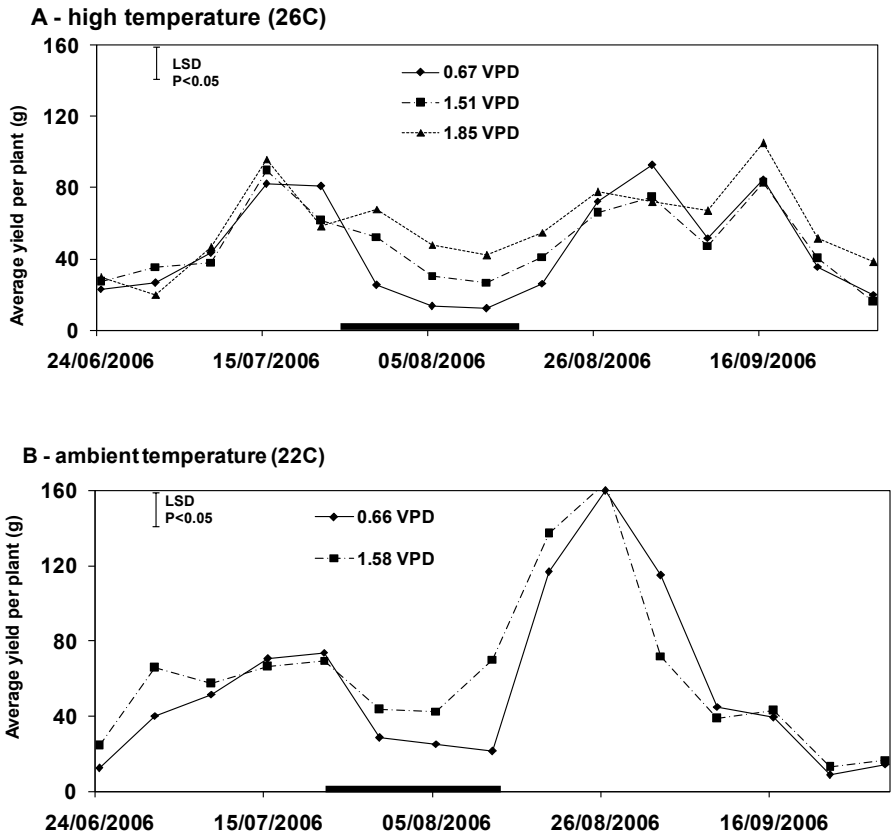


Figure 13: Mean fruit fresh weight per plant in 'Everest' in response to vapor pressure deficit (VPD). A; three VPD's at 26°C and B; two VPD's at 22°C. The thick lines on the x axis indicate the treatment application period. The LSD of the means is 9.07 (*P*<0.05).

In order to quantify the environmental stress of vapour pressure deficit on the plants, chlorophyll fluorescence measurements were conducted 5 days after transfer of the plants into controlled environment cabinets. The lower the value of Fv/Fm dropped below 0.75, the higher the stress level perceived by the plant (Papageorgiou and Govindjee, 2004). The least stressed plants were found in the high VPD treatment of the 26°C cabinet with an increase in stress level with decrease in VPD within this temperature environment (Fig. 14). At 22°C, in comparison, a relatively lower VPD resulted in less stressed plants.

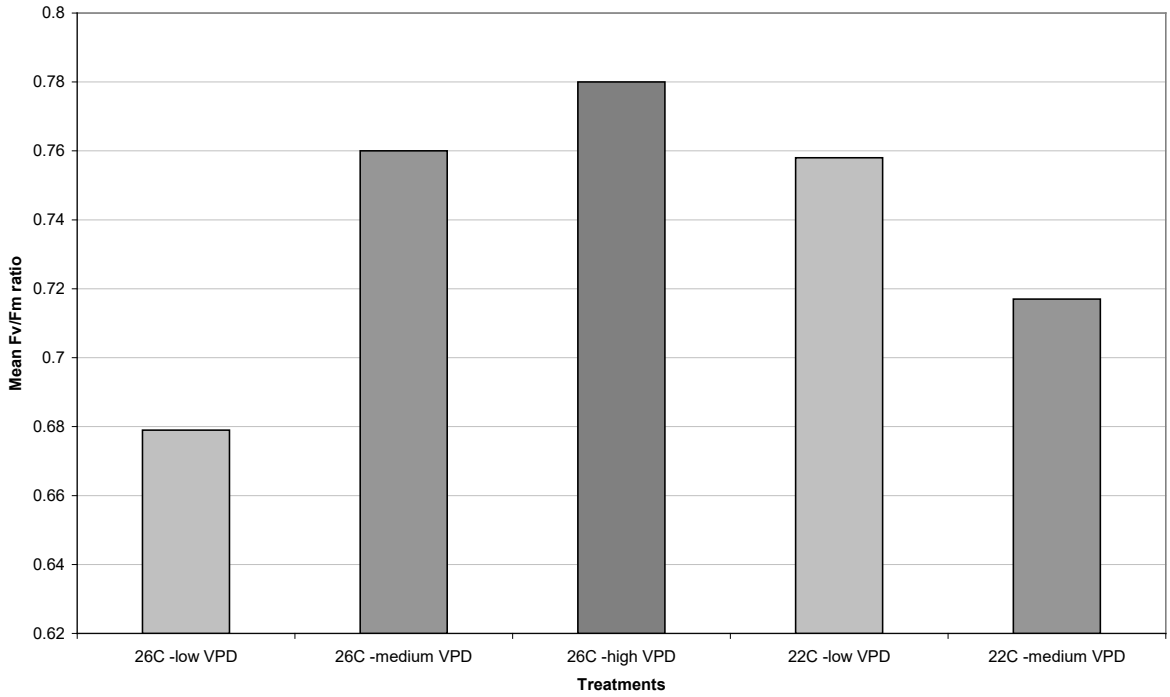


Figure 14: Mean Fv/Fm ratio for the 5 treatments (for treatment details see table 1 in the methods section). The Fv/Fm ratio is a measure of how stressed a plant is according to the chlorophyll fluorescence measurement and relates to photosystem II (Papageorgiou and Govindjee, 2004); an Fv/Fm ratio of 0.75 and above suggests that the plant is healthy, not stressed.

3.2. Effects of different temperature conditions on the stomatal opening patterns, flowering and cropping of two everbearing strawberry varieties.

In the third experiment 'Everest' and 'Diamante' plants were grown in glasshouse compartments at three temperatures (14ºC, 22ºC, 26ºC) to investigate differences in daily transpiration patterns.

In general, 'Everest' produced higher flower numbers per week than 'Diamante' (P<0.05) (Fig. 15). There was a trend indicating that 'Diamante' plants produced higher flower numbers under the cool temperature (14°C) whereas the highest flower numbers were produced at 22°C in 'Everest' but these differences were not statistically significant.

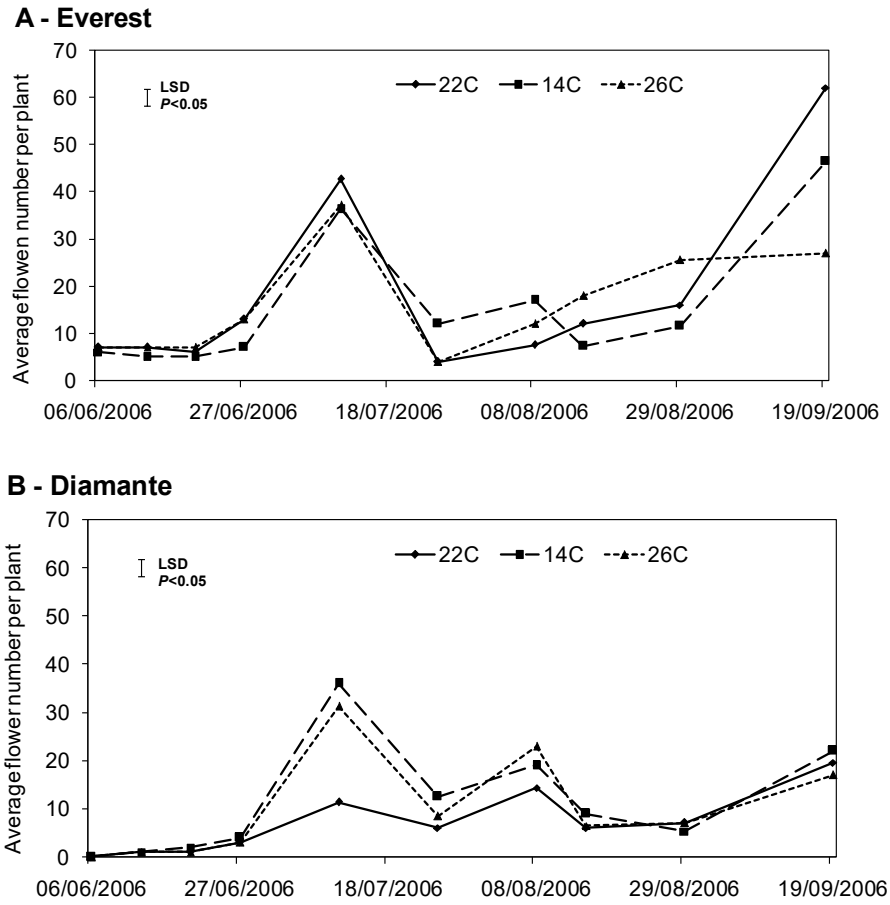


Figure 15: Flowering patterns (expressed as mean weekly flower no / plant) against date for the 3 temperature treatments (14°C, 22°C and 26ºC) in the varieties 'Everest' (A) and 'Diamante' (B) across the season. The LSD of the means for the temperature factor is 3.69 (*P*<0.05).

This pattern was also reflected in fruiting in both varieties with a sharp fall in mean fruit weight per plant during August and a recovery at the beginning of September (Fig. 16) In general, fruit fresh weight was significantly higher in 'Everest' than in 'Diamante', as seen in experiment 1. Air temperature had a significant effect on yield of both varieties (P< 0.001) (Fig. 16). There appeared to be thermodormancy at 22ºC as well as at 26ºC in both varieties. High ambient temperatures in July and August resulted in an increase above the set-point temperatures of 22ºC and 26ºC, as these compartments relied solely on venting and heating to maintain air temperatures, whereas the 14ºC compartment had additional control via air conditioning. Therefore the increase in temperature in the 22ºC and 26ºC-treatments resulted in reduced yields in August compared to yields of the 14ºC-treatment in both varieties. This dip was more pronounced in 'Everest', whereas 'Diamante' showed more even cropping patterns.

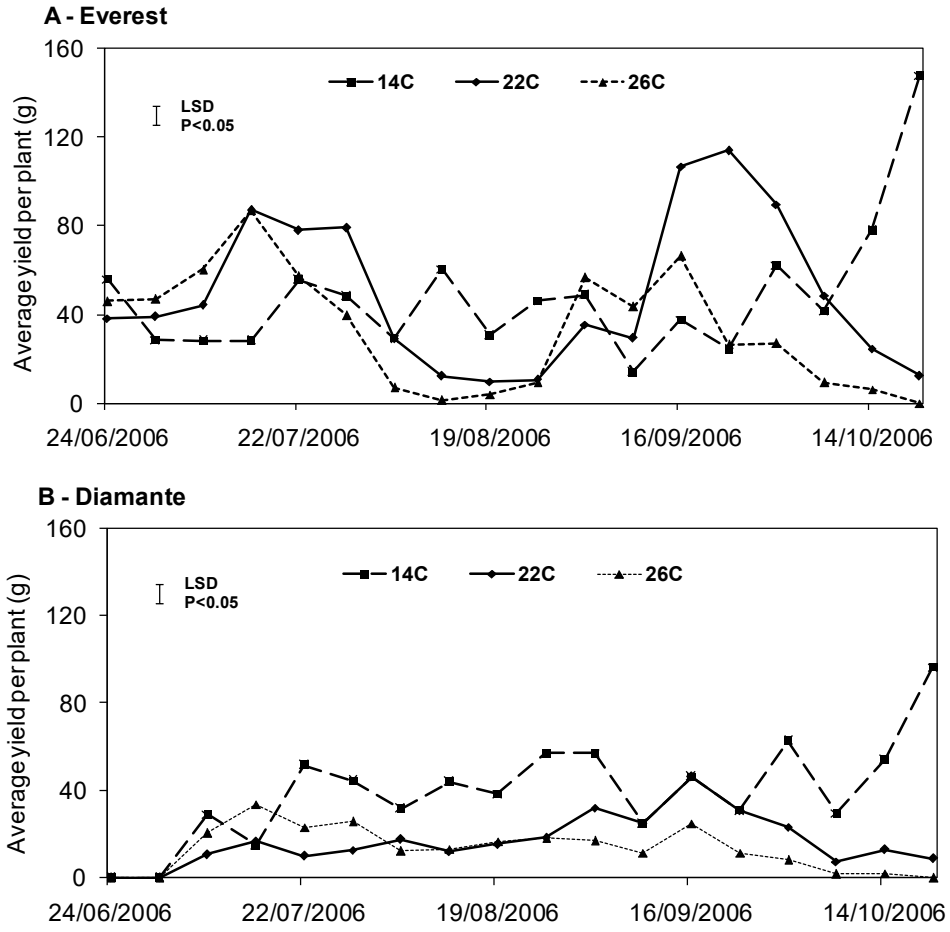


Figure 16: Cropping patterns (expressed as mean weekly fruit weight (g) / plant) against date for the 3 temperature treatments (14°C, 22°C and 26ºC) in the varieties 'Everest' (A) and 'Diamante' (B) across the season. The LSD of the means for the temperature factor is 8.85 (*P*<0.05).

In both varieties, stomatal conductance (and so transpiration) was greatest at 22ºC with a peak around midday (1pm) and lower values towards the end of the day period (around 6pm) (Fig. 17)

Although no significant temperature effect was apparent on stomatal conductance patterns across the season (Fig. 18), there was a large drop in stomatal conductance during mid-August observed in all treatments in both varieties..

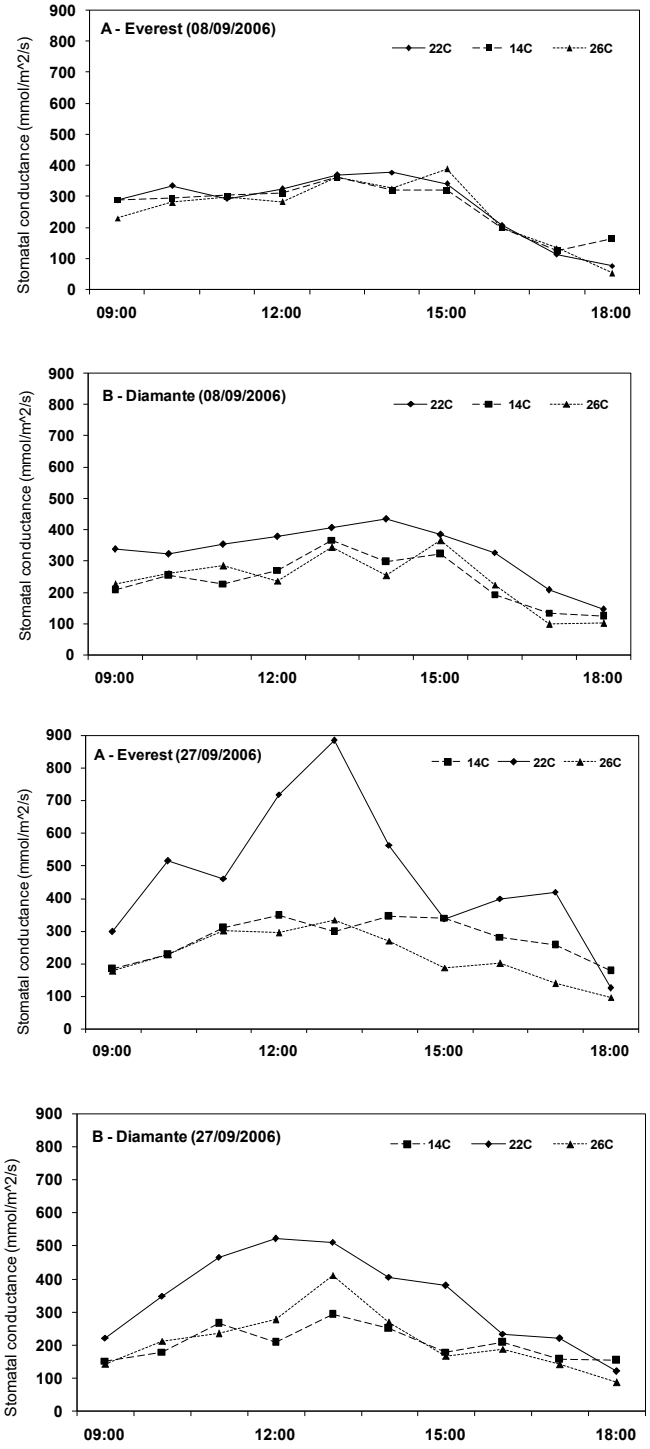


Figure 17: Daily stomatal patterns in stomatal conductance of 2 varieties. A – 'Everest'. B – 'Diamante' between 9 am and 6pm at three temperatures (14°, 22°C and 26°C).

Temperature significantly affected total yield per plant in both varieties (P<0.05) (Fig. 19). In 'Everest', yields were similar at 14°C and 22°C but declined by approximately 30% at 26°C. 'Diamante' appeared to have a lower optimum temperature than 'Everest' with approximately 60% lower yields at 26°C and 22°C compared with 14°C.

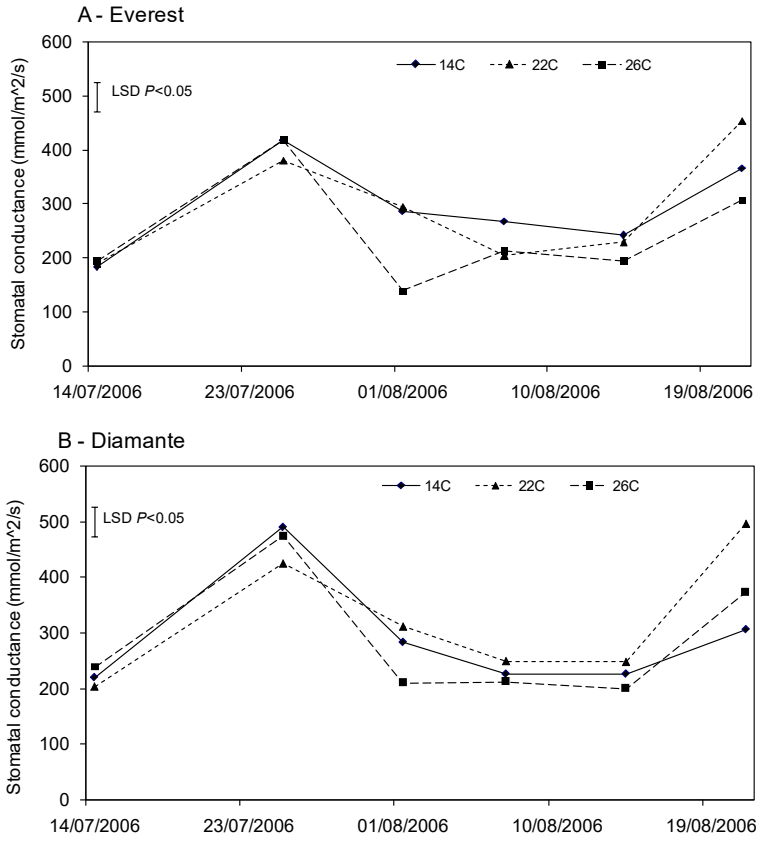


Figure 18: Mean stomatal conductance per plant against date across the season for the three temperature treatments. A – 'Everest', B – 'Diamante'. The LSD for the means for the temperature factor is 54.29 (*P*<0.05).

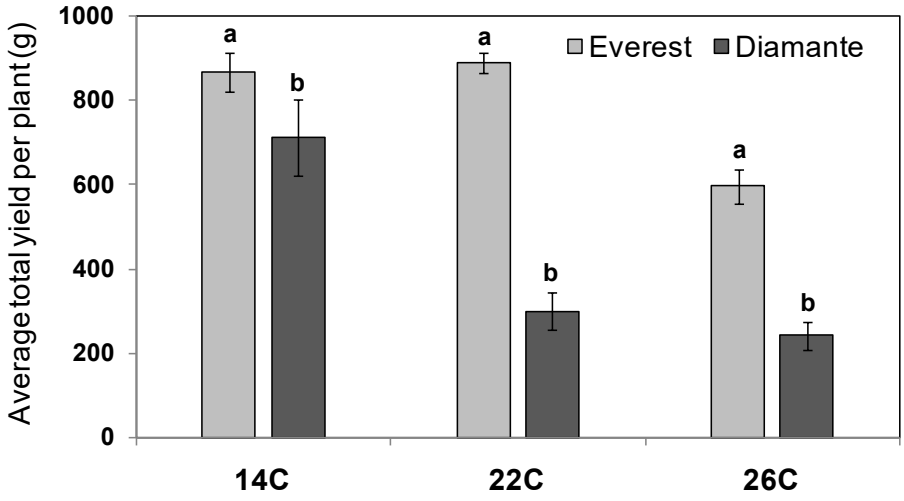
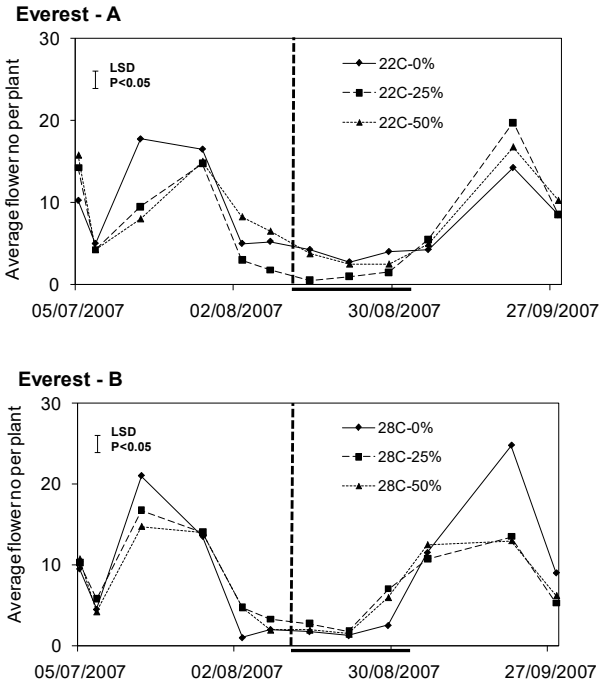


Figure 19: Average total yield (g) per plant for the three temperature treatments and the two varieties. The LSD of the means is 148 (*P*<0.05). Columns marked with different letters are significantly different within each temperature treatment (*P*<0.05). The vertical bars show the ±Standard Errors of the means (*P*<0.05).

3.3 The potentially ameliorating effect of crop load reduction during periods of high temperature on the severity of the thermodormancy response in two everbearing strawberry varieties.

Plants were exposed to either 22°C or 28°C for 20 days from 13/08/2007. At the same time, crop load treatments were applied with three levels of truss removal: 0%, 25% and 50%.

Crop load treatments had no significant effect on flowering patterns (based on flower number) (Fig. 20). Differences in flowering patterns between the two temperature treatments were also not significant. However, flowering patterns were significantly different between the two varieties with 'Everest' showing higher flower numbers at the start and end of the experiment (P<0.01). During treatment application, 'Everest' exhibited a significant dip in flower numbers in all treatments which lasted for four weeks with no more than 8 flowers per plant per week (compared to other periods with more than 15 flowers per plant per week). In 'Diamante', on the other hand, no significant dips in flowering were observed. However, towards the end of the season, the rates in the 50% truss removal treatment in the high temperature treatment were higher.



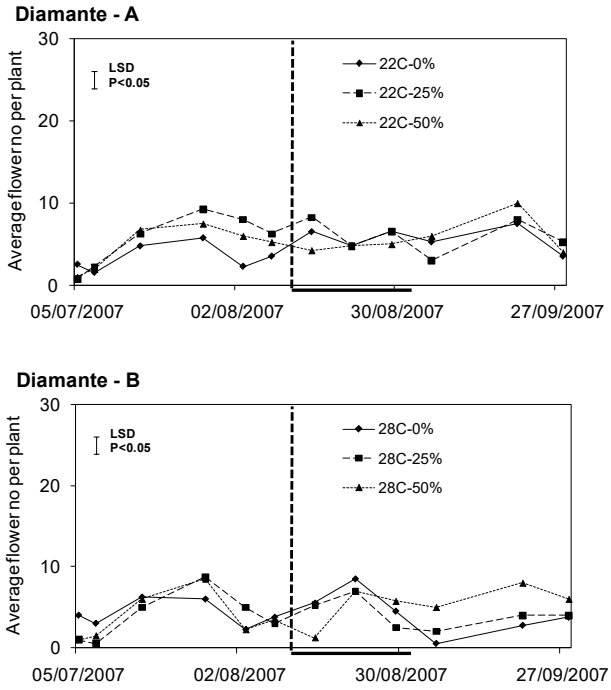


Figure 20: Weekly flower number per plant in 'Everest' and 'Diamante' as affected by 3 truss removal treatments (0%, 25% and 50%) under the two temperatures (22°C,and 28°C) across the season. The LSD of the means for the interaction of the three factors is 2.174 (*P*<0.05). The vertical dashed lines indicate the point at which trusses were removed. The thickened lines on the x axis indicate the temperature treatment application period.

Crop load treatments had no significant effect on subsequent cropping even though cropping rate would have been expected due to truss removal in the reduced crop load treatments. In 'Everest', the yield of the plants with an unaltered crop load was higher in both temperature treatments; however, towards the end of the season, in the control temperature (22°C) the two truss-removal treatments gave higher yield. In 'Diamante', in the high temperature treatment (28°C), plants that were treated with 50% truss removal gave higher yield towards the end of the experimental period (Fig. 21).

Temperature had a significant effect on fruit fresh weight in both varieties (*P*<0.05) following exposure to 28°C compared to the 22°C control. Variety choice had a significant effect on cropping (*P*<0.01). Both varieties produced the highest weekly yields in the 22°C treatment with 'Everest' averaging 35.4 g/plant/week and 'Diamante' averaging 25.1 g/plant/week (LSD=7.9, *P*<0.01).

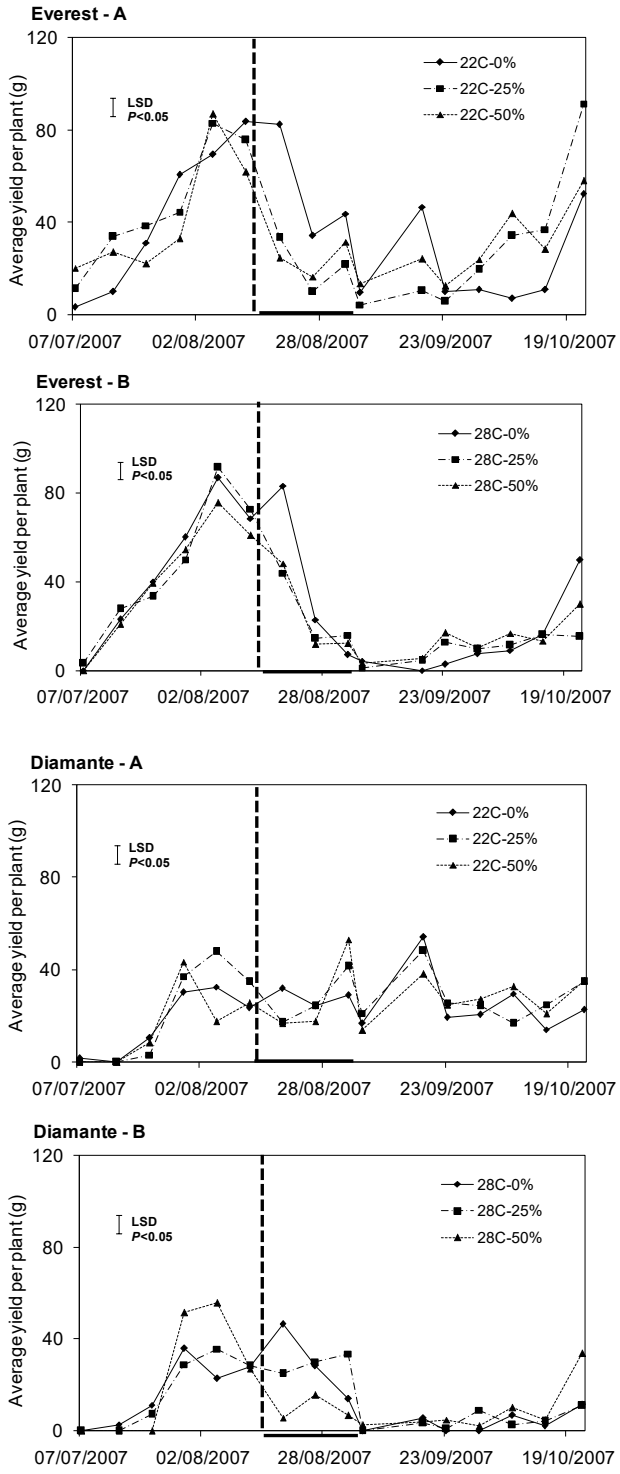


Figure 21: Cropping patterns expressed as mean weekly fruit fresh weight (g) per plant) in varieties 'Everest' and 'Diamante' as affected by 3 truss removal treatments (0%, 25% and 50%) under the two temperatures (22°C,and 28°C) across the season. The LSD of the means for the interaction of the three factors is 7.98 (P<0.05). The vertical dashed lines indicate the point at which trusses were removed. The thickened lines on the x axis indicate the temperature treatment application period.

Total yield per plant was significantly different between the two varieties and between the two temperatures (P<0.05) with 'Everest' giving higher yields than 'Diamante'. Both varieties produced higher total yields per plant at 22ºC than at 28ºC (Fig. 22). Truss removal had no

significant effect on total yield per plant as the yields in the thinning treatments were very similar.

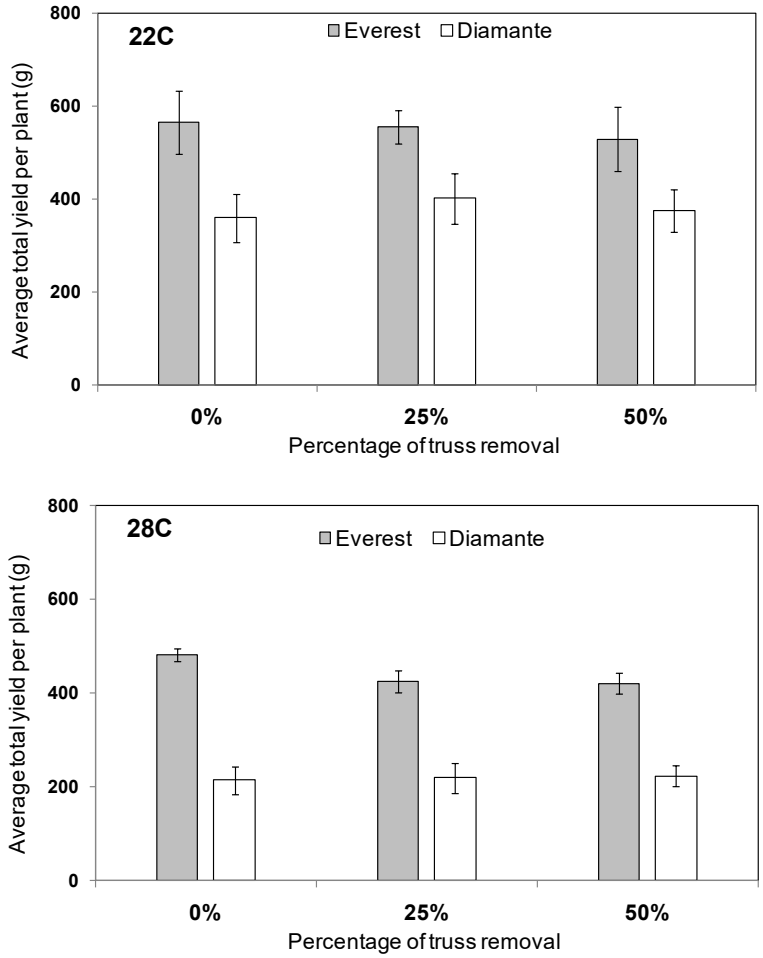


Figure 22: Average total yield (g) per plant for the three truss removal treatments, the two varieties and the two temperatures. The LSD of the means is 122.9 (P<0.05). The vertical bars show the ±standard errors of the means (P<0.05).

There were no significant pre-anthesis bud abortion rate differences across the season. Bud-to-flower conversions were above 75% in both varieties and both temperatures throughout the season (Table 4) apart from one week in Late August when some bud abortion was observed which coincided with a dip in flower numbers in 'Everest' (Fig. 20). In contrast, significant rates of post-anthesis flower abortion were observed over the season (measured as low flower-to-fruit conversion rates) (P<0.05) (Fig. 23), which is a typical symptom of thermodormancy. Following high temperature application, flower-to-fruit conversion rates in both varieties dropped below 50%. In 'Everest', in particular, a significant proportion of the flowers that bloomed during the mid-late September flowering peak, did not make it to fruits (Fig. 23 in comparison with Figs. 21 and 20). In addition there was also some post-anthesis abortion during the flowering peak in mid-late July (Fig. 23 in comparison with Figs. 21 and 20). This was observed in both temperatures and in all the truss removal treatments.

Table 4: Weekly bud-to-flower conversion percentages (%) in 'Everest' and 'Diamante' as affected by temperature.

	Everest		Diamante	
Date*	22°C	28°C	22°C	28°C
18.06.2007	100.00	75.00	100.00	-
26.06.2007	80.00	85.71	-	100.00
02.07.2007	82.86	88.89	85.71	82.35
06.07.2007	100.00	100.00	85.71	91.67
16.07.2007	88.00	87.04	94.12	93.75
26.07.2007	93.75	83.33	100.00	100.00
31.07.2007	100.00	100.00	100.00	100.00
06.08.2007	89.47	80.00	100.00	88.89
13.08.2007	93.33	100.00	96.43	100.00
20.08.2007	100.00	100.00	100.00	100.00
23.08.2007	62.50	00.00	61.54	50.00
29.08.2007	100.00	100.00	100.00	100.00
05.09.2007	100.00	100.00	100.00	100.00
20.09.2007	83.33	86.21	92.86	100.00
28.09.2007	100.00	100.00	100.00	100.00

* The dates given are those when buds were recorded. A bud was found to take 5 days on average to become a fully opened flower. The number of buds counted at each of the above dates was averaged and then the number of those which became fully opened flowers was also averaged and a percentage of conversion was calculated.

The plants replaced the trusses that were removed and the average number of trusses per plant by the end of the season (22/10/2007) was almost the same between plants of different truss removal treatments in both varieties and both temperatures (data not shown).

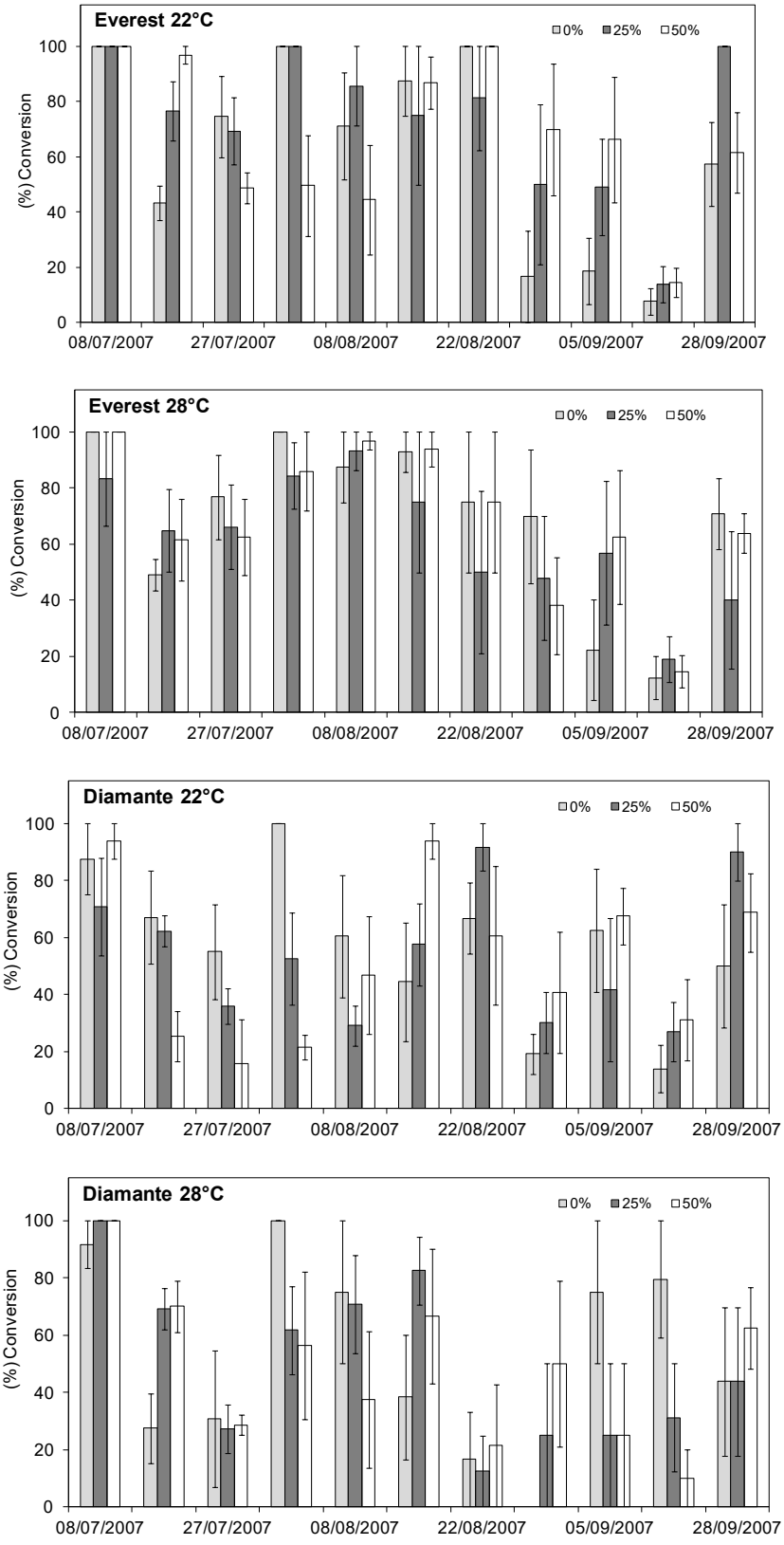


Figure 23: Average weekly flower-to-fruit conversion rates (%) per plant for the three truss removal treatments (0%, 25% and 50%) under the two temperatures (22°C, 28°C) for 'Everest' and 'Diamante' across the experimental period. The LSD of the means for the interaction of the three factors is 15.47 (P<0.05). The vertical bars show the ±standard errors of the means (P<0.05). Where no bars can be seen the values were 100%. The dates on the graphs are flowering dates.

3.4 Effects of high temperature on pollen performance of the everbearing strawberry varieties ‘Everest’ and ‘Diamante’.

These experiments investigated the effects of different day/night temperature regimes on pollen performance in terms of pollen viability, *in vitro* germination capacity and pollen tube growth. This was related to subsequent flowering and cropping patterns in two everbearing strawberry varieties. Two experimental replications were carried out during a seven-month period in 2007 and then a further experiment with slightly modified design conducted again in 2008.

2007 Experiments

Pollen measurements

Temperature and variety factors had a highly significant effect on pollen germination capacity ($P<0.01$). ‘Diamante’ was found to have different optimum germination rates than ’Everest’. Maximum *in vitro* germination rates recorded in ‘Diamante’ were higher than in ‘Everest’ in the optimum temperature treatment (control). In ‘Diamante’ it was 88.0% and in ‘Everest’ it was significantly lower with 72.5% ($P<0.01$) in experiment 1 and 82.3% and 45.2%, respectively ($P<0.01$) in experiment 2. Both varieties, however, showed a significant decrease in germination after the first 7 days of exposure to the high temperature treatment (30°C/19°C in experiment 1, 30°C/20°C in experiment 2) compared to the control plants (Fig. 24). As the high temperature event persisted, pollen germination rates continued to fall to 0% in both varieties in both experiments.

Pollen germination rates also decreased following exposure to the 26°C/11°C treatment, with ‘Everest’ being more responsive than ‘Diamante’. In experiment 1 ‘Everest’ germination rates fell to 35% and ‘Diamante’ fell to 50%. In experiment 2, ‘Everest’ germination rates fell to 13% whereas ‘Diamante’ was less responsive with the lowest value being 62%.

Pollen germination capacity in ‘Everest’ started to recover 20 days after the termination of the high temperature treatment in experiment 1 and after 7 days in experiment 2. Similarly, pollen germination capacity in ‘Diamante’ started to recover 14 days after the termination of the high temperature treatment in experiment 1 and after 26 days in experiment 2.

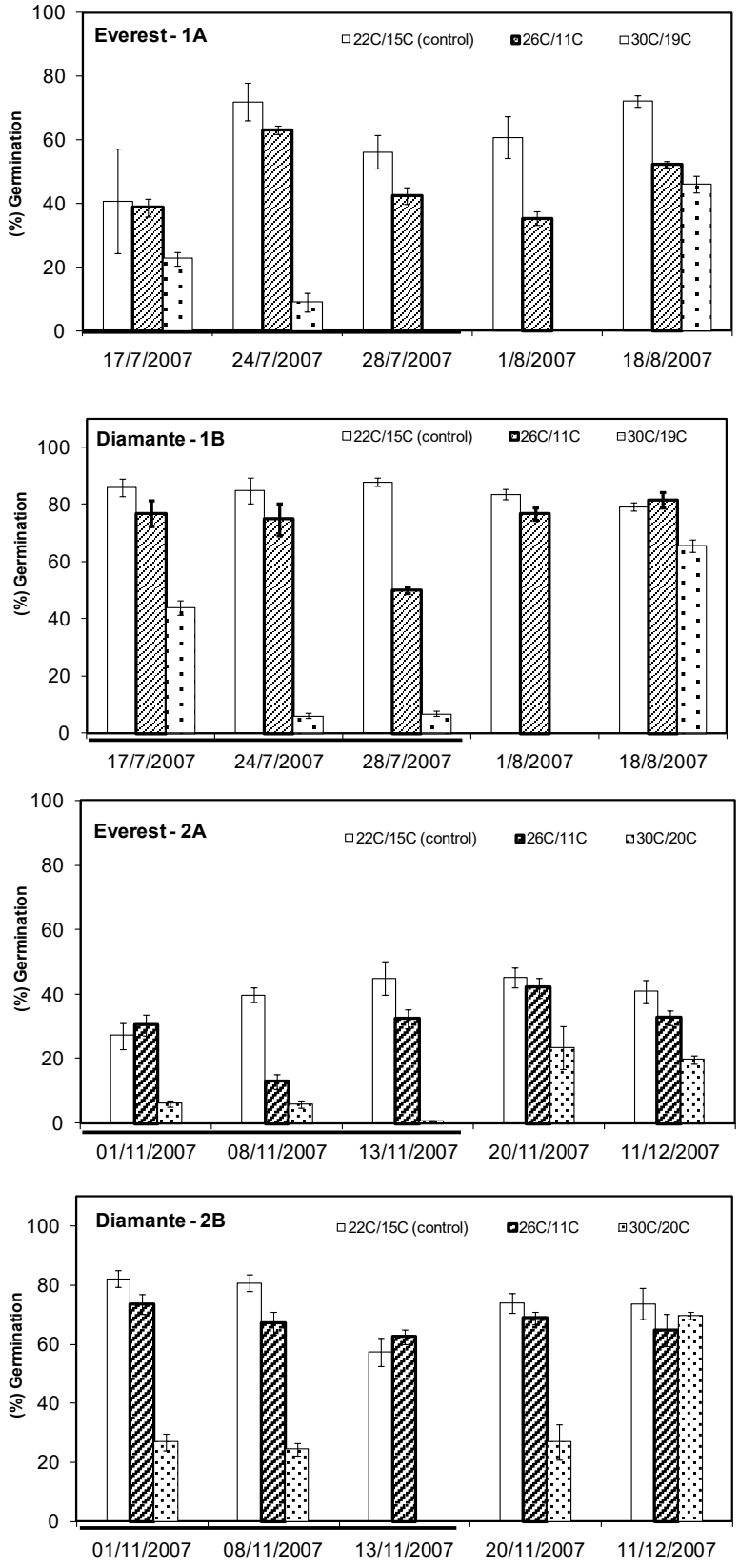


Figure 24: Average pollen germination rates for 'Everest' (A) and 'Diamante' (B) during and after three temperature regimes (22°C/15°C, 26°C/11°C and 30°C/19°C in experiment 1 and 30°C/20°C in experiment 2) for two experiments (1 and 2). Error bars show the standard errors of the means (P<0.05). The thickened lines on the x-axis indicate the treatment application period.

Choice of variety and temperature treatment significantly affected pollen viability (P<0.01) in experiment 1 with temperature showing a stronger effect on pollen viability than variety (Table 5). However, their interaction was not significant. 'Everest', pollen viability after the end of the high temperature event remained significantly lower than the control treatment, whereas in 'Diamante' it returned to normal levels towards the end of the experiment.

Table 5: Average pollen viability percentages in 'Everest' and 'Diamante' as affected by the three temperature treatments measured at fortnightly intervals across the season in experiment 1.

		Percentage of Pollen Viability (%)			
		Date			
Variety	Temperature	14/07/2007*	01/08/2007*	15/08/2007	28/08/2007
Everest	22°C/15°C	71.4 (4.35)	76.2 (4.94)	67.5 (3.23)	76.7 (2.69)
	26°C/11°C	81.8 (1.31)	74.7 (5.20)	55.0 (2.89)	71.0 (1.08)
	30°C/19°C	79.9 (1.82)	44.9 (3.36)	47.0 (3.39)	68.7 (1.31)
Diamante	22°C/15°C	86.4 (2.50)	91.5 (1.08)	90.9 (2.57)	81.0 (1.29)
	26°C/11°C	88.2 (2.56)	85.0 (2.36)	67.5 (2.84)	81.5 (2.53)
	30°C/19°C	76.6 (1.89)	48.9 (6.19)	66.2 (2.59)	81.5 (1.32)

The LSD (treatment*variety) of the means is 7.99 (P<0.05).
* These dates were during the high treatment period.
Numbers in parentheses are the Standard Errors of the means.

The length of the pollen tube, as measured on the fixed germinated samples, was found to be affected by variety as well as temperature treatments (P<0.05). Differences between dates of measurement, however, were not significant. In other words, the length of the pollen tube was different between the two varieties and it was also affected by temperature, but the rate of its growth did not change as the season progressed. As shown in Table 6 pollen tubes in 'Diamante' were longer than in 'Everest', and they were shorter in the high temperature treatment in both varieties during as well as after treatment application.

Flowering and cropping patterns

'Everest' showed higher flowering rates than 'Diamante' in both experiments (P<0.01) (Fig. 25). The high temperature event reduced subsequent flower numbers in 'Everest' in all temperatures two weeks after the termination of the treatments in both experiments. This effect was more marked in the 30°C/19°C and the 26°C/11°C treatments than in the control in the first experiment and in the 30°C/20°C treatment in the second. However, flowering in the second experiment recovered better in all treatments (P<0.05). In 'Diamante', on the other hand, flower numbers were only significantly reduced following high temperature

treatment in the second experiment (P<0.05) (although a trend could be seen in the first experiment for the 30ºC/19ºC treatment when fewer flowers were produced for two weeks following treatment application).

Table 6: Average pollen tube length (µm) per plant in 'Everest' and 'Diamante' as affected by the three temperature treatments measured before, during and after treatment application in experiment 2.

Variety	Temperature	Pollen tube length (µm)		
		5 days before treatment application	At 10 day exposure	5 days after treatment termination
Everest	22°C / 15°C	329.4 (26.2)ad	324.2 (27.6)ad	276.7 (43.1)ab
	26°C / 11°C	-	257.9 (19.1)ab	297.1 (49.2)ab
	30°C / 20°C	-	229.8 (15.9)be	215.8 (24.8)be
Diamante	22°C / 15°C	455.3 (28.3)c	403.8 (20.4)cd	529.2 (28.6)c
	26°C / 11°C	-	371.0 (21.7)d	366.7 (33.0)d
	30°C / 20°C	-	353.3 (32.7)ad	142.5 (19.1)e

Before and after treatment application all plants received 22°C/15°C day/night temperatures
Numbers in parentheses are the standard rrrors of the means. Values with the same letter are not significantly different from each other (P<0.05). The overall LSD (date*treatment*variety) of the means is 89.03 (P<0.05).

The temperature treatments had a significant effect on cropping patterns in both varieties (P<0.01) (Fig. 26). Fruit fresh weights were significantly reduced following high temperature treatments in both varieties in both experiments (30ºC/19ºC and 30ºC/20ºC respectively) (P<0.01). This effect was more pronounced in 'Everest' than in 'Diamante', but even 4 weeks after the end of the high temperature treatments, cropping was still low in both varieties in both experiments. The low cropping observed in both experiments correlated with the dates when pollen showed decreased germination capacity. Variety differences were also significant. 'Everest' produced, on average, three times the weekly yield per plant of 'Diamante' (41.3 g/plant/week, compared to 'Diamante' with 13.5 g/plant/week) in the first experiment and twice the weekly yield per plant in the second (33.2 g/plant/week, compared to 'Diamante' with 15 g/plant/week) (P<0.01). The higher fruit yields in 'Everest' were

correlated with its higher flowering rates. The highest fruit fresh weights (g) were found in the 26°C/11°C treatment in the first experiment, and in the 22°C/15°C treatment in the second experiment (Fig. 27).

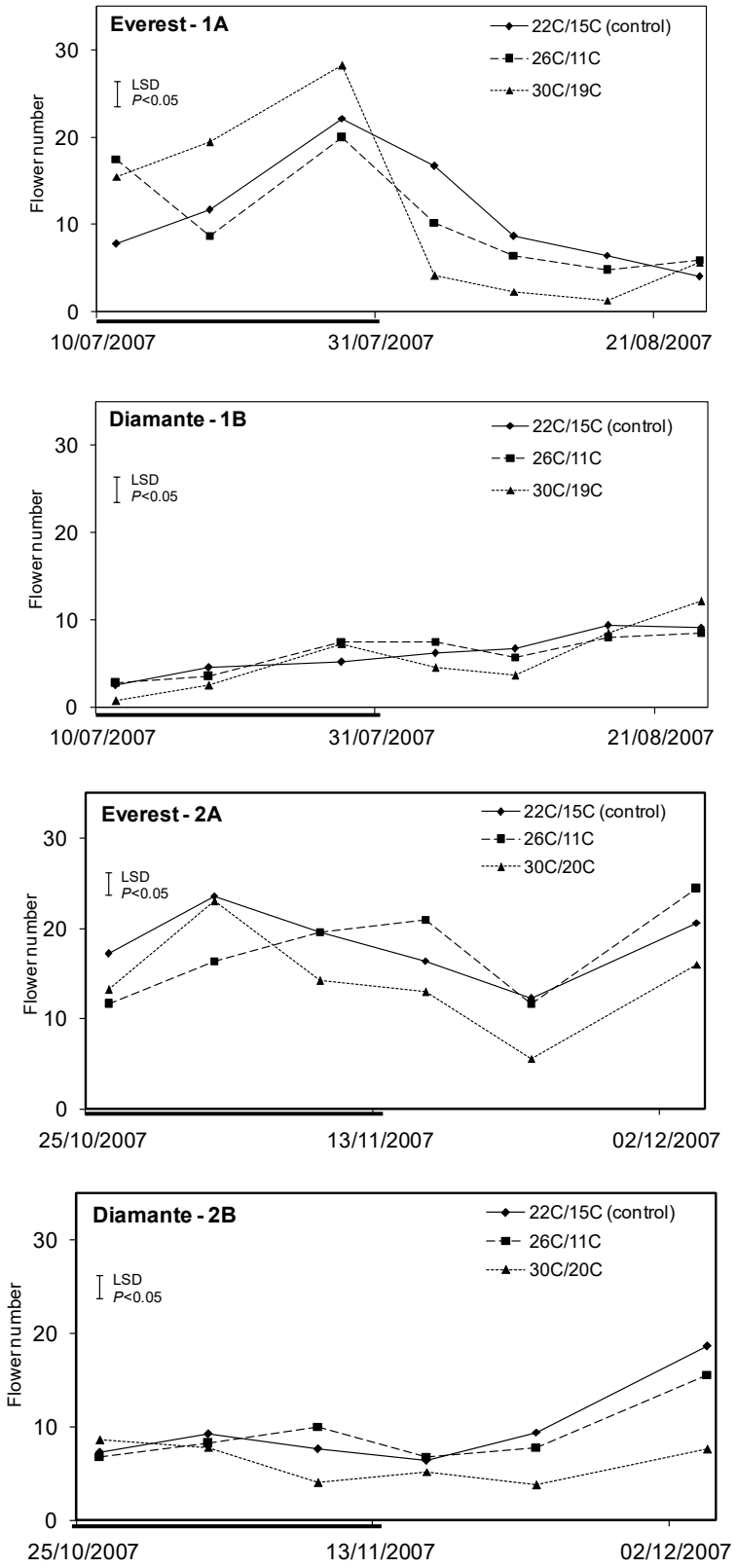


Figure 25: Flower numbers per plant over the season in 'Everest' (A) and 'Diamante' (B) as affected by the three temperature treatments in two separate experiments (1 and 2). The

LSD of the means is 2.9 for the first experiment and 2.55 for the second (P<0.05). The thickened lines on the x-axis show the treatment application period.

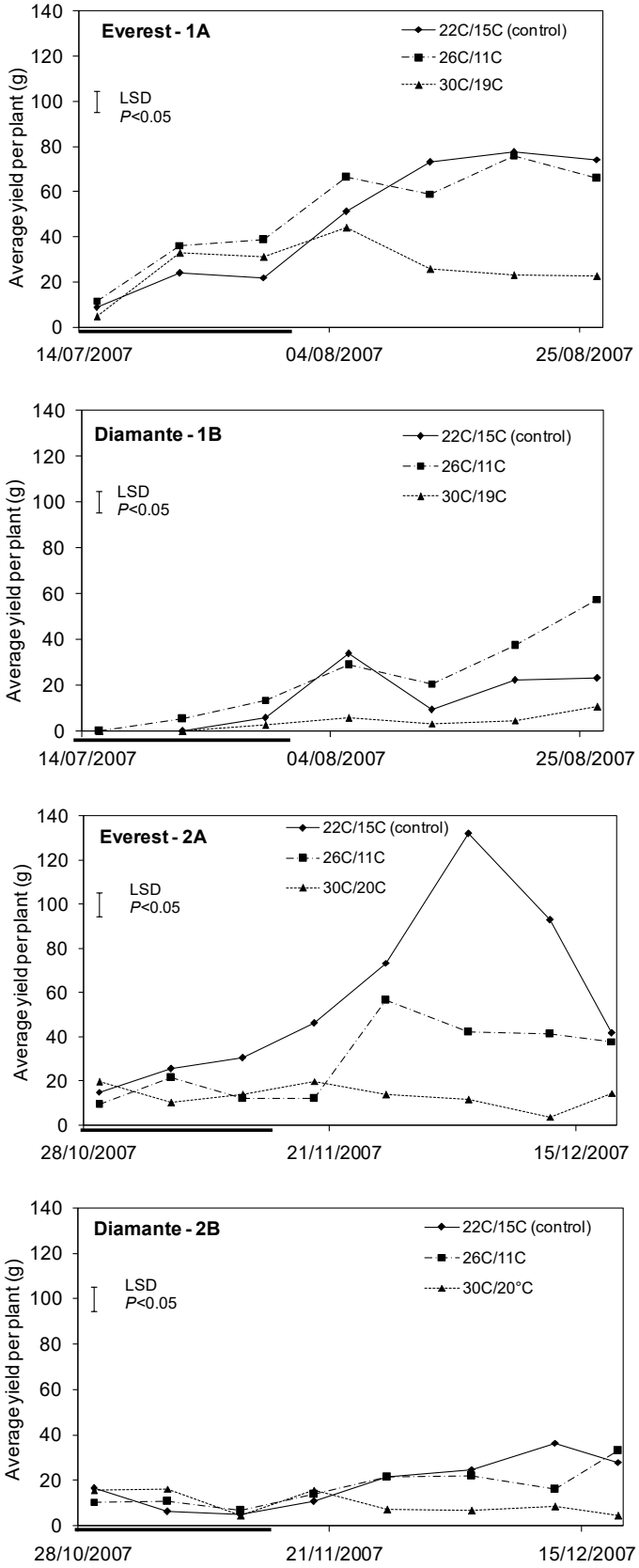


Figure 26: Cropping patterns expressed as mean weekly fruit fresh weight (g) per plant in 'Everest' (A) and 'Diamante' (B) as affected by the three temperature treatments and in two separate experiments (1 and 2). The overall LSD of the means is 9.34 for the first

experiment and 10.82 for the second (P<0.05). The thickened lines on the x-axis show the treatment application period.

Conversions of flower-to-fruit number were significantly reduced during high temperature application in both varieties in the first experiment, implying a typical thermodormancy response (Table 7). The flower-to-fruit conversion rates fell below 40% after 13 days of exposure to high temperature in both varieties which correlates with the reduced pollen germination capacity rates observed at the same time. This means that more than 60% of the flowers of both varieties that reached anthesis during high temperature application and therefore had low pollen germination capacity did not develop into fruits. However, in 'Everest', 5 days after the termination of the high temperature treatment 80% of the flowers that reached anthesis at that point did develop into fruits whereas in 'Diamante' only 25% of the flowers that reached anthesis at that same time point developed into fruits. In the second experiment low flower-to-fruit conversion rates were observed during high temperature application similarly to experiment 1 which recovered to higher levels after the termination of the high temperature event. Differences between temperature treatments were found, but variety differences were small.

Table 7: Average flower-to-fruit conversion proportions (%) per plant in 'Everest' and 'Diamante' as affected by the three temperature treatments in experiments 1 and 2. The treatment application periods were for experiment 1, 11 – 30/07/2007; and for experiment 2, 30/10 – 15/11/2007. Numbers in brackets are the ± standard errors of the means (P<0.05). The dates given correspond to flowering dates. A percentage of conversion was calculated from the number of flowers counted per plant at each of the dates compared to the number of subsequent fruits.

Experiment 1

	Everest			Diamante		
Date	22°C/15°C	26°C/11°C	30°C/19°C	22°C/15°C	26°C/11°C	30°C/19°C
03.07.2007	72.29 (11.97)	100.0 (0.00)	100.0 (0.00)	100.0 (0.00)	100.0 (0.00)	100.0 (0.00)
*11.07.2007	81.88 (7.32)	56.94 (10.25)	70.83 (18.16)	100.0 (0.00)	72.22 (27.78)	100.0 (0.00)
*18.07.2007	79.13 (6.85)	97.92 (2.08)	36.68 (6.11)	62.50 (19.09)	65.56 (16.14)	72.92 (17.80)
*28.07.2007	65.46 (10.22)	52.28 (4.57)	33.88 (8.77)	40.63 (12.01)	24.40 (15.44)	22.92 (14.80)
04.08.2007	81.94 (9.45)	97.09 (2.94)	80.00 (20.0)	36.46 (8.90)	53.87 (19.38)	25.00 (2.50)

Experiment 2

Date	Everest			Diamante		
	22°C/15°C	26°C/11°C	30°C/20°C	22°C/15°C	26°C/11°C	30°C/20°C
*02.11.2007	68.94 (11.21)	59.89 (8.33)	30.11 (13.00)	35.16 (17.19)	36.16 (12.72)	66.97 (15.57)
*09.11.2007	100.0 (0.00)	48.28 (13.71)	62.38 (11.28)	73.33 (13.79)	27.86 (14.91)	75.00 (25.00)
16.11.2007	98.57 (1.43)	67.23 (10.23)	12.22 (7.54)	97.14 (2.86)	70.00 (13.33)	87.50 (12.50)
23.11.2007	95.00 (3.06)	98.33 (1.67)	92.50 (7.50)	33.50 (15.32)	77.48 (7.08)	47.50 (22.50)

*The measurements at these dates occurred during treatment application.

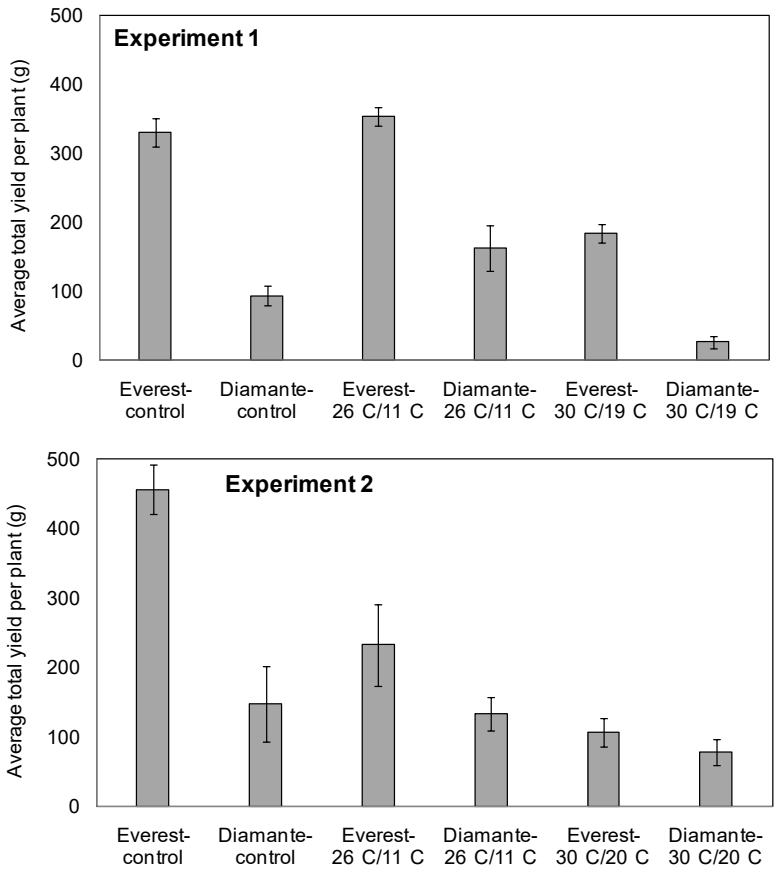


Figure 27: Average total yield (g) per plant for all six treatments in both experiments. The vertical bars show the standard errors of the means (P<0.05).

2008 Experiments

Pollen measurements

Pollen germination rates in both varieties began to decrease within four days of the start of the high temperature episode (30°C/20°C D/N) and continued so that, after a period of two weeks, germination rates were less than 15% for 'Everest' and less than 35% for 'Diamante' (Fig. 28). This decline continued so that no pollen germination was detected in 'Everest' and only 6.5% germination was recorded in 'Diamante' after 24 days of high temperature conditions (20 days under 30°C/20°C + 4 days under 35°C/20°C D/N). Meanwhile, germination rates of pollen from the control plants continued at high levels. In common with the results of the 2007 experiments optimal *in vitro* germination rates recorded in '2008 for Diamante' were significantly higher than in 'Everest'.

Again, in agreement with the 2007 experiments pollen germination rates recovered rapidly following the end of the high temperature episode so that germination rate in 'Everest' increased from 0% to 28% and increased in 'Diamante' from 6.5% to 78% (P<0.05) in 26 days?

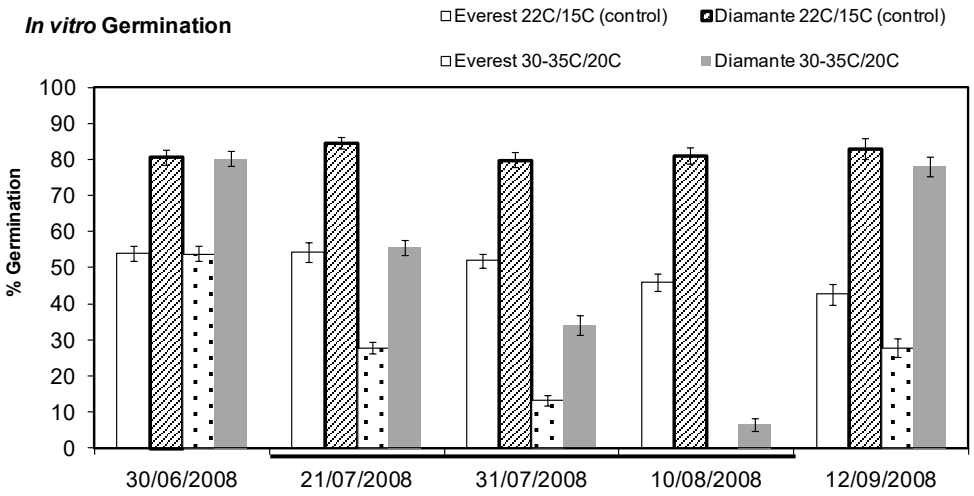


Figure 28: Average *in vitro* pollen germination (expressed in percentages) per plant for the two temperature treatments (22°C/15°C and 30°C-35°C/20°C) for 'Everest' and 'Diamante' before, during and after treatment application. The vertical bars show the ±Standard Errors of the means (P<0.05). The thickened line on the x-axis indicates the treatment application period.

No significant decrease was observed in pollen viability as a result of high temperature and the rates were not significantly different between the two varieties. Average pollen viability per plant was consistently above 60% in both varieties throughout (data not shown).

Pollen produced significantly shorter pollen tubes during the high temperature episode in both varieties (P<0.05) (Fig. 29). However, 26 days (graph suggests more than 26 days between measurements) after the termination of the high temperature event, pollen tube length from the high temperature treated plants had returned to the same length as that of control plants in both varieties. Pollen tubes length was generally greater in 'Diamante' than in 'Everest'.

Flowering and cropping patterns

The high temperature episode significantly reducing flower numbers in both varieties compared to the control plants (not subjected to the high temperature event) (P<0.05) (Fig. 30). Flowering subsequently recovered in both varieties towards the end of the experiment following the termination of the high temperature treatment. As in previous experiments, 'Everest' produced a greater number of flowers than 'Diamante' (P<0.05).

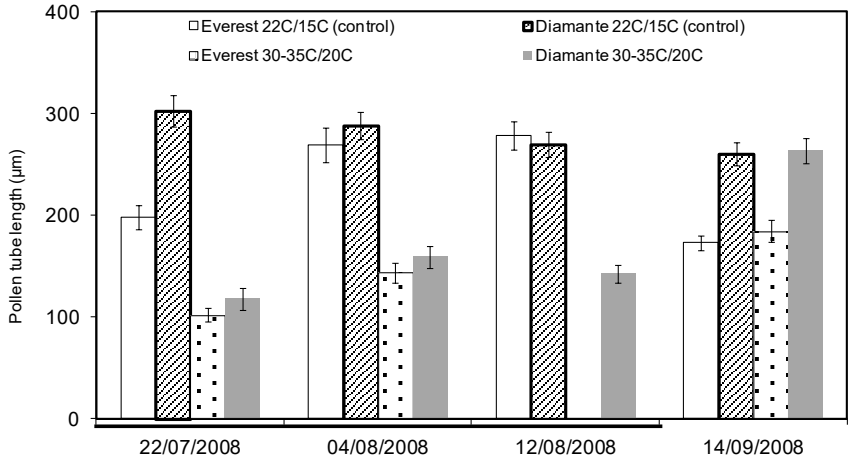


Figure 29: Average pollen tube length (µm) per plant for 'Everest' and 'Diamante' as affected by the two temperature treatments (22°C/15°C and 30°C-35°C/20°C) measured during and after treatment application. The vertical bars show the standard errors of the means (P<0.05). The thickened line on the x-axis indicates the treatment application period.

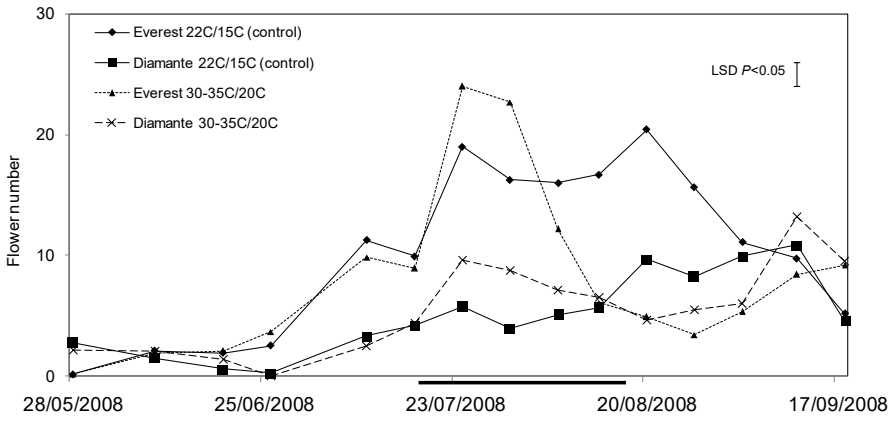


Figure 30: Average flower numbers per plant over the experimental period in 'Everest' and 'Diamante' as affected by the two temperature treatments (22°C/15°C and 30°C-35°C/20°C). The LSD of the means (treatment*variety) is 2.017 (P<0.05). The thickened line on the x-axis indicates the treatment application period.

Yield per plant was generally lower in 'Diamante' compared to 'Everest', confirming yield responses recorded in previous experiments. Following the high temperature episode, weekly fruit yield per plant declined significantly in both varieties resulting in very low yields in September compared to the control plants (Fig. 31). In agreement with the 2007 experiments, flowers produced immediately prior to the observed low cropping period in September had decreased pollen germination rates.

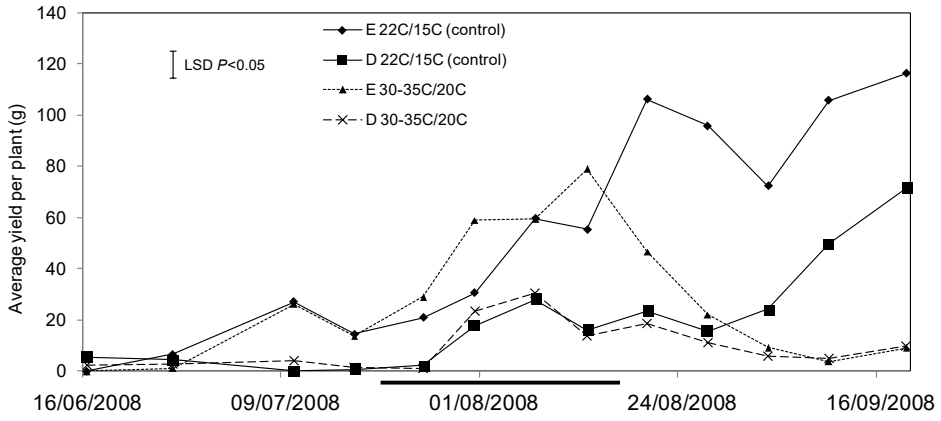


Figure 31: Cropping patterns expressed as mean weekly fruit fresh weight (g) per plant in 'Everest' and 'Diamante' under two temperature treatments (22°C/15°C and 30°C-35°C/20°C). The LSD of the means is 10.68 (P<0.05). The thickened line on the x-axis indicates the high temperature event.

Flower-to-fruit conversions rates declined significantly during the high temperature event in both varieties (P<0.05). The decline was evident 10 days after the beginning of the high temperature episode. This corresponded to the observed reduction in pollen germination and confirms observations made during the 2007 experiments. Flower-to-fruit conversion rates recovered during early September in both varieties (Fig. 32).

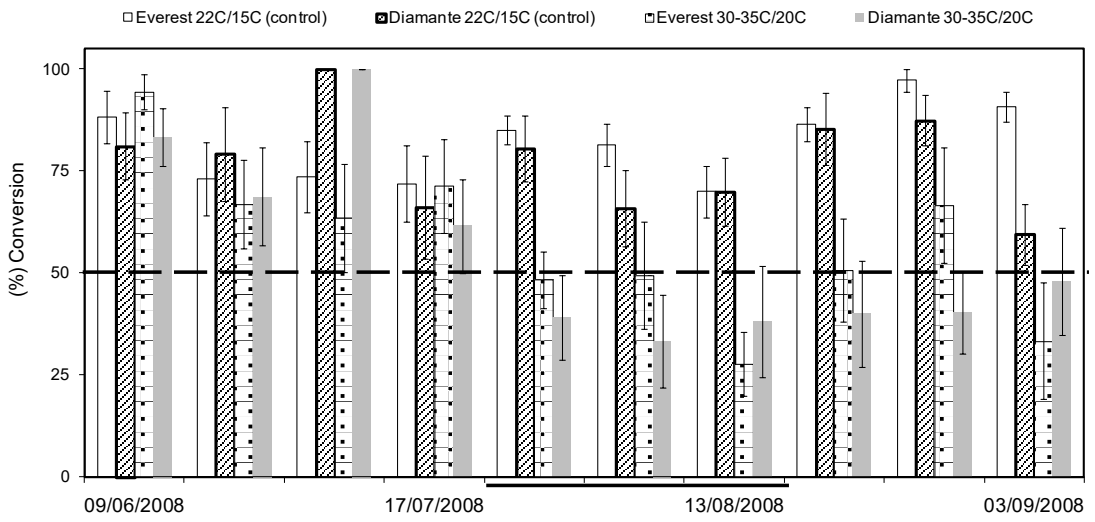


Figure 32: Mean flower-to-fruit conversion percentages in 'Everest' and 'Diamante' under two temperature treatments (22°C/15°C and 30°C-35°C/20°C). The thickened line on the x-axis indicates the high temperature event. The vertical bars show the Standard Errors of the means (P<0.05). The dates given correspond to flowering dates.

The high temperature event reduced overall yields in both varieties by approximately 50% (P<0.05) (Fig. 33). As observed in earlier experiments, yield of 'Everest' was significantly higher than that in 'Diamante'. Mean yields of 'Everest' under control temperature conditions was approximately 700g compared with only 300g for 'Diamante'.

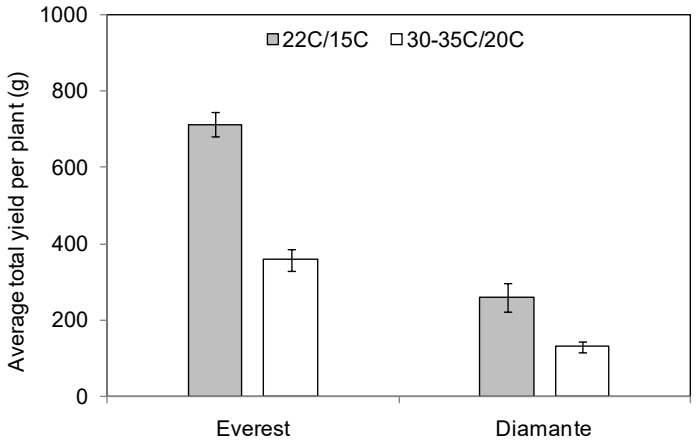


Figure 33: Mean total yield (g) per plant for the two temperature treatments in 'Everest' and 'Diamante'. The vertical bars show the ±Standard Errors of the means (P<0.05).

3.4 Evaluation of the potential of different heat control films (tunnel cladding materials) to ameliorate high temperature induced reductions in flowering and cropping of the everbearing strawberry varieties 'Everest' and 'Albion'.

The main flowering peak was observed from mid-late July until late August in all film treatments in both varieties (Fig. 34). 'Everest' plants generally produced higher flower numbers than 'Albion' (P<0.05). Plants of both varieties growing under the two clear film treatments had the highest flower numbers throughout (P<0.05). 'Everest' plants growing under the standard Luminance THB film produced the same flower numbers as under the clear film.

Cropping reached a peak during mid August and through September in both varieties (Fig. 35). 'Everest' produced almost double the fruit fresh weight per plant than 'Albion' (P<0.05). Cropping in 'Everest' was higher under the three non-shading films (2 Controls + Luminance THB) through August (P<0.05). However, through September plants growing under the two films with shading (Luminance THB25, Luminance THB33) produced higher yield levels per plant (P<0.05).

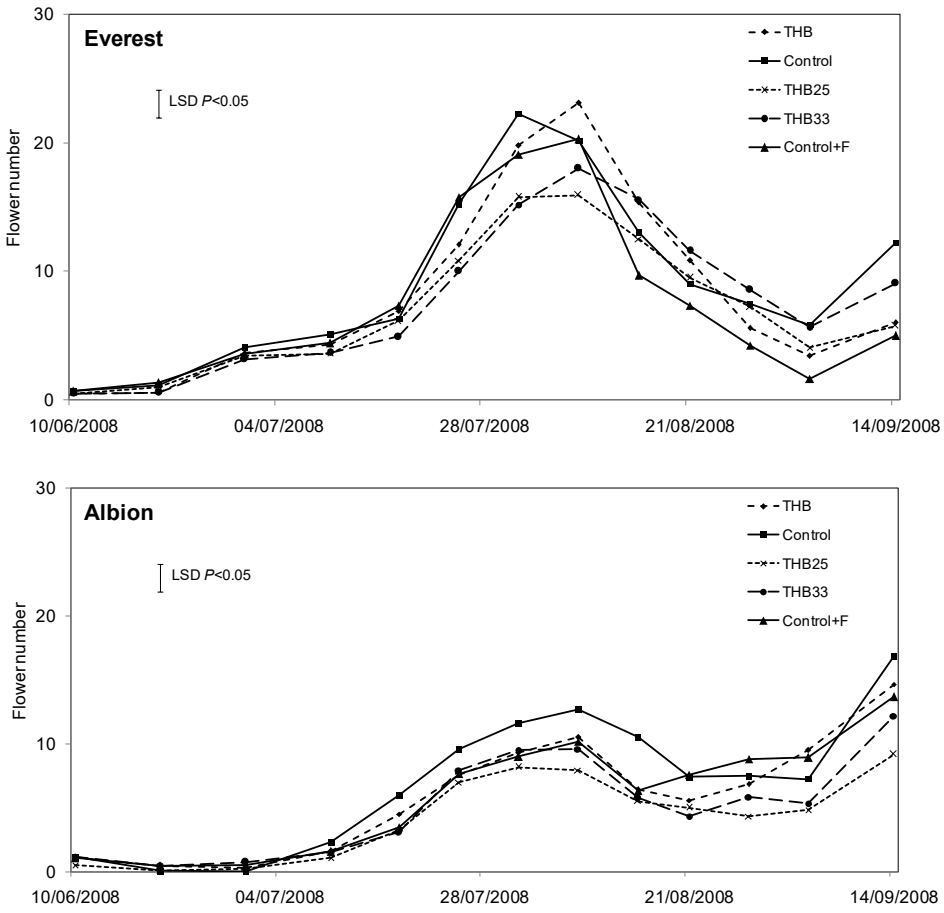


Figure 34: Average flower numbers per plant over the experimental season in 'Everest' and 'Albion' as affected by the five film treatments (Control = Clear film, Control+F = Clear + fans, THB = Luminance THB, THB25 = Luminance THB+25% shade and THB33 = Luminance THB+33% shade). The LSD of the means is 2.134 (P<0.05).

Flower-to-fruit conversions rates were consistently above 50% throughout the season in both varieties (Fig. 36). There was some flower abortion observed in 'Albion' during August but fruit set did not drop below 50%.

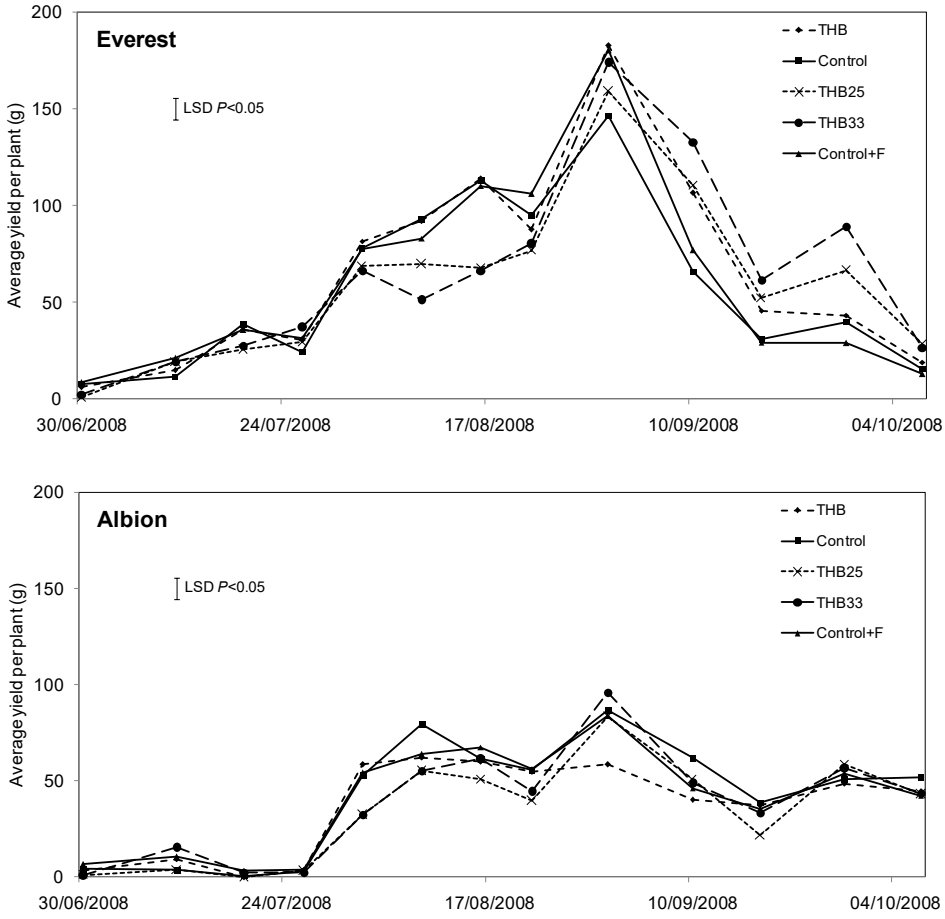


Figure 36: Cropping patterns expressed as mean weekly fruit fresh weight (g) per plant across the experimental season in 'Everest' and 'Albion' as affected by the five film treatments (Control = Clear film, Control+F = Clear + fans, THB = Luminance THB, THB25 = Luminance THB+25% shade and THB33 = Luminance THB+33% shade). The LSD of the means is 11.206 (P<0.05).

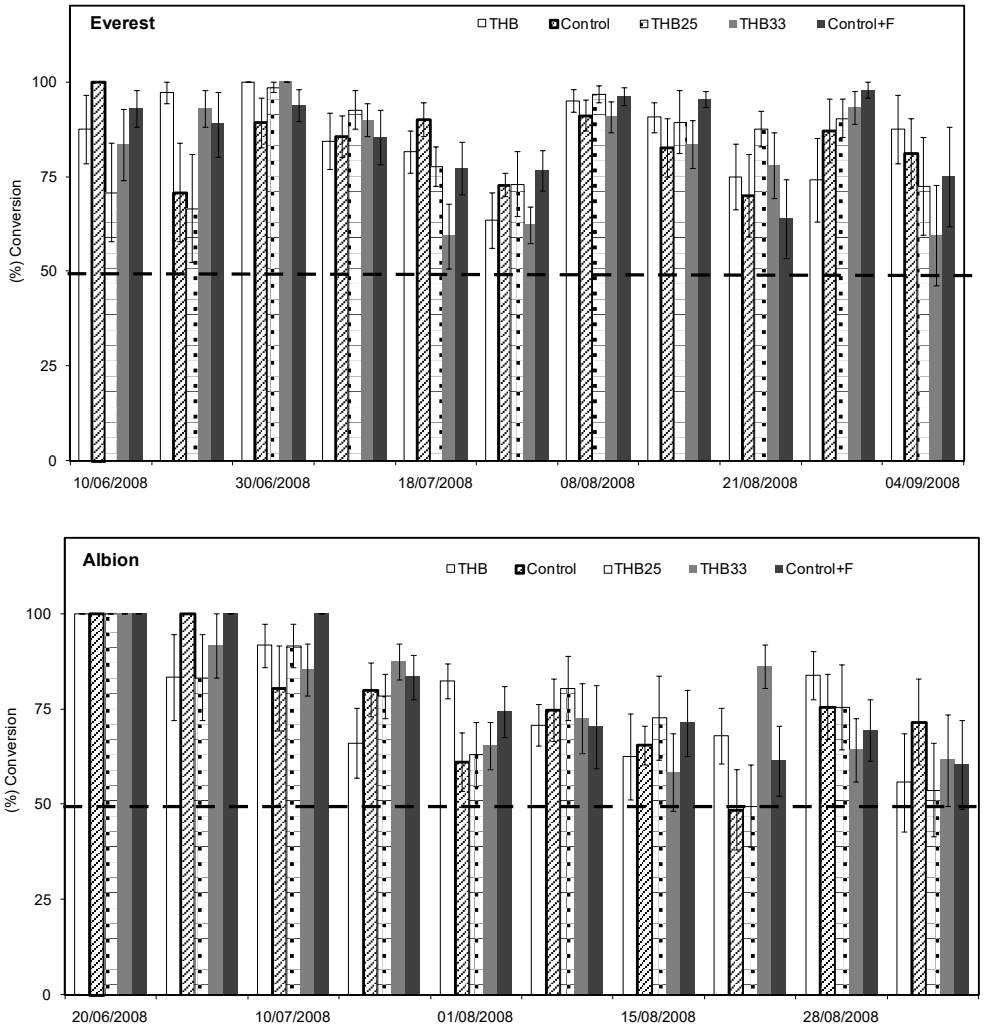


Figure 36: Average flower-to-fruit conversion proportions (%) per plant in 'Everest' and 'Albion' as affected by the five film treatments (Control = Clear film, Control+F = Clear + fans, THB = Luminance THB, THB25 = Luminance THB+25% shade and THB33 = Luminance THB+33% shade). The vertical bars show the standard errors of the means (P<0.05). The dates given correspond to flowering dates.

Average total yield per plant was only significantly affected by variety, with 'Everest' plants having higher values than 'Albion' in all plastic film treatments (P<0.05) (Fig. 37).

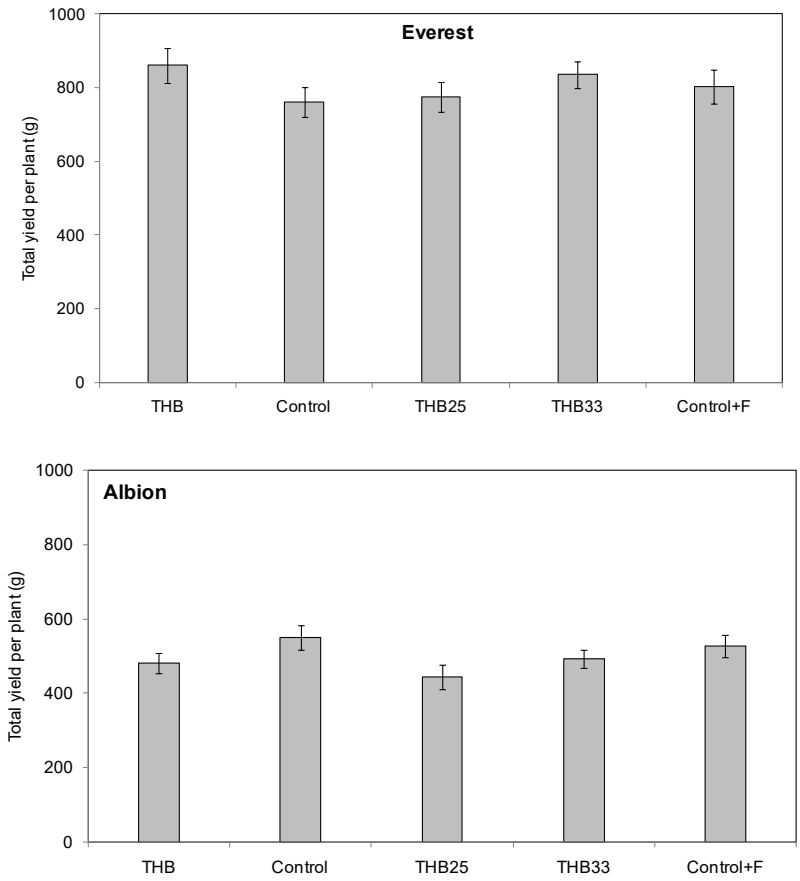
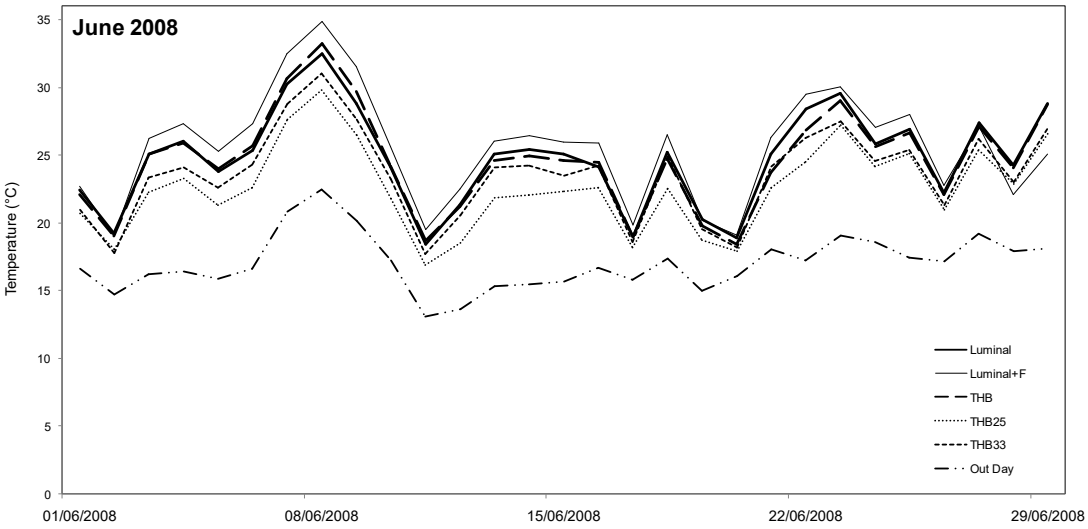
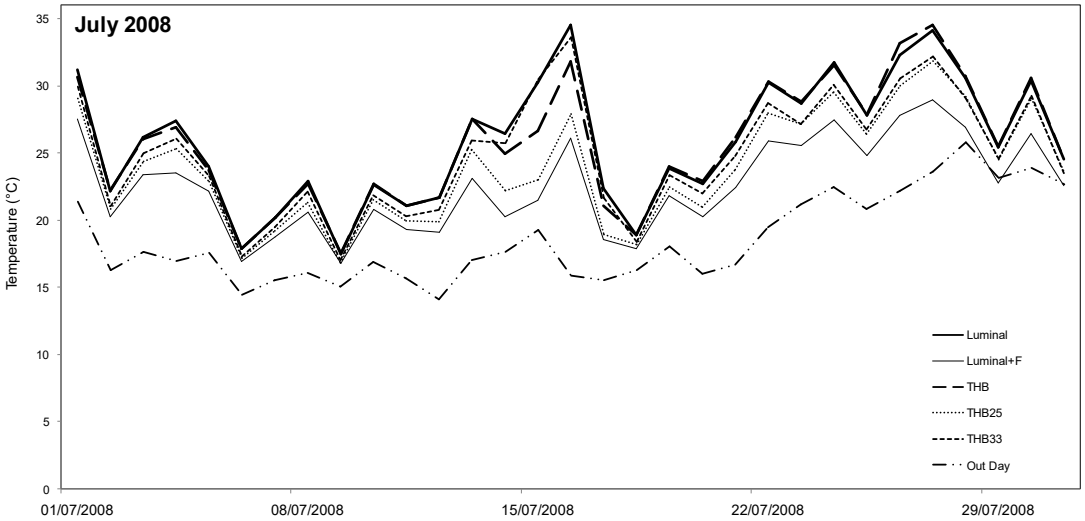
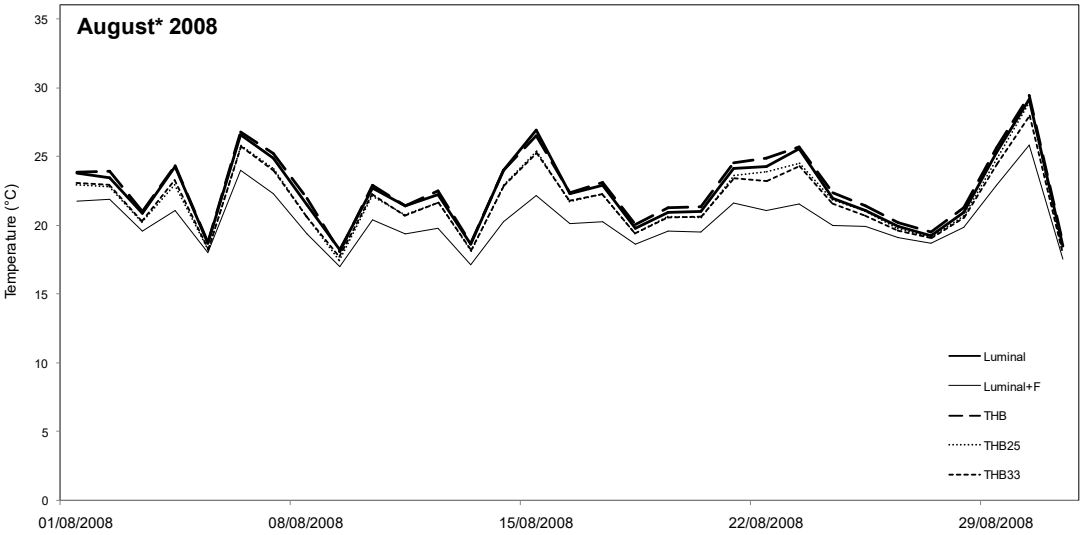


Figure 37: Average total yield (g) per plant for the five film treatments (Control = Clear film, Control+F = Clear + fans, THB = Luminance THB, THB25 = Luminance THB+25% shade and THB33 = Luminance THB+33% shade) in 'Everest' and 'Albion'. The vertical bars show the ±Standard Errors of the means (P<0.05).

The incorporation of the shading element into the modified Luminance THB films reduced the average air temperature inside the covered tunnels. Average air temperature was higher under the films without any internal shading throughout the experimental season (Figure 39). However, the application of forced air circulation (fitted fans) in two of the four tunnels with clear films also reduced the average air temperature in the tunnel: after the 1st of July 2008 (fans put into use) the tunnels with the fans showed the lowest temperature values.
Therefore in the June 2008 graph I find it difficult to justify including the + fan treatment in the graph when the fans were not on!







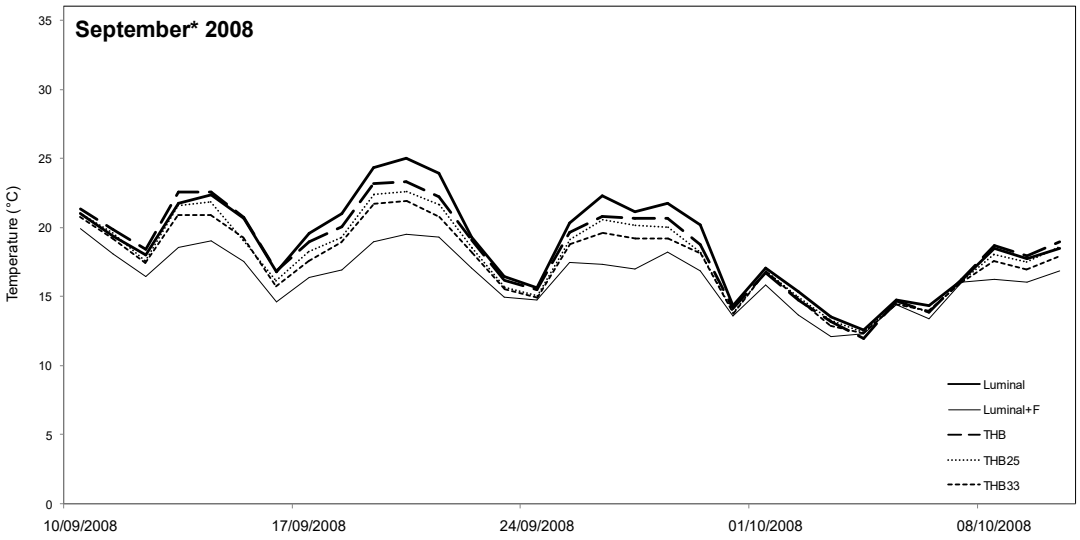


Figure 38: Average daily temperatures (°C) (summarized from 2-minutely readings during daytime) for every day across the experimental season (June-July-August and 10 September – 10 October) inside the polytunnels plus the outside temperature (averaged in the same way) for June and July. The five film treatments (Control = Clear film, Control+F = Clear + fans, THB = Luminance THB, THB25 = Luminance THB+25% shade and THB33 = Luminance THB+33% shade) were summarized from both experimental blocks combined.
* Due to technical constraints it was not possible to have an accurate estimate of the outside temperature for the months August and September.

4. Discussion

During this 3 year project four main areas of experimental work were studied. First, experiments were designed to investigate the interaction of high temperature with other parameters of the growing environment, specifically EC of the feeding solution and varying levels of relative humidity. The possibility that manipulating either of these two parameters could ameliorate the negative impact of high temperature was investigated by monitoring flowering, cropping and plant growth and stress status. In a separate experiment transpiration was assessed by monitoring stomatal conductance.

The three feed-level (EC level of the feeding solution) treatments applied during high temperature treatment in July had no significant effect on the flowering response. Yield patterns, however, were affected and a trend was observed for higher average weekly yield in the low-feed treatment in both 'Flamenco' and 'Diamante' and a higher total yield in 'Everest' in the high-feed treatment. A lack of any clear cut response could be attributed to the plants' capacity for osmotic adjustment to compensate for the change in salt level in the feed (Jones, 1992). It also suggests a need to use a wider range of electrical conductivities in the feed solution to establish a physiological response. For example, photosynthesis levels in potted apple were found to be little affected by 14 days of water stress, when moderate stress levels were applied, but were strongly affected in a severe stress treatment (Jones, 1992).

'Everest' plants at 22ºC were found to have significantly higher yields than those at 26ºC irrespective of vapour pressure deficit treatments, which were given by varying relative humidity levels in controlled environment cabinets. A trend could be observed, however, of increased yields in August when plants had been exposed to high vapour pressure deficits at both 22ºC and 26ºC for most of July. In non heat-stressed strawberry plants, Lieten (2002) showed that high humidity levels in the growing environment stimulated vegetative growth and fruit size, but a significant response usually required long term exposure to altered relative humidity levels. In the current work, in comparison, periods of exposure were relatively short (23 days). Variety differences were also found, demonstrating the relevance of genetic background. Experiment 3, for example, showed 22ºC to be the temperature with highest transpiration levels in both 'Everest' and 'Diamante', which was in agreement with optimum yield production in 'Everest' only. 'Diamante' produced its highest yields at 14ºC.

In the second area of experimental work, the impact of reducing crop load during periods of high temperature and thermodormancy was studied. Whilst the results of this experiment did not suggest crop load reduction could have a beneficial impact on thermodormancy reduction, experiments in the second and third year on the third main area, pollen performance, were very promising. Pollen performance, measured in terms of germination

rate, pollen viability and pollen tube growth, was significantly reduced in both ‘Everest’ and ‘Diamante’ in response to high temperatures. Therefore, low cropping levels and poor fruit set observed in the current study can be attributed, at least partly, to a reduction in pollen performance. Cropping dips following exposure to 30ºC/19ºC were more pronounced in ‘Everest’ than in ‘Diamante’. These findings are in agreement with previous work conducted on ‘Everest’, where significant dips in cropping were found following exposure to high temperature and were correlated with high flower abortion rates post-anthesis (Wagstaffe and Battey, 2006 a + b).

Pollen germination declined to zero in both strawberry varieties following high day/night temperature application in 2007. Similarly in 2008, pollen germination declined to zero in ‘Everest’ and to 6.5% in ‘Diamante’ following a high temperature episode. Similar declines in strawberry pollen germination in response to high temperature have been noted in recent work where germination rates fell to very low levels (< 20%) (Ledesma and Sugiyama, 2005; Koyuncu, 2006; Leech et al., 2002). Pollen performance in strawberry also appears generally to be variety dependent (Ledesma and Sugiyama, 2005; Koyuncu, 2006; Hortynski and Żebrowska, 1991).

In all experiments, both varieties exhibited a strong capacity to recover pollen germination rates after the termination of high temperature events. This implies that, provided the high temperature event is not prolonged, no permanent damage to the plant’s pollen system is caused. This may also be due to the perpetual flowering habit of these varieties (Durner et al., 1984; Hancock 1999). Thus, if flower buds develop when the plant is not under thermal stress, then the flowers produce viable pollen. In addition, under high day temperature conditions where night temperature was low (for example, 26°C/11°C D/N), the lower night temperature appeared to ameliorate the negative impact of high temperature effect on pollen performance as the germination rates did not decline to zero in any of the varieties. This finding is in agreement with previous work on ‘Everest’ where cool night temperatures had an ameliorating effect on the severity of the thermodormancy response (Wagstaffe and Battey, 2006 a + b). Thus, even though both 26°C/11°C and 30-35°C/19-20°C temperature regimes had a negative effect on pollen germination rate, only the highest temperature treatment had a significant negative effect on yield.

On a return to lower temperature conditions, pollen performance increased rapidly, but this recovery appeared to be variety dependant. In the 2007 experiments in ‘Everest’ most flowers that opened within 5 days after the termination of the high temperature treatment completed fruit set. This implies a strong capacity of ‘Everest’ plants to recover flower quality. However, in ‘Diamante’ few flowers emerging at the same time completed fruit set and flower quality improved more slowly.

The reduction in pollen performance therefore appears to be a major contributory factor to flower abortion and low fruit set and thus it probably forms a significant part of the thermodormancy response. Nonetheless, reduced pollen performance may not the only cause of the lower yield in thermodormant plants. Other studies in Japanese everbearers suggest that high temperature may reduce rates of flower initiation (Oda and Yanagi, 1993; Yanagi and Oda, 1990, 1992 and 1993; Kumakura et al., 2005). In the current study, reduced flower numbers per week in both 'Everest' and 'Diamante' following high temperatures could have been due either to reduced flower initiation, high rates of pre-anthesis flower abortion, or to both factors. Previous studies did not, however, show any clear effect of high temperature on flower initiation in 'Everest' (Wagstaffe and Battey, 2006b).

The key role of pollen performance within the thermodormancy response implies that crop husbandry techniques that aid in temperature reduction, such as ventilation and, potentially heat control cladding materials, may be of value in reducing thermodormancy in everbearing strawberry. Therefore, the fourth major experimental programme concerned the potential of heat control films to reduce high temperature impacts on everbearer strawberry production.

Because of the relatively cool outside temperatures over the summer of 2008 in the UK, no significant thermodormancy induction was evident in any treatment. The flower-to-fruit conversion data confirm this. There was some flower abortion observed in variety 'Albion' in August but the levels were not representative of a typical thermodormancy response.

Plants growing under the shaded films produced lower flower numbers. Cropping in 'Everest' was also significantly lower during August under the two diffusive films with internal shading but through September average plant weekly yield under these films was significantly higher. This observation has implications for late season fruit production in this variety. In 'Albion' on the other hand, differences in cropping patterns between the film treatments were not apparent. Differences in flowering, cropping and plant growth between the clear films with and without forced air circulation were also not so marked.

Although outside temperature conditions were not high enough to induce thermodormancy conditions, internal shading incorporated into the plastic film reduced the temperature inside the tunnels compared to the control. Moreover, mean air temperature in the tunnels with forced air circulation was lower than in the tunnels covered with the shading plastic films. This suggests that both approaches could be beneficial in reducing thermodormancy in the event of high outside temperature conditions.

5. Overall Conclusions

The work in this project aimed to shed light on the physiological basis of thermodormancy to enable the development of practical methods to alleviate its severity on a commercial scale. Two physiological components have been revealed by the research: first, the potential importance of pollen performance. In flowers which bloom under high temperatures, pollen has reduced germination capacity. This is likely to be a contributory factor to the observed post-anthesis flower abortion, which means that significant proportions of flowers do not set fruit. As a result, the final crop does not reflect the number of flowers produced by the plant and there is a high temperature-induced cropping trough.

The second physiological component is the reduced number of flowers. This reduction, though not clear-cut, has been inferred from the data from all 3 years of the project and could be due to reduced flower initiation following the high temperature event. There is some evidence from other work that high temperature may reduce flower initiation in everbearing strawberries, but the picture is still far from clear, and effects may be variety-dependent.

As far as practical methods are concerned, the interaction of other environmental factors with temperature was not found to alleviate the adverse effects of high temperature on cropping. The factors studied were vapour pressure deficit in the growth environment, and osmotic potential of the substrate (EC levels of the feed solution). Although indications were found of temperature effects on photosynthetic efficiency and patterns of stomatal opening, no clear connection could be made to the symptoms of thermodormancy. Crop load reduction (i.e. truss removal) was also studied as a potential means of alleviating high-temperature induced cropping troughs, but the cropping patterns of treated plants did not differ significantly from those of the non-treated plants.

Finally, tunnel cooling techniques were tested. Frustratingly, temperatures during the 2008 growing season were insufficiently high to create thermodormancy. However, there were some useful findings from the work. The primary method of temperature reduction tested was internal shading incorporated into the polytunnel cladding material. A secondary method was forced air circulation in a polytunnel covered with clear plastic film. The internal shading element positively affected yield levels in September in one of the two varieties tested (‘Everest’). This could have implications for late season fruit production using this variety. A further finding was that forced air circulation could be an effective greenhouse cooling strategy, along with shading. The combination of the two should provide maximum temperature control during high temperature episodes.

In the future, a further season’s work is needed to test methods for controlling thermodormancy in a semi-commercial system; cool summer conditions in 2008 prevented

such a test during the course of this project. Artificial application of good quality (stored) pollen, along with the temperature control strategies described above, could alleviate the negative effects of high temperature on pollen performance and fruit set, and so reduce the impact of thermodormancy on crop production.

5 Technology Transfer

Open day: HDC Soft Fruit Panel visit 28th February 2008

Commercial publication: Wagstaffe, A. and Karapatzak, E.K. (2006). Studentship tackles strawberry dormancy. *HDC News*. **125**, 5.

Peer reviewed papers: Two papers are currently in preparation.

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