Project Title	The development of methods to alleviate thermodormancy in everbearing strawberry and secure season extension in the UK (HDC Studentship)
Project number:	CP35
Project leader:	Dr Alexandra Wagstaffe, Prof Nick Battey, the University of Reading
Report:	Annual Report, December 2007
Previous report	Annual Report, December 2006
Key staff:	Eleftherios Karapatzak (PhD student) Dr Alexandra Wagstaffe Prof Nick Battey
Location of project:	Plant Sciences Laboratory, School of Biological Sciences, the University of Reading, Reading RG6 6AS
Project coordinator:	Dr Alexandra Wagstaffe, Soft Fruit Technology Group, Plant Sciences Laboratory, School of Biological Sciences, the University of Reading, Reading RG6 6AS
Date project commenced:	1st January 2006
Date completion due:	31st March 2009 (duration 3 years and 3 months)
Key words:	Everbearing strawberry, thermodormancy, controlled environment cabinets, pollen viability, pollen germination, pollen tube length, crop load, bud-to-flower conversion rates, flower-to-fruit conversion rates, pre/ post-anthesis abortion

Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

The contents of this publication are strictly private to HDC members. No part of this publication may be copied or reproduced in any form or by any means without prior written permission of the Horticultural Development Council.

The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

© 2007 Horticultural Development Council

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.

Dr Alexandra Wagstaffe
Manager Soft Fruit Technology Group & PhD student Supervisor
School of Biological Sciences, the University of Reading

Signature ..... Date .....

Prof. Nick Battey
Head of School & PhD student Supervisor
School of Biological Sciences, the University of Reading

Signature ..... Date .....

Report authorised by:

[Name]
[Position]
[Organisation]

Signature ..... Date .....

[Name]
[Position]
[Organisation]

Signature ..... Date .....

© 2007 Horticultural Development Council

CONTENTS

© 2007 Horticultural Development Council

Grower Summary

Headline

- Work in year 2 determined the effect of day/night temperatures on pollen/flower development and the technique of truss removal as a method of ameliorating the severity of thermodormancy.

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 cultivars 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.

Key findings of the first year of the current study (2006) were that significant rates of flower abortion were observed post-anthesis following high temperature events in July. A further key finding was that no significant signs of plant stress were observed based on measurements of stomatal conductance and chlorophyll fluorescence. In the second year of the study (2007), factors which may cause flower abortion at high temperatures, such as reduced pollen viability, pollen germination and pollen tube growth were investigated. Moreover, truss-removal during periods of high temperature, as a potential technique to ameliorate the severity of the thermodormancy response, was assessed.

© 2007 Horticultural Development Council

Over the three years of this project, triggers for thermodormancy will be determined as well as interactive effects of cultivar choice, relative humidity, feed regimes/ concentration, crop load, temperature integrals and periods of exposure. Importantly, the establishment of the cause and nature of thermodormancy triggers will enable crop husbandry methods to be adapted. Ultimately, this 3-year HDC funded PhD studentship is expected to develop methods to alleviate the severity of the thermodormancy response in everbearing strawberry.

Summary of the project and main conclusions

Three experiments were conducted in 2007, two of which are described here. The third experiment was a replicate of Experiment 1. Data collection and analysis are ongoing and key findings will be presented at the HDC soft fruit panel meeting in February 2008.

Experiment 1 – Pollen Performance

Controlled environment cabinets were used to expose 'Everest' and 'Diamante' to three day/night temperature treatments (22°C/15°C - Control; 26°C/11°C and 30°C/19°C), based on previous work. All cabinets were kept at 22°C/15°C for the plants to establish. The high temperature treatments were then applied for 19 days in July. Thereafter, temperatures in all cabinets resumed 22°C/15°C, to assess rates of recovery. Measurements of pollen viability and pollen germination capacity in vitro, pollen tube growth, anther dehiscence (release of pollen) in situ, flowering and cropping patterns (labelling) and bud-to-flower and flower-to-fruit conversion rates were collected. This experiment is currently being replicated (Experiment 3).

Key findings:

- The germination capacity of the pollen (in vitro) significantly decreased in both cultivars with a 7 day exposure to high temperature.

- The in situ dehiscence of the anthers started to decrease after a 10 day exposure to high temperature.

- The pollen germination rates dropped to 0% after a 15 day exposure to 30°C/19°C.

- Both cultivars showed a capacity to recover, and pollen germination rates and anther dehiscence returned to normal levels in less than a month from the end of the high temperature event.

© 2007 Horticultural Development Council

- Pollen viability rates were significantly higher than the germination rates throughout, but reduced levels of viability occurred at the same time as those found for germination. The fact that pollen viability never dropped to 0% implies that, in these cultivars, affected pollen may be viable but with no ability to germinate.

- The length of the pollen tube was also affected by temperature; however, cultivar differences in pollen tube length were also significant.

- A reduction in pollen performance may therefore have resulted in subsequent low yields observed in the 30°C/19°C treatment in both cultivars. However, cropping dips were more pronounced in 'Everest' than in 'Diamante'.

- Both varieties show similarities in terms of pollen performance following exposure to high temperatures. However, this similarity ends when comparing subsequent flowering and cropping patterns, and implies that pollen performance is only part of the cause of thermodormancy, which differs significantly between cultivars.

- The difference in expression of thermodormancy between cultivars is a key issue and requires further investigation in the third year of the study.

Experiment 2 – Crop Load Effects

In 'Everest' and 'Diamante' a reduction in crop load by manual truss-removal was applied during a high temperature event, and was investigated as a potential tool to ameliorate the severity of thermodormancy. Three levels of crop load (0%, 25% and 50% truss removal) and two set-point temperatures (22°C – Control and 28°C) were applied for two weeks in August within glasshouse compartments.

The second experiment also investigated whether flower abortion is a pre-anthesis (pre-blossom) response as well as a post-anthesis (post-blossom) response. Therefore, bud-to-flower and flower-to-fruit conversions were recorded throughout.

Key findings:

© 2007 Horticultural Development Council

- Further analysis of this year's truss removal data will be required to evaluate whether a reduction in crop load can ameliorate a thermodormancy response, as initial analysis showed that the cropping rates of the reduced crop load treatments did not differ significantly to those of the full crop load treatment following high temperature exposure.

- However, this does imply that plants with a reduced crop load (truss removal) were capable of restoring full cropping. Depending on the outcome of the final data analysis (presented to HDC soft fruit panel in February 2008), some of this work may be carried forward to year 3.

- Flower abortion due to high temperature (28ºC) predominantly occurred post-anthesis rather than beforehand (pre-anthesis) in both cultivars.

- This resulted in 0% flower-to-fruit conversions, and suggests that when flowers are fully opened, they tend to be more sensitive to high temperatures; possibly due to the sensitivity of pollen to high temperatures (as determined in Experiment 1).

- However, at the peak of high-temperature exposure (late August) some of the flower buds recorded in 'Everest' were prevented from developing into flowers and very few subsequently turned into fruits.

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.

Further collaboration with major growers and polytunnel producers (Well-Pict European Ltd.; Haygrove Tunnels Ltd.; BPI Agri) in the 3rd Year will allow methods of alleviation of the thermodormancy response to be tested and validated. Ultimately, information will be provided that will assist consultants and growers in making cultivar and husbandry decisions.

© 2007 Horticultural Development Council

Action points for growers

The current work describes the second year of a three-year PhD studentship, and as such the results of this year require validation as well as transfer to field-scale cropping systems to warrant any conclusions for a commercial production system to be drawn.

The following are key action points for the current project rather than action points for growers. The latter will be determined at a more advanced stage of this project and summarised in the Final Project Report (end March 2009).

- The abortion of flowers post-anthesis (and only in some cases pre-anthesis) has been found to be a key expression of thermodormancy. Methods that reduce the level of flower death and therefore increase the level of flower to fruit conversion will be central in alleviating the severity of the thermodormancy response.

- More cultivars will be included in year 3, as this year's results showed that the response of flowering and fruiting to high temperature was cultivar dependent, and may in part be affected by differences in pollen performance between cultivars.

- Novel crop husbandry techniques that aid in temperature reduction will be tested in year 3, such as retrieval shade netting, venting and the use of novel heat control films (BPI Agri).

© 2007 Horticultural Development Council

Science Section

Introduction

The phenomenon of thermodormancy in everbearing strawberries was studied for the first time in the UK within a four-year DEFRA-Hortlink project at the University of Reading by Wagstaffe and Battey (2000-2004). One of the key findings of this work was that 26°C day/night temperature was capable of inducing thermodormancy following a 5 day exposure. However, cooler night temperatures (13°C) had an ameliorating effect on the severity of the cropping trough (Wagstaffe and Battey, 2006 a + b). In UK horticulture, the term 'thermodormancy' has been used when describing plants without visible flowers and fruits following a period of exposure to high temperatures. The economic consequence is a yield reduction by up to 30% compared to normal cropping levels (HDC News, 2006; Grower, 2003).

The flowering habit of everbearing strawberries in response to temperature has also been extensively studied in Japan, where high numbers of flower buds were found to abort when plants of everbearing cultivars 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 (Durner *et al*., 1984; Dennis *et al*., 1970). Knowledge of regional and microclimate as well as of crop husbandry practices therefore is of importance in these types of study (Jones, 1992; Jenks and Hasegawa, 2005).

In the first year of the current study (2006), a high temperature event during anthesis resulted in significant rates of flower abortion. This finding is in agreement with work by Wagstaffe and Battey (2006 a + b) and is considered a key symptom of thermodormancy. Another important finding in the first year was that the plants did not show significant signs of stress following treatment application, when measuring stomatal conductance rates and chlorophyll fluorescence.

© 2007 Horticultural Development Council

These findings suggest that it is important to identify the specific cause of the occurrence and onset of flower abortion, so as to deepen our understanding on the phenomenon of thermodormancy which will help to identify potential routes to ameliorate the severity of its effects on cropping on small and large scales of cultivation. It is considered critical that the stage at which high temperature causes detrimental physiological effects (i.e. flower development, pollination, pollen tube growth, flower abortion, assimilate partitioning) must be identified in order to elucidate the physiological basis of thermodormancy. Moreover, whether abortion can also be found pre-anthesis needs to be established, or whether it is specifically a post-anthesis response.

Having a strong genetic factor in a plant-environment interaction study is important as many of the abiotic stress response pathways are determined by the genetic background of the plant (Jenks and Hasegawa, 2005; Aitken, 1974; Jones *et al*., 1989). In terms of high temperature responses, in particular, different plant species, populations and even individuals show different ways of responding to high temperature (Larkindale *et al*., 2005; Kakani *et al*., 2002). Plants of a different genetic background usually have different adaptation mechanisms to a thermal environment (Mahan *et al*., 1995). In strawberry, flower induction is controlled by the interaction of temperature, daylength and genotype (Darnell *et al*., 2003). The temperature effects on growth and development are various, and high temperature effects can be quite adverse with different types and cultivars responding in a different way. For example, in Junebearing cultivars a hot thermal environment was found to have adverse effects on the rate of flower initiation, the onset of dormancy, as well as on vegetative growth and fruit production (Le Mière *et al*., 1996 and 1998).

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 (Hopkins and Hüner, 2004; Stanley and Linskens, 1974). The same holds true for strawberries (Žebrowska, 1998; Hancock, 1999). Thus the production of viable pollen, successful pollination and pollen tube growth are 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 (Dickinson, 1987); 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 (Stanley and Linskens, 1974). After the successful production of pollen and provided that successful pollination has taken place the pollen should germinate successfully and ultimately fertilize the ovules of the flower (Faegri *et al*., 1992). 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

© 2007 Horticultural Development Council

discharge (Heslop-Harisson, 1987). Throughout all these stages the tube interacts with several substances like the stigma surface materials, the underlying cells, the secretions in the transmitting tissue and finally with the female gametophyte (Heslop-Harisson, 1971) making the process of fertilization naturally complex; additional external stresses, such as high temperatures, can therefore 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 cultivars, 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 also been found in tomato (Pressman *et al*., 2002; Song *et al*., 1999), in which the response of pollen to heat treatments can also 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).

Crop load of most fruiting plants affects growth and development; according to such studies, fruit thinning can significantly enhance yield and quality of the product on crops like pecan, sweet pepper and orange (Smith *et al*., 1993; Fukumoto *et al*., 2004; Syvertsen *et al*., 2003 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 were also found to interact with high temperature stress in Japanese plum cultivars causing significant decreases in yield potential (Naor *et al*., 2004). In addition, a high flower load has also been found to affect subsequent crop load and consequently yield and quality of fruit in European plums (Meland, 2007).

Consequently, the effect of high temperature on pollen viability and pollen germination after pollination and the successful growth of the tube were key processes investigated in the second year of the current study. The hypothesis of the first experiment was that if the day and night temperatures in the growth environment rise when the plants are at anthesis, then the production, viability and performance of the pollen may be negatively affected. This would cause the plants to produce less fruit and fruit of a lesser quality. Thus, the objective of the

© 2007 Horticultural Development Council

first experiment was to establish the effects of different day/night temperature regimes on the viability of the pollen, its *in vitro* germination capacity and pollen tube growth, and to relate these to subsequent flowering and cropping patterns. Two everbearing strawberry varieties, Everest and Diamante, with a genetically different heritage were used as in the 2006 experiments.

The hypothesis of the second experiment was that if the crop load of a strawberry plant is manually reduced during a high temperature event, then this may have an ameliorating effect on the severity of a possible cropping trough due to a thermodormancy response. Thus, the objective of the second experiment was to establish the effects of high temperature in interaction with crop load (number of trusses) on subsequent flowering and cropping patterns of plants of the same everbearing cultivars used in the first experiment. The second experiment also investigated whether flower abortion is a pre-anthesis response as well as a post-anthesis response.

The third experiment is a replication of the first of the summer's experiments (pollen performance); data collection has just been finished (Sep-Dec 2007) and data analysis is ongoing.

Materials and Methods

Experiment 1 – Pollen Performance

This experiment investigated the effects of different day/night temperature regimes on pollen viability, as well as on its *in vitro* germination capacity and pollen tube growth. This was related to subsequent flowering and cropping patterns in two everbearing strawberry varieties.

Plant material

The everbearing varieties used were 'Everest' (bred in the UK) and 'Diamante' (bred in the US) and were chosen due to the fact that they are genetically distant, as they do not share any parents (Meiosis, 2006; Baruzzi *et al*., 2006). Twenty four tray plants of 'Everest' and twenty four bare rooted, cold stored plants of 'Diamante' were planted in 2-litre pots (5th May 2007) using standard strawberry peat compost (Bulrush Horticulture Ltd. UK). Drip irrigation (Field Ltd. UK) was used with the use of timing adjusted pumps. The feed pH was kept between 5.8 and 6.2 by application of nitric acid (with the use of a pH adjusted pump). A commercial, ready made, soft fruit fertilizer mix was used ('Soft Fruit Mix' by Avoncrop Ltd UK, 3-1-6 plus micronutrients). A stock solution of the mix was used and it was dosed into a bigger tank up to the desired EC. Standard pest and disease control methods were used as standard in the experimental grounds of the School of Biological Sciences.

© 2007 Horticultural Development Council

Controlled environment facilities and treatments

Controlled environment cabinets (Saxcils – Saxton Ltd. UK) were used to control day and night temperatures (Figure 1). The plants remained in the cabinets for the entire duration of the experiment; 12/06/2007 – 31/08/2007. The high-temperature treatments, however, were only applied for 19 days in July 2007.

The temperature regimes consisted of an optimum treatment (Control), which was 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 treatment 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 integral than the Control treatment and was used to asses the effect of a cool night temperature. The third temperature treatment was 30°C day / 19°C night (16 h light / 8 h dark) and was used to provide the high temperature treatment (average 26.4°C), which is above the previously found threshold-temperature for inducing thermodormancy (Wagstaffe and Battey, 2006a + b). All cabinets were kept at the same temperature as the Control before as well as after high-temperature treatment application, so as to allow for plant establishment and plant recovery, respectively. Treatment details are summarised in Table 1.

Figure 1: The controlled environment cabinets (Saxcils).

Table 1: Treatment details for experiment 1.

© 2007 Horticultural Development Council

Cultivar	Temperature Regime D/N (°C)
Everest	**22/15**
	26/11
	30/19
Diamante	**22/15**
	26/11
	30/19

Flowering and cropping patterns

Weekly flowering and cropping measurements were taken throughout the duration of the experiment. Flower number, fruit number and fruit weight (g) / plant were recorded in order to determine patterns over time. Flowers were tagged weekly so as to monitor the duration of development until fruiting and to record flower-to-fruit conversion rates.

The flowering and cropping data were collected manually in a list format and afterwards were converted to an electronic format with the use of Microsoft Excel spreadsheet software (Microsoft Corp. USA) to enable further calculations and analysis.

Pollen measurements

Pollen viability, pollen germination capacity and pollen tube growth were determined *in vitro* on a weekly basis throughout the duration of the experiment.

The methods used for these measurements are simple, well established, *in vitro* pollen viability and germination tests. To measure pollen viability acetocarmine staining solution (1%) was used (Ledesma and Sugiyama, 2005). Pollen was collected from the plants in a Petri dish and taken to the lab where it was placed onto normal microscope slides and mixed with the stain. After a period of approx. 15 minutes at ambient room temperature the numbers of stained against non-stained grains were recorded under a light microscope. 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).

For the germination test, the 'sitting drop culture method' was used (Shivanna and Rangaswamy, 1992), which is a fast, simple and accurate method. The germination medium (Brewbaker and Kwack, 1963) is well established 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). It consisted of: 100

© 2007 Horticultural Development Council

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 up to 4 hours incubation and no significant differences 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. The numbers of stained against non-stained and germinated against non-germinated pollen grains were recorded from 6-10 random microscopic fields under a 100x magnification. A percentage of stained or germinated grains was calculated:

$$\text{germination/viability (\%)} = \frac{\text{no of germinated/stained grains within the counted fields}}{\text{total no of grains counted}} \times 100$$

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. A possible effect of population size was statistically tested.

The length of the pollen tubes was measured with the use of a micrometer slide (Graticules Ltd. UK) and a Graticule eyepiece under a high-powered microscope (140x magnification). A 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.

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 in order to determine if there was a significant population effect (Shivanna and Rangaswamy, 1992); see Appendix.

Comparisons of means and Analyses of Variance (ANOVAs) were used also on the flowering and cropping data in order to determine the level of significance of factor effects. The statistical software used was Genstat 8.1 from VSN international and Minitab 15 (Minitab Inc.). Graphs were drawn in Microsoft Excel and Minitab's graph facilities.

Experiment 2 – Crop Load Effects

This experiment investigated the potentially ameliorating effect of a reduction in crop load during periods of high temperature on the severity of the thermodormancy response in two everbearing strawberry cultivars. The bud-to-flower and flower-to-fruit conversions were also recorded.

Plant material

Cultivars '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 2) 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 were the same with experiment 1.

Experimental design and treatment application

Treatment application took place for 20 days in August 2007 from 13/08/2007 when plants were exposed to a set point temperature of 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 (13/08/07).

© 2007 Horticultural Development Council

There were two temperature treatments x three truss removal treatments x two cultivars (thus 12 treatments in total). There were six replicate plants per treatment resulting in a total of 72 plants (36 per cultivar). The statistical design was an incomplete randomised block design.

The glasshouse compartments used were factorials and thus there was a good degree of temperature control. The treatment details are summarised in Table 2.

Figure 2: The temperature controlled glasshouse compartments.

Table 2: Treatment details for experiment 2.

Temperature compartment	Cultivar	% 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, bud numbers were recorded and tagged. As a result, the phenology of the flowering per plant was numerically described. The buds were recorded and labelled, they were then 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

© 2007 Horticultural Development Council

twice a week throughout the season. As a result it was possible to calculate the bud-to-flower conversion rates and their subsequent flower-to-fruit conversion rates for every plant, as well as to determine the time needed between the stages of development. These observations were of importance when comparing optimum to high growth temperatures in the two cultivars. Therefore, these measurements were taken only on plants with an unaffected crop load.

Data management and analysis of the flowering and cropping measurements was conducted as described for experiment 1.

Results

Experiment 1 – Pollen Performance

Pollen measurements

Treatment and cultivar factors had a highly significant effect on pollen germination capacity ($P<0.01$). Cultivar 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 % on average ($P<0.01$). Both cultivars, however, showed a significant decrease in germination after the first 7 days of exposure to the 30°C/19°C temperature treatment (Figure 3). As the high temperature event persisted germination rates continued to fall to 0% germination in both cultivars.

Germination rates also decreased following exposure to the 26°C/11°C treatment, with 'Everest' being more responsive than 'Diamante' its germination rates fell to 5.3%; however, they never reached 0% as in the 30°C/19°C treatment.

Pollen germination capacity started to recover 14 days after the termination of the high-temperature treatments when germination rates increased in cultivar Diamante; although levels were still lower than in the other treatments, they had risen above 50%. Similarly in 'Everest', pollen germination rates significantly increased again 20 days after the termination of the high-temperature treatments.

© 2007 Horticultural Development Council

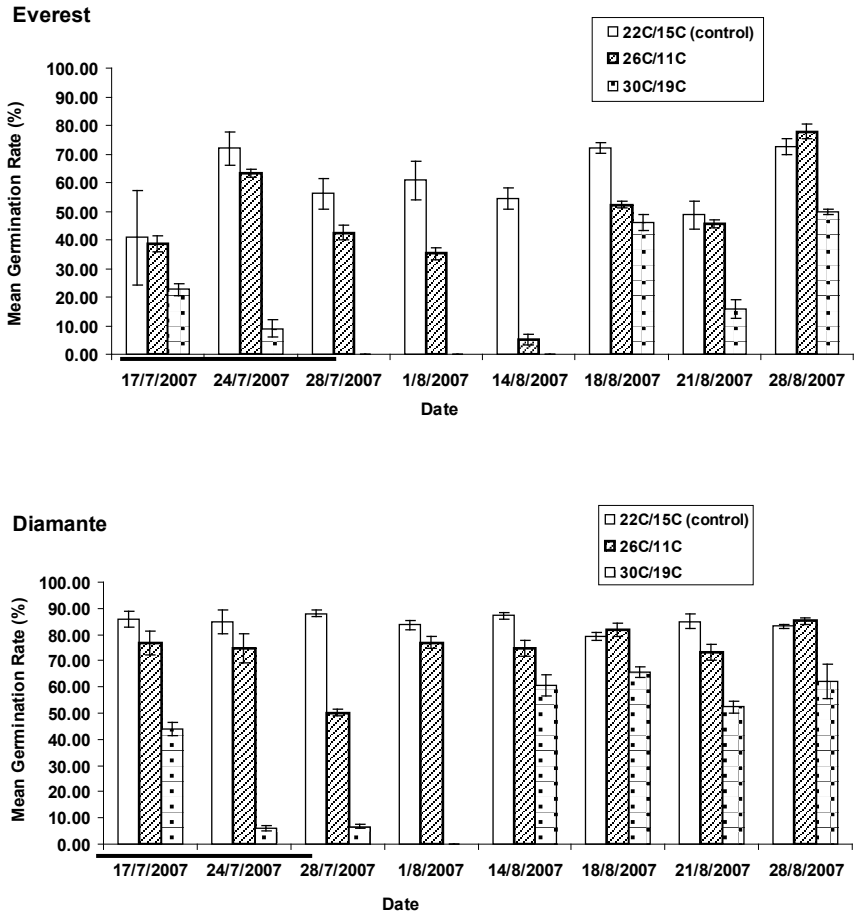

Figure 3: Average pollen germination rates for the three temperature treatments across the season in Everest and Diamante. Where no bars can be seen for the 30C/19C treatment, germination rates had reached 0%. Error bars indicate the Standard Errors of the means (P<0.05). The thickened lines on the x-axis indicate the treatment application period.

The choice of cultivar and temperature treatments significantly affected pollen viability (P<0.01). Their interaction, however, was not significant. Temperature had a stronger effect on pollen viability than on cultivar (Table 3). Moreover, in 'Everest' pollen viability after the end of treatment application remained significantly lower than the control, whereas in 'Diamante' it returned to normal levels towards the end of the experiment.

Table 3: Pollen viability percentages in Everest and Diamante as affected by the three temperature treatments measured at fortnightly intervals across the season.

		Percentage Pollen Viability (%)			
		Date			
Cultivar	Temp regime	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)

© 2007 Horticultural Development Council

	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 overall LSD (treatment*cultivar) of these means is 7.99 % (P<0.05).
* These dates are during treatment application.
Numbers in brackets 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 cultivar 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 cultivars and it was also affected by temperature, but the rate of its growth did not change as the season progressed. According to Table 4, pollen tubes in 'Diamante' were longer than in 'Everest', and they were shorter in the high temperature treatment in both cultivars during as well as after treatment application.

Table 4: Mean pollen tube length (µm) in 'Everest' and 'Diamante' before, during and after temperature treatment application.

Cultivar	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 / 19°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 / 19°C	-	353.3 (32.7)ad	142.5 (19.1)e

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

Flowering and cropping patterns

The effects of the different temperature treatments on flowering patterns were not found to be significant in both cultivars. Flowering was, however, affected by choice of cultivar (P<0.01) (Figure 4). A significant reduction in flowering was observed in 'Everest' in all temperatures two weeks after the termination of the treatments; this effect was more marked in the 30°C/19°C and the 26°C/11°C treatments than in the Control. In 'Diamante', on the other hand, no significant reductions in flower number were observed following treatment

© 2007 Horticultural Development Council

application, but a trend could be found for the 30°C/19°C treatment to produce fewer flowers for two weeks following treatment application.

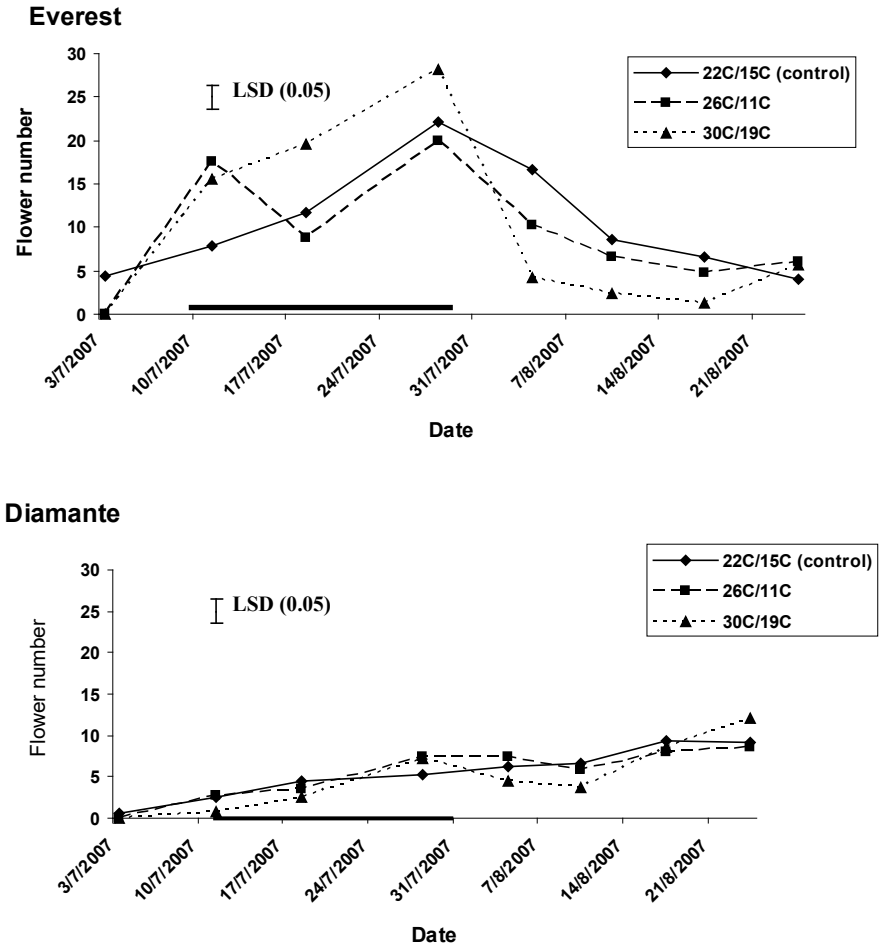

Figure 4: Flower numbers over the season in Everest and Diamante as affected by the three temperature treatments. The LSD of the means (treatment*cultivar) is 2.9 (P<0.05). The thickened lines on the x-axis indicate the treatment application period.

The temperature treatments had a significant effect on cropping patterns in both cultivars (P<0.01) (Figure 5). Cultivar differences were also significant (P<0.01). Everest produced, on average, double the weekly yield of Diamante (41.3 g/plant/week compared to Diamante with 13.5 g/plant/week). The highest fruit fresh weights (g) were found in the 26°C/11°C treatment. Fruit fresh weights were significantly reduced in the 30°C/19°C treatment. This effect was more pronounced in 'Everest' than in 'Diamante' and even after 4 weeks of the termination of the 30°C/19°C treatment cropping was still low in both cultivars.

© 2007 Horticultural Development Council

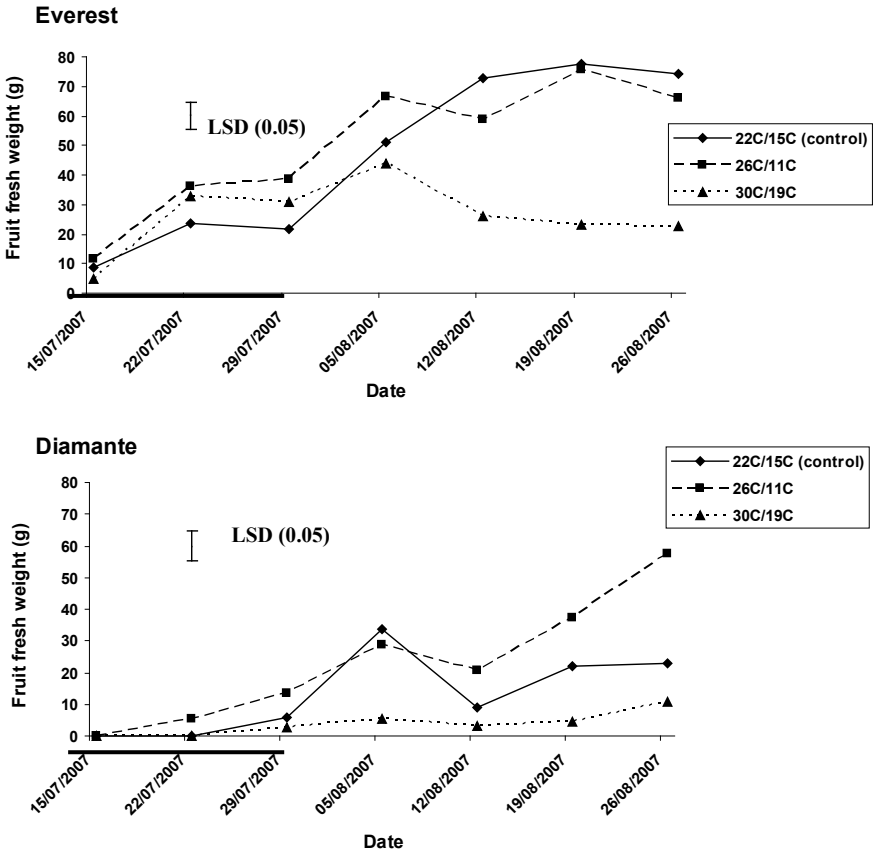

Figure 5: Cropping patterns expressed as mean weekly fruit fresh weight (g) per plant for the three temperature treatments for each cultivar. The overall LSD of the means (treatment*cultivar) is 9.34 (P<0.05). The thickened lines on the x-axis indicate the treatment application period.

Experiment 2 – Crop Load Effects

Flowering and cropping measurements

The crop load treatments on flowering patterns were insignificant (based on flower number) (Figure 6). Differences in flowering patterns between the two temperature treatments were also not significant. Flowering patterns, however, were significantly different between cultivars 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 week (compared to other periods with more than 15 flowers 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 found to be higher.

© 2007 Horticultural Development Council

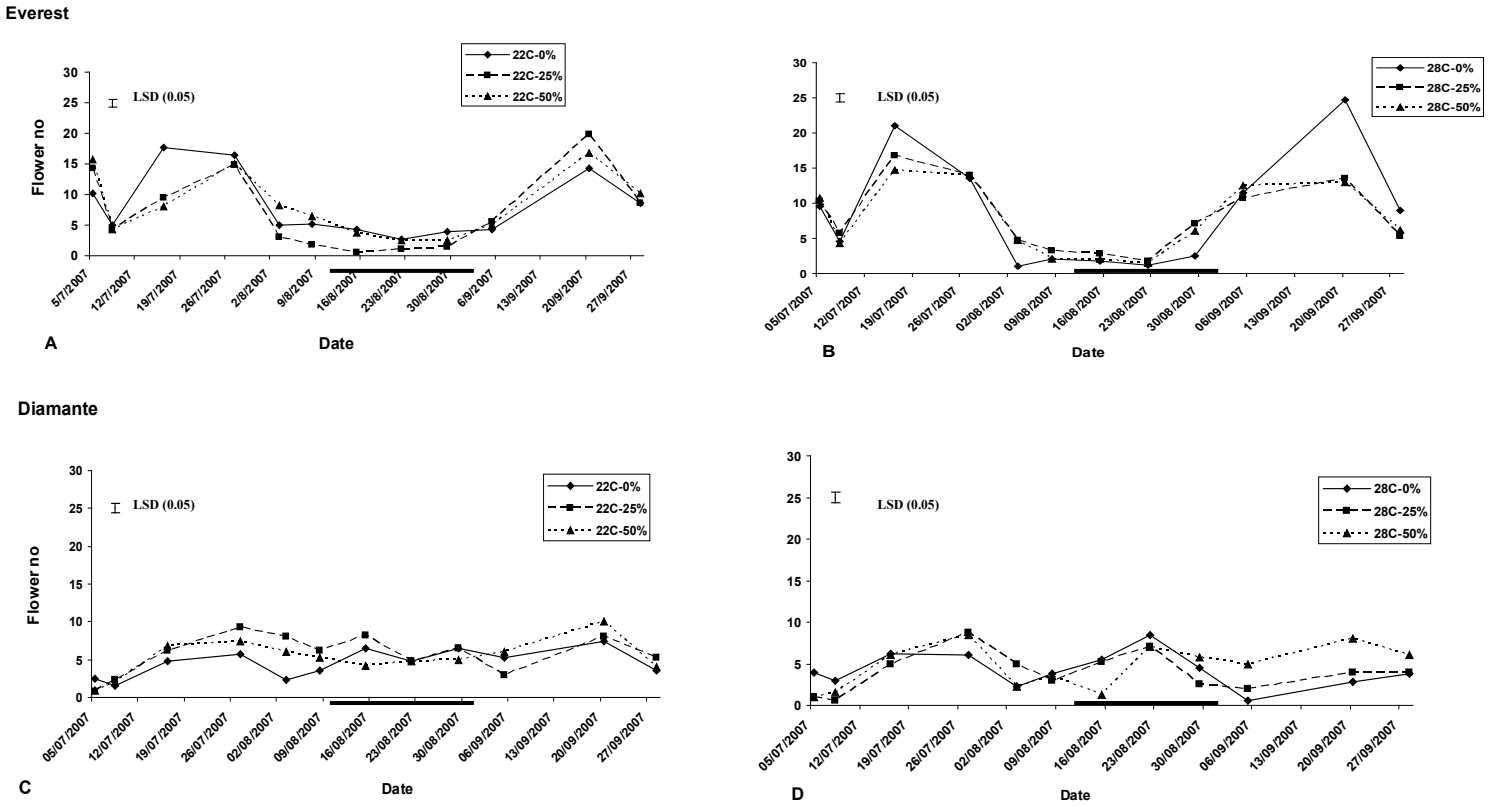

Figure 6: Weekly flower numbers per plant in 'Everest' and 'Diamante' as affected by truss removal and temperature treatments across the season. The LSD of the means is 1.25 (P<0.05). The thickened lines on the x-axis indicate the temperature and truss removal treatment application period. The legends give temperature and truss removal treatments. A: Everest, 22ºC, B: Everest, 28ºC. C: Diamante, 22ºC, D: Diamante, 28ºC.

© 2007 Horticultural Development Council

Initial analysis found the effects of crop load treatments on subsequent cropping not to be significant (fruit fresh weight (g)) (Figure 7), even though reduced cropping rates would initially be expected due to truss removal in the reduced crop load treatments. This data will require further analysis (in December 2007 / January 2008) to include a corrective factor which will take account of the preceding levels of truss removal. This analysis will then enable a clearer view of the potential rate at which cropping recovers in truss removal treatments, and whether there is scope for this as a technique to ameliorate the severity of thermodormancy. In 'Everest', for example, yield of the two truss removal treatments at the end of the season were higher than in the no-truss removal treatment at 22ºC.

Temperature had a significant effect on fruit fresh weight (g) in both cultivars ($P<0.05$) and decreased following exposure to 28°C compared to the 22°C Control. Cultivar choice had a highly significant effect on cropping ($P<0.01$). Both cultivars produced their highest weekly yields in the 22°C treatment, with Everest averaging 35.4 g/plant/week and Diamante averaging 25.12 g/plant/week (LSD = 7.9, $P<0.05$).

© 2007 Horticultural Development Council

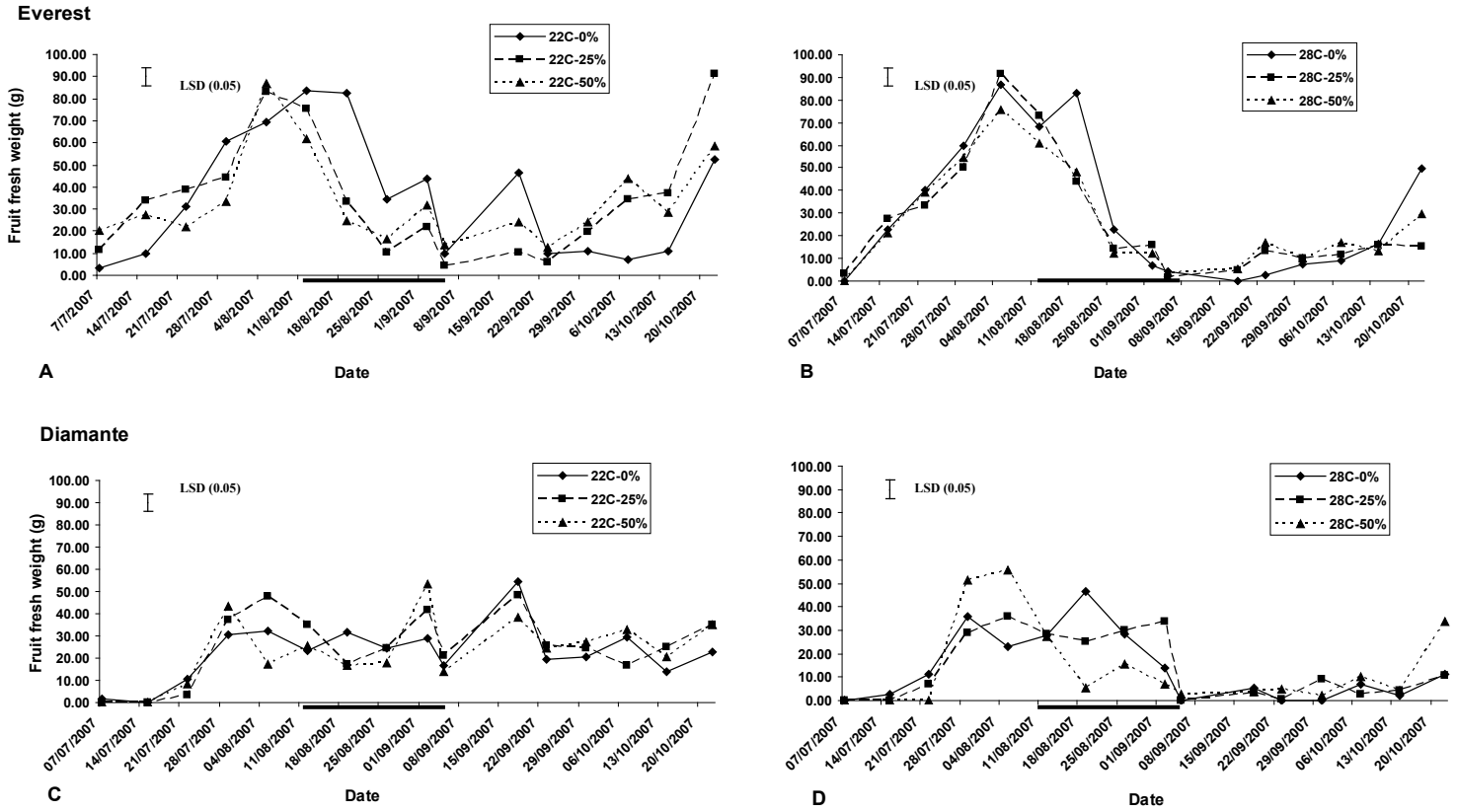

Figure 7: Cropping patterns expressed as fruit fresh weight (g) per plant in 'Everest' and 'Diamante' as affected by two temperature and three crop load treatments across the season. The LSD of the means is 8 (P<0.05). The thickened lines on the x-axis indicate the treatment application period. The legends give temperature and truss removal treatments. A: Everest, 22ºC. B: Everest, 28ºC. C: Diamante, 22ºC. D: Diamante, 28ºC.

© 2007 Horticultural Development Council

There were no significant rates of bud abortion pre-anthesis across the season. Bud-to-flower conversion rates were above 75% in both cultivars and both temperatures throughout the season (Table 2 – Appendix); apart, however, from one week when some bud abortion rates were observed and this coincided with the dip in flower numbers in 'Everest' (Figure 6).

In contrast, significant rates of flower abortion post-anthesis were observed over the season (Table 3 – Appendix), which is a typical symptom of thermodormancy. Following high temperature application, flower-to-fruit conversion rates in both cultivars dropped below 50% and down to 0% on, 15/08/2007 and between 29/08/2007 to 28/09/2007, even though flower numbers per plant may have been high on those dates (with the exception of 15/08/2007; Figure 6).

Discussion and Conclusion

A key observation this year was that both cultivars significantly reduced pollen performance in response to high temperatures in terms of *in vitro* germination capacity, pollen viability and pollen tube growth. This reduction in pollen performance may have resulted in subsequent low yields observed in the high temperature treatments in both cultivars. However, 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 high flower abortion rates post-anthesis were observed (Wagstaffe and Battey, 2006 a + b).

This means, both varieties show similarities in terms of pollen performance following exposure to high temperatures. However, this similarity ends when comparing subsequent flowering and cropping patterns, and implies that pollen performance is only part of the cause of thermodormancy, which differs significantly between cultivars. For example, other studies in everbearers suggest that high temperature in the growth environment reduces flower initiation in the first place (Oda and Yanagi, 1993; Yanagi and Oda, 1990, 1992 and 1993; Kumakura *et al*., 2005); no evidence for this, however, was found for 'Everest' (Wagstaffe and Battey, 2006b). The difference in expression of thermodormancy between cultivars is a key issue and requires further investigation in the third year of the study.

Following high temperature application, germination percentages of pollen from both cultivars fell to 0%. Similar responses of strawberry pollen to high temperatures were shown in recent work where germination rates dropped below 20% (Ledesma and Sugiyama, 2005;

© 2007 Horticultural Development Council

Koyuncu, 2006; Leech *et al*., 2002). It was reported that pollen performance may be cultivar dependent (Ledesma and Sugiyama, 2005; Koyuncu, 2006; Hortynski and Żebrowska, 1991). In general, pollen ecology has been shown to have different responses to temperature depending on both genetic and ecological factors (Stanley and Linskens, 1974; Heslop-Harisson, 1971).

In the current study, pollen viability rates were significantly higher than the germination rates, but reduced levels of viability occurred at the same time as those found for germination. The fact that pollen viability never dropped to 0% implies that, in these cultivars, affected pollen may be viable but with no ability to germinate. Moreover, the acetocarmine staining method also has well known disadvantages (Heslop-Harrison *et al*., 1984), such as the occasional occurrence of staining dead pollen as well as living pollen, resulting in potentially high and misleading estimates of viability in several species (Parfitt and Ganeshan, 1989; Vassileva *et al*., 1991; Parameswar and Venugopal, 1974). It can be concluded that acetocarmine staining can be used for assessing pollen viability, but only in conjunction with germination capacity so as to achieve greater accuracy (Leech *et al*., 2002).

The length of the pollen tube was found to be significantly affected by temperature. Shorter tubes were observed following high day/night temperature exposure of plants in both cultivars. Cultivar choice, however, had a more significant effect on pollen tube length. The lengths observed in ‘Everest’ ranged from 215.8 µm to 329.4 µm and in ‘Diamante’ from 142.5 µm to 529.2 µm. Similar values have been reported for other European strawberry cultivars (Koyuncu, 2006). However, among Japanese cultivars, pollen tube length was 4 times longer (Ledesma and Sugiyama, 2005), supporting the suggestion that genetic factors affect pollen systems. Furthermore, Jayaprakash and Sarla (2001) in pigeonpea reported that if the plants had fewer flowers at the time of measurements, the *in vitro* pollen germination percentage tended to be higher, which implies that at the peak of flowering, nutrients play an important role and can be a limiting factor. This observation is in accordance with the results of the current study, where even though cultivar Diamante had lower flowering rates than ‘Everest’, its optimum germination rates were significantly higher and the pollen tubes tended to be longer. However, it cannot be concluded that this was due to differences in nutrient availability.

No significant flower abortion pre-anthesis was observed in both cultivars even following high-temperature application. However, significant rates of flower abortion post-anthesis were observed in both cultivars. These were more profound in the high-temperature treatment (28ºC) and resulted in 0% flower-to-fruit conversions. This suggests that when flowers are fully opened, they tend to be more sensitive to high temperatures; this could be a

result of the sensitivity of pollen to high temperatures. There was, however, one week during high temperature exposure (late August) when few of the flower buds recorded in 'Everest' turned into flowers and very few subsequently turned into fruits; which is in agreement with studies in which bud production was inhibited and high rates of bud abortion were observed following exposure to temperatures above 26ºC (Kumakura and Shishido, 1995; Taimatsu *et al*., 1991).

Further analysis of this year's data will be required to evaluate whether a reduction in crop load can ameliorate a thermodormancy response. Initial analysis showed that the cropping rates of the reduced crop load treatments did not differ significantly to those of the full crop load treatment following high temperature exposure. This does, however, imply that plants with a reduced crop load (truss removal) were capable of restoring full cropping. In other fruit crops, reduced crop load has been found to enhance cropping (Naor *et al*., 2004; Smith *et al*., 1993; Fukumoto *et al*., 2004; Syvertsen *et al*., 2003). Depending on the outcome of the final data analysis, some of this work may be carried forward to year 3.

In conclusion, the results of this year's work show that high pollen quality and successful pollination are important for a good fruit set, and can be affected by periods of high temperature. This implies that pollinator presence is important as well as the use of crop husbandry techniques that aid in temperature reduction, such as venting. It will be beneficial to include more cultivars in year 3, as the results of year 2 showed that the response of flowering and fruiting to high temperature was cultivar dependent, and may in part be affected by differences in pollen performance between cultivars. For example, 'Everest' consistently had higher flowering rates and cropping rates than 'Diamante', but was more sensitive to high temperatures. In addition, novel crop husbandry techniques that aid in temperature reduction will be tested in year 3, such as retrieval shade netting and the use of novel heat control films (BPI Agri).

References

Abdul-Baki, A.A. and Stommel, J.R. (1995). Pollen viability and fruit set of tomato genotypes under optimum and high-temperature regimes. *HortScience*. **30**(1), 115-117.

Aitken, Y. (1974). Flowering time, Climate and Genotype. Melbourne University Press.

Arya, J.C. and Lardner, R.W. (1979). Mathematics for the Biological Sciences. Prentice-Hall, INC Eds.

Baruzzi, G., Magnani, S. and Della Strada, G. (2006). Strawberry varieties released from 1980 to 2004.

URL$_1$: http://www.agraria.it/isf/attivit/fragola/ishs/varieties [22/09/2007]

URL$_2$: http://www.ucop.edu/ott/strawberry/Diamantecultivar.htm [04/12/2007]

Brewbaker, J.L. and Kwack, B.H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany*. **50**(9), 859-865.

Darnell, R.L., Cantliffe, D.J., Kirschbaum, D.S. (2003). The physiology of flowering in strawberry. In: *Horticultural Reviews*, Vol. 28. (Edited by Janick J.) Wiley and sons Inc Pp 325-349.

Dennis, F.G., Lipecki, J. Jr. and Kiang, C. (1970). Effects of photoperiod and other factors upon flowering and runner development of three strawberry cultivars. *Journal of the American Society for Horticultural Science.* **95**(6), 750-754.

Dickinson, H.G. (1987). The physiology and biochemistry of meiosis in the anther. *International Review of Cytology*, **107**. Pollen, Cytology and Development (Edited by Jeon, K.W. and Friedlander M.). Academic Press. Pp 79-109.

Durner, E.F., Barden, J.A., Himerlick, D.G. and Poling, E.B. (1984). Photoperiod and temperature effects on flower and runner development in day-neutral, Junebearing and everbearing strawberries. *Journal of the American Society for Horticultural Science.* **109** (3), 396-400.

Faegri, K., Kaland, P.E. and Krzywinski, K. (1992). Textbook of pollen analysis. 4th ed. John Wiley & sons pub.

Feder, W.A. (1990). Simplifying pollen tube measurements without reducing tube growth potential. *Plant Cell Incompatibility Newsletter*. **22**, 11-12.

Fukumoto, Y., Nishimura, Y. and Shimasaki, K. (2004). Effects of the fruit load on fruit set and bearing habit in sweet pepper (*Capsicum annum* L.). *Journal of the Japanese Society for Horticultural Science.* **73**(2), 171-177.

Hancock, J.F. (1999). Strawberries. CABI publishing.

Hedhly, A., Hormaza, J.I. and Herrero, M. (2005). The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. *Plant Biology*. **7**(5), 476-483.

Heslop-Harisson, J. (1971). Pollen development and physiology.

Heslop-Harisson, J. (1987). Pollen germination and pollen tube growth. *International Review of Cytology*, **107**. Pollen, Cytology and Development (Edited by Jeon, K.W. and Friedlander M.). Academic Press. Pp 1-77.

Heslop-Harisson, J., Heslop-Harisson, Y. and Shivanna, K.R. (1984). The evaluation of pollen quality, and a further appraisal of the fluorochromatic (FCR) test procedure. *Theoretical and Applied Genetics*. **67**, 367-375.

© 2007 Horticultural Development Council

Honsho, C., Somsri, S., Tetsumura, T., Yamashita, K., Yapwattanaphun, C. and Yonemori, K. (2007). Characterisation of male reproductive organs in durian; anther dehiscence and pollen longevity. *Journal of the Japanese Society for Horticultural Science*. **76**(2), 120-124.

Hopkins, W.G. and Hüner, N.P.A. (2004). Introduction to Plant Physiology. 3rd Ed. John Wiley and Sons Inc.

Hortynski, J.A. and Žebrowska, J. (1991). The effect of different air temperatures on *in vitro* pollen germination of selected strawberry cultivars (*Fragaria ananassa* Duch.). *Folia Horticulturae*. **3**(3), 107-113.

Jagadish, S.V.K., Craufurd, P.Q. and Wheeler T.R. (2007). High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *Journal of Experimental Botany*. **58**(7), 1627-1635.

Jayaprakash, P. and Sarla, N. (2001). Development of an improved medium for germination of *Cajanus cajan* (L.) Millsp. pollen *in vitro*. *Journal of Experimental Botany*. **52**(357), 851-855.

Jenks, M.A. and Hasegawa, P.M. (2005). Plant abiotic stress. Biological Sciences Series. Blackwell Publishing.

John, K. and Prabhakara, R. (2005). Pollen viability and meiotic studies in muskmelon (*Cucumis melo* L.). Agricultural Science Digest. Agricultural Communication Centre, Karnal, India. **25**(3), 157-161.

Jones, H.G. (1992). Plants and microclimate: a quantitative approach to environmental plant physiology. 2nd Ed. Cambridge University Press.

Jones, H.G., Flowers, T.J. and Jones M.B. (1989). Plants under stress. Biochemistry, physiology and ecology and their application to plant improvement. Society for Experimental Biology, Seminar Series 39. Cambridge University Press.

Kakani, V.G., Prasad, P.V.V., Craufurd, P.Q. and Wheeler, T.R. (2002). Response of *in vitro* pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. *Plant, Cell and Environment*. **25**, 1651-1661.

Koyuncu, F. (2006). Response of in vitro pollen germination and pollen tube growth of strawberry cultivars to temperature. *European Journal of Horticultural Science*. **71**(3), 125-128.

Kumakura, H. and Shishido, Y. (1995). Effect of temperature and photoperiod on flower bud initiation in everbearing type strawberry cultivars. *Journal of the Japanese Society for Horticultural Science*. **64**(1), 85-94.

Kumakura, H., Fujiwara, T. and Ikeda, T. (2005). Inflorescence development in strawberry cv. Sachinoka exposed to flower inducing runner cooling and subsequent exposure to high temperature. *Bulletin of the National Agricultural Research Centre for Western Region, Japan*. **5**, 1-18.

Larkindale, J., Mishkind, M. and Vierling, E. (2005). Plant responses to high temperature. In: Plant Abiotic Stress (Edited by M.A. Jenks and P.M. Hasegawa). Blackwell Publishing. Pp 100-144.

Leech, L., Simpson, D. and Whitehouse, A.B. (2002). Effect of temperature and relative humidity on pollen germination in four strawberry cultivars. *Acta Horticulturae*. **567**(1), 261-263.

Le Mière, P., Hadley, P., Darby, J. and Battey, N.H. (1996). The effect of temperature and photoperiod on the rate of flower initiation and the onset of dormancy in the strawberry (*Fragaria* x *ananassa* Duch.). *Journal of Horticultural Science*. **71**(3), 361-371.

Le Mière, P., Hadley, P., Darby, J. and Battey, N.H. (1998). The effect of thermal environment, planting date and crown size on growth, development and yield of *Fragaria* x *ananassa* Duch. cv. Elsanta. *Journal of Horticultural Science & Biotechnology.* **73**(6), 786-795.

Ledesma, N. and Sugiyama, N. (2005). Pollen quality and performance in strawberry plants exposed to high-temperature stress. *Journal of the American society of Horticultural science*. **130**(3), 341-347.

Lovelidge, B. (2003). Thermodormancy is a real threat to berry supplies. *Grower*. August 21, 2003, 6-7.

Mahan, J.R., McMichael, B.L. and Wanjura, D.F. (1995). Methods for reducing the adverse effects of temperature stress on plants: a review. *Environmental and Experimental Botany*. **35**, 251-258.

Mead, R., Curnow, R.N. and Hasted, A.M. (2003). Statistical methods in Agriculture and experimental Biology. 3rd ed. Chapman & Hall/CRC Eds.

Meiosis, (2006). Everest and Flamenco breeder's description.
URL: http://www.meiosis.co.uk/fruit/everest.htm [22/09/2007]
URL:http://www.meiosis.co.uk/fruit/flamenco.htm [22/09/2007]

Meland, M. (2007). Efficacy of chemical bloom thinning agents to European plums. *Acta Agriculturae Scandinavica. Section-B Soil and Plant Science*. **57**(3), 235-242.

Mercado, J.A., Fernández-Muñoz, R. and Quesada, M.A. (1994). *In vitro* germination of pepper pollen in liquid medium. *Scientia Horticulturae*. **57,** 273-281.

Mercado, J.A., Mar Trigo, M., Reid, M.S., Valpuesta, V. and Quesada, M.A. (1997). Effects of low temperature on pepper pollen morphology and fertility: Evidence of cold induced exine alterations. *Journal of Horticultural Science*. **72**(2), 317-326.

Naor, A., Peres, M., Greenblat, Y., Gal, Y. and Ben Arie, R. (2004). Effects of pre harvest irrigation and crop level on yield, fruit size distribution and fruit quality of field grown 'Black-Amber' Japanese plum. *Journal of Horticultural Science and Biotechnology.* **79**(2), 281-288.

© 2007 Horticultural Development Council

Oda, Y. and Yanagi, T. (1993). Effect of climatic condition on the floral initiation at the runner tip of everbearing strawberry cultivar (*Fragaria ananassa* Duch.). *Acta Horticulturae*. **345**, 67-72.

Parameswar, N.S. and Venugopal, R. (1974). Evaluation of different methods of determining pollen viability in cardamom. Current Research, Bangalore, India. **3**(9), 108-109.

Parfitt, D.E. and Ganeshan, S. (1989). Comparison of procedures for estimating viability of *Prunus* pollen. *HortScience*. **24**(2), 354-356.

Pressman, E., Peet, M.M. and Pharr, D.M. (2002). The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers. *Annals of Botany*. **90**, 631-636.

Shivanna, K.R. and Heslop-Harisson, J. (1981). Membrane state and pollen viability. *Annals of Botany*. **47**, 759-770.

Shivanna, K.R., Linskens, H.F. and Cresti, M. (1991). Pollen viability and pollen vigor. *Theoretical and Applied Genetics*. **81**, 38-42.

Shivanna, K.R. and Rangaswamy, N.S. (1992). Pollen Biology. A laboratory manual. Sringer-Verlag Eds. 1992.

Singh, A.K., Mann, S.S. and Raghbir, S. (2004). Studies on pollen characteristics and pollination in relation to fruit set in different pear cultivars. *Indian Journal of Horticulture*. **61**(4), 352-353.

Smith, MW., Reid, W., Carroll, B. and Cheary, B. (1993). Mechanical fruit thinning influences fruit quality, yield, return fruit set and cold injury of pecan. *HortScience*. **28**(11), 1081-1084.

Song, J., Nada, K. and Tachibana, S. (1999). Ameliorative effect of polyamines on the high temperature inhibition of *in vitro* pollen germination in tomato (*Lycopersicum esculentum* Mill.). *Scientia Horticulturae*. **80**, 203-212.

Stanley, R.G. and Linskens, H.F. (1974). Pollen. Biology, biochemistry, management. Springer-Verlag.

Syvertsen, J.P., Goni, C. and Otero, A. (2003). Fruit load and canopy shading affect leaf characteristics and net gas exchange of 'Spring' navel orange trees. *Tree physiology*. **23**(13), 899-906.

Taimatsu, T., Yoshida, N. and Nishimoto, T. (1991). The habit of flower bud formation and flowering in everbearing strawberries. *Bulletin of the Nara Agricultural Experimental Station*. Japan. **22**, 35-42.

Van Bavel, C.H.M., Nakayama, F.S. and Ehrler W.L. (1965). Measuring transpiration resistance of leaves. *Plant Physiology*. **40**, 535-540.

Vara-Prasad, P.V., Craufurd, P.Q. and Summerfield, R.J. (1999). Fruit number in relation to pollen production and viability in groundnut expose to short episodes of heat stress. *Annals of Botany*. **84**, 381-386.

© 2007 Horticultural Development Council

Vara-Prasad, P.V., Craufurd, P.Q., Kakani, V.J., Wheeler, T.R. and Boote, K.J. (2001). Influence of high temperature during pre- and post-anthesis stages of floral development on fruit set and pollen germination in peanut. *Australian Journal of Plant Physiology*. **28**, 233-240.

Vasil, I.K. (1960a). Studies on pollen germination of certain Cucurbitaceae. *American Journal of Botany*. **47**(4), 239-247.

Vasil, I.K. (1960b). Pollen germination in some Graminae: *Pennisetum typhoideum*. *Nature*. **187**, 1134-1135.

Vasil, I.K. (1987). Physiology and culture of pollen. *International Review of Cytology*, **107**. Pollen, Cytology and Development (Edited by Jeon, K.W. and Friedlander M.). Academic Press. Pp 127-173.

Vassileva, R., Lidanski, T. and Tseckova, Z. (1991). Acetocarmine test and pollen viability in genus Helianthus. Comptes Rendus de L'Academie Bulgare des Sciences. **44**(1), 71-72.

Voyiatzis, D.G. and Paraskevopoulou-Paroussi, G. (2002). Factors affecting the quality and *in vitro* germination capacity of strawberry pollen. *Journal of Horticultural Science and Biotechnology*. **77**(2), 200-203.

Wagstaffe, A. and Battey, N.H. (2004). Overcoming the loss of Methyl Bromide with a competitive and sustainable soil-less strawberry production system. DEFRA, HortLINK Project Report 215.

Wagstaffe, A. and Battey, N.H. (2006a). The optimum temperature for long-season cropping in the everbearing strawberry 'Everest'. ISHS conference presentation. *Acta Horticulturae*. **708**, 45-49.

Wagstaffe, A. and Battey, N.H. (2006b). Characterization of the thermodormancy response in the everbearing strawberry 'Everest'. *Journal of Horticultural Science & Biotechnology.* **81**(6), 1086-1092.

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

Wheeler, M.A. and McComb, J.A. (2006). *In vitro* pollen viability and pollen storage in *Eucalyptus marginata* (Myrtaceae). *Australian Forestry*. **69**(1), 32-37.

Woodson, W.R. (2002). Pollination signals and flower senescence. In: Plant Reproduction. Annual plant reviews. Vol 6. (Edited by: O'Neill, S.D. and Roberts, J.A.). CRC Press, 2002. Pp 279-295.

Yanagi, T. and Oda, Y. (1990). Effects of chilling history on successive flowering and runner development of everbearing and non-everbearing strawberry cultivars. *Journal of the Japanese Society for Horticultural Science*. **59**(2), 357-363.

Yanagi, T. and Oda, Y. (1992). Effects of winter chilling and summer temperature on inflorescence and runner production during summer season in cultivated strawberries

(*Fragaria* x *ananassa* Duch). *Journal of the Japanese Society for Horticultural Science*. **60**(4), 889-895.

Yanagi, T. and Oda, Y. (1993). Effects of photoperiod and chilling on floral formation of intermediate types between June- and everbearing strawberries. *Acta Horticulturae*. **348**, 339-346.

Youmbi, E., Akoa, A. and Eteme, R.A. (2004). *In vitro* germination and effects of drying time on the preservation of *Canarium schweinfurthii* pollen. *Journal of Tropical Forest Science*. Forest Research Institute Malaysia, Kuala Lumpur. **16**(3), 357-362.

Žebrowska, J. (1995). The viability and storage of strawberry pollen. *Plant Breeding*. **114**, 469-470.

Žebrowska, J. (1998). Influence of pollination modes on yield components in strawberry *(Fragaria* x *ananassa* Duch.). *Plant Breeding*. **117**, 255-260.

© 2007 Horticultural Development Council

Appendices

Table I: Binomial distribution tests for pollen germination frequencies observed in 'Everest' and 'Diamante' as affected by three temperature treatments.

Treatment	Grains germinating	Observed frequency	Expected frequency	$(O - E)^2 / E$
22°C/15°C Everest				
	0	0	0.95	0.95
	1	0	0.31	0.31
	2	1	0.2	3.2
	3	3	0.069	124.5
	4 or 5	1	0.056	15.91
				$X^2 = 144.87$ (P<0.1)
22°C/15°C Diamante				
	0	0	0.00018	0.00018
	1	0	0.0043	0.0043
	2	0	0.039	0.039
	3	0	0.178	0.178
	4 or 5	5	0.406	51.1
				$X^2 = 52.203$ (P<0.1)
26°C/11°C Everest				
	0	0	0.0205	0.0205
	1	0	0.12	0.12
	2	3	0.283	26.08
	3	1	0.33	1.36
	4 or 5	1	0.195	3.32
				$X^2 = 30.9$ (P<0.1)
26°C/11°C Diamante				
	0	0	0.00032	0.00032
	1	0	0.0064	0.0064
	2	0	0.0512	0.0512
	3	1	0.205	3.08
	4 or 5	4	0.41	31.43
				$X^2 = 34.57$ (P<0.1)
30°C/19°C Everest				
	0	1	0.253	2.2
	1	2	0.4	6.4
	2	1	0.253	2.2
	3	1	0.08	10.6
	4 or 5	0	0.012	0.012
				$X^2 = 21.41$ (P<0.1)
30°C/19°C Diamante				
	0	2	0.084	43.7
	1	0	0.27	0.27
	2	1	0.345	1.24
	3	1	0.22	2.76
	4 or 5	1	0.07	12.35
				$X^2 = 60.32$ (P<0.1)

5 classes of germinating pollen grains were set for testing the binomial distribution fit. Zero to 4 or 5 grains germinating out of a total of 5 (this is why the observed frequencies always add up to 5). The frequencies are taken from all the measured dates of each treatment. The percentage of germination observed for each measured date was taken and divided by 10; for example a germination rate of 80.5 % = 8.05 grains germinating out of 10 = 4.025 (≈ 4) grains germinating out of 5. The total number of grains '5' has been used in order to avoid very large factorial terms (5! = 120). The degrees of freedom for any binomial distribution dataset are the number of classes minus 2; thus here they are 3. The observed X^2 values were compared to the P-value of the Chi-squared distribution at the 99.9% confidence level for 3 d.f., which is 16.3.

© 2007 Horticultural Development Council

The density of the grains within the culture sample was critical, as our results showed that there was a significant population effect within the culture samples. Pollen grains of many species exhibit a population effect, with larger populations tending to germinate better due to the presence of a pollen growth factor (Shivanna and Rangaswamy, 1992). Consequently, a population effect should be expected which means that the total population sizes within the sample cultures need to be large enough so as to have adequate germination, as provided in the current study.

Table 2: 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	0.00	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.

Table 3: Weekly flower-to-fruit conversion percentages (%) in 'Everest' and 'Diamante' as affected by temperature.

© 2007 Horticultural Development Council

	Everest		Diamante	
Date*	22°C	28°C	22°C	28°C
05/07/2007	78.05	76.32	50.00	75.00
16/07/2007	45.07	50.00	47.37	32.00
27/07/2007	78.79	75.93	60.87	70.83
03/08/2007	70.00	100.00	66.67	100.00
08/08/2007	100.00	87.50	78.57	60.00
15/08/2007	23.53	57.14	34.62	0.00
22/08/2007	100.00	0.00	89.47	17.65
29/08/2007	25.00	0.00	15.38	0.00
05/09/2007	23.53	17.39	19.05	0.00
20/09/2007	17.54	16.16	33.33	54.55
28/09/2007	85.29	66.67	42.86	53.33

* The dates given are those when flowers were recorded. The number of flowers counted at each of the above dates was averaged and then the number of those which became fruits was also averaged and a percentage of conversion was calculated.

© 2007 Horticultural Development Council