Using the Floral Status of Strawberry Plants, as Determined by Stereomicroscopy and Scanning Electron Microscopy, to Survey the Phenology of Commercial Crops

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Abstract. **Field surveys were conducted on cultivated strawberries (*Fragaria* ×*ananassa* Duch.) to determine the time of flower initiation and its relation to maximum and minimum temperatures and daylength. Stereomicroscopy and scanning electron microscopy (SEM) were compared. Flower initiation in 'Torrey' strawberry was more dependent on minimum temperature than on daylength or maximum temperature. Flower initiation in the day-neutral 'Aptos' occurred regardless of daylength or temperature during sampling. For the study of flower initiation and inflorescence development, SEM gave more detail than stereomicroscopy.**

The transition from vegetative growth to flowering is one of the most important periods of ontogenesis. It requires the structural and physiological preparation of the entire plant and occurs at a definite time of the year and under definite conditions (Aksennova et al., 1980). Scanning electron microscopy (SEM) is a well established technique which has allowed a considerable advancement in studies on floral morphogenesis. The development of flowers of a number of tropical and subtropical fruit and nut species (Moncur, 1988) has been documented using this technique. The use of SEM to study flower development in strawberry has not been reported.

Studies in controlled environments have shown that flowering in strawberry in daylength responsive lines can be induced either by short days (Guttridge, 1969; Ito and Saito, 1962) or low temperature (Guttridge, 1985; Heide, 1977), with a strong interaction between daylength and temperature (Durner et al., 1984; Went, 1957). Flower induction in day-neutral types can be modified by temperature (Durner et al., 1984; Himelrick, 1985). Flower initiation and/or flower development of strawberry in the field has been studied using a microscopic dissecting technique (Durner and Poling, 1985; Jahn and Dana, 1970).

Two surveys in the commercial growing area around Sydney, Australia, were conducted to study the correlation between daylength and temperature, and flower initiation of short-day and day-neutral types. Stereomicroscopy and SEM were used and compared to study flower initiation and development. This study aimed to obtain information on the time of flower initiation in field grown plants of both strawberry types.

Materials and Methods

Greenhouse studies. Plants were grown from fresh runners of the short-day cultivar Torrey. On 24 July 1990, runners were transplanted into pots (15 cm depth and 14 cm width) containing a potting mix consisting of 1 peat : 1 coarse sand : 1 loamy sand soil (by volume). A complete fertilizer was also mixed with the soil. Plants were grown for 1 month under natural light at 21/16 °C (day/night temperature, 12 h each) at the Darlington greenhouse, Univ. of Sydney. During this time plants were watered twice daily and fertilized with liquid fertilizer. At the end of August, plants were divided into six groups and grown at six different day/night temperatures (15/10, 18/13, 21/16, 24/19, 27/22, and 30/25 °C) under natural photoperiods. Six uniform plants were sampled periodically between the end of September and the middle of November 1990. This was to ensure that apices at stages from vegetative to floral initiation to floral development were sampled.

Field studies. Farmers in New South Wales, Australia, usually plant cold stored runners from January to the end of February. Short-day 'Torrey' was planted in February 1988 at Tahmoor, Australia, ≈107 km southwest of Sydney (34.13S, 150.54E, 168 m above sea level). Ten crowns each of 1-year-old and newly planted cold-stored runners were sampled at 2-week intervals for 1 year starting in February. The day-neutral 'Aptos' and the short-day 'Pajaro' were planted in March 1989 at Kellyville ≈30 km northwest of Sydney (33.43S, 150.57E, 60 m above sea level). Ten crowns each of both cultivars were sampled at 2-week intervals from summer to the end of fall. The samples were dissected under a Wild Heerbrugg M5 stereomicroscope at a magnification of 6 to 50× and the percentages of floral apices of first- and second-year plants were recorded. The climatic data at the Tahmoor site was obtained from the Bureau of Meteorology. A portable weather station (Environdata Mark 3) was set up at the Kellyville site and maximum and minimum temperatures were recorded daily during the sampling period. Daylengths include civil twilight, and were obtained from the Sydney Observatory.

Examination of flowering. Apices were examined under a Wild Heerbrugg M5 stereomicroscope (6 to 50× magnification). For SEM, apices were fixed in 1% glutaraldehyde in 0.1 M phosphate buffer, pH 6.8, by adding fixative during dissection and then exposing to fixative for 2 h, during which time they were continuously rotated. The apices were then washed with three changes of 0.1 M phosphate buffer pH 6.8. Postfixation was with 1% osmium tetroxide in phosphate buffer pH 6.8 for 1 h. After washing in three changes of distilled water and dehydration using a graded ethanol series (30% to 100%), apices were critical-point-dried through carbon dioxide and stored in a desiccator for 2 d before being mounted on aluminium SEM stubs. The stubs were

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dagged with dag solution 154. The apices were examined and photographed using a Jeol JSM 35C scanning electron microscope operating at an accelerating voltage of 15 kV coupled with Robinson backscattered electrons (Faulk, 1980)

Correlation analysis was used to determine the relationship between climatic data and plant measurements. However, to avoid carry over effects of previous initiation in the newly planted cold-stored runners of 'Torrey', results in the first month and a half were not used in the correlation analysis for the first-year plants at Tahmoor.

Results

Determination of the flower or vegetative status by stereo-microscopy and SEM. Apices without pronounced swelling were considered vegetative (Fig. 1A). The first evidence of flower initiation appeared as a mounding of the apex (Fig. 1B). Signs of the start of sepal development on primary flower primordium were then seen (Fig. 1C). As sepals grew, petal initiation was noted (Fig. 1D). The sepals began to enclose the bud at about the time the stamens began to develop. Then the epidermal hairs developed and began to cover the flowers. At about this time pistil differentiation began (Fig. 1 E and F). The SEM technique showed most detail, however, the stereo microscope technique was quicker and was more appropriate for determining flower initiation in strawberry plants grown in the field.

Response to greenhouse temperature. The average percentage of initiated apices from the greenhouse grown plants was 79.0% at 15/10 °C, 82.5% at 18/13 °C, 45.5% at 21/16 °C, 15.1% at 24/19 °C and 13.7% at 30/25 °C. The percentage initiated at any given temperature did not show any clear trend over the 6-week sampling period. Thus initiation was high at the lower temperatures, low at the higher temperatures, and fell sharply with increasing temperature between 18/13 and 24/19 °C.

Field surveys. Lowest mean daily maximum and minimum temperatures were 17.4 and 1.5 °C, respectively, in July at Tahmoor (Fig. 2). The shortest day was 10.8 h (including civil twilight) on 21 June. The highest mean daily maximum and minimum temperatures, 30.2 and 17.3 °C, were in November and December, respectively. The longest day was 15.4 h on 22 Dec.

All first-year 'Torrey' plants at Tahmoor were found to be floral on 27 Feb. (Fig. 3). The percentage of apices in the floral state then dropped but started to increase again on 7 May when the daylength was 11.5 h and maximum and minimum temperatures were 23.1 and 12.6 °C, respectively. The percentage floral apices continued to be high until the 19 Nov. when the daylength was 14.9 h and maximum and minimum temperatures were 23.5 and 11.7 °C. In second-year plants, the percentage of floral apices increased sharply on 23 Apr. when daylength and maximum and minimum temperatures were 11.8 h, 23.1, and 12.5 °C, respectively. However, the percentage of second-year plants induced to flower dropped in September, but increased again in October. The second-year plants were not well fertilized, and were not as vigorous as the first-year plants. Thus in the spring (September) in these plants there was severe competition for nutrients between flower initiation, and the vigorous growth of leaves and inflorescences, the latter responding to the after effects of winter chilling and the favorable growing temperatures. By October, the spring flush was less strong, and flower initiation resumed.

There was a fall in percentage induced in both first- and second-year plants in December to a low in January. Thus, 'Torrey' grown in the Tahmoor area was found to be induced from the middle of fall until early summer. In other words flowering was induced when the daylength was about or below 11.8 h and maximum and minimum temperatures were at or below 23.1 and 12.5 °C, respectively (17.8 °C on average). The percentage of apices that were florally induced began to fall when the daylength was 15 h or above and maximum and minimum temperatures were 26.0 and 13.8 °C (19.9 °C average), which was in December (Figs. 2 and 3).

The percentage of induced apices of 'Aptos' taken from Kellyville did not correlate with the maximum and minimum temperatures or daylength (Fig. 4). Whereas, the percentage of induced apices of 'Pajaro' increased sharply

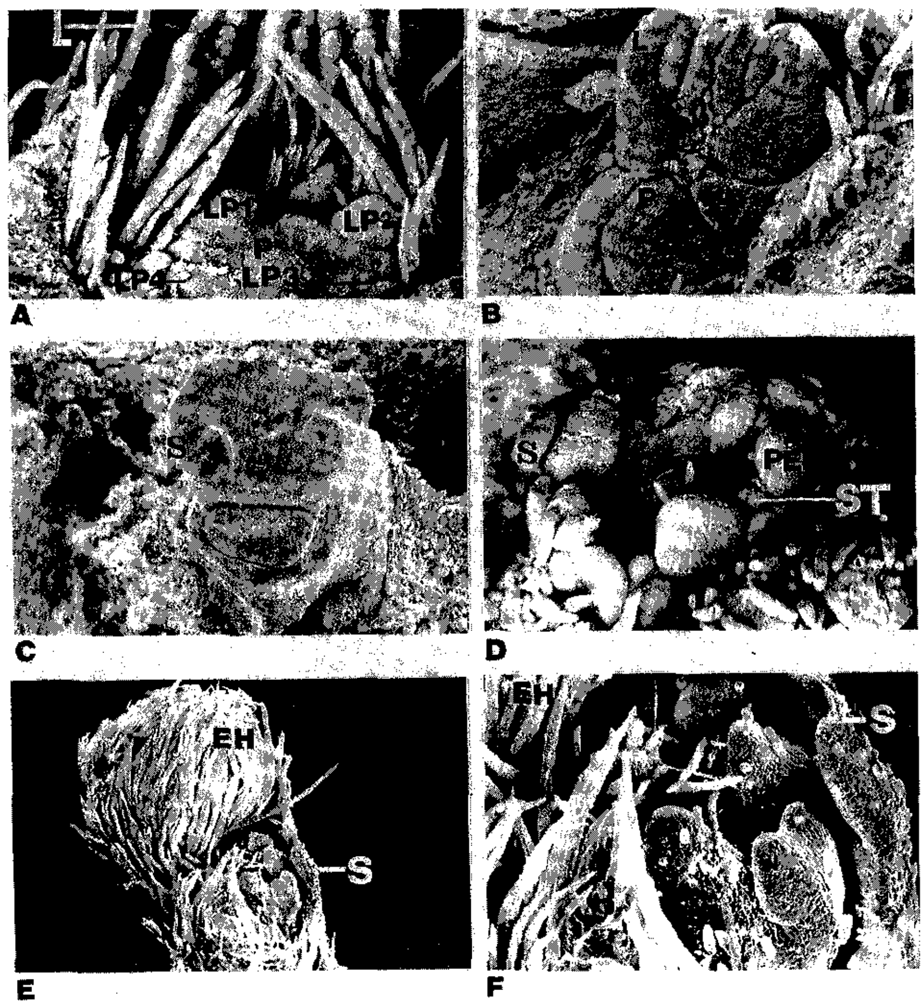


Fig. 1. Flower development in strawberry observed using scanning electron microscopy. (**A**) Vegetative apex showing the last trifoliolate (L), nonswelling apex (primordium) (P), and the 2/5 spiral arrangement of leaf primordia (LP). (**B**) Initial phase of change from vegetative to reproductive stage showing mounding of the apex and last young trifoliolate leaf (L). (**C**) Early stage of sepal (S) development showing the start of sepal initiation. (**D**) The development of sepals (S), petals (PE), and stamen (ST). This is about the time of pistil differentiation. (**E–F**) Later stages of flower development showing the elongation of sepals (S) and epidermal hairs (EH).

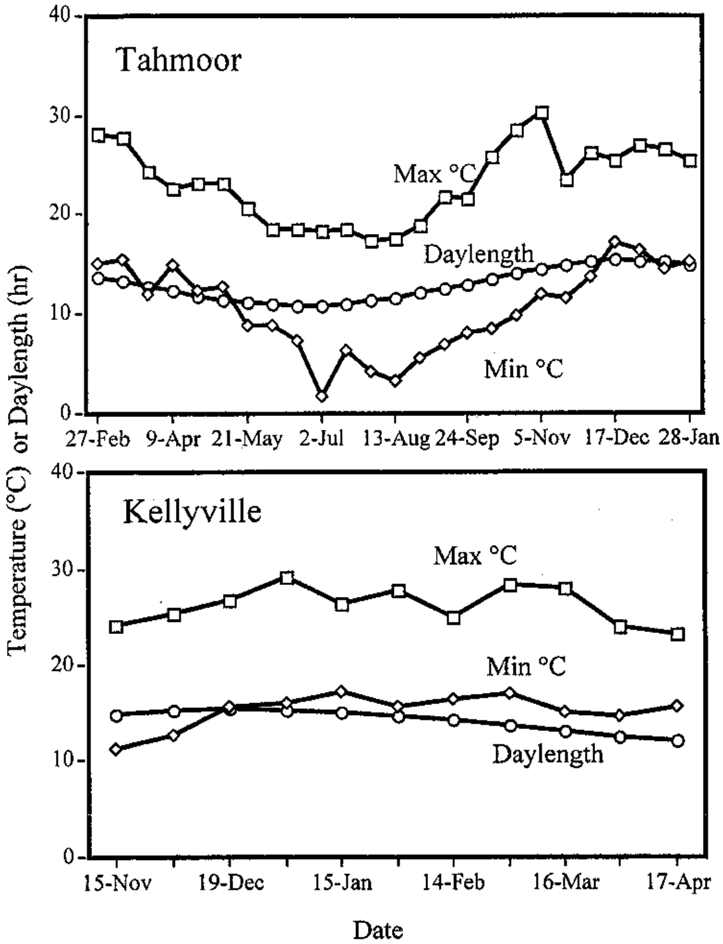


Fig. 2. The mean daily maximum and minimum temperatures and daylength at fortnightly intervals from 14 Feb. 1988 to 11 Feb. 1989 at Tahmoor, and from 1 Nov. 1989 to 2 May 1990 recorded in the field at Kellyville, Australia. The date is the starting date of the fortnight.

on 2 Apr. 1990 when the daylength had fallen to 12.5 h and maximum and minimum temperatures were 23.9 and 14.7 °C, respectively (19.3 °C average).

CORRELATION BETWEEN FLOWER INDUCTION AND CLIMATIC DATA. The percentage induced plants of 'Torrey' was negatively correlated with temperature and daylength (Table 1). Minimum temperatures showed the highest correlation with percentage induction of first- and second-year plants, followed by daylength and maximum temperature, respectively. Multiple regression analysis showed highly significant differences ($P < 0.001$), when daylength and mean daily minimum temperature were used as independent variables, and percentage induction of first-year plants as the dependent variable. Furthermore, mean daily minimum temperature was highly significant ($P < 0.001$) while daylength was not statistically significant ($P = 0.45$). In contrast, when considering the induction of second-year plants, although the multiple regression was significant ($P < 0.05$), the mean daily minimum temperature and daylength were not significant ($P = 0.26$ and 0.43, respectively). Therefore, the percentage induction of first-year plants is more dependent on the minimum temperature than on the daylength. On the other hand, the percentage induction of second-year plants is likely to be an additive effect of the minimum temperature and daylength, with the minimum temperature having the greater effect. However the percentage induction of second-year plants was positively correlated with percentage induction of the first-year plants.

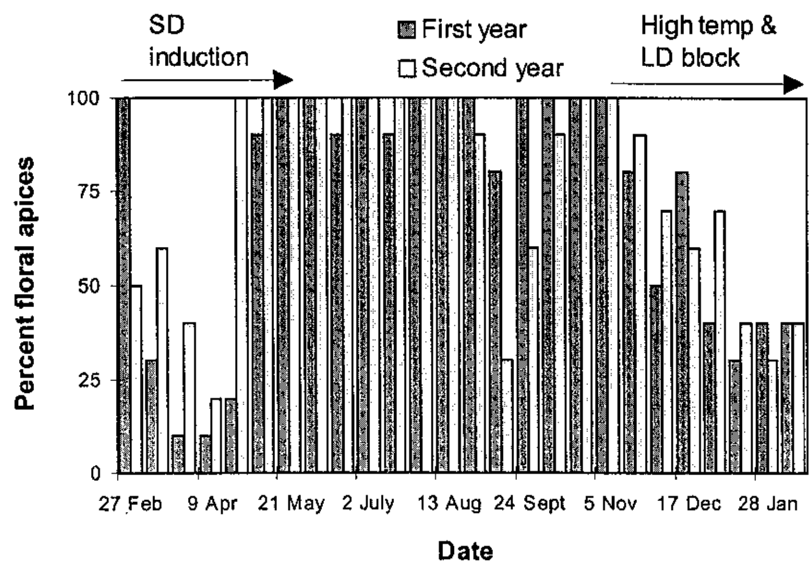
The percentage induced plants of 'Aptos' was not correlated with temperature or daylength (Table 2). The percentage induced plants of 'Pajaro' was highly negatively correlated with daylength and maximum temperature respectively. However, there was no correlation between the percentage induced plants of 'Pajaro' and the minimum temperature.

Discussion

THE INITIATION AND DEVELOPMENT OF THE STRAWBERRY FLOWER. SEM enables the demonstration of the detailed changes associated with flower development in strawberry much more effectively than stereomicroscopy. This is due to the advanced technique of sample preparation and the higher magnifications possible in SEM (Moncur, 1981). Using light microscopy Jahn and Dana (1970) and Durner and Poling (1985) saw only the last trifoliolate leaf and apices without pronounced swellings at the vegetative stage. Using the SEM the 2/5 spiral arrangement of the leaf primordia can be seen, as Arney (1953) found using a microtome technique. Enlargement and elongation of the apex is the first evidence of flower initiation after induction (Fig. lB) and is much more clearly seen using SEM than stereo microscopy. The last trifoliolate leaf is also very noticeable. In the more advanced stage (Fig. lD) as sepals grow and petals are initiated and develop, we can clearly see the difference between these two organs. Stamen initiation can also be seen. The last stages of flower development are shown by SEM (Fig. 2 E and F). They are poorly visible in light microscopy. However, the stereomicroscope dissecting technique is more appropriate to determine flower initiation of strawberry plants grown in the field. Apices at the stages shown in Figs. 1 C and D were considered floral when using the stereomicroscope technique (Durner and Poling, 1985).

RESPONSE TO TEMPERATURE AND DAYLENGTH. The sensitivity of flower induction in short-day strawberries to temperature is well

Fig. 3. Percentage of induced apices of first- and second-year 'Torrey' strawberry plants grown in the field at Tahmoor, Australia, and dissected each fortnight.



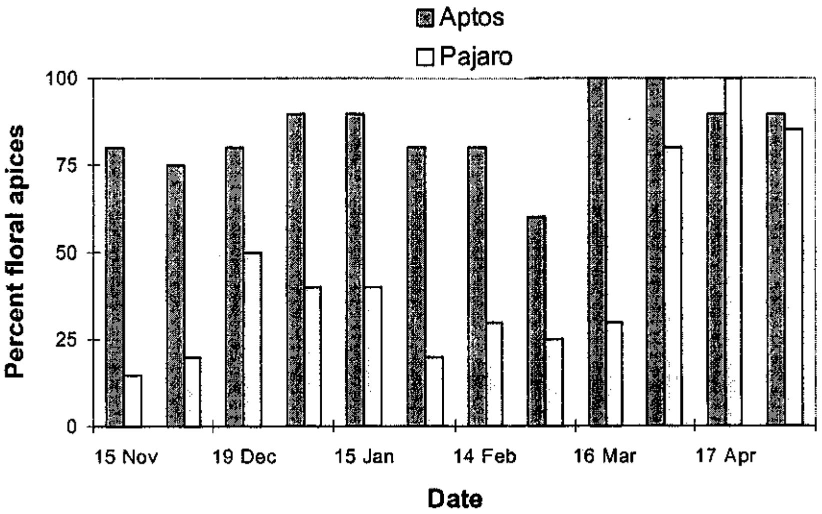


Fig. 4. Percentage of floral apices of 'Aptos' and 'Pajaro' strawberry plants grown in the field at Kellyville, Australia, and dissected every 2 weeks from 15 Nov. 1989 to 2 May 1990.

established (e.g., Guttridge, 1985). The greenhouse study on 'Torrey' conducted in October and early November (natural daylengths 13.30 to 14.80 HR) showed a response to temperature in agreement with previous work, with a fall in initiated apices with increased growing temperatures over the range 18/13 to 24/19 °C day/night temperature. These night temperatures are in agreement with the critical temperatures found by Heide (1977).

The survey at Tahmoor indicated that flower induction in the short-day 'Torrey' occurred in the field from mid-fall to early summer. The induction began when the daylength was 11.8 h and maximum and minimum temperatures were 23.1 and 12.5 or 17.8 °C on average. The percentage induced fell when the daylength was >14.9 h and maximum and minimum temperatures were 26.0 and 13.8 or 19.9 °C on average. The percentages of first- and second-year plants induced were more strongly correlated to minimum temperature than to daylength, and the critical minimal mean fortnightly temperature for maximal flower induction (12.5 to 13.8 °C) was in agreement with the minimum night temperature found in the greenhouse trial to give maximal flower induction (13 °C). There was a positive correlation between the percentage of induced apices on first- and second-year plants.

That short days and/or low temperatures induce flowering in short-day type strawberry cultivars is well documented (Guttridge, 1969, 1985; Heide, 1977). Flower induction occurs when daylength falls below a threshold value of ≈14 h (Darrow and Waldo, 1934) and/or temperature between 12 and 18 °C (Heide, 1977). However, the response of flower induction to the photoperiod × temperature interaction is cultivar-dependent (Darrow, 1966; Heide, 1977). The field observations here indicate that the critical daylength for flower induction in 'Torrey' is 14.9 h with minimum mean temperatures of 12.5 to 13.8 °C. Went (1957) found that the lower the temperature the longer the daylength at which flowers can be induced (at 14, 10, and 6 °C, inductive daylengths are 8, 16, and 24 h respectively).

Flower induction in short-day types is more strongly correlated to the minimum temperature than to the maximum or average temperature. The initial morphological changes leading to flowering in the buds of olive are associated with the number of hours that the olives are exposed to low temperatures (Hackett and Hartmann, 1963). Thus, minimum temperatures that usually occur at night could possibly determine flower induction in 'Torrey'. The positive correlation between percentage of induced first- and second-year plants indicates that the pattern of flower induction in this cultivar generally follows the same trend regardless of plant age. The slightly earlier induction of second-year plants in the mid-fall is probably due to the effect of fruit load on the first-year plants. The first-year plants were bearing a heavy fruit load resulting from flower initiation from mid to the end of March. In strawberry, the growth reducing effects of fruiting are pronounced. Effects of crop load on quality characteristics have been found by Jahn and Dana (1966) and Lenz and Bunemann (1967). Hence, the reduction or delay in vegetative plant growth could in turn delay flower induction.

Unlike the short-day cultivars, flower induction in the day-neutral 'Aptos' at Kellyville was not correlated with either temperature or daylength. However, induction in 'Pajaro' (short-day type) was more negatively correlated with daylength than maximum temperature, and did not show any correlation with minimum temperature. Flower induction in day-neutral types is independent of daylength, but can be modified by temperature. At temperatures of 26/22 °C and above they become longday types, but give poor quality flowers and fruit (Himelrick, 1985). Since the minimum temperature over the sampling period did not exceed 22 °C (between 10.7 and 14.2 or 12.4 °C on average) and the average maximum temperature did not exceed 26 °C (between 22.0 and 29.0 or 25.5 °C on average), the effect of temperature was only examined over a small range. Hence we did not find any correlation between flower induction and climate. Furthermore, the minimum temperature did not show any correlation with flower induction in 'Pajaro' as would have been expected following the results with 'Torrey' at the Tahmoor site. This was because of the different periods of sampling. At Tahmoor, we took samples for 1 year while at Kellyville it was only the warmer 6 months, November to April. Hence, the effect of low temperatures, which play a major role in the induction of flowering from mid-fall to early summer, was absent. However, flower

Table 1. Correlation coefficients for the relation between percentage of apices floral of first- and second-year plants of 'Torrey' strawberry and daylength, maximum and minimum temperature.

	Percentage floral	
	First-year plants	Second-year plants
Daylength (h)	0.488*	0.521*
Maximum temperature (°C)	0.423*	0.377
Minimum temperature (°C)	0.704***	0.660***
First-year plants induced (%)	---	0.660***

*,**,***Significant at $P = 0.05$, 0.01, and 0.001, respectively.

Table 2. The correlation between percentage of apices floral of 'Aptos' and 'Pajaro' strawberry plants and daylength and maximum and minimum temperature.

	Percentage floral	
	Aptos	Pajaro
Daylength (h)	0.399	0.744**
Max temperature (°C)	0.247	0.634*
Min temperature (°C)	0.097	0.096

*,**,***Significant at $P = 0.05$, 0.01, and 0.001, respectively.

induction in this cultivar did start in mid-fall when the daylength was short (12.5 h) and average minimum temperature was 14.6 °C, which is in approximate agreement with 'Torrey' at the Tahmoor site (11.8 h, 12.5 °C).

These results lead to the conclusion that flower initiation of the strawberry 'Torrey' is very responsive to temperature, daylength and also their interactions. However, the correlation was stronger between flower initiation and minimum temperature than with daylength or maximum temperature. The critical minimum temperature and daylength for the summer repression of flower initiation in 'Torrey' grown in the Sydney region is 13.8 °C at 14.9 h. Hence flower initiation of this cultivar stops in early summer and resumes in mid-fall. Flower initiation in 'Aptos' occurred regardless of daylength and temperature during the period of sampling. To recommend production procedures for maximum off season production in the field, we need a precise knowledge of the temperature and daylength effects on flower development, inflorescence emergence and also fruit set. The effect of plant vigor due to fertilization, which affects nutrition levels of the plant and probably affects flower induction, also needs to be further investigated.

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