Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Effect of temperature on plant growth and yield in ever-bearing strawberry *Fragaria* x *ananassa,* cv. Florentina

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Degree Project • 30 hec
Horticultural Science – Master's Programme
Alnarp 2020

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Inverkan av temperatur på tillväxt och skörd hos remonterande jordgubb *Fragaria x ananassa*, sort Florentina

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Credits: 30 hec
Level: A2E, master's thesis
Course title: Independent project in Horticultural Science, A2E- Horticultural Science – Master's programme
Course code: EX0948
Programme: Horticultural Science – Master's programme

Place of publication: Alnarp
Year of publication: 2020
Cover Art: Denis Butare

Keywords: Strawberry, ever-bearing, temperature, flower mapping, photoperiod, flowering, plant development, yield.

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Acknowledgements

I would like to acknowledge the support and guidance of my main supervisor Samia Samad, who carried out the flower mapping and modified the key reference used in this thesis. Thank you for providing with scientific knowledge and training in this field.

Furthermore, I would like to thank my co-supervisors Sammar Khalil and Salla Marttila for their guidance and mentorship through this training period.

I also convey my gratitude to the Swedish Institute that has facilitated my studies through Swedish Institute scholarship program.

Finally, my appreciation to my family and friends, for encouraging me and being there for me throughout the entire process.

Abstract

Strawberry (*Fragaria* x *ananassa* Duch.) growth and yield are under genetic control and regulated by environmental factors such as temperature and photoperiod. The major objective of this study was to determine the effects of temperature under short-day treatment on plant development and flowering in the ever-bearing strawberry cv. Florentina in the greenhouse. Plants from the strawberry cv. Florentina were subjected to three temperature treatments 20°C, 25°C and 30°C under short-day conditions. Flowering was enhanced at low temperature of 20°C. The highest number of fruits occurred at 20°C and the lowest at 30°C. The largest fruit size was obtained from plants at 20°C while there was no significant difference between 25°C and 30°C. Vegetative growth was enhanced under high temperatures. At intermediate temperature 25°C, plants achieved the highest height while at 30°C they had the highest number of leaves. There was no significant difference in fresh and dry weight between 25°C and 30°C but significantly higher than at 20°C. Few runners were produced under 25°C and 30°C but none at 20°C. Growing Florentina at 20°C was better with respect to yield than the other temperature treatments tested in this study.

Popular science summary

Strawberry fruits are consumed fresh, in desserts and as juice. Consumption of strawberries is increasing due to their nutritional benefits. This has led the growers to produce more strawberries for the market. Production of strawberries is controlled by environmental conditions such as light and temperature. When these conditions are suitable, the strawberry plant flowers that is fertilized to form fruits. On the other hand, if the conditions are not suitable, flowering will not occur and thus no fruits. Understanding the effects of these conditions on strawberries would provide insight into maximizing the production. Strawberry production occurs in different geographical locations around the world. These locations have diverse environmental conditions that affect flowering in the strawberry plants. Strawberries are grouped according to their flowering response to light conditions. June-bearing strawberries produce flowers in short day conditions and usually produce a single harvest in a season. Everbearing strawberries flower frequently and produce more than one harvest. This means that these varieties can extend the growing season and thus, there is a growing interest to study the behaviour of these varieties. Temperature is one of the factors that affect the behaviour of these varieties. The growth of some ever-bearing varieties tends to be more vegetative than reproductive, which reduces the production of flowers and, hence affecting the yield. It is thus of interest to study the behaviour of the ever-bearing varieties under different temperature conditions. The study results indicated that strawberries were more vegetative at high temperatures with a lower yield. On the other hand, plants under lower temperature produced high yield and less were less vegetative.

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1. Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) is one of the widely consumed fruits around the world, whereby 8 million tonnes was globally produced, and European countries contributed 20.5% in 2017 (FAOSTAT, 2020). Strawberry production in Sweden has been rising steadily since the strawberry is a popular fruit and a part of summer. In 2019, the strawberry crop was cultivated on a total area of about 2,400 hectares producing 16,000 tonnes (Jordbruksverket., 2019). Strawberry production is affected by a range of factors such as altitude, day length, temperature and other factors (Kadir *et al.*, 2006; Palencia *et al.*, 2013). Cultivation in the northern climates such as the Scandinavian region, lasts from June to August due to harsh weather conditions. Extending the growing season has become possible by cultivation in tunnels (Sønsteby and Karhu, 2005). However, there is a challenge that some ever-bearing cultivars tend to invest more energy in vegetative than generative growth when grown in tunnels, thus affecting the yield (Sønsteby and Heide, 2009). Ever-bearing cultivars have a frequent flowering habit (Brown and Wareing, 1965). Vegetative and generative growth are oppositely influenced by temperature and/or photoperiod (Hytönen and Elomaa, 2011). However, temperature overrides the photoperiodic effect on strawberry (Hartmann, 1947). Temperature is a significant factor influencing most of plant growth processes such as photosynthesis, respiration and flowering (Badeck *et al.*, 2004). Strawberry growth and yield is also determined by dry matter accumulation in different parts of the plant (Pérez de Camacaro *et al.*, 2002). Thus, the effects of temperature on ever-bearing strawberry cultivar would give insight into the plant growth behaviour.

1.1. Objectives

The overall objective of the study is to understand the growth behaviour and yield of ever-bearing strawberry cultivar under different temperature regimes. The following research questions were formulated;

1- How do different temperature regimes affect vegetative growth and flowering behaviour of ever-bearing strawberry cultivars?

2- Can the flower mapping technique be used as a tool to estimate the yield of ever-bearing cultivars?

1.2. Limitations

The practical work of this thesis is restricted to one ever-bearing cultivar of *Fragaria* x *ananassa* Florentina. This cultivar was used to conduct flower mapping and to study the growth behaviour of an ever-bearing cultivar under different temperature regimes. Flower mapping data was collected on only one timepoint. Potential effects of pests and pathogens were not included in this study.

2. Literature review

2.1. The strawberry

Strawberry (*Fragaria* × *ananassa* Duch.) is a herbaceous perennial species that belongs to the genus *Fragaria* and is a member of the family Rosaceae. The family includes over 100 genera and about 3100 species. The family contains economically important edible fruits such as apple, blackberry, raspberry and apricots (Hummer and Janick, 2009). The garden cultivated strawberry originated from France in the 17th century. The origin was first documented by the botanist Antoine Nicholas Duchesne after several years of research on strawberry classification and history. He published his results in the book *Histoire naturalle des fraises* in 1766. Duchesne concluded that the modern cultivated strawberry is a hybrid between *Fragaria chilonensis* and *Fragaria virginiana.* The parental species, *F. virginiana* and *F. chiloensis* are of North and South American origin (Darrow, 1966).

2.2. Plant morphology

The strawberry has a rosette "crown" as its main stem with short internodes. The leaves are trifoliate and are spirally attached to the stem axis (Darrow, 1966; Savini *et al.*, 2005). Between the crown and each leaf, there is an axillary meristem (bud). The axillary buds can either remain dormant or grow into branch crowns or stolons (Heide *et al.*, 2013). A branch crown is a side stem originating from the leaf axil. Stolon (runner) is a stem with two long internodes terminating with a daughter plant at the second internode (Darrow, 1966; Savini *et al.*, 2005). The inflorescence develops on the terminal point of the crown (fig 1). The inflorescence on the terminal point of the crown is the primary flower. Branches emerge from the crown producing secondary flowers to which further lateral branches develop producing tertiary flowers (Darrow, 1929). The sepal encloses the flower at a bud stage. The stamens are the male part that delivers the pollen to the pistil, the female organs. The pistils are borne on the dome-shaped receptacle. The main parts of the strawberry flower are shown in figure 2.

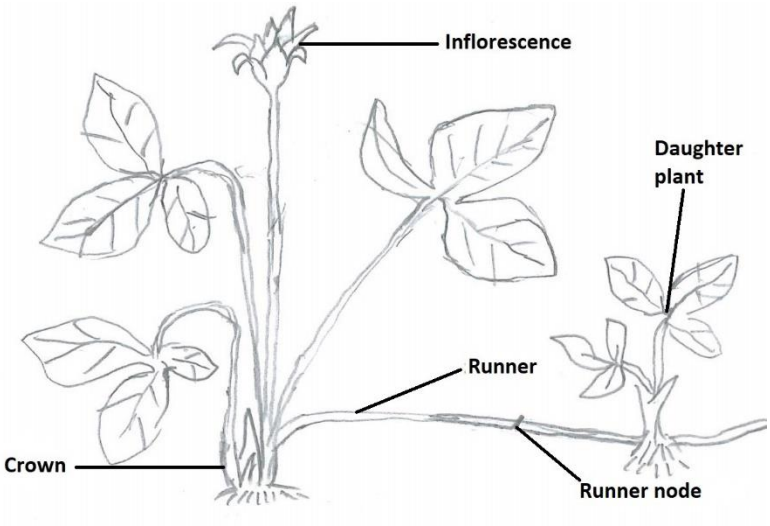

Figure 1. Strawberry plant.

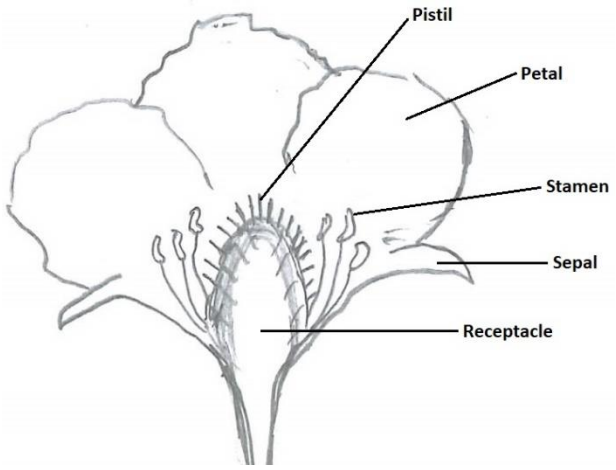

Figure 2. Principal parts of the strawberry flower.

2.3. Flowering habit and development

Flowering is a vital development stage in the life cycle of the plant associated with fruit production. The flowering process is defined in three main stages of development: floral induction, initiation and differentiation (Taylor, 2002). Floral induction is the process that involves the activation of flowering genes in the leaves triggered by environmental cues producing a signal that is then translocated to the shoot apical meristem (SAM) to initiate a transition to reproductive growth. SAM is a group of expanding cells located on the shoot tip (Aukerman and Amasino, 1998). Taylor *et al.* (1997) described that flower initiation is observed by the expansion and cell differentiation of the SAM. The SAM develops into the primary flower and initial bracts. The sepals appear first then followed by petals, stamens and carpels. Floral

differentiation is the development of flower organs, flowers and inflorescence in the bud (Galletta and Bringhurst, 1990).

2.3.1. Environmental factors regulating growth

Photoperiod

One of the major environmental factors affecting growth and flowering in plants is the photoperiod, the daily daylength. Garner and Allard (1920) were the first to discover how the photoperiod influenced the growth and reproductive stages in plants. They found out that flowering can be induced in some plants only in certain limits of the day length. Plants are categorized based on their flowering response to photoperiod. Short-day (SD) plants are plants that require a photoperiod that is shorter than certain daylength to initiate flowers. Long-day plants (LD) are plants that require a longer photoperiod to initiate flowers (Thomas and Vince-Prue, 1997). Day-neutral are plants that initiate flowers regardless of the photoperiod (Bringhurst and Voth, 1980). SD and LD plants are further categorized into two types based on their specific response to photoperiod; Obligate (qualitative) and facultative (quantitative) types (Thomas and Vince-Prue, 1997). Obligate types require a specific photoperiod to initiate flowering. On the other hand, in the facultative types, flower initiation is accelerated by a particular photoperiod.

Strawberry vegetative growth is also strongly influenced by photoperiod. Vegetative growth expressed as stolon growth, leaf petiole and leaf size are enhanced when there is an increase in photoperiod (Darrow and Waldo, 1934), followed with shorter and small leaves in late summer and autumn (Darrow, 1966). Heide (1977) studied the photoperiod effect on four SD cultivars Senga Sengana, Zefyr, Jonsok and Glima. The vegetative characteristics such as petiole length, stolons and leaf area were enhanced as photoperiod increased from 10 to 16 hrs. However, the photoperiod for enhancing vegetative growth varies with cultivar.

Photoperiod x **Temperature interaction**

Strawberry cultivars are classified based on their flowering response to photoperiod. Several studies have demonstrated that there is also a strong interaction of temperature and photoperiod in regulating strawberry flower induction (Ito and Saito, 1962; Heide, 1977). High air temperatures promote respiration rate in warm regions and cause the inhibition of plant growth and fruit formation due to reduced photosynthesis (Hancock, 1999).

Flower induction varies with cultivar and is temperature-photoperiod dependent (Stewart and Folta, 2010). A study by Ito and Saito (1962) demonstrated that flower initiation was induced in SD cv. Robinson under both SD and LD conditions at 9°C. At a high temperature of 30°C, Robinson failed to initiate flowers. Nishiyama and Kanahama (2002) demonstrated that flowering was inhibited in ever-bearing cv. Summerberry at high temperatures (30°C/26°C) under SD conditions (8 hours). High temperatures reduce fruit size. Miura *et al.* (1994) demonstrated that the fruit size was smaller at 19°C than at 15°C. Similar results were shown by Kumakura and Shishido (1994) where the fruits were largest at 15°C as compared to 20°C and 25°C. Kumakura and Shishido (1994) reported that a high total dry matter accumulation was recorded at lower temperature 15°C compared to higher temperatures 20°C and 25°C.

2.3.2. June-bearing cultivars

Darrow and Waldo (1934) describe that June-bearing cultivars are short-day (SD) plants that initiate flower buds in the autumn when the photoperiod is short at low temperatures. June-bearers are also known as seasonal flowering genotypes, and are the most cultivated varieties in Europe. June-bearers initiate flowers in spring and give fruit in mid-summer (Brown and Wareing, 1965).

The flowering response to photoperiod varies between cultivars. For instance, the photoperiodic effect on flower initiation in June-bearers can be influenced by temperature interaction. Flower initiation is also induced under long days with low temperature (Taylor, 2002). Hancock (1999) reviewed flower induction in SD plants to occur in the range of 7-24 days of photoperiod depending on the temperature. Under high temperatures, more photoinductive periods are needed for floral induction. However, it is cultivar dependent.

2.3.3. Ever-bearing cultivars

Darrow and Waldo (1934) described ever-bearing cultivars as long-day plants that initiate flower buds under the long days of summer. LD plants flower when they are exposed to light for a period longer than critical daylength (Jarillo *et al.*, 2008). Darrow and Waldo (1934) defined LD cultivars as plants which require daylength that is more than 14 hours for flower initiation to occur. LD plants produce more than one harvest in a year (Massetani *et al.*, 2011). In contrast to June-bearing cultivars, ever-bearers initiate flowers and give fruits in spring, summer and autumn (Brown and Wareing, 1965). Although ever-bearing cultivars are termed as LD plants, they initiate flowers also under SD conditions but in few numbers compared to LD conditions (Guttridge, 1969). Nishiyama *et al.* (2006) demonstrated that ever-bearing cv. Summerberry initiated more flowers under LD conditions (15-16 hours) as compared to SD conditions (12 hours).

2.4. Generative growth

In June-bearing cultivars, generative growth is promoted under SD conditions and low temperatures. June-bearing cultivars are SD plants that also initiate flowers under LD conditions (Darrow, 1966). Many cultivars initiate flowers qualitatively under SD conditions at temperature 15°C and above, while lower temperatures the photoperiodic effect is reduced (Ito and Saito, 1962). However, the temperature and photoperiod vary between cultivars (Heide, 1977).

The environmental regulation of flowering in June-bearing cultivars has been well studied, whereas in ever-bearing cultivars this has been a challenge. These cultivars do not exhibit the true behaviour of LD plants (Stewart and Folta, 2010). Nishiyama and Kanahama (2002) demonstrated that flowering was inhibited in ever-bearing cv. Summerberry at high temperature (30°C/25°C) in SD conditions. Later this cultivar resumed flowering when subjected to 20°C/15°C under SD and LD conditions. Similar results were reported by Sønsteby and Heide (2007a) in ever-bearing cv. Elan. They concluded that ever-bearing cultivars, in general, behave as qualitative LD plants at high temperature (27°C) and quantitative LD plants at intermediate temperature (15°C-21°C).

2.5. Vegetative growth

Vegetative growth expressed as petiole length and leaf area is promoted under LD conditions at high temperatures (Heide *et al.*, 2013). Petiole length increased as photoperiod increased from 10 to 24 hours. Leaf area also increased in a similar manner from 10 to 16 hours (Heide, 1977). The leaf growth rate is related to vegetative growth but oppositely related to generative growth (Durner and Poling, 1987). The axillary meristems in the leaf axil can differentiate into branch crowns or runners (Heide *et al.*, 2013). Branch crowns formation occurs after runnering has ceased when the day length is shorter SD cultivars (Darrow and Waldo, 1934).

Runners are usually produced in long days (>10 hours) and in temperature ranges 12-26°C in SD cultivars (Smeets, 1956; Heide, 1977). According to Smeets (1956), runner formation was dependent on temperature. A high number of runners was produced at 26°C than 17°C. Similar results were reported by Heide (1977) with more runners at 24°C than at 18°C and 12°C. On the other hand, long-day stimulated runner formation. The number of runners increased as the photoperiod increased from 10 to 16 hours. A high number of runners was produced at 26°C than 17°C. Similar results were reported by Heide (1977) with more runners at 24°C than at 18°C and 12°C. Vegetative growth in ever-bearing cultivars is promoted at high temperatures. Sønsteby and Heide (2007b) reported that in LD cv. Elan, runner formation and the number of leaves were enhanced at 27°C compared to 15°C.

2.6. Flower mapping

Improving crop yield has been a focus of breeders and growers due to the increasing consumer demand of the strawberry fruit. Strawberry yield depends on a range of major environmental conditions such as temperature and photoperiod. Modifying these environmental conditions can modify the plant growth and yield potential by promoting or delaying flower induction. The yield potential is evaluated by dissecting plants to observe the number of potential flowers and their development stages (Massetani and Neri, 2016). Flower mapping is a method of dissecting plants under a microscope to identify the fate and position of the meristems (Durner, 2018). Dissection of the plants involves breaking down the plant into functional units. Each leaf is removed from the crown to identify the bud present in the leaf axil. The fate of the buds in the leaf axil is identified as either reproductive or vegetative (Massetani and Neri, 2016). A scale has been developed to describe the distinct features of the developing stages (Massetani *et al.*, 2011).

Scales developmental stage descriptions frequently referenced to include the studies by Jahn and Dana (1970), Taylor *et al.* (1997), Aspuria and Fujime (1995) and a recent one by Massetani and Neri (2016). However, the scaling and description of development stages vary between these authors. Jahn and Dana (1970), Aspuria and Fujime (1995), Taylor *et al.* (1997) and, Massetani and Neri (2016) describe the earliest stage before flower initiation as a vegetative apex. This stage is defined by a flat surface of the apex, whereas the subsequent stages are described differently. The last stage is characterised as a fully open flower (Taylor *et al.*, 1997). Aspuria and Fujime (1995) classified the earliest stage as stage 0 to stage 6 as the last stage. Massetani and Neri (2016) classified stages from 1 to 9.

3. Materials and methods

3.1. Plant material

Strawberry (A+ frigo) plants *Fragaria* x *ananassa* Duch. cv. Florentina were obtained from Flevoberry. Florentina is an ever-bearing variety, with traits such as firm texture, less bruise damage, excellent flavour and high yield. It was developed by Goossens Flevoplants's breeding programme (Flevoberry., 2020). Florentina selected due to its excellent traits such as good shelf life and continuous flowering.

3.2. Experimental setup

Plants were delivered on 18 December 2019 and stored in cold storage. Four plants were planted on 23 December 2019 in each 11 L plastic pots filled with peat substrate. The plants were grown in a greenhouse in three different chambers at temperatures of 20°C, 25°C and 30°C at Swedish University of Agricultural Sciences at Alnarp. In this study, the effect of temperature was investigated because temperature overrides photoperiod in most varieties. Plants were grown under short-day conditions. Plants in the temperature chamber at 20°C and those at 25°C had 6 hours of photoperiod while at 30°C the photoperiod was 9 hours. The short-day conditions were set to simulate the light patterns of autumn or winter of Sweden during short days. Plants were fertilized every week, starting from week 4. The fertilizer solution used was a mixture of Superba Red (14-4-21% NPK) and Calcinit (15% N, 19% Ca).

3.3. Data collection

Every 2-3 weeks morphological measurements were determined by recording plant height, the number of leaves and number of stolons. Plant height was measured by a ruler from the root to where most of the canopy formed. The units of measurement were in centimetres (cm).

The number of plants with newly formed flower buds was recorded each week. Ripe berries were harvested, and the number and the total weight of all berries, were recorded per week from each temperature chamber. Towards the end of the project, 20 fruits from each treatment were randomly selected to approximately measure the fruit size. The size (cm) was measured with a ruler from the pedicel to the apex of the fruit. Fruit size was measured as the length from the pedicel to the apex.

Sixteen plants from temperature treatment 30°C, 25°C and 20°C were harvested on 9, 14 and 20 April 2020. Plants were separated into shoot and root. The fresh weight (FW) was recorded. Thereafter, plants were dried at 90°C for 96 hours, and dry weight (DW) was recorded. The total dry matter (DM) (%) was calculated by the following formula;

$$DM = \frac{DW}{FW} \times 100$$

3.4. Flower mapping

Seven plants were randomly selected in week 6 from each treatment for flower mapping. Plants were dissected under a microscope (Olympus VMT 4F) to classify the meristems into 9 developmental stages using the reference scale (Table 1). The stages 1-3 were considered vegetative because there was no observable distinction under the microscope used. The following stages 4-9 show the distinctive features in floral organs development. Twelve plants were dissected and photographed using stereomicroscope Leica M165 FC.

Table 1. Reference scale for flower mapping

Meristem stage	Identification
3	Vegetative apex
4	Cup-shaped with flat carpel
5	Stamen taller than flat carpel
6	Colourless/ white stamen taller than slightly domed shaped carpel
7	Pistil starting to differentiate into individual stigma and visible small colourless petals.
8	Differentiated pistil taller than yellow stamen and white petals
9	Open flower

The statistical analysis was performed in Minitab. Graphs were made in Microsoft Excel. Plant height and number of leaves were subjected to ANOVA and Tukey's Pairwise comparison test. The number of flowering plants each week per temperature was subjected to Cross tabulation and fisher's Exact test. Differences were considered significant at $P<0.05$.

4. Results

4.1. Morphological measurements

The following line graphs indicate a linear increase in plant height and number of leaves over time in all temperature treatments. According to figure 3 and 4, the average plant height and number of leaves in 20°C treatment was the lowest among the treatments. Plant height in 30°C treatment was the greatest from week 5 to week 8, when it was surpassed by 25°C treatment. Plant height in 20°C treatment was the lowest among the treatments.

Plants at 30°C treatment had an average height of 9.86 cm per plant, which was significantly higher ($P<0.05$) than plants at 25°C (7.51cm) and 20°C (7.31cm) treatments in week 5. In week 8, there was no significant difference in height between plants under 30°C and 25°C treatment but were significantly higher ($P<0.05$) than the 20°C treatment. In week 10, 12 and 14, there were significant differences between the treatments, plants under 25°C treatment were significantly taller ($P<0.05$) than the rest of treatments. No significant difference in number of leaves was observed at 30°C and 25°C in week 5,12 and 14 whereas the number of leaves was significantly higher in week 8 and 10 (Table 2).

There were 5 and 7 runners at 25°C and 30°C, respectively but no runners in 20°C treatment (fig 5).

Figure 6 illustrates the percentage of dry matter was higher at 20°C compared to other treatments; however, there was no significant difference statistically between temperature treatments ($p>0.005$).

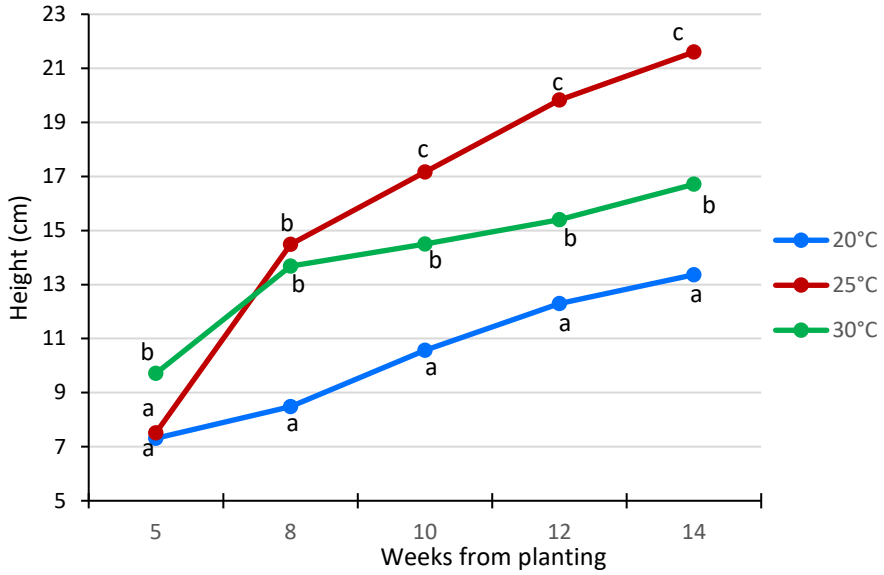

Figure 3. Average plant height (in cms) in three temperature treatments strawberry cv. Florentina. Different letters at each time indicates significant differences between treatments according to Tukey's test (P<0.05)

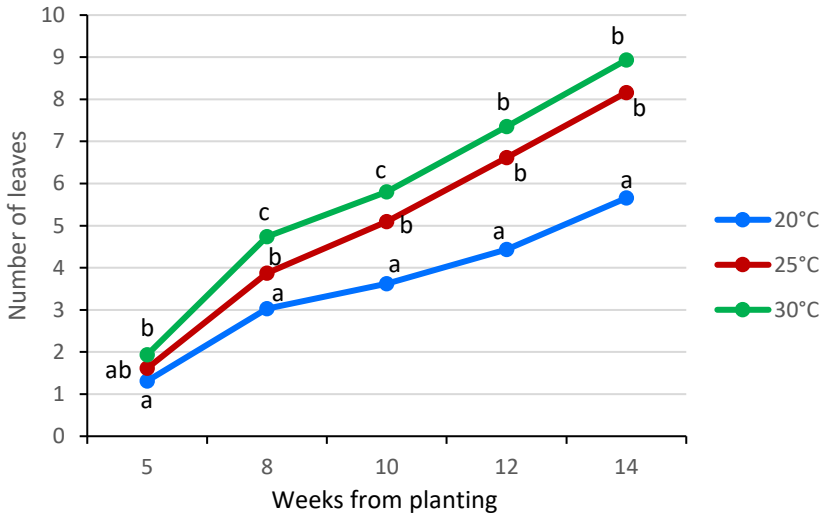

Figure 4. Average number of leaves per plant in strawberry cv. Florentina. Different letters denote significant difference according to Tukey's test at P<0.05.

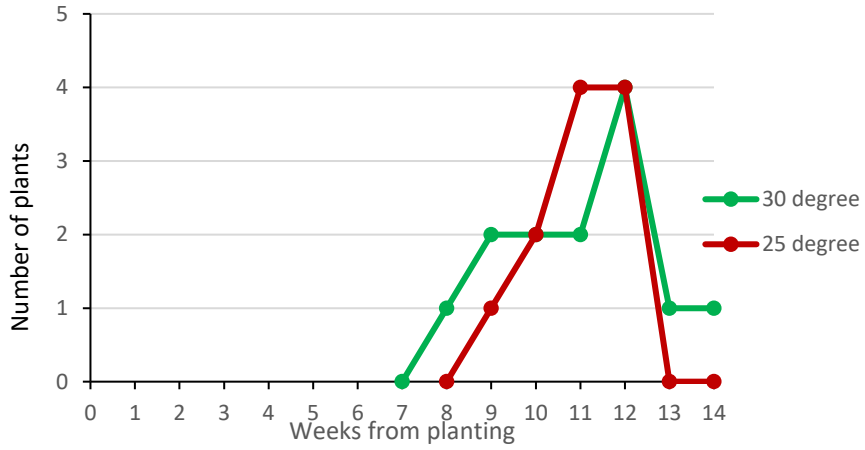

Figure 5. Cumulative number of plants with runners at 25°C and 30°C. There were no runners formation at 20°C in ever-bearing cv. Florentina.

Table 2. Effect of temperature on morphological traits of ever-bearing strawberry cv. Florentina. Means that do not share a grouping letter are significant different according to Tukey's significance test at P <0.05. The analysis was performed in Minitab 18.

	Plant height						**Number of leaves**					
	Treatment	**N**	**Mean**	**Grouping**			**Treatment**	**N**	**Mean**	**Grouping**		
Week 5	30°C	31	9.861	A			30°C	31	1.935	A		
	25°C	31	7.516		B		25°C	31	1.613	A	B	
	20°C	32	7.313		B		20°C	32	1.313		B	
Week 8	25°C	31	14.48	A			30°C	31	4.742	A		
	30°C	31	13.69	A			25°C	31	3.871		B	
	20°C	32	8.484		B		20°C	32	3.031			C
week 10	25°C	31	17.17	A			30°C	31	5.806	A		
	30°C	31	14.5		B		25°C	31	5.097		B	
	20°C	32	10.57			C	20°C	32	3.625			C
Week 12	25°C	31	19.82	A			30°C	31	7.355	A		
	30°C	31	15.4		B		25°C	31	6.613	A		
	20°C	32	12.3			C	20°C	32	4.438		B	
Week 14	25°C	31	21.6	A			30°C	31	8.935	A		
	30°C	31	16.71		B		25°C	31	8.161	A		
	20°C	32	13.36			C	20°C	32	5.656		B	

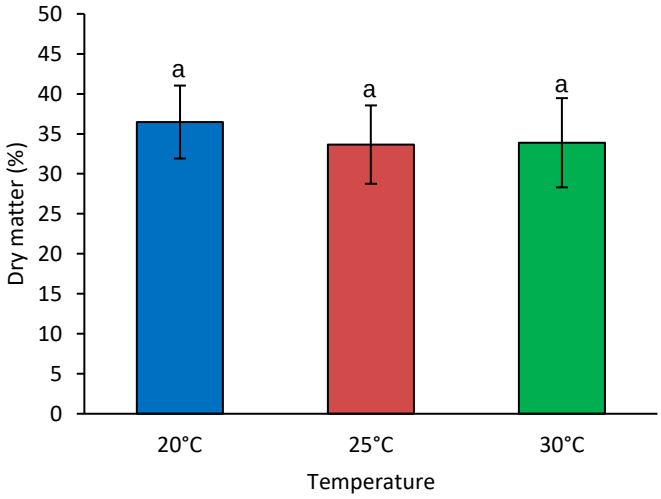

Figure 6 Percentage dry matter in ever-bearing cv. Florentina. Vertical error bars denote standard deviation. Different letters indicate significant differences by Tukey's significance test (P <0.05). The analysis was performed in Minitab 18. (n=16).

4.2. Flowering plants

The flowering pattern in 30°C and 25°C treatments were similar but different compared to 20°C treatment (fig 7). A flush of flowering at 30°C and 25°C treatment was observed between week 1 to week 4. The second was not clear. There was a flush of flowers in plants in 20°C treatment between week 1 to week 6.

Table 3 illustrates there were significant differences in flowering plants in each week. There were no significant differences in weeks 3,6,7,9 and 12 (P>0.05). The percentage of flowering plants at 20°C were significantly higher (P <0.05) than the rest of the treatments in week 4,5,11,13 and 14. The percentage of flowering plants at 30°C were significantly higher (P <0.05) than other treatments in week 1 and 2.

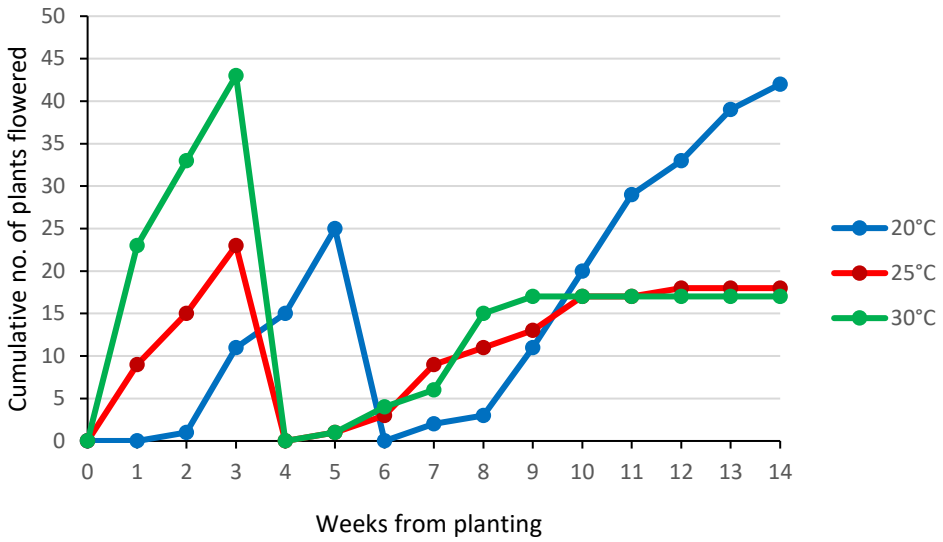

Figure 7. Cumulative number of plants that flowered in three temperature treatments.

Table 3. Effect of temperature on flowering plants (%) in ever-bearing strawberry cv. Florentina. The percentage that does not share a grouping letter is significantly different, according to Fisher's Exact test at P <0.05. The analysis was performed in Minitab 18.

	% of plants that flowered in each week per temperature		
Weeks from planting	20°C	25°C	30°C
1	0a	18.75b	47.92c
2	2.08a	12.5ab	20.83b
3	20.83a	16.67a	20.83a
4	8.33a	0b	0b
5	20.83a	2.08b	2.08b
6	0a	4.17a	6.25a
7	4.88a	15.79a	5a
8	2.44a	4.88a	21.95b
9	19.51a	5.26a	5a
10	21.95a	10.53ab	0b
11	21.95a	0b	0b
12	9.76a	2.63a	0a
13	17.65a	0b	0b
14	8.82a	0b	0b

4.3. Fruit yield, size and number

The fruit harvest was between week 11 and week 17 (fig 8). For plants under 20°C treatment, no harvest was observed in week 11, 12 and 13 while the plants in 30°C and 25°C temperature treatments produced fruits. Fruits were harvested from 20°C treatment in week 14 and onwards. The total fruit yield in 30°C, 25°C and 20°C temperature treatments was 195.2 g, 331 g and

1130.8 g respectively. Higher cumulative number of berries were obtained with 20°C treatment than the other treatments (fig 9). 20°C treatment had higher total fruit number than the other treatments. 20°C, 25°C and 30°C treatment produced 120, 44 and 32 fruits respectively.

Fruit size in 20°C was the highest compared to 25°C and 30°C treatments (fig 10). The average fruit size measured as the fruit length (cm) was 3.38, 2.77, and 2.65 cm in 20°C, 25°C and 30°C treatments respectively. Figure 10 shows that there was no significant difference in fruit size between 30°C and 25°C treatments but significantly lower ($P<0.05$) than 20°C (see table 4 in appendix).

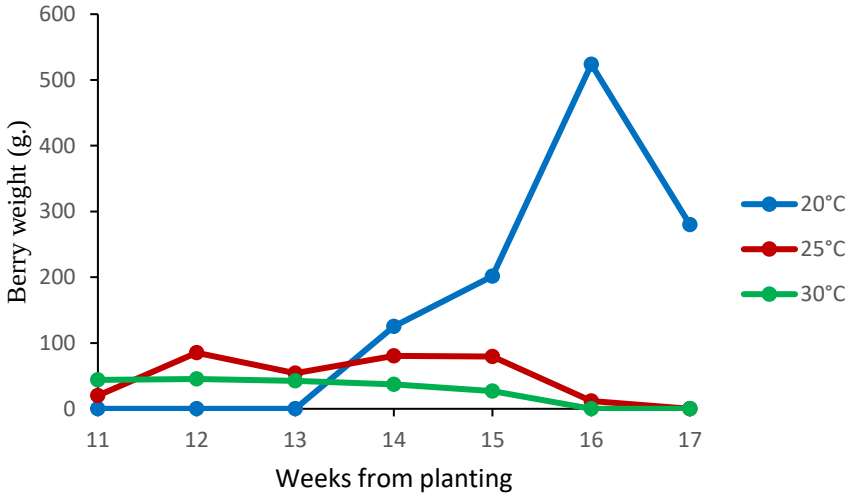

Figure 8. Fruit yield (g) in three temperature treatments.

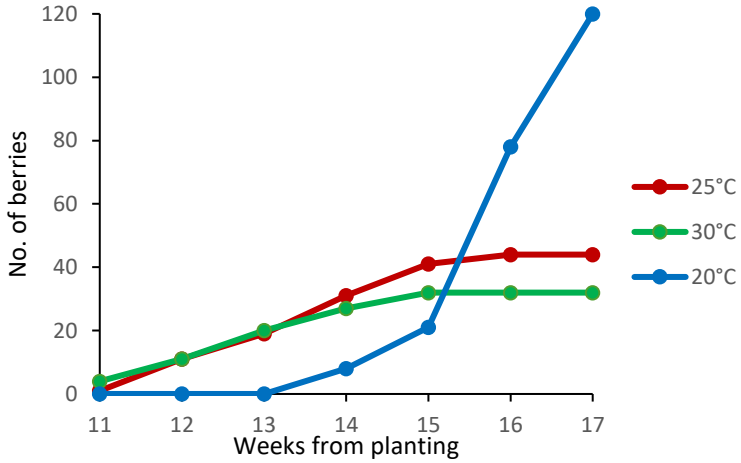

Figure 9. The cumulative number of berries in each treatment. Berries were picked each week.

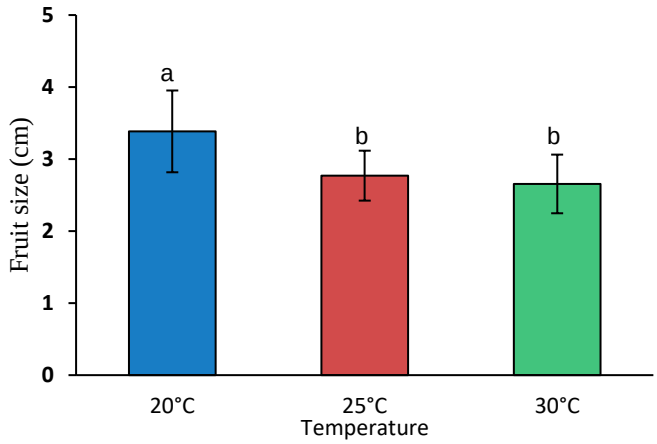

Figure 10. Fruit size in three temperature treatments (n= 20). Vertical error bars denote standard deviation. Different letters indicate statistically significant differences according to Tukey's significance test at P <0.05.

4.4. Flower mapping

Flower mapping was conducted in week 6 from planting. The development stages of the dissected plants were classified according to a reference scale (table 1) presented in the materials and methods section. Figure 11 shows the development stages of the flowers.

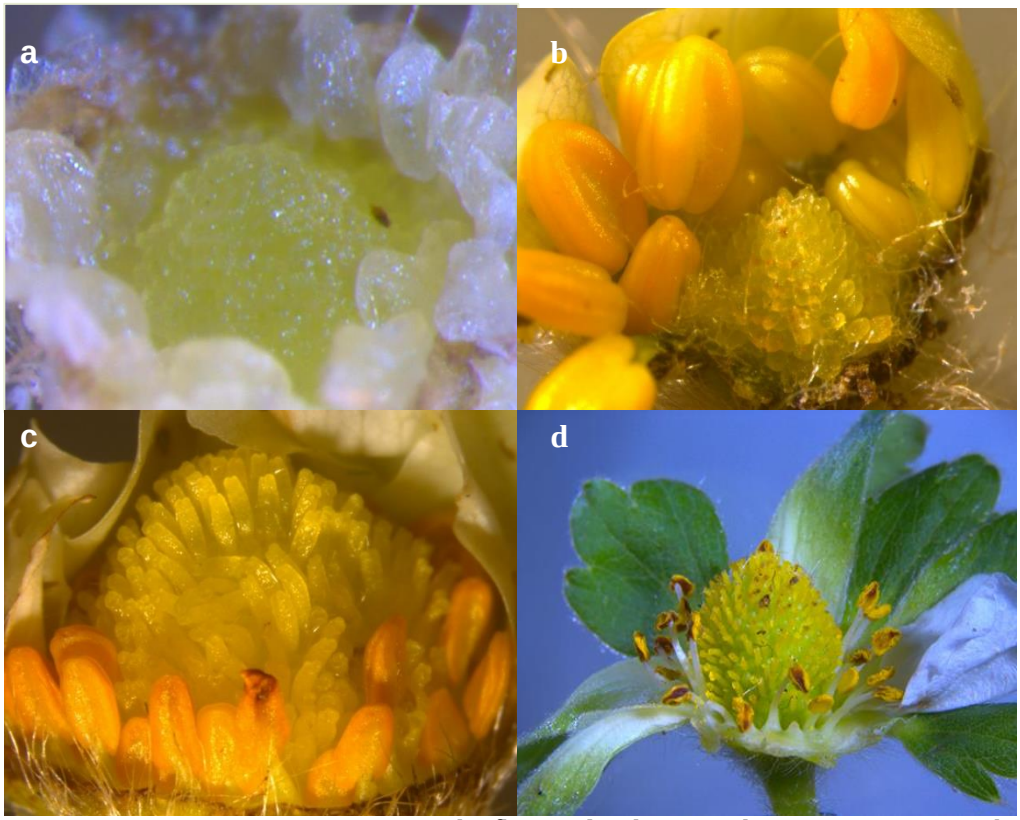

Figure 11. Fragaria x *ananassa* Duch. *flower developmental stages.* ***a*** *Stage 6 the stamens, the male reproductive organs, are white and are taller than the dome-shaped carpel. The pistils have not started to differentiate.* ***b*** *Stage 7 The stamens are yellow. The pistils are starting to differentiate into individual stigma, and the petals are white.* ***c*** *stage 8 The pistils are growing taller than the stamens. Pistils cover the dome-shaped receptacle.* ***d*** *Stage 9 the flower is fully open with dehisced anthers. Photographed using stereomicroscope Leica M165 FC Photo: D. Butare*

Figure 12 illustrates the sum of buds in the three temperature treatments. A higher number of buds (28) were at stage 3 at 30°C compared to other buds. A higher number of buds were at stage 5 followed by stage 3 and stage 4 at 25°C. A higher number of buds were of stage 9 followed by stage 5 and stage 3 at 20°C treatment. Buds at 20°C with 42 of 49 meristems being flower buds were more developed than the buds in plants at 30°C and 25°C. Buds at 25°C treatment with 32 of 44 meristem being flower buds were more developed than buds in plants at 30°C. Meristems of plants under 30°C treatment were less developed with 18 of 46 meristems being flower buds.

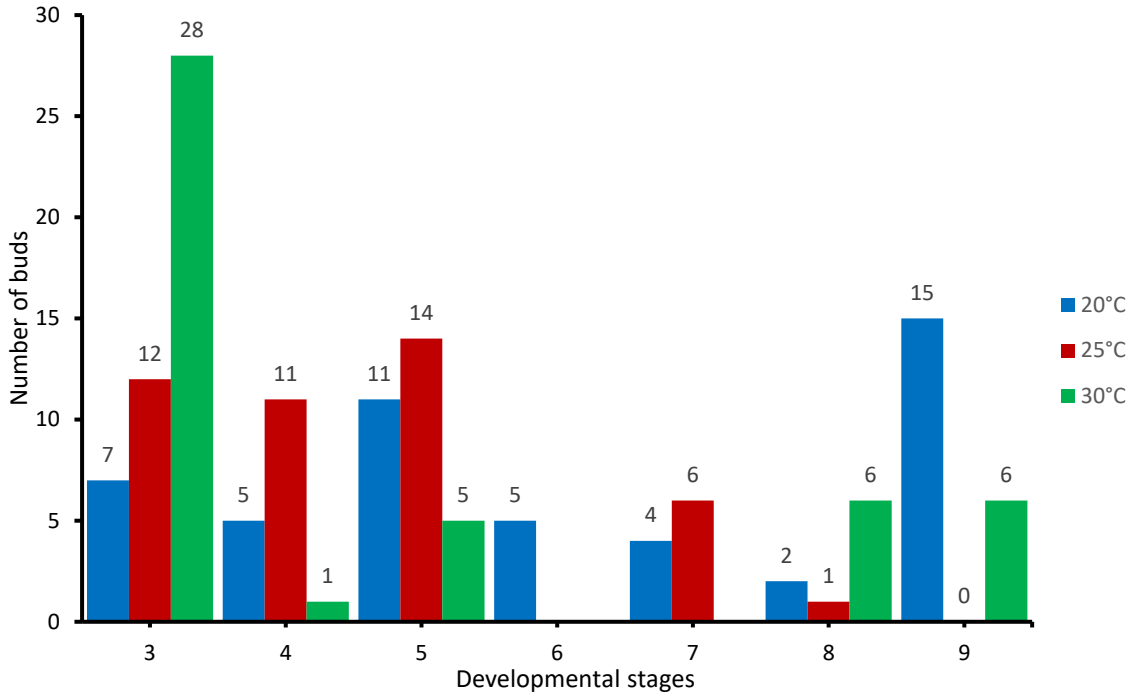

Figure 12. Sum of buds and their development stages of plants selected from each temperature treatment (n=7). These results are derived by Flower mapping that was performed on 11/02/2020. Stage 3 is vegetative and stages 4-9 are generative.

5. Discussion

This study was undertaken to investigate the effects of different temperature regimes on plant growth and flowering in an ever-bearing strawberry cv. Florentina in the greenhouse. The obtained results of the study will now be discussed.

5.1. Plant growth and yield

Plant height was shown to be significantly affected by temperature in all treatments. Plants at 25°C recorded the highest mean plant height and the lowest at 20°C treatment. Plant height at 30°C was the highest in all treatment until week 7 when mean plant height fell below that of 25°C treatment. Plant height in the strawberry is based on the rosette leaves. High temperatures slow down plant growth by promoting respiration rate and reducing photosynthesis (Hancock, 1999). A significant increase in the number of leaves per plant was observed as temperatures increased. Higher temperatures recorded the highest number of leaves. These results reflect those of Sønsteby and Heide (2007b) who also found that the number of leaves per plant was higher at 27°C compared to 15°C.

There were a few runners produced, 7 and 5 runners at 30°C and 25°C while at 20°C no runners were produced. Runner formation in ever-bearing cultivars is enhanced under SD conditions at high temperatures. Sønsteby and Heide (2007a) suggested that the continuous flowering was the cause of poor runner formation in ever-bearing cultivars.

Flowering started earlier in 30°C and 25°C treatments than in 20°C. The 30°C treatment enhanced earlier flowering than the other treatments. When 95% of the plants had flowered in 30°C treatment, 50% and 26% of the plants flowered in 25°C and 20°C treatments respectively. These results agree with the study carried out by Sønsteby and Heide (2007b), where LD cv. Elan grown at temperatures 27 °C flowered earlier than those at 21°C. High temperatures could be associated with the earlier flowering. High temperature accelerates cell growth and thus plants reaching the maturity stage earlier (Badeck *et al.*, 2004). At 30°C, the lighting in the greenhouse was 3 more hours than the other treatments; this could have also affected the rate of flowering. Originally the lighting duration was supposed 6 hours in temperature treatment. It was later realized as a mistake that the 30°C treatment was 9 hours. The short-day conditions were selected to simulate the light patterns of autumn or winter of Sweden. Fruits at 25°C and 30°C treatments

ripened earlier than those in 20°C treatment. The number of fruits harvested in 25°C and 30°C treatments was almost the same until week 13 when 25°C produced more fruits than in 30°C treatments. Fruit harvest in 20°C treatment started in week 14, two weeks later than the other treatments.

The total yield and fruit number were higher at 20°C than at 25°C and 30°C. A higher number of fruits was recorded at 20°C than 30°C and 25°C treatments. This finding is consistent with that of Wagstaffe and Battey (2006) who reported that fruit yield and number of fruits in ever-bearing strawberry cv. Everest was greatest at 23°C compared to 27°C. Least number of fruits were recorded at 30°C. High temperatures reduce pollen viability. Karapatzak *et al.* (2012) demonstrated that the pollen failed to germinate when ever-bearing cv. Everest and Diamante were subjected to high temperature (30°C/20°C). Fruit size is affected by high temperatures. Fruit size was significantly enhanced by lower temperature. Kumakura and Shishido (1994) reported similar results where the strawberry fruit size was bigger at 20°C compared to 25°C. The fruit size measured in this study was the length from the pedicel to apex, it would also be interesting to measure the width of the fruit. There was a presence of some pest insects and pathogens in the greenhouse, which may have affected the yield to some extent.

There was no significant difference in dry matter between temperature treatments. This contrasts with earlier studies which indicate dry matter decrease with high temperatures (Kumakura and Shishido, 1994; Wang and Camp, 2000).

5.2. Flower mapping

Flower mapping results indicate that the flower development stages were more advanced at 20°C than at 25°C and least developed at 30°C. This is shown by a higher number of fruits produced at 20°C compared to 25°C and 30°C. Furthermore, floral buds at 20°C were at several different development stages at the chosen timepoint compared to other treatments. This could imply that the flowers will emerge at several different times hence a longer period of flowering. Flower mapping can provide an understanding of yield potential by providing information on the potential number of flowers (Massetani and Neri, 2016). These flowers are likely to turn into fruits in the future. Furthermore, flower mapping can give insight into the duration of inflorescence emergence based on the flower developmental stages. Even though flower mapping

provided some information on flowering potential, attempts to assess flower mapping as a tool to estimate the yield was not possible, because data was collected on only one occasion. It is a challenge to decide when to carry out flower mapping in ever-bearing cultivars. In addition, the process is labour intensive and time-consuming. There is need to collect enough data that would help in the estimation of the yield. It is important that the data is collected more than once to follow plant development. Other researchers have carried out flower mapping on several occasions and larger sample size (Savini *et al.*, 2006; Bosc *et al.*, 2012). It would have been interesting to test a correlation analysis between the flower bud development stages and the emerged flowers and fruits. This analysis would require more data sets, larger sample size and frequent flower mapping. In addition, more training on flower mapping is needed to produce good results.

To increase the fruit yield is important to avoid the high temperatures. The use of lower temperatures in the tunnel would beneficial to farmers during the high temperatures in the open field. The effect of high temperature on the increase of the vegetative growth could be the reason for reductions in total yield. Reproductive growth in plants is more sensitive to high temperatures. High temperature 30°C would contribute to early production and low yield. On the other hand, low temperature 20°C contribute to delayed production and with high yield. The results from this study indicate that everbearing strawberry cv. Florentina yield was greatest at lower temperature 20°C under short-day conditions (6 hours) in the greenhouse. This cultivar can perform in the open field from autumn when days are getting shorter and temperatures are dropping. However, using tunnels for summer production would not be beneficial because of the high temperatures inside the tunnel that affects the yield.

6. Conclusion

The present study indicates that temperature has a significant effect on growth and yield of strawberry cv. Florentina. High temperatures promoted vegetative growth. Lower temperature enhanced flowering and yield. High temperatures accelerated flower development, whereas the number of fruits was reduced. The results indicate growing plants at 20°C is considered better than other temperature treatments.

In conclusion, flowering and yield can be controlled by adjusting the temperature under greenhouse conditions to obtain a high yield. In the future, more research on the environmental factor on flower initiation is needed to obtain optimal yield. It would also be interesting to study the temperature effect on plant behaviour under long-day conditions to compare the yield with short-day conditions. Flower mapping technique can give insight on yield potential and flowering period, but more extensive material is needed than in this study.

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8. Appendix

Table 4. The effect of temperature on fruit size (n=20). Means that do not share a grouping letter are significantly different according to Tukey's significance test at P <0.05. Analysis performed in Minitab.

One-way ANOVA: Fruit size in treatments 20°C, 25°C, 30°C

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Means

Treatment	N	Mean	StDev	95% CI
20°C	20	3.385	0.568	(3.183, 3.587)
25°C	20	2.7700	0.3466	(2.5684, 2.9716)
30°C	20	2.6550	0.4071	(2.4534, 2.8566)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping	
20°C	20	3.385	A	
25°C	20	2.7700		B
30°C	20	2.6550		B