Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v36i1.17285

Phenology and leaf accumulation in vernalized and non-vernalized strawberry seedlings in neutral-days

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ABSTRACT. Phenology and phyllochron are parameters that help in characterizing vegetal growth and development. This study is an evaluation of the performance of the vernalized and non-vernalized Albion strawberry cultivar in relation to phenology and phyllochron in substrate. The experiments were developed in the greenhouse of the Horticulture Sector of the Universidade de Passo Fundo, Rio Grande do Sul State. The plants were placed in bags spread over wooden benches and filled with substrate formed by carbonized rice hulls and Mec Plant Horta 2®. The experimental design used for both experiments was randomized blocks with 4 repetitions and 8 plants per parcel. In each parcel, 4 plants were randomly marked and evaluated for phenology and phyllochron. For phenology, the dates of onset of flowering and fruiting and the beginning and the end of the harvest were registered. For phyllochron, a weekly count of the number of leaves was performed. A linear regression of the number of leaves (NL) in the main corona and the accumulated thermal time (Att) was performed. Vernalization predicts the cycle of the Albion cultivar, the leaf appearance rate and the phyllochron when compared to those without vernalization.

Keywords: *Fragaria* x *ananassa* Duch., phenologic stages, thermal time.

Fenologia e acúmulo de folhas em mudas vernalizadas e não vernalizadas em morangueiro de dias neutros

RESUMO. A fenologia e o filocrono são parâmetros que auxiliam na caracterização do crescimento e desenvolvimento vegetal. O objetivo do trabalho foi avaliar o desempenho da cultivar de morangueiro Albion com e sem vernalização quanto à fenologia e ao filocrono, em substrato. Os experimentos foram desenvolvidos em estufa agrícola no Setor de Horticultura da Universidade de Passo Fundo, Estado do Rio Grande do Sul. As plantas foram dispostas em sacolas distribuídas sobre bancadas de madeira, preenchidas com substrato formado por casca de arroz carbonizada e Mec Plant Horta 2®. O delineamento experimental utilizado para os dois experimentos foi de blocos casualizados, com quatro repetições e oito plantas por parcela. Em cada parcela foram marcadas ao acaso quatro plantas, nas quais foi realizada as avaliações de fenologia e filocrono. Para fenologia registrou-se datas do início do florescimento e da frutificação, início e final de colheita. Para filocrono semanalmente realizou-se a contagem do número de folhas. Foi realizada regressão linear entre o número de folhas (NF) na coroa principal e a soma térmica acumulada (STa). A vernalização antecipa o ciclo da cultivar de morangueiro Albion, a taxa de aparecimento de folhas e o filocrono quando se compara com as sem vernalização.

Palavras-chave: *Fragaria* x *ananassa* Duch., estádios fenológicos, soma térmica.

Introduction

The price variation of commercial strawberries *in natura* is noticeable. The better products are obtained between April and June, when the supply is reduced due to climactic conditions and features of the cultivars explored. From July on, with the arrival of fruits from other regions in Brazil, mainly Minas Gerais State, prices fall until the end of the crop (January and February). Previously, most strawberry cultivars in Brazil responded to photoperiod, termed Short-Day (SD). In recent years, Neutral-Day cultivars (ND), indifferent to photoperiod and flowering in 10-20ºC temperature, such as cv. Albion, gained market share in regions of milder summers, such as the Rio Grande do Sul State sierra region, which allows production during intercrop periods. Options such as soilless cultivation in greenhouses have been employed for out-of-season production, possibly advancing or delaying harvest periods in addition to incrementing yield per area (CALVETE et al., 2007).

The vernalization of seedlings is a method that provides plants with artificial climactic conditions for gemma differentiation into flower buds at times when these conditions do not occur naturally, simulating natural vernalization (OLIVEIRA et al., 2006). The strawberry seedlings may be fresh (non-vernalized) or frigo (vernalized). According to Oliveira et al. (2007), vernalized seedlings remain approximately 24 days in a cold chamber at 4 ± 1°C temperature and 94 ± 2% relative humidity.

Precocity of production and maturation vary according to cultivar and plant handling (DIAS et al., 2009). In strawberry (*Fragaria* X *ananassa* Duch.), the growth and development of leaves, flowers and stolons depend on the interactions between temperature, photoperiod and temperature range (SILVA et al., 2007).

Antunes et al. (2006) determined strawberry phenology based on Oso grande, Tudla, Chandler and Dover cultivars in Passo Fundo, Brazil. Nine stages were observed, from the flower bud appearance (stage 1) to the maturation of fruits with 75-100% red surface (stage 9). The duration of the 9 stages varied between 36.4 days for cv. Tudla to 40 days for Oso Grande. Stage 4, characterized by the fall of petals, was the longest for the 4 cultivars studied, lasting approximately 11.3 days. Dover had the fastest onset of flowering and fruiting (ANTUNES et al., 2006).

Another parameter in the vegetative development of cultures is the estimation of leaf appearance rate (LAR) and of the phyllochron (necessary time for the appearance of successive leaves), which is important for the calculation of number of accumulated leaves in the plant's main stem (STRECK et al., 2005, 2007; XUE et al., 2004). One way to calculate the number of leaves (NL) in mathematical models is the phyllochron concept, i.e., the interval between the appearance of two successive leaves in one stem (WILHELM; MCMASTER, 1995; XUE et al., 2004). The time in the phyllochron unit is usually expressed as thermal time in °C days, defined as the daily sum of thermal units above a base temperature, below which the plant did not develop or showed negligible development. Thus, the phyllochron is expressed in °C days leaf^{-1} (RUSSELE et al., 1984; STRECK et al., 2005, 2007).

Despite the existence of information on phenology of flowering and maturation of strawberry fruits, its performance in each cultivation site must be identified. To determine phenology, the site's thermal time in degree-days, the difference between accumulation of mean daily temperature and base temperature, above which the plant is unable to perform its physiological functions, must be calculated (CARVALHO et al., 2005). For strawberry cultivars, base temperature is estimated in 7°C (RIEGER, 2007).

Although the phyllochron and the phenology are important metrics and are studied for olericuls (HERMES et al., 2001; MALDANER et al., 2009; PIVETTA et al., 2007), few studies have been devoted to strawberry, and the existing studies are aimed at distinct cultivars and conditions (MENDONÇA et al., 2012). Therefore, this study provides an evaluation of phenology as well as an estimation of leaf appearance rate and the phyllochron in fresh and vernalized strawberry seedlings (frigo) cultivated in substrate.

Material and methods

The experiments were developed in a semicircular 420 m^2 greenhouse, set in the NE-SE direction. The structure, constructed of galvanized steel, was covered with low density polyethylene (LDPE), with anti-ultraviolet additive and a 150 micra thickness. On the inside, an aluminum thermal-reflective screen of 60% shading was installed. The structure was located in the School of Agronomy and Veterinary (FAMV) Horticulture Sector, Universidade de Passo Fundo (UPF).

The strawberry cultivar used both for vernalized (frigo – 24 days in cold chamber of 4 ± 1°C temperature and 94 ± 2% relative humidity) (OLIVEIRA et al., 2007) and non-vernalized (fresh) seedlings was Chilean Albion (LLAHUEN plant nursery); each seedling source comprised one experiment with uniform characteristics and environment. Vernalized seedlings were transplanted on August 25, 2009, and non-vernalized seedlings were transplanted on June 21, 2010. The seedlings were transported in 150 mm white tubular LDPE bags with an ultra-violet additive of 1 m length and 0.30 m breadth and filled with 75% carbonized rice hull and 25% Mec Plant Horta 2® commercial substrate. Each bag represented two plantation lines, spaced 0.20 m between plants and 0.10 m between rows. A randomized block design was used for both experiments, with four replications and eight plants per parcel.

Parcels were irrigated independently through a drip irrigation hose inside the bags and nozzles at 15 cm apart. Fertirrigation was affected weekly according to the formula described by Calvete et al. (2007).

In each parcel, four plants were randomly chosen to assess phenology and phyllochron. In phenology evaluations, the onset dates of flowering and fruiting and the beginning and the end of the harvest were recorded. The onset of flowering was recorded when 50% of plants in each parcel presented at least one open flower. The onset of fruiting was determined after the end of flowering (after all petals had fallen). Fruits showing 75% of red-colored epidermis were considered ripe.

Minimum and maximum air temperatures were measured with a Sato thermohygrograph located inside the greenhouse at 1.5 m height. Mean daily temperature (Mdt) was obtained through the following equation:

$$Mdt = (t0+t2+t4...t18+t20)/12;$$

which calculates the arithmetic mean of temperatures (°C) registered by the thermohygrograph every two hours. Daily thermal time (DTT) was calculated as proposed by Gilmore e Rogers (1958) and Arnold (1960) using the following equation:

$$DTT = (Mdt-Bt)\ [°C\ day^{-1}]$$

where Mdt is mean daily temperature, and Bt is base temperature, defined as minimum temperature necessary for leaf appearance. The Bt was 7°C. The DTT was accumulated based on seedling transplantation, resulting in the accumulated thermal time (Att):

$$Att=\Sigma\ DTT.$$

To determine the phyllochron for both vernalized and non-vernalized seedlings, Leaf Number (LN) was counted twice per week, from the beginning of leaf appearance until the second flowering (October for vernalized and September for non-vernalized plants). A new leaf appearance was counted when it was visible and approximately 1 cm in length. Linear regressions between leaf number (LN) in the main corona and the Att were performed. The phyllochron (°C day leaf^{-1}) was estimated as the inverse of the angular coefficient of linear regression.

Results and discussion

The phenology results for the cv. Albion strawberry (Table 1) show that for vernalized seedlings, flowering began 10 days after transplantation (DAT), with fruiting and harvest at 31 and 49 DAT, respectively. For non-vernalized seedlings, flowering onset occurred at 30 DAT, fruiting at 59 DAT and harvest at 72 DAT.

These results show that vernalized seedlings demonstrate greater precocity in their production cycle than non-vernalized seedlings. Other studies carried out in the same site using cv. Albion from fresh, non-vernalized seedlings produced similar results; Mendonça et al. (2012) observed a later onset of flowering for this cultivar (63 DAT) compared to other cultivars assessed and harvest at 94 DAT.

Table 1. Phenology of strawberry cv. Albion originating from vernalization and non-vernalization - Passo Fundo/RS, FAMV-UPF, 2009-2010.

Seedling origin	Transplant date	Onset of flowering	Onset of fruiting	Onset of harvest
	DAT (Days after transplantation)			
Vernalized (frigo)	Aug 8 2009	10	31	49
Non-vernalized (fresh)	Jun 6 2010	30	59	72

Vernalized seedlings produced in the Southern Hemisphere, more specifically in Chile and Argentina, need a lower accumulation of cold hours for floral induction (PERTUZÉ et al., 2006), resulting in conditions which allow producers to anticipate the supply of fruit on the market.

A study by Verdial et al. (2007) assessing the phenologic behavior of vernalized and non-vernalized strawberry seedlings showed that flowering was directly influenced by the process. Cultivars Sweet Charlie and Cartuno presented 88.70 and 80.91% of plants with flowers, respectively, after 28 days at 10°C and 8-hour photoperiod, while tests (fresh seedlings) did not present flowers in this period. Fruiting was also anticipated for these cultivars. Forty-five days after transplanting 3.76% of Sweet Charlie plants presented fruits ready for harvest, while Cartuno presented 25.29%.

Minimum and maximum absolute temperatures in the greenhouse during the evaluation period for flower appearance in vernalized seedlings were 3.7 and 50°C at 263 (September 20, 2009) and 277 (October 4, 2009) Julian Days, respectively. Six days had minimum temperatures equal to or lower than the base of 7°C, and mean temperature was 20.6°C inside the greenhouse on these days (Figure 1A).

During the evaluation period for flower appearance in non-vernalized seedlings, minimum and maximum absolute temperatures in the greenhouse were -2 and 44°C at 195 (July 14, 2010) and 219 (August 18, 2010) Julian Days, respectively. A total of 21 days were recorded with minimum temperatures less than or equal to 7°C, and on these days, the mean temperature inside the greenhouse was 20.6°C (Figure 1B).

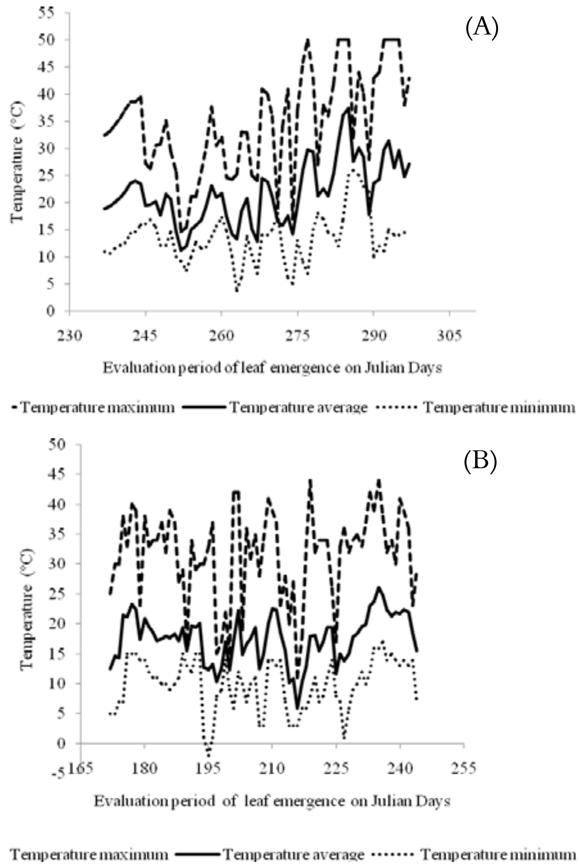

Figure 1. Maximum, minimum and mean temperatures recorded in greenhouse during evaluation of flower appearance in vernalized (A) and non-vernalized seedlings (B) - Passo Fundo/RS, FAMV-UPF, 2009.

When estimating leaf appearance rate (LAR) for cultivar Albion (Figure 2), a variation of 0.03 for vernalized seedlings and 0.005 for non-vernalized seedlings was observed for leaf appeared at each accumulated °C day presenting a phyllochron of 93.3°C day leaf^{-1} for successive leaf appearance in vernalized seedlings, while for the non-vernalized ones, a phyllochron of 117.8°C day leaf^{-1} was observed. Mendonça et al. (2012), working with cv. Albion in an intercropping system with the fig tree, found 0.007 leaf each accumulated °C day and phyllochron of 149.35 ± 31.3°C day leaf^{-1}. In the single production system, Albion required 199.96°C day leaf^{-1}. In both systems, Mendonça et al. (2012) observed an increased degree-day requirement for the appearance of each leaf compared with this study.

During the observation period for fresh seedlings, a mean temperature of 17.5°C was observed inside the greenhouses. However, Mendonça et al. (2012) observed mean temperatures of 15.5°C in the consortium and 16.4°C in the single cultivation system, which explains the lower phyllochron values (117.8°C day leaf^{-1}) in this study because the Att necessary for leaf appearance is more readily reached.

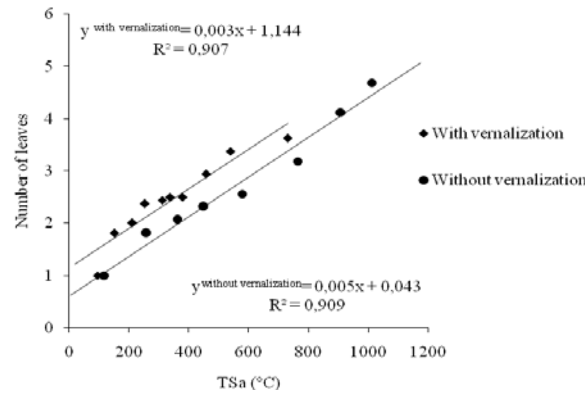

Figure 2. Phyllochron of vernalized and non-vernalized cv. Albion strawberry in the greenhouse - Passo Fundo/RS, FAMV-UPF, 2009

In regard to the two types of vernalized seedlings used in this study, one explanation for the observed difference may be the vernalization treatment conferred to the seedlings in anticipation of the culture cycle. Another explanation could be the cultivation period for fresh seedlings (May-September), in which mean temperatures were lower compared to the evaluation period for vernalized seedlings (August-October).

In lilies and hay, the main effect of vernalization is the shortening of the plant development cycle (RAWSON et al. 1998; ROH, 1984). Schuh et al. (2005), assessing the influence of vernalization of lily bulbs on the plants' phyllochron, concluded that vernalization affects the phyllochron. The plants with little or no vernalization have a higher phyllochron than those completely vernalized. In hay, there is evidence that non-vernalized plants present a lower rate of flower appearance, i.e., a greater phyllochron (RAWSON et al. 1998).

According to literature, the period after the appearance of two successive leaves for strawberry culture is 8-12 days, and temperature is the decisive factor in this physiologic process (GALLETA; HIMELRICK, 1990). In this study, vernalized seedlings required 5-10 days with mean temperature of 20.6°C for the appearance of two leaves, while non-vernalized (fresh) seedlings required 9-24 days with mean temperature of 17.5°C.

Lutchoomun (1999) affirmed that vernalized seedlings present higher quantities of accumulated stock in the corona and the roots, were more developed and rapidly moved after planting for the production of new leaves (ROUDEILLAC; VESCHAMBRE, 1987).

The linear relationship with a high coefficient of determination between the number of leaves and accumulated thermal time (Figure 2) shows that air temperature is one of the decisive factors for leaf appearance in Albion. Similar results were obtained for tomato (PIVETTA et al., 2007) and strawberry (MENDONÇA et al., 2012). The linear response shows that the culture occurred in due time (STRECK et al., 2007), providing evidence of adaptation to cultivation conditions.

Conclusion

Vernalized seedlings of the cv. Albion are more precocious than fresh seedlings due to the greater accumulation of leaves per time interval.

The phyllochron is influenced by mean daily air temperature during the period of leaf appearance.

With the increase in air temperature, the accumulated thermal time (Att) is accelerated, and lower phyllochron values are observed.

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Received on May 17, 2012.
Accepted on August 21, 2012.