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PHENOLOGY AND GROWING DEGREE DAYS OF FESTIVAL STRAWBERRY GROWN ON RED VOLCANIC ROCK AT TWO PLANT DENSITIES

FENOLOGÍA Y GRADOS DÍAS DE DESARROLLO DE FRESA FESTIVAL CULTIVADA EN TEZONTLE EN DOS DENSIDADES DE POBLACIÓN

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SUMMARY

Due to their correlation with temperature, growing degree days (GDD) allow to analyze the effect of climate change on plant phenology, while being also useful for scheduling activities in a cultivation system. Fruit cropping systems in general lack enough information in this regard. This research aimed to determine GDD during the phenology of Festival strawberry (*Fragaria × ananassa* Duch.) grown on two substrates at two plant densities under a macro-tunnel. Previously chilled (4 °C/720 h), one-leaf plants were grown from August 2016 to March 2017 either in hydroponics on red volcanic rock or in soil with fertigation at 10 or 15 plants m^{-2}. A Steiner nutrient solution reduced to 50% of its original concentration was used. GDD were counted daily after planting using the single sine curve method with a base temperature of 3 °C. A high correlation coefficient ($r > 0.964$) was found between GDD and days after planting (DAP) for the phenological stages studied. The phyllochron was not affected by treatments, and 597 GDD were required as an average for the onset of leafing and a high correlation between air temperature and the emission of leaves number 2 to 10 ($r = 0.9754$; $P \leq 0.0001$). The substrate did not affect the chronology of the reproductive phenological stages. The onset of anthesis was delayed by 17 days in plants grown at 15 plants m^{-2} (2039.4 GDD) compared to those grown at 10 plants m^{-2} (1829.7 GDD), but there was no effect of plant density for the sequent phenological stages. It is concluded that GDD have a high predictive value in Festival strawberry for DAP of the phenological stages studied; likewise, the phyllochron and reproductive phenology were not affected by substrate cultivation; however, planting at high densities could delay the onset of anthesis.

Index words: *Fragaria × ananassa* Duch., anthesis, phenology, phyllochron, plant density, red volcanic rock.

RESUMEN

Por su correlación con la temperatura, los grados días de desarrollo (GDD) permiten analizar el efecto del cambio climático en la fitofenología y también son útiles para programar actividades en un sistema de cultivo. La fruticultura en general carece de suficiente información al respecto. El objetivo de esta investigación fue determinar los GDD en la fenología de fresa (*Fragaria × ananassa* Duch.) Festival cultivada en dos sustratos a dos densidades de población bajo un macro-túnel. Plantas con una hoja y previamente enfriadas (4 °C/720 h) se cultivaron de agosto 2016 a marzo 2017 en hidroponía con tezontle o en suelo con fertirriego a densidades de 10 o 15 plantas m^{-2}. Se utilizó una solución nutritiva Steiner reducida al 50 % de su concentración original. Los GDD se determinaron diariamente a partir de la plantación mediante el método del seno simple con temperatura base de 3.0 °C. Se encontró una alta correlación ($r > 0.964$) entre los GDD y los días después de plantación (DDP) para las fenofases estudiadas. El filocrono no fue afectado por los tratamientos, requiriéndose en promedio 597 GDD para iniciar la foliación y existió una correlación alta entre la temperatura del aire y la emisión de las hojas 2 a 10 ($r = 0.9754$; $P \leq 0.0001$). El sustrato no afectó la cronología de las fenofases reproductivas. El inicio de la antesis se atrasó en 17 días en plantas cultivadas a 15 plantas m^{-2} (2039.4 GDD) en comparación con aquellas cultivadas a 10 plantas m^{-2} (1829.7 GDD), pero no hubo efecto de la densidad de población para las siguientes fenofases. Se concluye que en fresa Festival los GDD tienen un alto valor predictivo para los DDP de las fenofases estudiadas; igualmente, el filocrono y la fenología reproductiva no son afectados por el cultivo en sustrato, aunque plantar a altas densidades podría atrasar el inicio de la antesis.

Palabras clave: *Fragaria × ananassa* Duch., antesis, densidad de plantación, fenología, filocrono, tezontle.

INTRODUCTION

Phenology, the study of the timing of recurrent life cycle events and the causes of their temporary interrelation with biotic and abiotic factors, is now acquiring great relevance for the planning of cropping systems. Phenological information can be used to determine the proper time to carry out activities such as pruning, application of chemicals or planning the harvest date (Bisognin *et al*., 2015; Rahman *et al*., 2016). In addition, plant phenology is getting environmental interest as more evidences show that chronology of stages like dormancy release, flowering and harvest in some crop species are being altered due to climate change (Bethere *et al*., 2016; Funes *et al*., 2016; Guo *et al*., 2015). In this regard, the chronology of phenological phases in plants based on growing degree days (GDD) seems more practical and accurate than that based on natural days, as the former involves air temperature in the computations; thus, resulting in a better analysis of plant phenology (Elnesr y Alazba, 2016; Parra-Coronado

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et al., 2015). Phenology based on GDD can be used to generate prediction models either for precision horticulture or cropping systems in a specific region affected by climate change (Bethere *et al*., 2016; Caffarra *et al*., 2012; Parthasarathi *et al*., 2013); nevertheless, these models require enough phenological data from different regions to be constructed and to render trustable outputs, and this information is rarely available (Caffarra *et al*., 2012; Grab and Craparo, 2011; Mendoza *et al*., 2017; Olesen, 2011). Phenological records based on GDD become important to the fruit crops not only to schedule activities during the growing cycle, but also to project policies related to the regional distribution of species in a future affected by climate change.

Phenological analyses are essential for protected horticulture due to manipulation of environmental factors around the crop and the search for maximum and faster returns from the system. Regarding strawberry cultivation, there is an evident interaction between temperature and photoperiod that affects not only floral induction, but also the periods of leaf and fruit development, thus, modifying the harvest time (Krüger *et al*., 2012; Sønsteby and Heide, 2006); despite the fact that this interaction has been addressed in several reports, there is not enough studies quantifying the GDD at the beginning of phenological phases in this crop (Bethere *et al*., 2016; da Costa *et al*., 2014; Diel *et al*., 2017a; Krüger *et al*., 2012; Rosa *et al*., 2011).

Previous research has shown that organic substrates could affect strawberry phenology (Diel *et al*., 2017a), but not much is known about plant density and inorganic substrates affecting phenology and GDD requirements in this crop. Considering the importance of phenological records based on thermal needs of strawberry under protected systems, this research aimed to determine the effect of volcanic red rock and plant density on foliar phenology (phyllochron) and reproductive phenology of the Festival strawberry growing under a polyethene macro-tunnel while comparing the days after planting (DAP) to GDD as chronological methods for phenology.

MATERIALS AND METHODS

Plant material

Strawberry plants (*Fragaria* × *ananassa* Duch.) cv. Festival from a commercial nursery in central Mexico were planted on August 26, 2016 inside a polyethylene macro-tunnel (34.0 m length, 5.0 m width, 2.9 m height) located at 19° 20' N, 98° 53' W and 2250 masl. Temperature records inside the tunnel are shown in Figure 1. Plants were chilled at 4 °C for one month and then planted on beds (20.0 m length, 1.1 m width, 0.35 m height) filled with either red volcanic rock or local agricultural soil. Beds were previously disinfected with Metalaxil-M for soil-borne diseases control following commercial recommendations. There were three rows of plants per bed with 30 cm spacing between rows and 20 or 30 cm between plants, resulting in plant densities of 15 and 10 plants m^{-2}, respectively.

Cultivation system

A Steiner mineral solution (Steiner, 1984) adjusted to 50 % of its original concentration, pH 5.5 to 6.0 and electric conductivity (EC) of 1.2 to 2.0 dS m^{-1} was used in a fertigation system with three drip lines and compensated emitters (3.5 mL min^{-1}) at 20 cm separation. The system was set to allow five events per day from 9:00 am to 6:00 pm for a total of 16 min dripping for the red volcanic rock substrate and one event of 10-12 min every other day at 11:00 am for the soil. Volcanic red rock was < 1.0 cm particle diameter and came from local mines; physical and chemical properties for this substrate have been reported by Trejo-Tellez *et al*. (2013). Local agricultural soil was clay loam with pH 7.5, EC 0.5 dS m^{-1}, bulk density 1.23 g cm^{-3} and $CaCO_3$ 15.8 %. Pests and weeds were controlled following commercial recommendations for strawberry.

Phenological stages

Temperatures were recorded every hour from the day of planting by using a sensor placed 50 cm above ground level and connected to a Dataloguer (HOBO®, Onset Computer Corporation, Bourne, Massachusetts, USA). Daily growing degree days (GDD) after planting date were computed with temperature data and the cumulative GDD requirements for phenological stages, as decribed by Enz and Dachler (1997) for strawberry, were determined: early balloon stage (first flowers with petals forming a hollow ball), onset of anthesis (first flowers open), flowers fading (majority of petals fallen), receptacle protruding from sepal whorl, seed clearly visible on receptacle tissue, beginning of ripening (most fruits white in color), first fruits with cultivar-specific color. Daily GDD were computed using the single sine method (Equation 1) with lower and upper threshold temperatures of 3.0 and 30 °C, respectively (Elnesr and Alazba, 2016; Krüger *et al*., 2012;), fitting inside the range every temperature found outside the thresholds.

$$GDD = \frac{Tmax + Tmin}{2} - Tbase$$

Equation 1

Where GDD: daily growing degree days, Tmax: maximum temperature for the day, Tmin: minimum temperature for the day, Tbase: 3 °C.

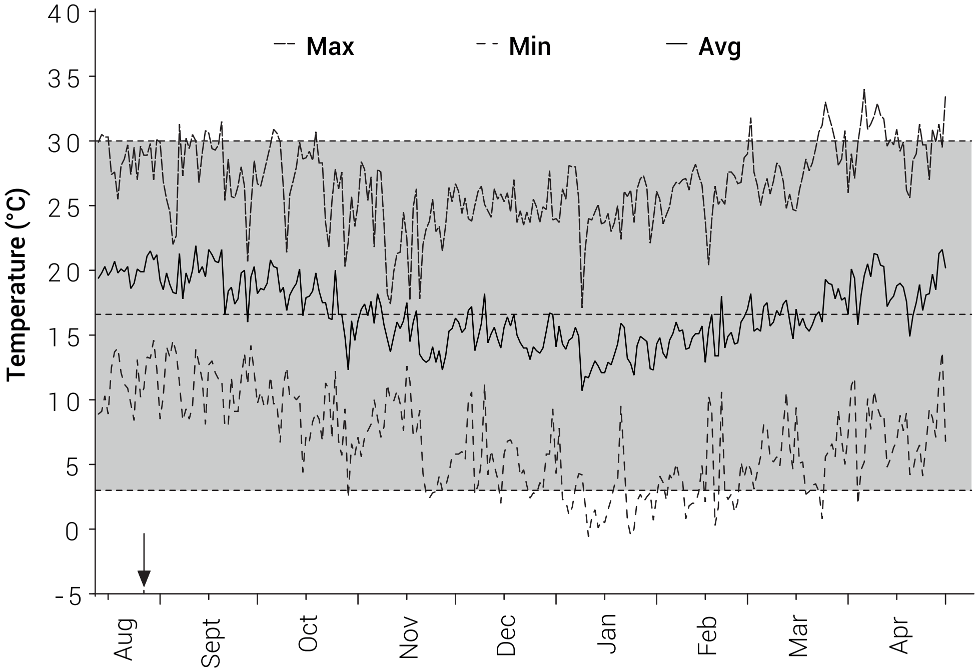

Figure 1. Temperature inside the plastic macro-tunnel. Highlighted section shows the temperature used for the growing degree days (GDD) computation. Dotted central line represents the average total temperature during the experiment; the arrow shows the planting date.

Experimental design and statistical analysis

The experiment was conducted under a completely randomized design and split-plot arrangement with factor substrate (red volcanic rock and soil) as the main plots and factor plant density (10 or 15 plants m^{-2}) as the split plot with three replications (beds). Distribution of treatment resulted from dividing three beds corresponding to each substrate into two equal sections and randomly assigning plant density to each section. Twenty plants from the central part of each replication were labeled and used to collect data. Resulting data were submitted to analysis of variance by using the GLM procedure of the SAS v9.0 program and mean separation by the LSD test ($P \leq 0.05$) to compare treatment means. Linear regression was performed to predict the phyllochron and the DAP in every phase of reproductive phenology using the GDD. Prior to the regression analysis, natural logarithm transformation of the independent variable was performed to estimate the best linear fit between the involved variables (Ott and Longnecker, 2001).

RESULTS AND DISCUSSION

Growing degree days (GDD) *vs.* days after planting (DAP)

High correlation coefficients ($r > 0.964$) between GDD and DAP were found for each phenological stage considered in this study (Table 1). Confidence interval (95 % confidence) for the slope was under a 20-day range. The decision to choose one or the other method to count the time to reach a specific phenological phase will depend on the benefits of each one. Parra-Coronado *et al.* (2015) found that GDD can reliably predict the days to the onset of various phenological stages in feijoa [*Acca sellowiana* (O. Berg) Burret] grown in different natural environments. Chronology to beginning of the phenological phases of cultivated fruit species has traditionally been reported in calendar days; however, because of the influence of temperature on plant phenology, these values could vary significantly for the same cultivar grown in different environments. In contrast, GDD-based phenology becomes important in determininig the onset of phenological stages by providing a more reliable tool in scheduling activities in a specific crop cycle (Krüger *et al*., 2012; Parra-Coronado *et al*., 2015; Parthasarathi *et al*., 2013; Rahman *et al*., 2016).

Leafing phenology (Phyllochron)

The analysis of this process becomes relevant since the productivity of the Festival strawberry is highly correlated with its foliar development (Menzel and Smith, 2014) and the data from these type of analysis might be used to generate models that facilitate detailed scheduling of

Table 1. Linear regression analysis to estimate days after planting (DAP) from the natural logarithm of growing degree days (GDD) in flowering and fruiting phenology for strawberry Festival grown in soil and red volcanic rock under a plastic macro-tunnel in the Mexican central highlands.

Variable	Equation	Standard error of the slope	Confidence interval of slope (α = 0.05)	R-Square	p-Value
Early balloon stage[†]	Y = 152.33X - 1009	29.13	144.3 to 160.3	0.978	< 0.0001
Onset of anthesis[†]	Y = 164.3X - 1100	25.05	157.5 to 171.1	0.985	< 0.0001
Flowers fading	Y = 169.9X - 1143	25.42	163.2 to 176.6	0.973	< 0.0001
Receptacle protruding from sepal whorl	Y = 174.0X - 1175	24.26	167.6 to 180.4	0.978	< 0.0001
Seed clearly visible on receptacle tissue	Y = 170.3X - 1147	23.68	164.1 to 176.5	0.978	< 0.0001
Beginning of fruit ripening (fruits white in color)	Y = 176.2X - 1193	32.48	1667.7 to 184.7	0.968	< 0.0001
First fruits with cultivar specific color	Y = 183.3X - 1246	37.42	173.4 to 193.1	0.964	< 0.0001

[†]Values only for plants grown in soil. Y: days after planting, X: growing degree days.

activities, thus increasing productivity. In this experiment, leaf formation was not different between treatments up to 163 DAP (February 4, 2017); afterwards, plants grown in soil formed more leaves than those grown on red volcanic rock (Figure 2). Compared to soil, red volcanic rock has low water retention and low water use efficiency (fruit mass per volume of water) (Ojodeagua *et al*., 2008; Trejo-Téllez *et al*., 2013). It seems that water availability was not a limiting factor for plants of any treatment during most of the growing season; however, during the last third of the season, as the plant accumulated more leaves, the water on red volcanic rock was clearly insufficient to maintain the same leafing rate compared to the soil, even though there were no obvious symptoms of water stress in plants of both substrates throughout the growing season. During the experiment, plant density had no effect on the number of leaves per plant and there was interaction between factors (substrate × plant density; P = 0.003) only on the last sampling date (March 22, 2017) resulting in more leaves (12.9) in the plants grown in soil at low plant density (10 plants m^{-2}).

Treatments had no significant effect on phyllochron in this experiment (Figure 3A). There was a requirement of 597 GDD for the onset of leafing and the second leaf to be evident. Other studies have demonstrated that phyllochron in strawberry may depend on the cultivar, the origin of the plant and the planting date or the interaction among these factors (Rosa *et al*., 2011; Tazzo *et al*., 2015; Thiesen *et al*., 2018); furthermore, the chilling treatment to the crown before planting may or may not have a significant effect on this variable (da Costa *et al*., 2014; Diel *et al*., 2017b); nevertheless, all these studies agreed on a high correspondence between air temperature and phyllochron, which was also found in this experiment ($R^2 = 0.9754$; Figure 3B). All these previous studies indicate that every period between leaves in the strawberry crown is highly genetically influenced yet significantly modulated by air temperature; in addition, crown carbohydrates, either stored or newly synthesized, are important for this modulation (da Costa *et al*., 2014; Rosa *et al*., 2011). Though the strawberry phyllochron and all the regulating factors should be carefully investigated, this experiment shows that despite the fact that cultivation on red volcanic rock could decrease the final number of leaves on the strawberry crown compared with cultivation in soil, its phyllochron based on GDD remains relatively constant.

Reproductive phenology

All phenological phases studied in this research are also considered in the BBCH code for strawberry (Enz and Dachler, 1997). Literature on strawberry phenology that considers the BBCH code and that is based on GDD is scarce. Data from this experiment are in the range reported in literature for strawberry Elsanta, Korona and Clery grown at different latitudes in Europe, from Italy to Norway, by Krüger *et al*. (2012), either for DAP to the onset of flowering and harvest (red fruit) or for GDD accumulated during the period between these two stages (Table 2). Strawberry reproductive phenology is genetically regulated and environmentally modulated by temperature, photoperiod, humidity, and plant nutrition (Diel *et al*., 2017a; Krüger *et al*., 2012; Rahman *et al*., 2016; Sønsteby and Heide, 2006; Wan *et al*., 2018); as a result, activities at the place of origin of the plantlets and during their cultivation could

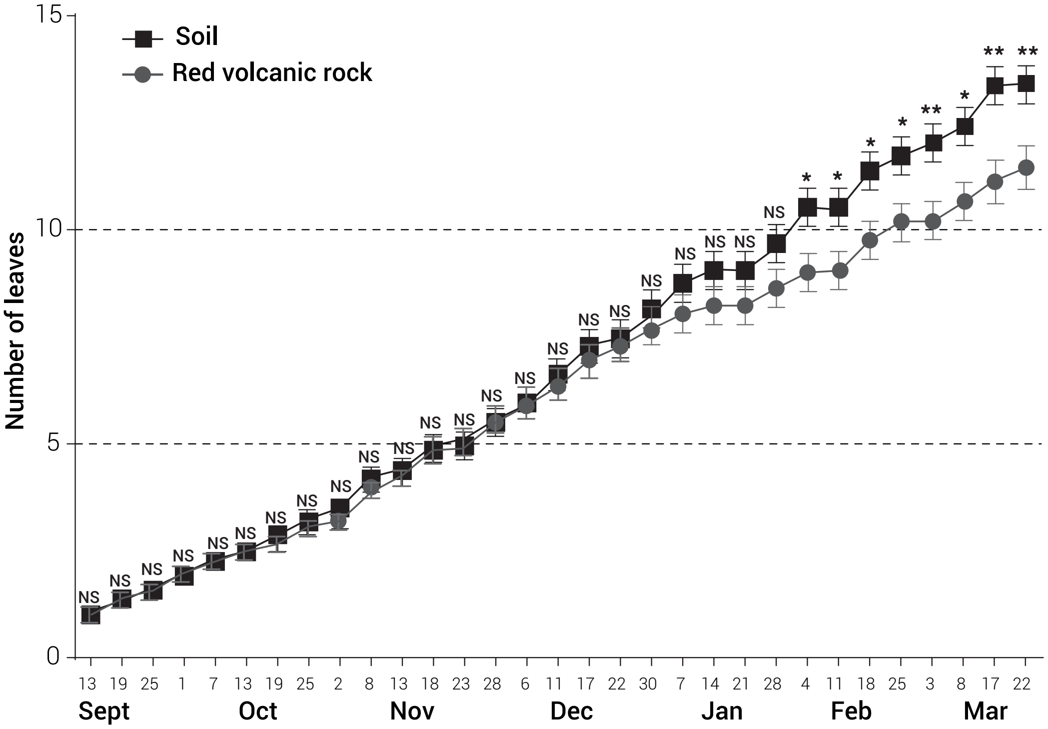

Figure 2. Leafing in Festival strawberry plants grown in soil or red volcanic rock in a plastic macro-tunnel in central Mexico. Difference between means is non-significant (NS), significant (*) or highly significant (**) according to the LSD test. Bars are the standard error of the mean (n = 6).

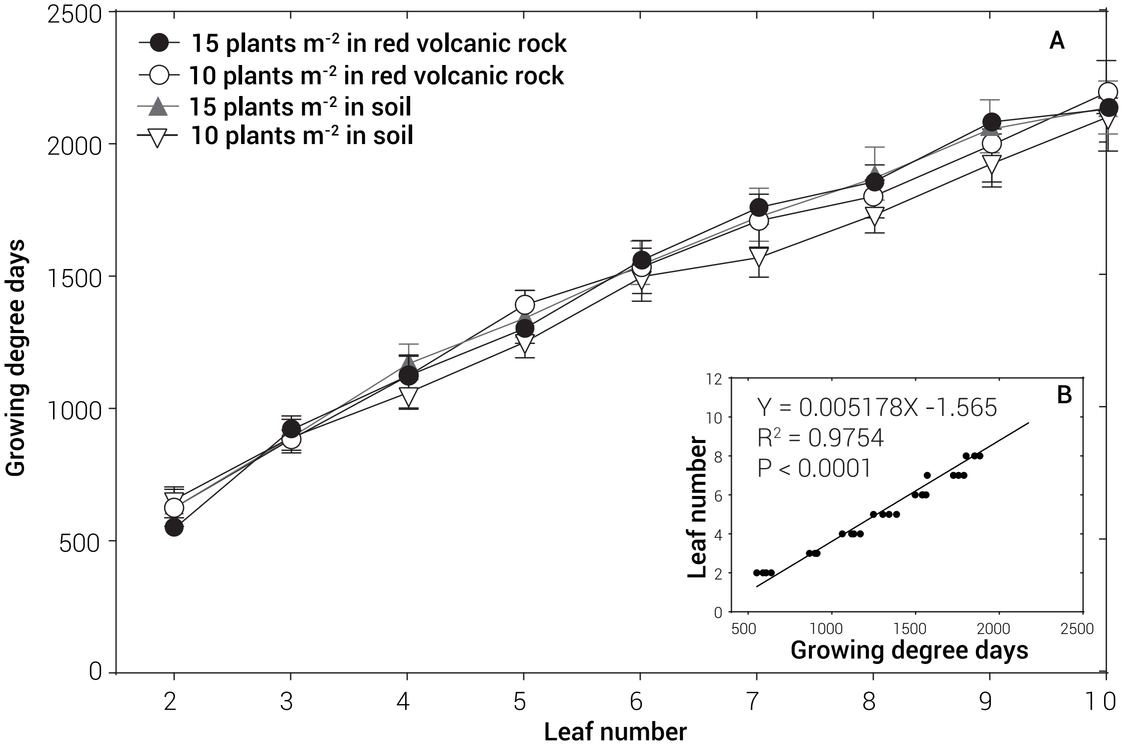

Figure 3. A) Growing degree days (GDD) to consecutive leaves formation in the crown (phyllochron), B) linear regression to predict leaf number from natural logarithm of GDD. Bars in A are the standard error of the mean (n = 3).

Table 2. Days after planting and growing degree days to flowering and fruiting stages in Festival strawberry grown in soil or red volcanic rock at two plant densities under a plastic macro-tunnel in the Mexican central highlands.

Factor	Level	Early balloon stage	Onset of anthesis	Flowers fading	Receptacle protruding from sepal whorl	Seed clearly visible on receptacle tissue	Beginning of fruit ripening (fruits white in color)	First fruits with cultivar specific color	Flower to fruit period[†]
		Days after planting (DAP)							
Substrate (s)	Soil	128.2	134.9	140.6	142.5	148.2	152.3	153.4	32.6
	Red volcanic rock	135.1	141.7	146.3	147.4	149.1	149.5	151.1	36.2
	Significance	NS	NS	NS	NS	NS	NS	NS	*
Plant density (d)	15 plants m^{-2}	140.6	147.4	151.3	149.9	153.2	155.5	155.5	35.2
	10 plants m^{-2}	123.1	129.7	136.2	140.6	144.9	147.8	150.2	33.4
	Significance	*	*	NS	NS	NS	NS	NS	NS
	s × d interaction	NS	NS	NS	NS	NS	NS	NS	NS
		Growing degree days (GDD)							
Substrate (s)	Soil	1809.2	1891.8	1958.6	1982.4	2052.5	2098.8	2109.6	385.6
	Red volcanic rock	1892.6	1972.1	2018.6	2024.2	2062.3	2063.7	2079.7	427.2
	Significance	NS	NS	NS	NS	NS	NS	NS	*
Plant density (d)	15 plants m^{-2}	1957.1	2039.4	2087.2	2070.1	2112.8	2138.4	2136.5	418.9
	10 plants m^{-2}	1749.6	1829.7	1906.1	1961.1	2012.7	2043.9	2069.6	392.6
	Significance	*	*	NS	NS	NS	NS	NS	NS
	s × d interaction	NS	NS	NS	NS	NS	NS	NS	NS

[†] Period from onset of anthesis to fruit red in color (harvest). NS: non-significant, *: statistical significatance (LSD test, P ≤ 0.05) within the respective factor (n = 3).

modify the reproductive phenology and the phyllochron while growing in substrates under plastic cover (Diel *et al*., 2017a; 2017b). In this study there was no significant effect of the substrate (soil *vs.* red volcanic rock) on reproductive phenology of Festival strawberry, meaning that plants had equivalent conditions before and after planting or at least not significant enough to generate differences between treatments for most of the registered variables.

Regarding plant density, there was a significant delay of 17 days to reach the early balloon stage and the onset of anthesis in plants at high density (15 plants m^{-2}) compared to plants at low density (10 plants m^{-2}), but this difference disappeared thereafter (Table 2). Nitrogen is a mineral that can affect floral differentiation in strawberry (Wan *et al*., 2018), and when applied three weeks after the beginning of short-day conditions it can advance flowering by eight days (Woznicki *et al*., 2018). The plants in this experiment were exposed to short days just one month after the planting date; thus, plants at high densities could have a stronger competition for nitrogen than those at low densities, which affects the first two reproductive stages considered in this study. The lack of effect of plant density on the later reproductive stages is probably due to the similar foliar development between treatments during most of the growing period and to the similar *de novo* synthesis of carbohydrate which apparently is determinant for reproductive phenology in strawberry (Menzel and Smith, 2014).

CONCLUSIONS

Growing Festival strawberry on red volcanic rock had no effect on plant phyllochron or its reproductive phenology compared to plants grown in soil. Plant density also had no effect on the strawberry phyllochron; however, high densities can delay the onset of the early balloon stage and the onset of anthesis. Growing degree days (GDD) and days after planting (DAP) are highly correlated and GDD may predict DAP within a high-confidece interval. Phyllochron is highly correlated with air temperature and GDD can reliably predict leaf protrusion.

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