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# Phenology, phyllochron, and gas exchanges in frigo and fresh strawberry (*Fragaria* × *ananassa* Duch.) plants of cv. Albion

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#### Abstract

Aiming to identify a possible precocity in frigo and fresh strawberries plants of cv. Albion, in greenhouse, the cycle and the rate of leaf appearance were evaluated and the photosynthetic behavior of these plants was analyzed. The experiments were performed in a greenhouse at University of Passo Fundo. The plants were disposed on stand after being transplanted in plastic bags filled with carbonized rice hull. For phenology, treatments consisted of two types of plants (frigo and fresh) while for determining the phyllochron, this factor plus the accumulated thermal sum were considered. For variables related to gas exchanges, evaluations were performed in frigo and fresh plants and three evaluation periods (8:00 a.m., noon and 4:00 p.m.) in completely random blocks design, four repetitions, and eight plants per plot. Relating phenology evaluations and phyllochron determinations, frigo plants were more precocious than fresh plants. However, even frigo plants presenting a lower leaf emission rate, the thermal sum needed for the emission of successive leaves is lower (93.9 °C day leaf<sup>-1</sup>). Therefore, our study allows the producers, who want to sell strawberries in offseason, use frigo plants. However, fresh plants are recommended for those who cultivate in the traditional season.

Keywords: photosynthesis; stomatal conduction; carbonized rice hull; accumulation of leaves.

**Abbreviations:** A-Photosynthesis; Bt-base temperature of culture; CRH- carbonized rice hull; DAT-days after the transplanting; Dmt-daily mean temperature; DN-day neutral; Dts-daily thermal; E-tanspiration; gs- stomatal conduction; JD-Julian Days; LAR- leaf appearance rate; LDPE-low-density polyethylene film; NL-number of leaves; PAR- photosynthetically active radiation; RH- relative humidity.

#### Introduction

Strawberry is highly important for the Horticultural sector in Brazil, mainly in the internal market, considered the main culture among red fruits both in consumption and cultivated area. In Brazil, the conventional system is still the main form of strawberry cultivation (Fragaria X ananassa Duch.), but other forms have been increasingly used, such as soilless cultivation in greenhouse associated to Day-neutral cultivars (DN). Vernalized plants are also used (frigo), which are keep approximately 24 days in cold chambers under low temperature (4  $\pm$  1 °C) and relative humidity of 94  $\pm$  2%, speeding the thermal sum process needed to activate floral induction (Oliveira and Scivittaro, 2006). Fresh plants still represent the main type of plants used for strawberry cultivation in Brazil, particularly in the South-Eastern region. On the other hand, frigo plants are now an important option for Southern producers, especially those aiming the internal and external market, early production and offseason. Production precocity and maturation vary according to the type of plant, cultivars and management (Dias et al., 2009) as well as the environmental conditions. Temperature and radiation are the main environmental factors impacting on the growth and development of DN strawberry cultivars. Fresh plants behavior in different Brazilian regions has already been studied in order to identify the best performance in each cultivar in relation to its management. However, frigo plant adaptability, particularly in the Northern region of the state of

Rio Grande do Sul, has not been studied vet. The technology of soilless cultivation has attracted producers due to its fertility control, the ability of increasing plants density, decrease of diseases and pests and the increment in the products quantity and quality (Afsharipoor and Roosta, 2010). The physical features of the substrate (organic and inorganic) directly affect the production. Air and water are important factors for productivity (Verdonk et al., 1981). Aerobic conditions increase the capillarity of roots due to even distribution of these two factors. Nevertheless, it must be said that plants produced in soilless cultivation in greenhouse can be exposed to sudden stress. The main cause of this is the limited volume of the substrate where the plants are grown. In general, water reserves are quickly exhausted and the plants suffer from water stress (Klamkowski et al., 2006).

Strawberry soilless cultivation has been widely used due to high productivity. But to achieve this goal, information on physiological behavior are important (leaf gas exchanges), mainly when developing in greenhouse, where the micrometeorological elements are modified in its interior. The reflexes of these alterations on the plant physiology are not defined, since the factors direct or indirectly influencing photosynthesis are many, including water deficit, thermal stress, internal and external gas concentration and light composition and intensity (Concenço et al., 2008). Temperature has been indicated as primary importance in plants gas exchanges, affecting both photosynthesis and respiration. In this sense, studies aiming improve the understanding of plants physiological processes are important to produce information about response patterns of CO<sub>2</sub> assimilation in plants and ecosystems. Transpiration of most vegetal species is determined by the evaporative demand related to sun radiation, physiological mechanisms linked to stomatic responses and environmental factors, leaf area index and water availability in soil or substrate (Taiz and Zeiger, 2004). Stomatal conduction, on its turn, is defined as the physiological mechanism that vascular terrestrial plants have to control transpiration (Messinger et al., 2006), being proportional to transpiration, liquid photosynthesis and leaf water potential (Naves-Barbiero et al., 2000). In this process, stomas act as regulating water loss through transpiration (Larcher, 2000). Strawberry plants have a high demand for water, since they possess superficial radicular systems, large leaf area and fruits with high water content. However, if the aim is precocity and facilitate the production staggering, allowing fruit offering to the market in offseason periods, studies on phonologic cycles are equally important. Knowing the vegetal and reproductive cycle of the plant, the estimation of leaf appearance rate (LAR) and phyllochron (necessary time for the appearance of successive leaves) are important to calculate the number of accumulated leaves in the plant's main stem (Xue et al., 2004; Streck et al., 2007). Therefore, with the objective of identifying a possible precocity in frigo strawberry plants of cv. Albion produced in greenhouse, the cycle and the rate of leaf appearance were assessed and the photosynthetic behavior were analyzed.

#### **Results and Discussion**

#### Phenology of fresh and frigo strawberry plants

Comparing frigo and fresh plants, all phenologic stages presented differences, although frigo plants of cv. Albion presents higher precocity in reproductive cycle than fresh plants (Fig. 1). In vernalized plants flowering began 10 days after the transplanting (DAT), fruiting and harvest occurred at 31 and 49 DAT, respectively. In fresh plants, flowering began at 30 DAT, fruiting at 59 DAT, and harvest at 72 DAT. Vernalized plants 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), what allow the producers to predict the fruit offers in the market. The cycle of Albion plants produced from fresh plants obtained by other authors are similar to those obtained in this work. In Mendonça et al. (2012), flowering occurred at 63 DAT, later than other cultivars, and harvest at 94 DAT.

#### Phyllochron of fresh and frigo strawberry plants

Frigo plants precocity was assessed in phyllochron evaluations (Fig. 2). A lower number of Celsius degrees per day were needed for the emission of each leaf, with a phyllochron of 93.9 °C day leaf<sup>-1</sup> for the appearance of successive leaves. For fresh plants, phyllochron was 117.8 °C day leaf<sup>-1</sup>. However, compared to other studies using the

same cultivar and fresh plants, even in other production systems, this study presented a lower thermal need for leaf appearance. Mendonça et al. (2012), found a phyllochron of 149.35°C day leaf<sup>-1</sup> (intercropping system) and 199.96°C day leaf<sup>1</sup> (single production system). Estimating the LAR (leaf appearance rate) for frigo plants, a variation of 0.0055 leaf per °C day is observed, whereas in fresh plants, the variation was 0.0044 leaf per °C day. Thus, when the thermal sum reaches 600°C day, the frigo plants present 3.9 leaves, and the fresh plants, 3.1. Comparing to the work developed by Mendonça et al. (2012), the number of leaves found in this study is lower in cv. Albion. A possible explanation is the difference between materials used, such as substrate. However, this cultivar needs a higher thermal sum to emit one leaf in relation to other cultivars. When thermal sum reached 800°C day, in Ventana cv., the number of leaves was 11, while in Albion cv. it was 6 (Mendonça et al., 2012). A high phyllochron value in a plant, such as Albion, indicated a lower leaf appearance rate, since leaves need a more degrees day for the emission of each leaf. Vernalized plants present more reserves accumulated in the corona and in the roots being rapidly mobilized after transplanting to produce new leaves (Roudeillac and Veschambre, 1987). The linear relation between number of leaves and accumulated thermal sum (Fig. 2) shows that air temperature was one of the decisive factors for leaf emission 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 was cultivated in the recommended period (Streck et al., 2007), adapting to cultivation conditions.

### Gas exchanges in fresh and frigo plants and micrometeorological parameters

The mean temperature inside the greenhouse (Fig. 3A) was 15°C and the highest temperature was 20°C at 2:00 p.m. at 297 JD. In the following day, the mean air temperature (Fig. 3A) was 24°C and the highest (34°C) was recorded at 2:00 p.m. RH was 39% at 6:00 p.m. (Fig. 3B), reaching 97% from 10:00 p.m. to midnight for the evaluation day of fresh plants, and 39% by noon, reaching 97% from 6:00 p.m. to midnight, when another group of plants was evaluated. The city of Passo Fundo, Rio Grande do Sul, where the study was conducted, has an altitude of 709 m above sea level, temperate climate with humid subtropical features according to Koppen's classification (Koppen and Geiger, 1928) with well distributed rains during the year. The climatologic normals (1961-1990) presented mean annual temperature of 17.5 °C and relative humidity of the air of 72%. Mean air temperatures inside the greenhouse remained within an optimum range for the photosynthetic process in most  $C_3$ plants, such as strawberry. They extend over a range of 15-30°C, with an upper limit of temperature for the absorption of CO<sub>2</sub> of 40-50°C. This temperature amplitude is typical of each species/cultivar and certainly goes through alternation due to external factors (Larcher, 2000). The PAR, considering the mean in cloudy days (Fig. 3C) was 92.23  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> and maximum of 319.83  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> at 3:00 p.m. The mean in sunny days was 331  $\mu mol~m^{-2}~s^{-1}$  and maximum of 490.36  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> at 1:00 p.m.

### Phenologic Calendar (Days after transplanting)



Fig 1. Phenological stages of frigo and fresh plants of strawberry cultivar Albion during the period of soil cultivation under greenhouse. Passo Fundo / RS, Brazil, 2009-2010



Fig 2. Phyllochron of frigo and fresh plants of strawberry cultivar Albion. Passo Fundo/RS, Brazil, 2009-2011.

Observations of CO<sub>2</sub> assimilation vary widely according cultivation conditions (environment and management) and the cultivar used. Fresh plants of Albion cv. in our work (Fig. 4 A) remained within a range of 15-25  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> established for strawberry in field cultivation (Hancock, 1999). The substrate cultivation system (horizontal columns) in greenhouse, the photosynthetic rate ( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) varying between 10.62 to 13.75 30 days after the transplanting and between 4.11 and 7.39 60 days after transplanting. This decrease was recorded due to the potential of water remaining below -1.5 MPa (Klamkowski et al., 2006). The results found in the three evaluation times showed an increase in photosynthesis proportional to the increase in radiation. At 8:00 a.m., there was a variation in assimilation from 10.02  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> in 200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> to 18.74  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> at air temperature of 13°C. By noon, it varied between 9.5 (200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) and 17.25  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> at 16°C. At 4:00 p.m.

assimilation was between 8.74 (200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) and 17.92  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> (1200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) when the temperature recorded was 19°C.

In relation to frigo plants (Fig. 4B), there was also an increase in photosynthesis in the three evaluation times, according to the increase in radiation. At 8:00 a.m, the maximum CO<sub>2</sub> assimilation value was 8.40 µmol m<sup>-2</sup> s<sup>-1</sup> (ambient temperature of 21°C); by noon, .34 µmol m<sup>-2</sup> s<sup>-1</sup> (30°C); and at 4:00 p.m. at 27°C the maximum photosynthetic rate was 7.33 µmol m<sup>-2</sup> s<sup>-1</sup>, in 1200 µmol m<sup>-2</sup> s<sup>-1</sup> light. These values were lower to those found for fresh plants, regardless of the evaluation time. The reduction was probably due to air temperature being higher than when fresh plants were evaluated. In most species, photosynthesis was reduced when the plant was submitted to high temperatures, its optimum value being



Fig 3. Temperatures (A), relative humidity (RU) of the air (B) and photosynthetically active radiation (PAR) in typical days (C) recorded in greenhouse during the days (temperature and RH) and month (PAR) of evaluation of foliar gas exchanges for in the greenhouse.

in intermediary temperatures (Hikosaca et al., 2006). On the other hand, chlorophyll contents may result in variability in CO<sub>2</sub> assimilation rates for different species. According to Blanke (2002), strawberry leaves presented chlorophyll values varying between 1.5 to 2.0 mg chlorophyll g<sup>-1</sup> MF. In addition to depending on chlorophyll contents on the leaves, this variability is determined by environmental conditions such as temperature, relative humidity, photosynthetically active radiation, growth habits and distinct varieties in relation to the plant's phenologic stage, nutrient availability and propagation method (Dale and Luby, 1990). In 800  $\mu mol$  $m^{-2} s^{-1}$  there was saturation of CO<sub>2</sub> for both fresh and frigo plants. In this radiation the maximum photosynthetic activity for fresh plants reached 18 µmol m<sup>-2</sup> s<sup>-1</sup>, while frigo plants were lower than 8  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. In the growing period of frigo plants the temperature varies from 21 to 34 °C. With this temperature, it was expected that photosynthesis were reduced. Other authors such Morgan (2006) has related the limitation of photosynthesis in strawberry with the same light intensity, however at mean temperature of 25 °C. In this case, CO<sub>2</sub> absorption speed is no longer limited by photochemical reactions, but actually by enzymatic reaction and CO2 availability (Larcher, 2000), which may have been reflected in the results found for frigo plants. For fresh plants, the diurnal temperature varied between 10 and 20°C, favoring

CO<sub>2</sub> assimilation. Environmental conditions when fresh plants are evaluated probably favor similar values to those obtained by other authors, such as Carlen et al. (2009), who also obtained saturation at 1400 µmol m<sup>-2</sup> s<sup>-1</sup> for Marmolda and Darselect cultivars, coinciding with the light range found for both types of plants in this work, although with CO<sub>2</sub> assimilation of 18 and 16  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, obtained by us in fresh plants. The luminous compensation point for fresh and frigo plants was next to 200 µmol m<sup>-2</sup> s<sup>-1</sup> for all times analyzed. These results correspond to the light rate in which the photosynthetic activity equals respiratory activity. This means that, in this point, the plant has consumed in respiration an amount of O<sub>2</sub> equaling that produced in photosynthesis or that it consumed in photosynthesis an amount of CO<sub>2</sub> equaling that released in respiration (Barbosa et al., 2010). Peixi et al. (2002) working with strawberry in greenhouse obtained 91.7 µmol m<sup>-2</sup> s<sup>-1</sup> for luminous compensation point. When fresh plants were submitted to control luminosity (0  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), only the reading performed at 4:00 p.m. presented a negative value of CO<sub>2</sub> assimilation (-0.95  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>). In other words, at this moment only accumulated reserves were consumed (Barbosa et al., 2010).



**Fig 4.** Photosynthetic behavior (A: $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) in fresh (A) and frigo (B) plants of strawberry cultivar Albion in greenhouse

On the other hand, frigo plants did not show negative values for photosynthesis when the plants were submitted to control luminosity. Zangh et al. (2007), assessing photosynthetic features of Tongzi 1 strawberry cultivar, found 54.2 µmol m<sup>-2</sup>  $s^{-1}$  for luminous compensation point and 916 µmol m<sup>-2</sup>s<sup>-1</sup> for  $CO_2$  saturation point. Peixi et al. (2002), working with strawberry in greenhouse, found 943.3 µmol m<sup>-2</sup> s<sup>-1</sup> light saturation point. These values are similar to the ones found in this study. The relation between stomatal conduction and transpiration was positive linear, presenting high coefficients of determination for both types of plants (Fig. 5). This shows a positive effect, since these factors are directly associated to photosynthetic rate (Vidal, 1997). The opening of stomatal pores is ensured by carbon gain and water loss by transpiration (Klamkowski and Treder, 2006). As water conductivity per m<sup>2</sup> and second increases in the stomas, there is an increment in the transpiration of leaves for fresh plants. In Fig. 5A it is shown that each mol of water via stomatal conduction corresponds to an increase of 6.7, 18.4 e 15.7 mols of water in fresh plants' leaf transpiration by stomatal conduction unit at times 8:00 a.m., noon, and 4:00 p.m. respectively. The values obtained for stomatal conduction in fresh plants' leaves were also found to be higher than the limit of 0.05 mol  $H_2O$  m<sup>-2</sup> s<sup>-1</sup> (Medrano et al., 2002), indicating only the involvement of stomatic effects as shown in Fig. 5A. At 4:00 p.m. stomatal conduction (mol H<sub>2</sub>O m<sup>-2</sup>-



**Fig 5.** Relation between stomatal conduction (gs:mol H<sub>2</sub>0 m<sup>-2</sup> s<sup>-1</sup>) and transpiration (E: mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) in fresh plants at 297 JD and frigo plants at 298 JD in 2010.

 $m^{-2} s^{1}$ ) doubled, going from 0.16 in 200 µmol  $m^{-2} s^{-1}$  radiation to 3.2 in 1400 PAR. For frigo plants (Fig. 5B), the relation between the two factors was also positive, but conductance values were lower than 0.05 mol  $H_2O \text{ m}^{-2} \text{ s}^1$  in all the times evaluated. According to Lorenzo-Minguez (1994), this fact induces an increase in leaf temperature. Therefore, with stomas almost closed, the photosynthesis/transpiration ratio rapidly declines, since CO2 entry suffers more restraints than stomatic transpiration, and the loss of water in vapor form continues through the cuticle (Larcher, 2000), which justifies the decrease in photosynthesis for these plants, since the mean leaf temperature during the evaluation varied from 33°C (8:00 a.m.) to 29°C (4:00 p.m.). Transpiration as physiologic variable has a great interest concerning the yield of the crops, since the water flow through the plant, induced by transpiration, is the transportation system for minerals absorbed by the roots moving through the transpiratory stream (Caird et al., 2007). Another important function is cooling the leaf surface and helping in the maintenance of the optimum turgor. Chaves et al. (2003) say that stomatal

closure protects the plants against the excessive loss of water, but also restrains CO<sub>2</sub> diffusion in the leaves, this being the main cause of photosynthesis reduction. When there is an increase in photosynthetic activity, the internal quantity of carbon dioxide tends to reduce, since it is being incorporated and, thus, there is the tendency to a greater stomatal opening, leading to greater conductance (Farguhar and Sharkey, 1982). phenology evaluations Relating and phyllochron determination with those of gas exchanges, frigo plants, which were more precocious than fresh plants, presented lower CO<sub>2</sub> assimilation. According to Aires et al. (2012), the liquid assimilation rate diminishes with the plant's growth, due to leaf shading. However, even frigo plants presenting a lower leaf emission rate than fresh plants, the thermal sum needed for the emission of successive leaves for them was lower (93.9 °C day leaf<sup>-1</sup>) with more leaves emitted in comparison to fresh plants, increasing their shading, which probably explains the reduction in CO<sub>2</sub> assimilation for these plants.

#### **Materials and Methods**

#### **Experiment** site

The experiment was carried out at the Horticulture Sector of the University of Passo Fundo (UPF), Rio Grande do Sul, Brazil, which present the following geographic coordinates: latitude 28°15' S, longitude 52°24' W and mean altitude 709 m. The normal annual mean of cold hours is 422 h with temperatures lower than or equal to 7.0°C, varying from 214 h to 554 h (Cunha, 2003).

The work was performed in a galvanized steel greenhouse with no acclimatization with semicircular roof and area of 420 m<sup>2</sup>, in the period ranging from august 2009 to march 2011. Fresh plants were transplanted to 172 Julian Days (JD) and the frigo plants at 237 JD. The structure is located in northeast-southeast direction and covered with low-density polyethylene film (LDPE) with anti-ultraviolet additive and thickness of 150 micra. In the inner upper part an aluminum reflective thermal screen of 50% shading was installed. The management of the portable curtains was adapted to the internal ambient temperature, remaining closed during the day while temperature remained lower than 12°C and higher than 30°C and during the night regardless of temperature.

#### Culture manegment and treatments

Plants of the Albion cultivar were transplanted to 150 micra white LDPE bags of tubular shape (1 m length, 0.30 m width) in a 0.20 m spacing line and 0.10 m interline, suspended on stand at 1.20 m over the soil.

The bags were filled with organic material originated from residues of a rice crop (carbonized rice hull - CRH). For the carbonization of the hull a 'carbonizer' was used, composed of a carbonizing cylinder, a docking station and a chimney, following the phases described by Kämpf (2005). Physical and chemical analyses were performed in the respective substrate, presenting 0.618 m<sup>3</sup> m<sup>-3</sup> airing space, 0.107m<sup>3</sup> m<sup>-3</sup> easily available water and 0.160 m<sup>3</sup> m<sup>-3</sup> reserve water, 6.8 pH, and 0.31mS cm<sup>3</sup> to EC. Irrigation was individualized in the bags and performed by drip irrigation system located in its interior, composed with drippers each 15 cm. Fertirrigation was used according to the formulation of Calvete et al. (2007). Cultural practices were performed according to the need of the strawberry; the main disease and pests were controlled, such as mycosphaerella

(Mycosphaerella fragariae (Tul.) Lindau), oidium (Sphaerotheca macularis), grey rot (Botrytis cinera L.), red spider mite (Tetranychus urticae), aphides (Capitophoru sfragaefolii; Cerosiphaforbesi) and thrips (Frankliniella occidentalis (Perg.)). Phenology treatments included two types of plants (frigo and fresh) while the phyllochron was determined by these factors plus the accumulated thermal sum.

#### Environment evaluations

Temperature and relative humidity (RH) of the air were monitored with a weekly recordings thermohygrograph (Sato) installed at 1.50 m height inside the greenhouse. The gas exchanges evaluation were performed at 297 JD for the experiment with fresh plants and at 298 JD for frigo plants in 2010. Photosynthetically active radiation (PAR) in typical days (cloudy and sunny) inside the greenhouse from 8 a.m. to 5 p.m., through a PAR Photon flux sensor (Q SO-S) and the reading was performed through ProCheck device. To evaluate the phyllochron, the mean temperature and the daily thermal sum were assessed according to the following equations:

Daily mean temperature (Dmt):

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

Which, calculates the arithmetic mean of temperatures (°C) recorded by the thermohygrographer each two hours. The daily thermal sum (Dts) was calculated according to Gilmore and Rogers (1958) and Arnold (1960):

 $Dts = (Dmt - Bt) [^{\circ}C ddy^{-1}].$ 

Where,

*Dmt*= Daily mean temperature;

Bt= Base temperature

Base temperature (Bt) is defined as the minimal temperature, below which there is no leaf appearance. The Bt considered was 7°C. The Dts was accumulated from transplantating, resulting in the accumulated thermal sum (Ats); in other words,  $ATs = \Sigma DTs$ .

#### Phenologic evaluations

Phenologic evaluations consisted of recordings on the onset of flowering, fruiting and beginning and end of the harvest. The onset of flowering was considered when 50% of the plants selected of each type of plants (frigo and fresh) presented at least one open flower. The onset of fruiting was determined after the end of flowering (all petals fallen). Fruits showing 75% of red-colored epidermis were considered ripe.

#### Phyllochron evaluation

Phyllochron was determined in both frigo and fresh plants by counting the number of leaves (NL), which was performed twice a week, from the onset of leaf emission to the second flowering. A new leaf was considered as emitted when it was visible with approximately 1 cm length.

#### Gas exchange evaluations

Evaluations related to gas exchanges were performed in three leaves at 297 and 298 JD in 2010 for fresh and frigo plants, respectively, considering the central leaflets. Evaluations consisted of photosynthesis measurements (A:  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), stomatal conduction (gs: mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) and transpiration (E: mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), using a infrared radiation gas analyzer

(IRGA) (LI-6400, LI-COR, Lincoln, USA) equipped with a closed top chamber, emitting light at 1400, 800, 600, 400, 200, 100, 0  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> during all the evaluations. The parameters analyzed (A, gs e E) were determined under CO<sub>2</sub> concentration environment.

#### Experimental design

For variables related to gas exchanges, evaluations were performed in the two types of plants and three evaluation periods (8 a.m., 12:00 and 4 p.m.) in randomized blocks design with four repetitions and eight plants per parcel.

#### Statistical analysis

Phylochron regression analysis was performed between the NL in the main corona and the STa . The phyllochron (°C days leaf<sup>-1</sup>) was estimated by the inverse of the angular coefficient of the linear regression. For gas exchange three leaves per plot were analyzed considering each wave length emitted by the IRGA. For data related to photosynthesis light response curves were performed. Stomatal conduction (gs: mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) and transpiration (E: mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) were analyzed by analysis of variance (ANOVA), significant at 5%, and regression.

#### Conclusions

For early production in the offseason proposes frigo plants. However, fresh plants are indicated to be cultivating in recommended agricultural period, for being more photosynthetically active.

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