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## Performance of day-neutral strawberry cultivars in soilless culture

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## Abstract

Cultivars of strawberry (*Fragaria* x *ananassa* Duch.) are classified as short-day (single-cropping) or day-neutral (DN; perpetualflowering), in term of flowering and according to the place and year of production. The performances of DN cultivars are little known, mainly in temperate and sub-tropical regions, such as those located in South America. The purpose of this study is to determine the performance of Albion cultivar from frigo seedlings in the off-season period in a soilless culture system, using two different materials as a base, namely carbonized rice husk (CRH) and the commercial substrate Mec Plant Horta 2<sup>®</sup>. The treatments consisted of substrates (main plot) and harvest time (subplot). The substrates comprised of 100, 75, 50, 25 and, 0% CRH (or 100% commercial substrate). We evaluated 13 harvest time to fruit production in terms of the number and total and commercial fresh mass per plant. In relation to quality, the sugar content/acidity (total soluble solids [TSS]/total titratable acidity [TTA]) and external fruit color were considered. The results showed that out of 19 months of cultivation, there was fruit production in 13 months, with an average productivity of 3.5 kg plant<sup>-1</sup>. In conclusion, it is possible to produce strawberries independent of the substrate used, in offseason periods, where they are harvested fruits with better taste (TSS/TTA).

Keywords: Fragaria x ananassa Duch.; production; quality; perpetual flowering vernalization; yield.

**Abbreviations:** EAW\_easily available water; AS\_aeration space; BW\_buffering water; CEC\_capacity exchange cation; CRH\_carbonized rice husk; DN\_ day-neutral; EC\_electrical conductivity; FBD\_flower bud differentiation; LD\_long-day; LDPE\_low density polyethylene; PAR\_photosynthetically active radiation; TP\_total porosity; TSS\_total soluble solids; TTA\_total titratable acidity.

## Introduction

Due to problems with soil diseases and the banning of fumigant compounds for disinfection, the cultivation of strawberries in soilless substrate has been a solution adopted by many countries. In response to the growing demand for strawberries on the market, researchers are seeking to cultivars that produce outside the normal growing season or more than once per year. Cultivars of strawberry (Fragaria x ananassa Duch.) can be classified into the mono-flowering (single-flowering) and perpetual flowering (everbearing) groups (Sønsteby and Heide, 2007). The latter are considered day-neutral (DN). Among the cultivars that belong to this group, different physiological behaviors are exhibited according to the temperature and photoperiod conditions. They behave as long-day (LD) qualitative with temperatures higher than 27°C. Meanwhile, at temperatures between 10 and 25°C, they are LD quantitative, and they can be considered DN below 10°C (Sønsteby and Heide, 2007); however, flowering in DN cultivars decreases with higher temperatures.

In previous research, Tribute cultivar plants were insensitive to a photoperiod of 14 hours at a temperature of  $23^{\circ}$ C, but required long days at higher temperatures (Bradford et al., 2010). The considered DN cultivars, more specifically, required lower temperatures of up to  $10^{\circ}$ C for flowering (Nishiyama and Kanahama, 2000; Heide and

Sønsteby, 2007), when they are less sensitive to day length (Sønsteby and Heide, 2007). This definition is based on the octoploid strawberry cultivars from the breeding programs of California, distinct from strawberry cultivars defined as DN in Gutridges (1985) review, which are genetically different (Sønsteby and Heide, 2007).

Strawberry cultivars that are classified as DN often vary in the degree of reflowering due to location and year of production (Strick, 2012). The transplantation of DN cultivars from frigo seedlings has been carried out in the spring/summer in regions of high altitude and mild summer, as well as during winter (Otto et al., 2009). However, there have been few reports in the literature on the behavior of these plants grown in a soilless culture system in a protected environment.

The productivity and fruit quality of strawberries grown in a soilless system depends largely on the substrate. Producers have been using commercial substrates mixed with carbonized rice husk, a low-cost byproduct that is available at the cultivation sites. Scientific research is important when it comes to encouraging the use of the derivative products industry as a substitute for the commonly used in horticulture. We used frigo DN Albion for the experiment. The objective was to assess whether it is possible to produce crops in the off-season or more than once crop per year in soilless cultivation systems using two different substrate materials, namely carbonized rice husk (CRH) and the commercial product Horta 2.

### Results

### Total marketable fruits

This study did not show a significant interaction between the yield (fresh weight) of the total and commercial strawberry fruit among the substrates and the 13 crops observed between October 2009 and April 2011. However, a significant difference was observed in isolation to harvesting dates (Fig. 1). It was possible to harvest for more than one season, starting the harvest (Oct. 2009) soon after transplantation (Aug. 2009). After gathering for 6 months, there was an interruption in production, with the fruit harvest beginning again in September 2010, one year after planting. The maximum fruit production occurred in January 2011 (about 526 g plant<sup>-1</sup>), although this was similar to the productions obtained in March, November, and December 2010.

If we consider a period of 19 months from the beginning until the end of harvest, plants produced fruits for 13 months. It should be emphasized that in the first 5 months of harvest, from October 2009 to February 2010, the total production was about 73% below that of the final seven months, from September 2010 to March 2011.

It was also observed in the elapsed period in the fruit production interval that plants exhibited increased stolon emission. Although there was an interruption, the harvest period could extend until the months of March and April, even with lower volumes, in the off-season. In addition, the PAR and the air temperature inside the greenhouse agriculture affected strawberry production in total and commercial fruits, as verified by the absence of harvest between March and August 2010.

In this period, the average PAR (Fig. 1A) reduced from 600  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>, average value presented in the production months, to 200  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> and the average relative temperature (Fig. 1B) from 28 °C to 16 °C, when the average in the production period was 24 °C.

#### Sugar content and external color of the fruit

Flavor was statistically analyzed in the period of greater fruit production (Nov. 2010 to Apr. 2011), looking at the relation between sugar and acidity in plants grown on different substrates. There was no influence of the substrate on the flavor of strawberry fruit, but differences were observed between the months of harvest. The behavior of this attribute followed a quadratic trend (Fig. 2), with the lowest flavor (more acidity) in January 2010 and increasing sugar content from that harvest. It was found that the harvest dates were responsible for 95% of the variations in flavor.

The quality characteristics evaluated by external coloring of the fruit, such as chroma and luminosity (L \*), determined by °Hue, showed no significant interaction between the substrates and months of harvest. ANOVA only showed a difference between the months of harvest.

The most intense coloring or saturation in strawberry fruits was obtained in January 2011, but these fruits were less bright (Fig. 3A). Fruits with °Hue closer to zero (Fig. 3B), with a more reddish aspect, were harvested in November 2010.

#### Discussion

This study represents the first report in Brazil showing the performance of a DN cultivar of strawberry for two consecutive harvests in substrate cultivation under protected ambient conditions. In this research, it was found that frigo cultivar Albion produced more than one crop in the offseason period, in which there was sharp sugar content at the end of that period. It was also observed that there were two crops to harvest, with a range between the same. It was evident that the harvest from the year after transplantation was 73% higher than in the first year. This response was due to the establishment of plants in the cropping system in question. At this stage, the energy accumulated in plants increased the photosynthate translocation for the production of flowers and fruits, unlike that observed in the first crop, where part of the energy was used for the establishment of the culture. Between crops, the plant passed through a dormancy period, characterized by the lack of production between the months of March and August 2010.

The stage that preceded this pause in production was characterized by average temperatures in the cultivation environment, at around 28°C. Temperatures above 28°C decreased or inhibited flowering in DN cultivars, and consequently, production. A previous work showed that the influence of temperature on flowering DN plants with 30°C day/25°C night and a photoperiod of 8 hours of light inhibited floral induction (Nishiyama and Kanahama, 2002), also in accordance with Kadir et al. (2006). The authors point out that in addition to the negative effect on flowering, there is a genotype factor. The cultivars classified as DN generated doubts because the degree of flowering varies depending on the place and year of cultivation. The genetic basis for this designation is being investigated.

Therefore, it can be inferred that it is insufficient to classify DN cultivars solely based on temperature (Stick, 2012). Thus, these strawberry plants resemble LD at all temperatures except those below  $10^{\circ}$ C, when they are less sensitive to day length (Sønsteby and Heide, 2007; Stick, 2012). This was considered, although the purpose of our study was not to evaluate temperature on flowering.

During the interval production period (corresponding to fall and winter), the plants exhibited increased stolon emission. When temperatures were close to 15°C, the metabolic activities of the plants decreased and were mobilized to store reserve substances, which were accumulated in plant roots, while development of the leaves was reduced, making the plant relatively dormant. When reduced temperatures occurred during the winter, there was an accumulation of the chilling hours, resulting in floral induction and vegetative growth. With the arrival of spring and the resumption of higher temperatures, production of fruit restarted. The cultivar used in this study exhibited this behavior, confirming its similarity to quantitative LD plants, which emit flowers or resume flowering at temperatures between 10 and 25°C (Nishiyama and Kanahama, 2000; Sønsteby and Heide, 2007). According to Brandford et al. (2010), temperatures and cultivars exert a strong influence on the rate of flower bud differentiation (FBD).

According to the literature, for development and satisfactory grow of plants, the solar radiation flux density above the limit of culture trophic and air temperature higher than the basic limit for their growth and development must

Table 1. The chemical analysis of substrates.

	CEC	pН	EC
Treatments	mmol <sub>c</sub> /kg		$(mS/cm^3)$
100%CRH	61.22	6.8	0.31
75%CRH+25% H2	176.25	6.1	0.38
50%CRH+50% H2	256.67	5.6	o.41
25%CRH+75% H2	302.69	5.6	0.51
100% H2	314.32	5.5	0.67

Method the beaker (IN 17, MAPA, 2008). Base moist. 65°C. CRH= carbonized rice husk.



**Fig 1.** Total and commercial fresh mass and photosynthetically active radiation (A); total and commercial fresh mass and average temperature (B) of monthly fruits produced by strawberry cv. Albion protected ambient. Means did not differ significantly according to the F test (\*\*p<0.01).

occur simultaneously (Wien, 1997). These factors may increase the production of assimilates and their availability for growth and fruit production (Andriolo, 2000). From the literature, it is clear that the production of flowers and strawberry fruits can be improved with light intensity of 400–450  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> of PAR (Kirschbaum, 1998). The plants included in our work were radiated under 517  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, close to the ideal values for the species. The aim of our work using a soilless system was to indicate a material or combination of materials for use as a substrate in the

cultivation of strawberries. However, it was not possible to identify this because the materials were similar in terms of the production and quality of fruits. Considering the mean of the five substrates, using physiological analyses of plants, it was found that frigo seedlings are less photosynthetically active but produces more compared to fresh plants, because they remain in cultivation for a longer time (Costa et al., 2014). The results only showed differences between the harvest seasons. Even with the production interval, it was possible to maintain the cultivation of plants for 19 months.



Fig 2. The relationship between total soluble solids (TSS) and total titratable acidity (TTA) of fruits of strawberry cv. Albion in a protected ambient environment.



Fig 3. Average values of the fruits' external color, L \*, chroma (A), and hue (B) of strawberry cv. Albion in protected ambient conditions (\*\* $p \le 0.01$ ).



Fig 4. The physical analysis of substrates.

This illustrates the potential to reduce costs related to the annual renovation of seedlings, particularly in regions that need to import seedlings such as southern Brazil. In this area, the seedlings are purchased from Argentina and Chile. They have high value and undergo a delay in delivery time, being transplanted later (in July) than the indicated period (May) for the region. It is noteworthy that although an interruption in the production of fruits occurred, it was possible to extend production in the off-season, until February, March, and April, even with lower volumes, representing financial gains for producers. Although the effect of substrates on increased production and quality has not been realized yet, the results did not invalidate our proposal, as they indicated that we could replace commercial products with materials obtained in the region as agricultural by-products. This is relevant information to producers, who will not need to formulate mixtures; thereby, reducing hand labor and enabling sustainable production.

In our experiment, we concluded that frigo seedlings of the DN cultivar have double the productive potential when grown in a soilless culture system. This finding is in accordance with the Carlen and Crespo (2012), who suggested that new production systems can increase profitability, reduce costs, and generate technologies for protected ambient conditions and allow crops to be extended in agricultural areas throughout the year for marketing of berries.

Techniques for checking the sugar content and acidity (Conti et al., 2002; Yommi et al. 2003) facilitate the identification of the tastiest strawberry fruit (Fig 2) at the end of the growing season (March–April). Therefore, despite the reduction in production, farmers will be able to reap a betterquality harvest in the off-season. A good relationship between TSS and TTA is dependent on the maturity stage, in which the fruit is harvested, and generally increases during ripening via the degradation of polysaccharides (Chitara and Chitara, 2005). According to Morgan (2006), the reference value for the equilibrium between sugar and acid content is between 9 and 13.5%. In our study, there was an increase reaching 15.3% starting in January. It was found that the relationship between sugar and acidity was 95% dependent on the harvest season.

It is possible to monitor the metabolic changes for each stage in the growth and ripening of fruits. For example, the formation of the pigment and flavor is linked to materials from the primary metabolism. The biosynthesis of amino acids is an important factor in the fruit quality of strawberries (Zhang et al., 2011). According to Zhang et al. (2011), flavor is the result of a complex mixture of many volatile compounds (over 300) and organoleptic properties combined with the texture. It not only depends on physiological processes, but also on the relationship between the climate and biological factors, with temperature particularly relevant (Kader, 2002). Similar to other variables, analyzing the color of fruits (chroma), brightness (L \*) (Fig. 3A) and °Hue (Fig. 3B) were only found to be influential at harvest time.

In the literature, studies on frigo and DN strawberry indicate luminosity values between 42 (Selva) and 49 (Aromas) (Yommi et al., 2003). Values close to 32 found for Albion are reliable using the methodology described by Conti et al. (2002). However, fruits with less intense brightness showed a dark red hue, agreeing with Shüneman (2009) and Conti et al. (2002).

In November 2010, redder fruits were collected (Fig. 3B). During this period, the average temperature was  $22.5^{\circ}$ C and the average radiation was  $331 \ \mu$ mol m<sup>2</sup> s<sup>-1</sup>. According to the literature, high temperatures and solar radiation over a long period cause premature ripening of the fruit and a reduced crop cycle (Palencia et al., 2013). In this study, the mean values of temperature and radiation found for this period (November 2010) were close to the range considered ideal for the C3 metabolism of plants (Larcher, 2002) to express their production potential and fruit quality. Fruits harvested in late

spring and early summer are more attractive for fresh consumption.

## **Materials and Methods**

#### Plant material

We used frigo of the Albion cultivar from the vivarium LLAHUEN ( $33^{\circ}50'15.41$  "S and  $70^{\circ}40'03.06$ " W) of Chilean Patagonia, whose headquarters were provided by the Breeding Program at the University of California. Propagation carried out at open soil after sterilization, spaced at 27 cm per plant (total of 62.000 plants in one ha). Two months after planting, the seedling were mechanically harvested and prepared via washing and selection (crown diameter). They were packed without leaves and kept in the cold for 2 months at a temperature of  $4\pm1^{\circ}$ C and a relative humidity of  $94\pm2\%$ . Seedlings were sent to Brazil in containers transported by ships and delivered after 10 to 12 weeks of quarantine.

#### Location and duration

The work was conducted from August 2009 to April 2011 (2009-2011) in an agricultural greenhouse of 420 m<sup>2</sup>, using 150 m<sup>2</sup> for the experiment. The greenhouse is located in the northeast-southeast direction in the Sector of Horticulture, Faculty of Agronomy and Veterinary Medicine of the University of Passo Fundo (UPF), Passo Fundo, Rio Grande do Sul, Brazil (28°15'39 "S, 52°24'33" W).

#### Cultivation

The plants were grown in substrate (soilless), then transplanted into containers constructed using a tubular film of low density polyethylene (LDPE) with white color and 150 micron thickness, called bags (1 m long by 0.30 m wide). They were spaced 0.20 m apart in a row and 0.10 m between rows, and suspended on stands of 1.20 m in height.

Irrigation was performed individually per plot via the drip system located inside the bags, comprising a flat hose with drippers every 15 cm. For the cultivation of the seedlings until early fruiting, each plot received 10 L of water once per week. After this period, the distribution was 10 L of water and distributed twice per week. With the beginning of harvest, irrigation management increased to 50 L of water weekly. On weekends during this period, irrigation was increased to over 10 L per plot. The fertigation method followed the formulation described by Calvete et al. (2007).

#### Experimental design

The experiment was set up in a factorial with a split plot; here, the substrates were the main plot and the harvest times were the subplot. The experiment employed a randomized block with four replications and eight plants per plot (total of 24 plants per treatment).

The substrates were made from five proportions of materials, comprising carbonized rice husk (CRH) and the commercial substrate Mec Plant H2. The proportions were as follows: T1: 100% CRH; T2: 75% CRH + 25% H2; T3: 50% CRH + 50% H2; T4: CRH 25% + 75% H2; and T5: 0% CRH (100% Horta 2). Considering the factor harvest seasons, they sampled the fruits of 13 months over the crop.

## Total marketable fruits

A weekly assessment was carried out for six plants per plot of the total and commercial fresh mass (g), as well as total and marketable fruit (fruit with a diameter greater than 22 mm) devoid of injuries, diseases, and deformations. The fruits were harvested when they were 3/4 to fully pigmented red.

#### Physical and chemical characteristics of the substrates

The substrates analyzed in the Soil Physics Laboratory (LAFAS), Faculty of Agronomy and Veterinary Medicine of UPF for the total porosity (TP), aeration space (AS), easily available water (AFD), and buffering water (AT) according to the methodology described by Klein (2005) (Fig. 4). The chemical characteristics were determined at the Soil Chemistry Laboratory of the same university (Table 1).

#### Sugar content and coloring of external fruits

Monthly, after weighing, the chemical characteristics of the total soluble solids (°Brix) and the external coloring of fruits were analyzed. The total soluble solid content was obtained from approximately 100 g of fresh fruit from each plot, expressed in Brix degrees, which determined using the digital refractometer model N-1E.

For the external coloration of fruits, the harvesting procedure was the same as described above. The determination of color on the surface of the fruits was performed only in commercial fruits at three distinct points located in the equatorial region of each fruit. A diffuse reflectance spectrophotometer (Hunter Lab), ColorQuest II model with an optical geometric sensor ball was used. This employed the Hunter color system, adjusted by the CIE, providing values for L\* (lightness) ranging from zero (black) to 100 (white), a\* (red to green), and b\* (blue to yellow) (Fallovo et al., 2009). These values were employed to calculate angle degree of hue ( $H^{\circ} = \arctan [b */a *]$ ), where  $0^{\circ}$  = red-purple;  $90^{\circ}$  = yellow;  $180^{\circ}$  = blue-green, and blue = 270° and the chroma (C \* =  $[a * 2 + b * 2] \frac{1}{2}$ ), which is indicative of the intensity or saturation of color (Conti et al., 2002).

#### Temperature and radiation

The environment was monitored according to the micrometeorological variables of temperature and relative humidity, recorded weekly using a Sato brand thermohygrograph, installed at a height of 1.50 m inside a greenhouse. The photosynthetically active radiation (PAR) was also evaluated using a sensor made by ProCheck (reading device). PAR was measured on typical clear and overcast days, monthly, inside and outside of the greenhouse.

#### Statistical analysis

The data relating to the harvest were grouped into four samples per month, totaling 13 evaluation periods. The variables were analyzed considering the split plot, comprising the main portion of the substrate and the sub-plot, and the harvest time. The values related to external color corresponded to nine periods of evaluations, and the Brix level was 10. These data were submitted to analysis of variance (ANOVA), and when there was a significant difference, the means were compared using the Tukey's test at 5% error probability with the CoStat statistical program (Cohort Software, 2003).

## Conclusions

Strawberry cultivar Albion frigo in substrate produced more than one crop period off-season. In all crop periods, it produces 3.4 kg plant<sup>-1</sup> fruit. The agricultural byproduct called CRH can be used by farmers to replace the commercial product Horta 2. Furthermore, in addition to crop in the off-season, fruits present best flavor (TSS/TTA).

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