Emergency and Growth of Passionfruit Following Different Seeding Dates and Stimulant Use, in Subtropical Autumn and Winter

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Summary

The yellow passionfruit (*Passiflora edulis* f. *flavicarpa*) is well adapted to tropical temperatures. Under subtropical conditions, winter temperatures limit its growth. Some treatments with biostimulants promote germination and seedling initial growth. The objective of this work was to evaluate emergency and growth of passionfruit seedlings under different seeding dates and seed treatments. Two stimulants were tested: gibberellic acid (GA) 1000 mg L^{-1} in water soaking and a stimulant (ST) composed of 90 mg L^{-1} kinetin, 50 mg L^{-1} gibberellic acid and 50 mg L^{-1} indolbutiric acid (IBA) applied without dilution (4 mL kg^{-1}). Seeding dates were May 2, May 22, June 11 and July 2 for 2013 and 2014. Emergency in the ST treatment was similar to the control but was reduced by GA treatment. GA increased the time needed for the seeds to emerge. Seedling growth evaluated on September 15, in two years, was not significantly modified by any stimulant. Seeding dates did not influence emergency percentage, but in the two latest dates the emergence was slower. The later the sowing dates, the smaller the seedling growth, and the models fitted to the response were different according to the characteristics evaluated and the year.

Keywords: *Passiflora edulis*, gibberellic acid, kinetin, IBA, seed

Emergencia y crecimiento de maracuyá con diferentes fechas de siembra y uso de estimulantes, en otoño e invierno subtropical

Resumen

El maracuyá amarillo (*Passiflora edulis* f. *flavicarpa*) se adapta bien a temperaturas tropicales. En climas subtropicales, las bajas temperaturas del invierno limitan su crecimiento. Algunos tratamientos con bioestimulantes promueven la germinación y el crecimiento inicial de la plántula. El objetivo del trabajo fue evaluar la emergencia y el crecimiento de plántulas de maracuyá en diferentes fechas de siembra y tratamientos de las semillas. Se evaluaron dos tratamientos: aplicaciones de ácido giberélico (AG) 1.000 mg L^{-1} en el agua de imbibición de las semillas, y un estimulante compuesto (ST) de 90 mg L^{-1} de cinetina, 50 mg L^{-1} de ácido giberélico y 50 mg L^{-1} de ácido indolbutírico (AIB) aplicado sin dilución en las semillas (4 mL kg^{-1}). Las fechas de siembra fueron 02/05, 22/05, 11/06 y 02/07, en 2013 y 2014. La tasa de emergencia con ST fue similar a la del testigo, pero se redujo con AG, que aumentó el tiempo desde la siembra hasta la emergencia. El crecimiento de plántulas, evaluado el 15 de septiembre de dos años, no se modificó significativamente con ningún estimulante. Las fechas de siembra no afectaron el porcentaje de emergencia, pero en las dos siembras más tardías la emergencia fue más lenta. El atraso en la siembra disminuyó el crecimiento de los plantines, y los modelos ajustados a la respuesta fueron diferentes en función de la característica evaluada y del año.

Palabras clave: *Passiflora edulis*, ácido giberélico, cinetina, AIB, semilla

Introduction

Passiflora edulis Sims is the main cultivated species in Passifloraceae family. Two *formae speciales* are known: the purple passionfruit (*P. edulis* Sims f. *edulis*) and the yellow passionfruit (*P. edulis* f. *flavicarpa* O. Deg.). *P. edulis* is probably originated in South-America, and the origin of the *P. edulis* f. *flavicarpa* is not determined (Pereira, 2008).

As a tropical plant, the passionfruit is well adapted to high temperatures. Low temperatures for a long time cause flower bud and fruitlet drop (Junqueira, 2009). Average annual temperatures between 23 and 27 ºC are ideal for its cultivation (Freitas, 2001). For seed germination, 20 to 30 ºC is the optimum range (Pereira & Andrade, 1994), and in tropical conditions, seeds germinate in three to four weeks (Ferreira et al., 2007; Oliveira et al., 2010).

The yellow passionfruit is the major species of Passifloraceae cultivated in Brazil. It is mainly grown in the tropics. Only 3 % of the cultivated area in 2013 was located in the South region (IBGE, 2015), where the climate is subtropical. In a big portion of that region, planting new orchards during autumn and winter (from mid-March to mid-September) is impracticable because of the risk of frost. However, growing passionfruit nursery plants in greenhouses during those seasons enables the obtainment of bigger plants (compared to the conventional ones) for planting in the spring. This approach increases the yield potential in the first crop (Laredo, 2013). Thus, seedlings production must count on cold protection through greenhouses to be planted at the end of the winter (Carvalho, Stenzel & Auler, 2015).

Passion fruit is usually propagated by seeds. Cloning through grafting and rooting is rarely used because it costs more and delays the process. Protocols for utilization of growth regulators in seeds, aiming the maximization of passionfruit seed germination, are already known. Using gibberellic acid (GA) improves seed germination and a stimulant compounded by kinetin, gibberellic acid, and indole butyric acid promotes initial plantlet growth (Echer et al., 2006; Lima et al., 2009). However, studies in low-temperature conditions have not been published, and it is known that interactions between environment temperature and doses of regulators occur (Malavasi, Dias & Malavasi, 2011). It means that the plant responses to exogenous regulators vary with temperature, and results cannot be widely extrapolated. On the other hand, more than one seed per pot is recommended to be seeded (Meletti, 1996; Siqueira & Pereira, 2001). So, a possibly lower germination rate at lower temperatures could be made up by the seeding of multiple seed sets in a pot. Therefore, the knowledge of the effects of regulator treatments on the growth of the seedlings is important to justify their use.

The objective of this work was to evaluate passionfruit seed emergence and seedling growth, in different sowing dates, and under seed treatments with compounds potentially stimulants of germination, under the conditions of a nursery in the subtropical autumn and winter of western Santa Catarina State, Brazil.

Material and Methods

The work was performed in Chapecó, Santa Catarina State (BRA) [27° 6' 24" S; 52° 36' 48" E]; hight = 670 m and Cfa climate of Köppen* (Wrege et al., 2011)]. All the experiments were conducted with the cultivar Redondo Amarelo (Isla LTDA) as genotype, from seeds bought in the formal market (seeds sold by a seed company fulfilling all legal requirements). The environment was a greenhouse covered with 10 mm alveolar polycarbonate, concrete ground, anti-aphid net and polyethylene curtains on the sides. Data on air temperature were logged trough a dispositive installed at 1.5 m of height and shaded. Daily degree-days accumulated were calculated according to Ometto (1981), considering 8 and 30 ºC as base temperatures (Veras, 1997).

The experimental treatments were the 12 combinations of four seeding dates (May 2, May 22, June 11 and July 2, in 2013 and 2014), two stimulants and one control. The two stimulants were gibberellic acid (GA) and the mixed compound Stimulate (ST). The GA was applied by immersion of the seeds in water solution of gibberellic acid 1 g L^{-1} during 96 h before seeding, following Lima et al. (2009), using a water solution of 10 g L^{-1} of Progibb® (Sumitomo Chemical do Brasil Representações Ltda, Brazil) (gibberellic acid 100 g kg^{-1}). The Stimulate® (Stoller do Brasil LTDA, Brazil) (90 mg L^{-1} kinetin, 50 mg L^{-1} gibberellic acid and 50 mg L^{-1} IBA) was applied directly to the seeds (4 mL kg^{-1} of seeds) immediately before seeding, according to Echer et al. (2006). The control received no treatment. The whole study consisted of the application of the treatments in three experiments: two in 2013 (Experiments 1 and 2) and one in 2014 (Experiment 3).

In Experiment 1, seeds were sown at the depth of 1 cm in the substrate, in plastic seeding trays with cells of 12.5 mL.

*Cfa, from the climate classification of Köppen, is a humid subtropical climate, with an average temperature in the coldest month between -1 and 18 ºC, no dry season, and an average temperature in the warmest month above 22 ºC (Pidwirny, 2016).

The substrate was Tecnomax HF fertilized with 15 % broiler litter (Brugnara, 2014). The trays were kept directly on the ground. In each seeding date, each treatment had three replicates of 80 seeds, 36 in the total. The seeds were observed daily during 40 days after seeding, and the number of plantlets emerged was registered. Data on the percentage of emergence were submitted to a 4 x 3 factorial analysis of variance, followed by a Tukey test for comparisons between stimulants, after a Box-Cox transformation ($\lambda = 2$). The effect of seeding date was evaluated by linear and nonlinear regressions. The data on daily emergence were also submitted to a survival analysis. The Kaplan-Meier method was utilized for estimating survival curves and median times to emergence (T_{50}). The function of survival (Equation 1) is estimated empirically by

$$\hat{S}(t) = \prod_{i=1}^{n}\left(1 - \frac{d_i}{n_i}\right) \quad (1)$$

where d_i is the number of seeds emerged in a given time and n_i is the number of seeds treated (Carvalho et al., 2011). With the objective of formally comparing the treatments, the log-rank test was utilized. It uses the chi-squared statistics to compare observed and expected values in each treatment under the hypothesis of an equal capacity of emergence among treatments. From this assumption called proportional risks, it was estimated the effect of the treatments without making assumptions about the probability distribution of the time for emergence dataset, using the Cox semiparametric model (Equation 2), as it follows:

$$\lambda_j(t)=\lambda_{0j}(t)e^{x\beta} \quad (2)$$

where $\lambda_j(t)$ is the function of risk, $\lambda_{0j}(t)$ is the base risk, x is the vector of co-variables (treatments) and β is the vector of parameters to be estimated. Cox model is flexible because it does not require the assumption of a particular distribution to the function of base risk [$\lambda_{0j}(t)$]. It just assumes that co-variables act multiplicatively over the risk, and this is the parametric part of the model (Carvalho et al., 2011). For the adjustment of the Cox model, the control was fixed as the reference for the comparisons. The analysis were performed with the statistical system R (R Core Team, 2014), using the procedures of the Survival package (Therneau, 2014).

Two experiments more (2 and 3) were performed in 2013 and 2014 to evaluate seedling growth. Both were carried out in a completely random design with three replicates, each compounded by four bags in 2013 and six in 2014. Polyethylene, perforated 1.5-L bags (Carvalho, Stenzel & Auler, 2015) were used, filled with Tecnomax HF substrate fertilized with 15 % broiler litter, disposed on the ground. Treatments were the same of Experiment 1. Thirty days after seeding, the plantlets in each bag were thinned leaving alive the biggest. Plants were evaluated for height, leaf number (just the ones with borders separated, except cotyledons), shoot dry matter, collar diameter and leaf area (using a CI 202 leaf area meter – CID-Bioscience, USA), on September 15, which is equivalent to 136, 116, 96 and 75 days after seeding, respectively. Data for each year were submitted to analysis of variance after a Box-Cox transformation ($\lambda = 0.1$ for height in 2013 and 2014; $\lambda = 0.1$ for leaf number in 2013 and $\lambda = 1$ in 2014; $\lambda = -0.2$ for collar diameter in 2013 and $\lambda = 2$ in 2014; $\lambda = 0.5$ for leaf area in 2013 and 2014; $\lambda = 0.1$ form dry matter in 2013 and $\lambda = 0.5$ in 2014), considering a 4 x 3 factorial scheme, complemented by Tukey test when effect of stimulants was significant.

The models adjusted were the first degree linear model for leaf number in both years; second degree for the other variables in 2013; and nonlinear model of Michaelis-Menten (Zeviani, 2013) in 2014: $y = b_1/(1+(x/b_2)\text{^}b_3)$, where

Table 1. Percentage of emergence and median time to emergence (T_{50}, median and confidence interval – C.I.) of the passion fruit seeds under different seeding dates and stimulant treatments, in a greenhouse and subtropical climate. Chapecó, SC, Brazil, 2013.

Seeding date	Stimulant	Emergence %	T_{50} Median	T_{50} C.I. 95 %
May 2	GA[1]	52.5b[2]	31	27-NE[3]
	ST[4]	71.9a	22	22-23
	Control	72.0a	22	21-23
May 22	GA	53.5b	30	24-NE
	ST	74.8a	21	21-22
	Control	72.1a	21	20-22
June 11	GA	64.0b	25	24-26
	ST	74.0a	25	24-25
	Control	71.3a	24	24-25
July 2	GA	57.1b	28.5	28-31
	ST	76.9a	25	25-26
	Control	78.1a	25	25-26

[1] Progibb® water solution 10g L^{-1}; [2] Means followed by the same letter do not differ by the Tukey test; [3] NE – not estimated, because less than 50 % of the seeds emerged; [4] 4 mL Stimulate® by kg of seed.

y = response variable; x = day of the year (ordinal); b_1 = maximum value of y; b_2 = number of days needed for reducing 50 % the maximum y observed; b_3 = parameter controlling the function form.

Results and Discussion

Experiment 1

The analysis of variance for the percentage of emergence revealed a significant effect of stimulants, on the contrary of seeding dates and the interaction seeding date x stimulants. The means for treatments with GA were significantly inferior to the others. ST and control did not differ from each other (Table 1). The percentage of emergence varied from 52.5 % to 64 % in the seeds treated with GA and in the other treatments, it was more than 71.3 %.

The median time for emergence (50 % of the plantlets, T_{50}) was higher with the stimulant GA (Table 1), except when seeding occurred on June 11, when the confidence intervals were coincident. The effect of the treatments in the time needed for emergence can be visualized in Figure 1, derived from the survival analysis, where the curve of the proportion of not emerged seeds in treatment GA was slower and remained higher than the others in all the dates. Also, it was estimated a proportional emergence risk equal to 0.65 ($p < 0.05$) for the seeds treated with GA in relation to the control, what means GA acted against the emergence.

The treatment Stimulate did not affect the median time to emergence, nor the proportional emergence risk, which was 0.99 ($p > 0.05$). It is possible that the higher T_{50} observed with the application of GA had been related to the reduction in the number of viable seeds. As a consequence, fewer seeds

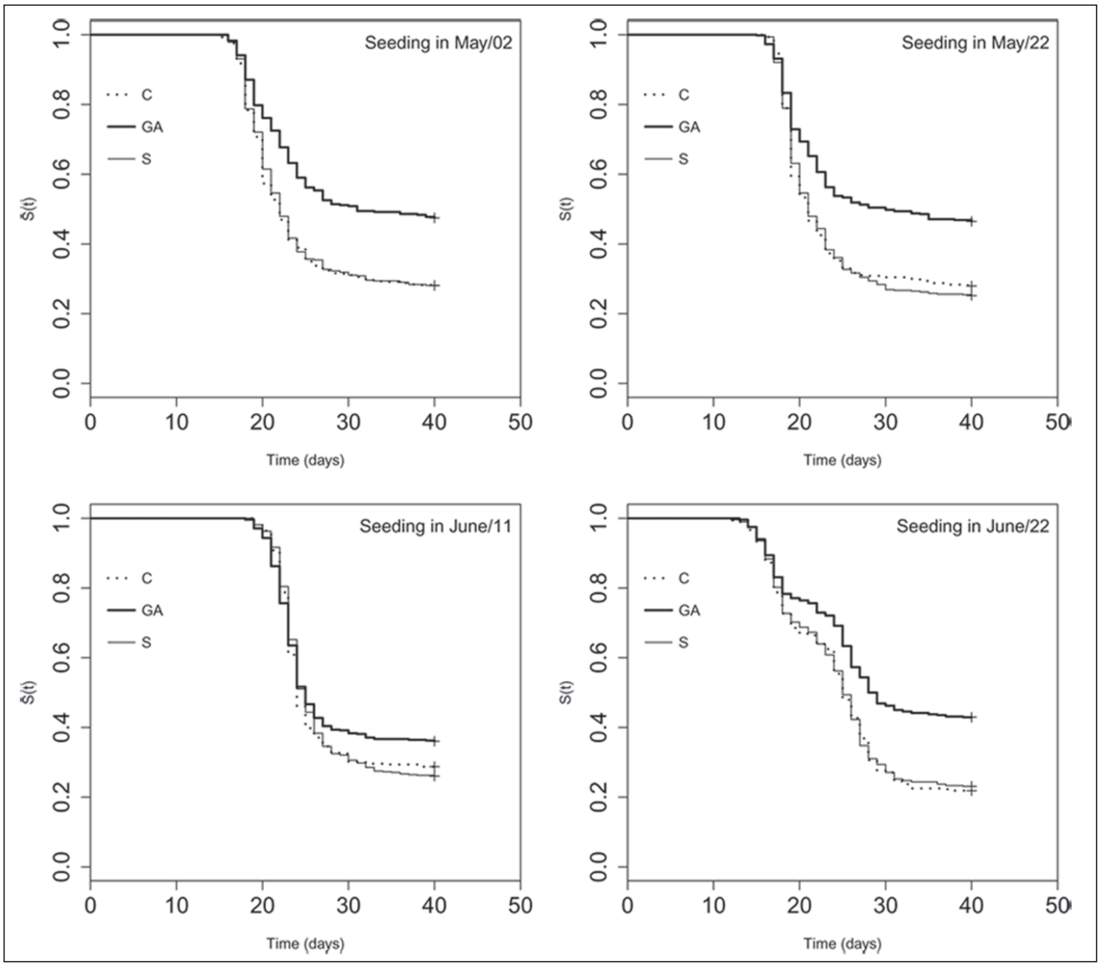

Figure 1. Estimate for Kaplan-Meier curves of the survival functions [S(t)] describing the time for the emergence of yellow passion fruit under treatments composed by seeding dates and stimulants (GA = Progibb® water solution 10 g L^{-1}; S = 4 mL of Stimulate® by kg of seed; C = control).

emerged along the whole period evaluated, delaying the attainment of 50 % of emergence.

The data presented revealed that immersing the seeds in GA 1.000 mg L^{-1} reduces the yellow passionfruit seeds emergence. This information contradicts the data published by Lima et al. (2009) who found an increase in germination until 1.000 mg L^{-1} and 96 h immersion. In another experiment in a greenhouse, doses up to 400 mg L^{-1} and immersion by 48 hours did not improve the germination (Cárdenas et al., 2013). Those conflicting results could be due to the interaction between doses and environmental conditions, as well as to the genotype studied (Souza et al., 2010; Malavasi, Dias & Malavasi, 2011).

The Stimulate (4 mL kg^{-1}) did not affect the emergence. Ferraz et al. (2014) reported an increase in emergence with doses up to 30 mL kg^{-1} of *P. edulis* seeds. However, for the supposed increase no statistical confidence level was presented. On the other hand, Benjamin (2009), soaking the seeds in water solutions with increasing concentrations from 0 to 32 mL L^{-1} of Stimulate, observed significant effects, but the difference from the control was not supported by any statistical test. The difficulty in stablishing a pattern of response can be due to the compound formulation, which is a mix of three regulators, whose interaction promotes diverse effects when the dose or concentration is changed. Still, Ferreira et al. (2007) verified a higher percentage of early emergence with 12 and 16 mL kg^{-1}, but 35 days after seeding the differences disappeared, which is in agreement with this work observations.

Seeding dates influenced all the variables. In the case of treatments with Stimulate and the control, T_{50} was smaller for the first two dates than for the two last ones, and the differences inside those pairs were not significant, given the overlapping of confidence intervals (Table 1). The difference occurred besides the similar amount of degree-days accumulated in the first three dates (Figure 2A). It is possible that temperatures inside the substrate had been different to the air, causing this discordance between degree-days and T_{50}. In the comparison of seeding dates inside treatment GA, the upper limit of the confidence intervals could not be estimated, and so some comparisons are imprecise. However, it is possible that on June 11 the T_{50} had been smaller than on May 2 and July 2.

Experiments 2 and 3

The application of GA and Stimulate in the seeds did not cause any alteration in dry matter, height, collar diameter, leaf number or leaf area of yellow passionfruit (Table 2). The

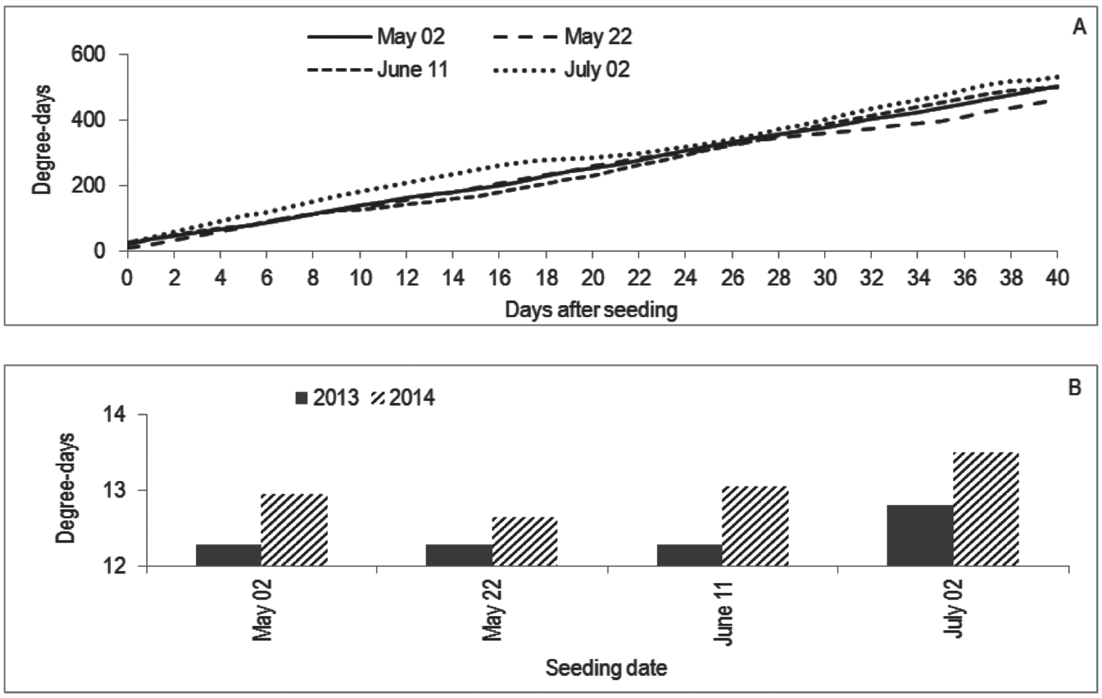

Figure 2. Degree-days for yellow passion fruit accumulated in a greenhouse located in Chapecó, SC, Brazil: (A) from 0 to 40 days after May 2, May 22, June 11 and July 2, 2013; and (B) mean daily accumulation from each of that dates and September 15 (B).

Table 2. Characteristics of yellow passion fruit seedlings evaluated on September 15, following the application of Progibb® (GA) and Stimulate® (ST) and in a control, in 2013 and 2014, in Chapecó, SC, Brazil. Means of four seeding dates.

Year	Stimulants	Dry matter (g plant^{-1})	Height (cm)	CD[4] (mm)	Leafs	Leaf area (cm^2 plant^{-1})
	GA[1]	2.06	30.66	2.77	11.21	402.90
2013	ST[2]	1.96	31.42	2.76	10.67	388.70
	Control	2.19	34.59	3.04	11.10	421.30
	p value[3]	0.27	0.21	0.18	0.35	0.30
	GA	2.67	41.76	3.96	10.37	417.10
2014	ST	2.91	47.18	3.95	10.72	435.40
	Control	2.87	47.05	3.89	10.60	422.80
	p value	0.67	0.67	0.81	0.47	0.78

[1] Progibb® water solution 10g L^{-1}; [2] 4 mL of Stimulate® by kg of seed; [3] Obtained in the analysis of variance after a Box-Cox transformation. [4] Collar diameter.

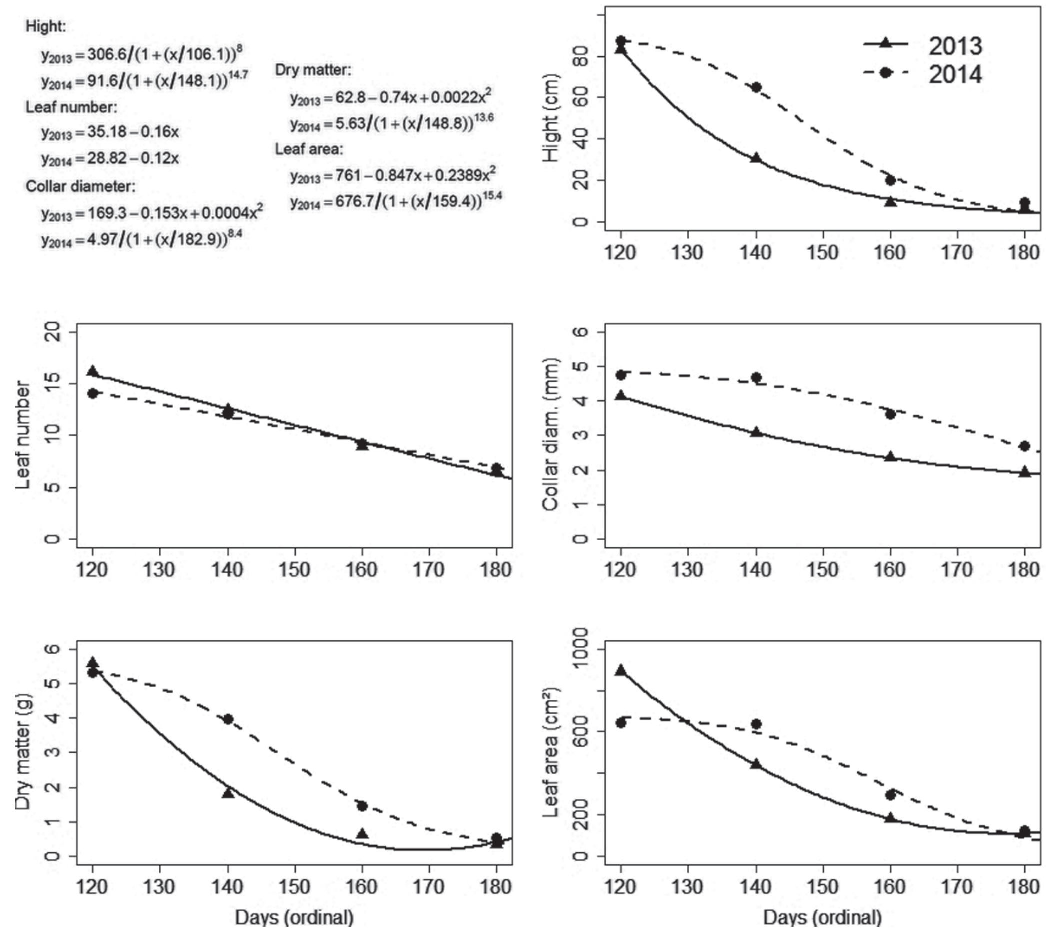

Figure 3. Models fitted to characteristics of yellow passion fruit seedlings, measured on September 15, 2013 and 2014, following the seeding dates May 2, May 22, June 11 and July 2 (121º, 141º, 161º and 182º day of the year, respectively), in Chapecó, SC, Brazil.

interaction between seeding dates and stimulants was neither significant. However, seeding dates alone caused a significant effect.

The seedlings seeded in May 2 (121º day of the year) had mean height of 83 and 87.2 cm in 2013 and 2014, respectively (Figure 3), which is smaller than the observed by Zaccheo et al. (2013) in 120-days old seedlings seeded on May 15 in Londrina, PR, Brazil. In that place, the climate is Cfa, what suggests the environment in Chapecó was limiting to the growth, as can be observed in Figure 4: some days had an accumulation of less than 10 degree-days.

Shoot growth in the seedlings was not affected by the stimulants, even though they are considered stimulants of initial growth (Echer et al., 2006; Santos, 2010). As three seeds were seeded in each bag and plantlets were further thinned, the selection of the biggest plantlet could have annulled the effect of the treatments. This explanation fits the effect of the GA, which delayed and decreased the emergence rates without influencing the final plant growth.

The simple effect of seeding date on seedlings growth was significant, observed in height, leaf number and area, collar diameter and dry matter, for both years (Figure 3). However, differences in the pattern of response happened among characteristics in the same year and reciprocally, too.

For plant height, collar diameter, dry matter and leaf area, in 2014, the responses followed the Michaelis-Menten model (Zeviani, 2013). The higher value of y (response variable) was observed at the first seeding date, followed by a fall, which was more intense between the second and third dates, and tending to stability at the end of the period (Figure 3). The same variable responded as a linear quadratic function in 2013 when the fall in y was more intense from the first to the second dates. Leaf number, on the other hand, responded as a simple linear first-degree function, in both years. The number of leaves was reduced by 0.16 and 0.12 each one-day delay in seeding, in 2013 and 2014, respectively. The differences in the pattern of response between leaf number and the other variables are due to the increase in the internodal space from the base to the apex of the shoot, i.e., small seedlings have a bigger number of leaves per centimeter of the shoot because the internodes are shorter.

The differences between years can be assigned to the different conditions of the environment. In Figure 4, it can be seen that for the same seeding date there were differences between years in the accumulation of degree-days in the days immediately subsequent to the seeding. In the cases with lower temperatures after the seeding (as after May 22, 2014) the initial daily growth rate is depressed, but it can be compensated by higher temperatures in the days ahead, along the growth period, resulting in similar sizes in the end of the experiment.

The effect of the seeding dates in the growth of the seedlings is due mainly to the differences in time (age of the plants in the end), but also to the differences in degree-days accumulation. As an example, seedlings seeded on June 11 and July 2, in 2014, suffered influences of a period of

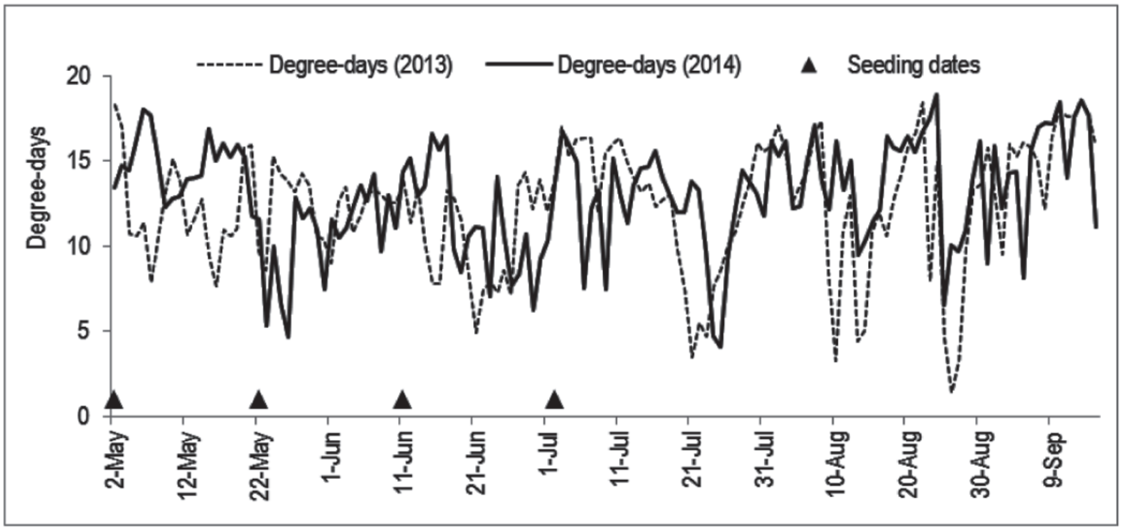

Figure 4. Daily accumulated degree-days (TB = 35 ºC; Tb = 15 ºC) in the greenhouse where yellow passion fruit seedlings from the Experiments 1, 2 and 3 were grown.

higher temperatures and accumulation of degree-days (Figure 2B) in comparison to the further seeding dates.

It is concluded that yellow passionfruit can be seeded anytime from May 2 to July 2 without losses in emergence percentage, which is about 70 %, even without a treatment with plant growth regulators. However, the earlier the seeding, the higher will be the seedlings to be transplanted in the field. Big-size seedlings generate more productive and precocious orchards in the first crop (Laredo, 2013). There is no biological advantage in using the stimulants tested when growing yellow passionfruit seedlings during subtropical autumn and winter of western Santa Catarina State; seeding from May 2 to 22 allows obtaining seedlings averaging 30 to 87 cm in height in mid of September.

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