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Phenological and quantitative plant development changes in soybean cultivars caused by sowing date and their relation to yield

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The study of soybean (*Glycine max*) biometry and phenology can detect characteristics that interfere with yield, and quantitative plant development may vary according to the interaction between the genotype and the environment, and in different phenological development stages of the plant. This study evaluated the quantitative changes and phenological development caused by sowing date and their relation to grain yield of four contrasting soybean cultivars. The experimental design was a split plot with three replications, being the sowing date allocated to plots and the cultivars to subplot. The height of plants, diameter and number of nodes of the main stem, number of the branches, and leaf area index (LAI) were determined at four growth stages: V4, V9, R2 and R5.3, and the number and dry matter (DM) of the nodules at R5.3 growth stage. There was a reduction of growth in all cultivars with delayed sowing dates; however, the cultivar of determined growth habit showed to be less responsive to different sowing dates than the other cultivars. The DM of nodules was higher when plants were sown in October, and the cultivar with larger LAI obtained more DM of nodules than the others cultivars. The definition of the final quantitative characteristic of the plants occurred after the growth stage V9, and the LAI was the character at R5.3 with highest correlation with grain yield.

Key words: Branches, *Glycine max*, leaf area index, nodulation, plants height.

INTRODUCTION

A plant phenological study is a tool that allows us to identify plant quantitative changes at determined growth stages associated with a series of necessities of the plants, which, if attended, will enable normal crop development and consequently high yields (Cruz et al., 2010).

The soybean (*Glycine max*) is a plant that is highly dependent on the interaction between the genotype and the environment; it can change its cycle and its vegetative development depending on this interaction. In the environment, the temperature and the photoperiod are the main factors responsible for the variation of the

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culture development (Sinclair et al., 2005). Thus, the sowing date is probably the most important crop management practice for soybean crop development, because it interferes directly with most of the environmental factors (De Bruin and Pedersen, 2008). Since different soybean genotypes respond differently to environment variation, experiments should be carried out to indicate the best sowing date for each cultivar (Pedersen and Lauer, 2004; Egli and Cornelius, 2009).

In the southern region of Brazil, winter crops like wheat, barley, triticale, and oats are widely cultivated and the soybean crop can be sown late. This late sowing may result in low canopy development and decreasing leaf area index (LAI), translocation of photoassimilates and accumulation of the biomass and consequently low yield (Rao et al., 2002; Setiyono et al., 2008). This was also verified in low south latitude regions (<25°), in research by Calviño et al. (2003) and Sinclair et al. (2005). Unfavorable temperatures out of the range between 24 and 34°C (Egli and Bruening, 2000), low solar radiation (Setiyono et al., 2010) and the short length of the photoperiod, which may affect the flowering (Han et al., 2006), are the abiotic factors commonly found at late sowing dates. These factors may interact with the genotype of the soybean plants, affecting the growth and development of this crop.

Key stages of growth and development of the soybean plants are present both at vegetative and reproductive stages (Setiyono et al., 2008); the duration of the development stages, especially post-flowering, also interfere in the growth and yield of soybean plants (Kantolic et al., 2007).

Therefore, it is important to study the growth of soybean cultivars with contrasting responses to the environment conducted under different environmental conditions. These studies can result in information that leads to an understanding of how the cultivars at different sowing dates result in a lower negative influence of the environment, and how quantitative plant characteristics could interfere with grain yield.

In this context, the present study analyzed the changes caused by different sowing dates on quantitative plant characteristics and phenology during the crop growth, and its relation to final grain yield of four soybean cultivars used in southern Brazil.

MATERIALS AND METHODS

The experiment was carried out in Guarapuava, Parana State, at 25°23'02" S, 51°29'43" W and 1,026 m of altitude. The climate of the region, according to classification of Köppen is temperate of altitude (Cfb) (Kottek et al., 2006).

The soil of the experimental area is classified as Latossolo Bruno (Clayey Oxisol) and the chemical characterization (0 to 20 cm), three months before the first sowing, revealed pH ($CaCl_2$) of 5.2; 42 g dm^{-3} of organic matter; levels of 3.98 $cmol_c$ dm^{-3} of Ca; 2.04 $cmol_c$ dm^{-3} of Mg; 0.18 $cmol_c$ dm^{-3} of K; 0.0 $cmol_c$ dm^{-3} of Al; and 6 mg dm^{-3} of P, with cation exchange capacity (pH 7.0) of 9.73 $cmol_c$ dm^{-3}; and base saturation of 62%. Compound fertilizer 05-25-25 was given at 310 kg ha^{-1} prior to sowing totaling 15.5 kg of N, 77.5 kg of P_2O_5 and 77.5 kg of K_2O. The seeds were inoculated with turfs inoculants at recommended dosage. The weeds, pests, and diseases were controlled according to the technical recommendations for growing soybeans under Brazilian conditions when needed.

The experimental design was a split-plot randomized complete block with three replications (that is, blocks). The main plots (environments) were formed by three sowing dates; October 21, November 18, and December 20, 2010. All within agricultural zoning recommendations for soybean crops in the region and commonly utilized by growers. The subplots (genotypes) were formed by four soybean cultivars largely utilized in the region: FPS Urano® RR, BMX Apolo® RR, BMX Energia® RR and BRS 284®. The first cultivar has determinate growth habit and the others have indeterminate growth habit; the maturity groups are respectively 6.2, 5.5, 5.0 and 6.5. Each subplot was formed by four rows, spaced by 0.4 m and with 11 m of length totaling 13.2 m².

Twenty seeds for linear meter were sown totaling a population of 500,000 plants ha^{-1} which at stage VC/V1 according to scale of Fehr and Caviness were thinned to final stand of 260,000 plants ha^{-1} (10.4 plants m^{-1}) for all cultivars within the recommendation given by the cultivar owners. Plants in 0.4 m of the two central rows of each subplot were sampled at each time totaling on average 8.32 plants per subplot, at four growth stages: V4, V9, R2 and R5.3. For the definition of growth stage, the cultivar FPS Urano was used as reference, that is, when this cultivar reached one of the four growth stages; plants of all four cultivars were sampled on the same day. To determine growth stage, daily observation of seven random plants in each subplot was made, and it was defined when 50% or more (four plants) were in the same stage. Between each sampling, a border was left of 0.4 m.

Plant height (cm), number of nodes ($plant^{-1}$), main stem diameter (mm), number of branches ($plant^{-1}$) and LAI was estimated by the software Image-J (Abramoff et al., 2004) through digital photos, and in last sampling, the number ($plant^{-1}$) and dry matter (DM, g m^{-2}) of nodules were determined and recorded at each growth stage. The DM was determined after drying in a forced aeration stove (70°C) until it reached constant mass.

The climatic data were from the meteorological station of Midwest Parana State University (Unicentro) / Agronomic Institute of Parana (Iapar), located approximately 100 m from the experiment. The data of all parameters of the study were submitted to analysis of homogeneity of variance (Box Cox) and if this prerequisite was confirmed, the analysis of variance (F test), was made considering the experimental design of split-plot with the main plots (environments) and the subplots (genotypes). ANOVA was conducted with SAS software. When statistical significance was verified ($F \leq 0.05$), the averages of the treatments were submitted to a Tukey test ($p \leq 0.05$). The analysis of the Pearson Correlation was performed between the quantitative plant characters and the final grain yield, and the correlation significance was evaluated by Student's t-test.

RESULTS AND DISCUSSION

The number of days in the studied intervals (that is VE-V4, V4-V9, V9-R2 and R2-R5.3) was reduced when plants were sown in December with the exception in the interval V4-V9 (Table 1). This reduction probably occurred because of the shortening of the photoperiod, as the sowing date was delayed, principally from V9 growth stage.

In general, average temperature was more than 2°C lower for plants sown earlier, until V9 growth stage;

Table 1. Climatic data for growth stage intervals and its duration of soybean plants sown on three dates.

Sowing date	Number of days in the interval	Growth stage intervals						
		VE-V4						
		T°Max.[a]	**T°Min.[b]**	**T°Ave.[c]**	**Ppt.[d]**	**Rad.[e]**	**ARad.[f]**	**Phot.[g]**
Oct. 21	30	25.5	13.0	18.5	141	624	20.8	13h19
Nov. 18	25	25.0	16.3	19.8	248	385	15.4	13h38
Dec. 20	24	26.9	17.0	21.0	276	437	18.2	13h37
					V4-V9			
Oct. 21	15	23.9	15.9	19.1	222	201	13.4	13h39
Nov. 18	15	26.7	17.1	21.0	47	270	18.0	13h42
Dec. 20	17	26.8	18.3	21.5	141	260	15.3	13h20
					V9-R2			
Oct. 21	18	26.4	17.0	20.8	52	322	17.9	13h42
Nov. 18	13	26.9	17.3	21.1	231	224	17.2	13h37
Dec. 20	8	24.9	18.2	20.6	73	111	13.9	13h05
					R2-R5.3			
Oct. 21	27	27.3	17.6	21.4	324	464	17.2	13h32
Nov. 18	31	26.5	18.2	21.3	242	471	15.2	13h15
Dec. 20	19	25.0	16.9	20.3	57	295	15.5	12h45

[a]Maximum temperature, [b]minimum temperature, [c]average temperature in °C day^{-1}, [d]rainfall in mm interval^{-1}, [e]radiation in MJ m^{-2} interval^{-1}, [f]average radiation in MJ m^{-2} day^{-1} and [g]average photoperiod in hours of light day^{-1}, for the respective intervals of soybean growth stages.

however, after R2 growth stage, the average temperature was more than 1°C higher for plants sown in October compared with December. During soybean growth, the precipitation was well distributed with only a reduction in the last growth stage intervals for the plants sown in December, however, without occurrence of severe drought. The average radiation was not low during the growth stage intervals with high rainfall evidencing the occurrence of cloudy days without precipitation.

Plant height until V9 growth stage was not an accurate parameter to estimate the final plant height at R5.3 (Table 2) because only after this growth stage, the interference of sowing dates in the final plants height was clear. At R2 growth stage, plants decreased height with a delayed sowing date so when sown in December, the plant height was lower than when sown in October and November for all cultivars.

There was significant interaction in last growth stage (R5.3) when plants from all cultivars sown in December showed a larger reduction in height compared to those in October with the exception of the cultivar Urano, which has a determined growth habit.

Comparing the data of plant height of the cultivars at R5.3 growth stage with the description given by its owners' companies, the cultivars Energia (average of 92 cm) and Urano (average of 73 cm) achieved approximately the height given by their owners when sown in October. For BRS 284 (average of 100 cm) and Apolo (average of 73 cm) it occurred when sown in November. For cultivars BRS 284 and Apolo in southern Brazil, the early sowing led to taller plants than that reported by the owner companies. This fact is more pronounced for cultivar BRS 284 which among the studied cultivars presented moderate risk to lodging according to the owner company and was the tallest cultivar in the present study. Therefore, for BRS 284 and other tall cultivars with indeterminate growth habit, the early sowing date can be a problem due to lodging.

The cultivar Urano had a reduction of plant height after R2 growth stage; however, the reduction of this cultivar was less than that of others. For Urano, more reduction occurs between V9 to R2 growth stages.This fact occurs due to the reduction in the number of days between V9 and R2 growth stages: 18, 13 and 8 days for sowing in October, November and December, respectively. The premature flowering of this cultivar was caused by the sensitivity to a short photoperiod combined with high temperatures with a delaying of the sowing date.

The interaction between treatments in plant height at R5.3 growth stage was probably due to the indeterminate growth habit of cultivar Urano, which at R2 growth stage reached approximately 75% of its final height in all dates of sowing. On the other hand, the others cultivars at R2 achieved 55, 60 and 65% of their final heights when sown in October, November and December, respectively. Bastidas et al. (2008) also verified a reduction in the final

Table 2. Height of plants[1] of four soybean cultivars, at four growth stages sown on three dates[2].

Cultivar / sowing date	V4			Average	V9			Average
	Oct. 21	Nov. 18	Dec. 20		Oct . 21	Nov. 18	Dec. 20	
Urano	15.8	13.9	14.0	14.6 [a]	30.8	29.7	36.2	32.3 [b]
Apolo	10.8	8.3	9.8	9.6 c	20.2	19.1	26.1	21.8 [d]
Energia	12.2	9.3	11.0	10.8 [b]	23.2	22.0	29.3	24.9 [c]
BRS 284	16.0	14.4	14.7	15.0 [a]	33.6	33.5	39.5	35.5 [a]
Average	13.7 [A]	11.5 [C]	12.4 [B]		27.0 [B]	26.1 [B]	32.8 [A]	
Date / cultivar	**R2**			**Average**	**R5.3**			**Average**
	Oct. 21	**Nov. 18**	**Dec. 20**		**Oct. 21**	**Nov. 18**	**Dec. 20**	
Urano	52.1	52.0	43.6	49.2 [b]	73.4 [Ac]	66.5 [Bc]	60.3 [Cb]	66.7
Apolo	46.1	44.8	35.2	42.0 [c]	92.2 [Ab]	73.3 [Bb]	56.1 [Cb]	73.9
Energia	52.5	46.0	37.6	45.4 [bc]	91.7 [Ab]	73.1 [Bb]	54.9 [Cb]	73.2
BRS 284	65.1	60.8	52.9	59.6 [a]	112.6 [Aa]	99.8 [Ba]	81.1 [Ca]	97.8
Average	53.9 [A]	50.9 [B]	42.3 [C]		92.5	78.2	63.1	

[1]In cm; [2]upper case letters compare averages in rows, and lower case in columns. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

Table 1. Diameter of main stem[1] of four soybean cultivars, at four growth stages sown on three dates[2].

Date / cultivar	V4			Average	V9			Average
	Oct. 21	Nov. 18	Dec. 20		Oct. 21	Nov. 18	Dec. 20	
Urano	5.5	5.0	5.5	5.3	7.4	7.7	8.2	7.8 [ab]
Apolo	5.4	4.6	5.5	5.2	7.8	7.9	8.0	7.9 [a]
Energia	5.4	5.1	5.6	5.4	7.9	7.9	8.3	8.0 [a]
BRS 284	4.8	4.7	5.4	5.0	6.8	7.2	8.1	7.4 [b]
Average	5.3	4.9	5.5		7.5 B	7.7 [B]	8.1 [A]	
Date / cultivar	**R2**			**Average**	**R5.3**			**Average**
	Oct. 21	**Nov. 18**	**Dec. 20**		**Oct. 21**	**Nov. 18**	**Dec. 20**	
Urano	9.4	9.8	8.9	9.4	11.3	9.8	8.6	9.9 [a]
Apolo	9.6	10.0	9.3	9.6	10.9	9.9	8.5	9.8 [ab]
Energia	9.8	10.1	8.7	9.5	10.3	9.3	8.4	9.3 [bc]
BRS 284	8.5	9.3	8.9	8.9	10.2	8.9	8.1	9.1 [c]
Average	9.3 [AB]	9.8 [A]	9.0 [B]		10.7 [A]	9.5 [B]	8.4 [C]	

[1]In mm; [2]upper case letters compare averages in rows, and lower case in columns. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

growth percentage achieved by soybean plants at a reproductive period when sown late. However, the authors did not observe interaction among the cultivars and the time of sowing.

In this paper, the authors utilized indeterminate growth habit cultivars and one semideterminate. Because of this, the results were probably different from the present study wherein the contrast among cultivars was higher.

There was no statistical difference in the diameter of the main stem caused by the date of sowing for cultivars at V4 growth stage (Table 3). Similar to plant height, the diameter of the plant main stem was greater in plants sown in December than those sown in October and November at V9 growth stage. However this changed at R2 and R5.3 growth stages, when the delay in sowing resulted in a smaller stem diameter, agreeing with the results of Marchiori et al. (1999).

There was an increase of 0.12, 0.12 and 0.13 mm of the main stem diameter for each 1 cm increase in plant height in the sowing of October, November, and December, respectively, at R5.3 growth stage, showing slight increments of stem diameter in relation to plant height with sowing delay. For cultivars, the increases of stem diameter with increases of plant height were: 0.15, 0.13, 0.13 and 0.09 for Urano, Apolo, Energia and BRS 284, respectively, showing a lower increase of stem

Table 4. Number of branches[1] of four soybean cultivars, at four growth stages sown on three dates[2].

Date / cultivar	V4			Average	V9			Average
	Oct. 21	Nov. 18	Dec. 20		Oct. 21	Nov. 18	Dec. 20	
Urano	1.3	1.0	1.1	1.1 [a]	2.1 C[a]	2.3 [Bab]	4.8 [Aa]	3.0
Apolo	1.0	1.0	1.0	1.0 [b]	1.7 [Ba]	3.1 [Aab]	3.5 [Ab]	2.8
Energia	1.0	1.0	1.0	1.0 [b]	2.4 [Ba]	3.8 [Aa]	3.5 [Ab]	3.2
BRS 284	1.0	1.0	1.0	1.0 [b]	1.3 [Ba]	2.7 [Ab]	2.5[Ab]	2.2
Average	1.1 [A]	1.0 [B]	1.0 [B]		1.9	3.0	3.6	
Date / cultivar	**R2**			**Average**	**R5.3**			**Average**
	Oct. 21	**Nov. 18**	**Dec. 20**		**Oct. 21**	**Nov. 18**	**Dec. 20**	
Urano	5.8	6.6	5.2	5.9 [a]	6.0	4.8	5.2	5.3 [a]
Apolo	6.1	6.5	6.0	6.2 [a]	6.2	4.6	5.6	5.4 [a]
Energia	6.6	6.7	5.5	6.3 [a]	6.0	4.7	5.7	5.5 [a]
BRS 284	3.5	3.5	3.6	4.5 [b]	3.8	2.0	3.0	3.0 [b]
Average	5.5	5.8	5.1		5.5 [A]	4.0 C	4.9 [B]	

[1]Plant^{-1}; [2]upper case letters compare averages in rows, and lower case in columns. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

diameter with increase of plant height for BRS 284, making this cultivar more susceptible to plant lodging. It was also noted that the change of sowing date did not result in an increase of stem diameter in relation to an increase of height in the cultivars Urano and BRS 284. However, the cultivars Apolo and Energia presented values of 0.11, 0.13 and 0.15 mm for sowing in October, November, and December, respectively.

In soybean crops the competition among plants decreases the stem diameter (Seiter and Altemose, 2004). These authors observed that at R5.5 growth stage, soybean plants showed the largest stem diameters at lower planting densities resulting in lower competition among plants. Therefore, smaller values on early sowing may be caused by higher competition between plants sown earlier when the plant height and the LAI were larger. Cultivars Urano and BRS 284 maintained the increase of stem diameter in relation to the increase of height for all sowing dates. Thus, we can suppose that for these cultivars, the competition among plants is similar independently of the sowing date. On the other hand, for cultivars Apolo and Energia, the competition among plants decreased in late sowing; it does not mean that late sowing dates are best for these cultivars, since if the competition is too small, the cultivation area can be underexploited and the yield reduced.

The number of branches at V4 growth stage was higher in plants sown in October than in November and December (Table 4). At R2 growth stage, the sowing date did not affect the number of branches; and at R5.3 growth stage, the number of branches was highest in plants sown in October, lowest on November, and intermediary in December. In addition, plants sown in November had a reduction in the number of branches at R5.3 relative to R2 growth stage. Among cultivars, BRS 284 showed a lower number of branches than the others cultivars. At V9 growth stage, there was significant interaction between sowing dates and cultivars. Cultivars increased the number of branches formed as the sowing dates were delayed, and in the cultivar Urano, this increase was higher than in the other cultivars.

The reduction in the number of branches in plants sown in November at R5.3 growth stage compared to at R2 growth stage (Table 4), was probably due to self-shading at the lower third of plant canopy. For the sowing of October, although plants lost formed branches, the largest photoperiod may have induced the new branch formation at the middle third of the plant canopy as cited by Jiang et al. (2011) and Han et al. (2006).This formation of new branches did not occur in plants sown in November due to a short photoperiod. Kantolic and Slafer (2001) explain that there is no difference in the number of branches in plants submitted to an extended two hours of artificial photoperiod. In December, because the self-shading was very low, there was probably less fall of branches at the lower third of the plant canopy.

Kantolic and Slafer (2001) verified differences among cultivars and sowing dates, where late maturing cultivars produced more branches, and the delay of sowing date reduced the number of branches of plants. In this study, cultivars of early and very early maturation group were studied and all had a reduction in the number of branches at R6 with the delay of sowing date.

In our study, the number of branches was also reduced with sowing delay. However, we realized that the late maturing cultivars (BRS 284) had the lowest number of branches. Indeed the number of branches is a characteristic of the cultivar and is not related to the maturity group. The cultivar BRS 284 showed the lowest number of branches, and it was inversely proportional to

Table 5. Number of nodes of the main stem[1] of four soybean cultivars, at four growth stages sown on three dates[2].

Date / cultivar	V4			Average	V9			Average
	Oct. 21	Nov. 18	Dec. 20		Oct. 21	Nov. 18	Dec. 20	
Urano	6.7 ^{Aa}	6.2 Ba	6.5 ^{ABa}	6.5	10.2	10.7	11.2	10.7 ^{a}
Apolo	6.1 ^{Ab}	5.6 Bb	6.0 ^{ABb}	5.9	9.4	9.8	10.5	9.9 ^{b}
Energia	6.0 ^{Ab}	5.9 Aab	6.0 ^{Ab}	6.0	9.3	9.6	10.6	9.9 ^{b}
BRS 284	6.0 ^{Ab}	6.2 Aa	6.2 ^{Aab}	6.2	10.2	10.6	11.5	10.8 ^{a}
Average	6.2	6.0	6.2		9.8 ^{B}	10.2 ^{B}	11.0 ^{A}	

Date / cultivar	R2			Average	R5.3			Average
	Oct. 21	Nov. 18	Dec. 20		Oct. 21	Nov. 18	Dec. 20	
Urano	15.1	15.0	14.0	14.7 ^{a}	17.8 ^{Ac}	17.4 Acb	16.0 ^{Ba}	17.1
Apolo	13.9	13.9	12.6	13.4 ^{b}	20.1 ^{Ab}	18.1 ^{Bb}	14.9 ^{Cb}	17.7
Energia	14.3	13.4	12.4	13.4 ^{b}	19.2 ^{Ab}	17.1 ^{Bc}	14.6 ^{Cb}	16.9
BRS 284	15.1	15.0	13.7	14.6 ^{a}	21.1 ^{Aa}	19.8 ^{Ba}	16.5 ^{Ca}	19.1
Average	14.6 ^{A}	14.3 ^{A}	13.2 ^{B}		19.5	18.1	15.5	

[1]Plant^{-1}, [2]upper case letters compare averages in rows, and lower case in columns. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

plant height. Heiffig et al. (2005) stated that the intra-specific competition in soybean plants may determine the plant size and its number of branches, these two characteristics being inversely proportional. The number of nodes (Table 5) of the main stem in general showed a behavior similar to the plant height (Table 2). At V9 growth stage, plants formed a higher number of nodes when sown in December, and at R2 growth stage the number of nodes decreased gradually with a delay in the date of sowing.

In the comparison among cultivars, Urano and BRS 284 had a higher number of nodes than cultivars Apolo and Energia except at R5.3 growth stage when the cultivar Urano showed a fewer number of nodes when sown in October compared to the other cultivars. Thus, the cultivar Urano was less affected by a delayed sowing date with a lower reduction in the number of nodes than other cultivars as the sowing date was delayed. This result corroborates with the findings of Setiyono et al. (2007), who verified an increase in the number of nodes with an increase in the interval in days from R1 until R5 growth stages for the indeterminate growth habit cultivars; this explains the larger number of nodes of the other cultivars in relation to the cultivar Urano, at the sowing in October at R5.3.

There is a positive correlation between the number of nodes and the temperature (Sinclair et al., 2005); thus, the higher number of nodes observed until V9 growth stage in plants sown in December may be explained by the increase of temperature in this interval (Table 1). From V9 growth stage, the temperature was lower at sowing in December and the number of days among stages decreased sharply with the delay of sowing partially explaining the higher number of nodes observed in plants on the two first sowing dates in relation to the last.

In the same way, Bastidas et al. (2008) verified significant interaction between sowing date and cultivar in the number of nodes. The authors explain this result affirming that cultivars with a predisposition to have a lower number of nodes tend to lose fewer nodes than cultivars with a predisposition to have a high number of nodes. Therefore, the present study corroborates these findings and states that the cultivar Urano with determinate growth habit, similar to the cultivar with lower loss in number of nodes in the study of the Bastidas et al. (2008), already achieved most of its growth development until R2 suffering less from the unfavorable environment that occurred after R2 growth stage. The authors also verified that the plants after V9 growth stage, presented a lower increase in the number of nodes with the sowing delay, in agreement with the results of our study.

The sowing date did not influence LAI of soybean plants at V9 growth stage, but at R2 growth stage, the lower LAI was found in the sowing of December (Table 6). At R5.3 growth stage, the highest LAI is clearly noted for plants sown in October, middle values in November and the lowest in December. Among cultivars, in the same growth stage, the highest final LAI was for cultivar BRS 284. There was significant interaction between cultivars and sowing date in LAI at V4 growth stage where the cultivar BRS 284 showed slow initial development, but more constant than others cultivars.

The decrease of LAI in plants at R5.3 growth stage with delaying the sowing date may be partially explained by the fewer number of days to plant development of 88, 83 and 67 days after emergence, for sowing in October, November, and December respectively. However, the major influence on this aspect was the photoperiod post-flowering that decreased with delaying the sowing date

Table 6. Leaf area index of four soybean cultivars, at four growth stages sown at three dates[1].

Date / cultivar	V4			Average	V9			Average
	Oct. 21	Nov. 18	Dec. 20		Oct. 21	Nov. 18	Dec. 20	
Urano	1.06 ^{Aa}	0.72 ^{Ca}	0.82 ^{Ba}	0.87	3.00	2.66	3.04	2.85 ^{a}
Apolo	0.80 ^{Abc}	0.49 ^{Cc}	0.63 ^{Bb}	0.64	2.17	2.35	2.13	2.22 ^{b}
Energia	0.88 ^{Ab}	0.53 ^{Cbc}	0.66 ^{Bb}	0.69	2.81	2.23	2.47	2.50 ^{ab}
BRS 284	0.73 ^{Ac}	0.62 ^{Cb}	0.68 ^{ABb}	0.68	2.47	2.46	2.86	2.60 ^{ab}
Average	0.87	0.59	0.70		2.57	2.42	2.63	
Date / cultivar	**R2**			**Average**	**R5.3**			**Average**
	Oct. 21	**Nov. 18**	**Dec. 20**		**Oct. 21**	**Nov. 18**	**Dec. 20**	
Urano	5.17	5.26	3.45	4.63	6.87	4.70	3.72	5.10 ^{ab}
Apolo	4.41	4.61	3.08	4.03	5.75	3.47	3.25	4.16 ^{c}
Energia	4.87	4.65	2.94	4.15	5.87	3.50	3.67	4.35 ^{bc}
BRS 284	4.44	5.04	3.65	4.38	7.56	4.88	3.84	5.43 ^{a}
Average	4.72 ^{A}	4.89 ^{A}	3.28 ^{B}		6.51 ^{A}	4.14 ^{B}	3.62 ^{C}	

[1] Upper case letters compare averages in rows, and lower case in columns. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

according to Han et al. (2006). The plants sown in October were at R2 growth stage in late December to early January when the days in these months have the largest photoperiod of the year.

The cultivar BRS 284 has high leaf production that assures high values of LAI even under unfavorable conditions. This characteristic is favorable because LAI around 5.5 in soybean plants ensures large light interception by the plant canopy both of short and long waves and with this LAI, the light amount incoming to the soil is very low, and thus, the soil water evaporation is also small (Sauer et al., 2007).

The interaction between treatments occurred at V4 growth stage for LAI; the high LAI of plants sown in October was probably due to the large number of days of plant development (30 DAE) and the highest average daily radiation among the sowing dates (Table 1). At this growth stage, plants from cultivars Apolo and Energia sown in November showed the lower LAI, probably caused by low average solar radiation (Table 1) as also explained by Calviño et al. (2003). It may be concluded that for these cultivars, high solar radiation is needed for leaf production.

Models for estimation of soybean plant growth, like that of Setiyono et al. (2010), utilized solar radiation as a parameter that drives vegetative growth. Models suggest a linear increase in biomass and LAI with an increase of radiation. At V4 growth stage, plants from cultivar Urano had the largest LAI in all sowing dates, showing higher early leaf development efficiency than other cultivars. The number of nodules (Figure 1a) was higher in plants sown in October and November, but the DM of nodules (Figure 1b) was higher in plants sown in October, demonstrating that plants sown in October formed larger nodules. Cultivar BRS 284 formed a higher number and DW of nodules than the other cultivars. The higher number of nodules of cultivar BRS 284 probably occurred because it is more able to nodulate and not because it is the cultivar with more vegetative development with a high amount of nodules to supply nitrogen.

According to Francisco Junior and Harper (1995), in a study with graft and rooting of the leaves and shoot of soybean with normal and mutant cultivars capable of hyper-nodulation, the leaves, in comparison with others organs of the soybean plants, have a greater influence on nodulation by producing a high portion of the physiological signs responsible for infection by *Rhyzobium.* However, it was not the amount of leaves that is the most responsible for the control of nodulation but the genotype of the plant.

Environmental conditions, i.e. soil moisture (King and Purcell, 2005) and soil temperatures below 25°C, limited soybean nodulation (Miransari and Smith, 2008); therefore there is risk of reducing soybean nodulation due to lower temperatures in southern Brazil in sowing before October. Zhang et al. (2003), studying yield of different cultivars and strains in Canadian soil, found nodules limitation due to low soil temperatures. The authors verified differences of mass and number of nodules among cultivars, and the greatest differences in the amounts of fixed nitrogen changed according to the strains utilized. Moreover, the authors verified that the bigger the amount of nodules (both number and mass), the higher the amount of nitrogen (kg ha^{-1}) produced by the shoots of the plants.

According to Salvagiotti et al. (2008), there is a positive linear correlation between nitrogen uptake in aboveground and soybean yield. Vollmann et al. (2011) emphasized the importance of nodulation in soybean plants, relating it with chlorophyll content of leaves, yield

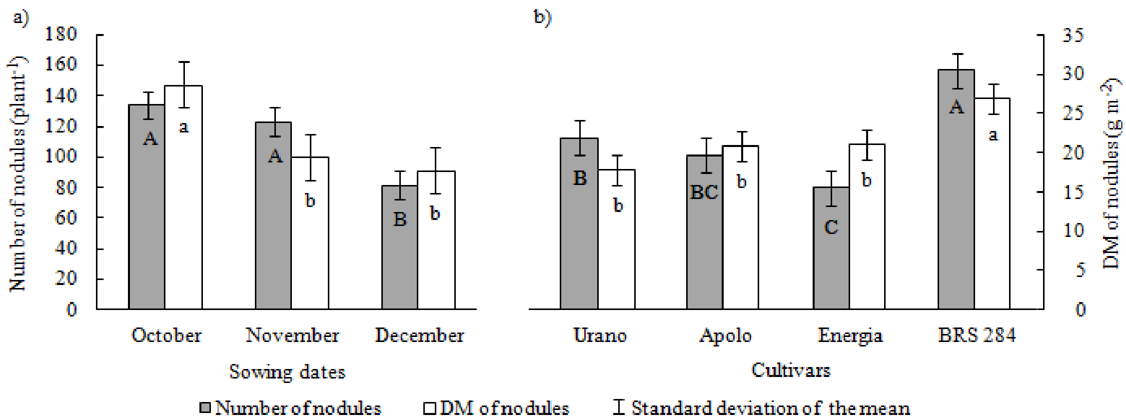

Figure 1. Number of nodules[1] and dry matter of nodules[2] at a) three sowing dates and b) four cultivars of soybean, at plant growth stage R5.3[3]. [1]Plant^{-1}; [2]g m^{-2}; [3]upper case letters compare averages of number of nodules, and lower case of dry matter (DM) of nodules. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

Table 7. Correlation coefficients between soybean quantitative plant characters analyzed and final grain yield of four cultivars sown on three dates.

Variable	Yield	Plant height	Nº of nodes	Nº of branches	Main stem diameter	Leaf area index	Nº of nodules
Plant height	0.469**						
Nº of nodes	0.486**	0.888**					
Nº of branches	0.242^{ns}	-0.427**	-0.203ns				
Main stem diameter	0.489**	0.394*	0.675**	0.428**			
Leaf area index	0.571**	0.680**	0.775**	0.090ns	0.713**		
Nº of nodules	0.072^{ns}	0.736**	0.726**	-0.474**	0.367*	0.640**	
DM of nodules	0.483**	0.807**	0.745**	0.067ns	0.472**	0.776**	0.705**

**: Significant at 1%, *: significant at 5%, and ^{ns}: not significant according to *t*-test.

and oil and protein content of seeds.Thus, it can be concluded that there are genotypes more able to nodulation, and that larger leaves are not responsible for higher nodulation, but the limitation of nodules may reduce the amount of nitrogen produced by the plants. Table 7 shows the correlation coefficients between studied quantitative plant characters and the final grain yield. Leaf area index was the plant character with the greatest influence on final grain yield. Other characters such as plant height and number of nodes, diameter of branches and DM of nodules also had an influence on yield, however lower than LAI. Sauer et al. (2007) verified a high correlation between LAI and plant yield.

In this study, we observed a reduction in LAI of 0.082 per day of delay in the sowing date, from October 21 to November 18. The grain yield in the same period decreased 24 kg ha^{-1} per day with a delay in the sowing date. Considering the period between November and December, the decrease in LAI was 0.016 day^{-1}, but we did not observe a reduction of grain yield. Therefore, the time of soybean sowing is essential to achieve elevated values of LAI and obtain high yields. The DM of nodules showed a higher influence on final grain yield than its number. The number of nodules and DM of nodules may change independently, as shown in Figure 1, so it is recommended that it DM rather than it number be observed more attentively if the nodulation is a parameter utilized in selection of soybean cultivars with high yield potential in breeding programs.

Conclusion

In southern Brazil, late sowing reduces the quantitative plant characteristics and phenological development of all soybean cultivars studied, independently of growth habit, but the cultivar with determined growth habit had the lowest reductions and is a good option for late sowing. Until the end of the vegetative period, cultivars did not show its final plant characteristics. Dry matter of nodules has more importance for cultivar selection than the number of nodules. Early sowing of soybean increases the LAI and can increase grain yield. Leaf area index has a high correlation with final grain yield.

Conflict of Interest

The authors have not declared any conflict of interest.

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