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Nitrogen fertilization on the establishment of *Arachis pintoi* cv. Belmonte

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ABSTRACT - The objective was to evaluate the effect of nitrogen fertilization on the establishment of forage peanut (*Arachis pintoi* cv. Belmonte) propagated vegetatively. The experiment was conducted in a greenhouse in a completely randomized design with treatments arranged in a 2 × 4 factorial design - two ages (70 and 85 days after planting) and four nitrogen doses (0, 40, 80 and 120 kg/ha) - with four replications. Morphogenetic and structural characteristics and production were evaluated. The nitrogen accelerated the establishment of the forage peanut with an increase in dry weight of green leaves and stolons. The greatest length of stolons (48.0 cm) was obtained with a dose equivalent to 86 kg N/ha and higher density of stolons (20 stolons/vase) between 78 and 82 kg N/ha. Nitrogen fertilization also reduced the phyllochron from 6.7 to 4.6 days/leaf. These data were more intense at 85 days, suggesting greater photosynthetic contribution during this period related to the large number of leaves after 70 days. Therefore, nitrogen can be an important tool to accelerate the establishment of pure stands of forage peanut.

Key Words: biomass partitioning, forage peanut, legume, morphogenesis

Introduction

The use of forage legumes has been an alternative to intensification of production of ruminants on pasture, improving and diversifying feed, and reducing costs. One of its most important contributions is its potential to fix atmospheric nitrogen, resulting in forage of better nutritional value and improved soil fertility (Miranda et al., 2003).

The forage peanut (*Arachis pintoi* cv. Belmonte), launched by Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), is a creeping legume, very aggressive on the ground cover due to its stolons, which are fixed in the soil for rooting of nodes, and their growing points are protected, giving them greater resistance to grazing (Jank et al., 2005).

According to Ramos et al. (2010), the establishment of forage peanut can be considered very slow, depending on soil fertility, water regime, spread mode and density of seedlings, thus becoming one of the major limitations of the species. According to these authors, propagation by seeds would be the most practical and efficient way, but the development of the fruits of plants of the genus *Arachis* occurs below the ground, which makes it difficult to harvest the seeds. Thus, the forage peanut is commonly propagated vegetatively. Therefore, appropriate management, combined with forage production strategies, such as fertilization, can assist in the rapid establishment of the pasture.

The production potential of a forage plant is genetically determined; however, the availability of nutrients and water modulate the productivity and quality of forage (Patês et al., 2007). A common practice for the increase in pasture biomass is nitrogen fertilization (Lopes et al., 2011; Santos et al., 2009; Alexandrino et al., 2004; Fagundes et al., 2006); however, it is not common to use nitrogen in legumes, since they are fixing this mineral. Moreover, being a constituent of several cellular components, nitrogen accelerates the growth, and this can be paramount in the speed of establishment of a cultivation.

According to Fagundes et al. (2006), beyond the availability of nutrients and appropriate choice of forage species, it is necessary to understand the morphophysiological mechanisms and their interaction with the management and environment, an important tool for assessing the growth dynamics of forage plants.

The objective of this study was to evaluate the effect of nitrogen fertilization on the establishment of *Arachis pintoi* cv. Belmonte propagated vegetatively.

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Material and Methods

The experiment was conducted in a greenhouse that belongs to the Laboratory of Forage and Pasture of Universidade Estadual do Sudeste da Bahia, UESB, Campus "Juvino Oliveira", in Itapetinga, state of Bahia, Brazil, in the period from July to October 2010. The trial was conducted in a 2 × 4 factorial arrangement, two ages (70 and 85 days after planting) and four nitrogen rates (0, 40, 80 and 120 kg/ha), arranged in a completely randomized design with four repetitions, totaling 32 plastic vases with a capacity of 10 dm^3.

The soil utilized was classified as an orthic Quartzarenic Neosol of loamy-sandy texture, collected at 0-20 cm depth on the campus of Universidade Estadual do Sudeste da Bahia, UESB. After passing through a 4 mm mesh sieve, the vase was filled and the material for the analysis of the ground was collected. The soil chemical analysis, performed at the Department of Agricultural Engineering and Soil Science of UESB, showed the following results: water pH = 5.7; P = 37 mg.dm^{-3}; K = 0.30 $\text{cmol}_c\text{.dm}^{-3}$; Ca^{2+} = 3.0 $\text{cmol}_c\text{.dm}^{-3}$; Mg^{2+} = 1.4 $\text{cmol}_c\text{.dm}^{-3}$; Al = 0.1 $\text{cmol}_c\text{.dm}^{-3}$; H = 2.1 $\text{cmol}_c\text{.dm}^{-3}$; base saturation = 4.7 $\text{cmol}_c\text{.dm}^{-3}$; T = 6.9 $\text{cmol}_c\text{.dm}^{-3}$; V = 68%; and organic matter = 29 g.dm^{-3}. Considering the results of soil analyses and the recommendations from the Soil Fertility Commission of the State of Minas Gerais (Alvarez & Ribeiro, 1999), there was no need for liming, because the value of base saturation was 68% in the layer of collected soil. For forage peanut, the values of P and K were also adequate, so there was no need for correction.

Forage peanut seedlings were grown from stolon pieces containing a node obtained from mother plants of Campus Juvino Oliveira. The planting of the cuttings was carried out on July 21st 2010 and, after shooting, four plants were maintained per vase. During the experiment, the water level in the soil was kept close to field capacity and all vases were weighed every 2 days.

For determination of the field capacity, all vases containing dry soil were weighed, saturated with water and, after flowing water, they were weighed again. The difference between wet and dry weight determined the maximum water holding capacity, which was of about 20%.

The nitrogen doses (0, 40, 80 and 120 kg/ha, corresponding to urea levels of 0; 0.44; 0.89 and 1.33 g/vase) were split in two applications: the first happened on the day of planting and the second after 20 days.

To study growth, a stolon was marked per vase with colored ribbons, resulting in four replicates per treatment. Measurements were made every two days during the experimental period. In each labeled stolon, the following traits were evaluated: appearance of leaf apex and complete exposure of the leaf, stolon length, number, length and width of folioles, number of secondary stolons and stolons per vase. From these data, morphogenectic (leaf appearance rate, phyllochron and stolon elongation rate) and structural (total number of green leaves and stolons, width and length of folioles and of stolons, number of stolons per vase and secondary stolons) traits were evaluated: *total number of green leaves per vase:* obtained by counting the number of expanding and expanded leaves per vase; *leaf appearance rate:* calculated by the number of leaves (petioles) emerging in the marked stolons divided by the number of days involved (leaves/stolons.day); *phyllochron:* calculated as the opposite of the leaf appearance rate (days/leaf); *stolong elongation rate (mm/stolons.day):* estimated by the difference between final length (cm) and initial length of stolons (cm) divided by the interval of days between measurements; *final length of stolons (cm):* obtained by the difference between the initial and final measurement of stolons in the experimental period; *number of secondary stolons:* obtained by counting the lateral stolons emerging in the period; *number of stolons per vase:* estimated by counting the number of primary stolons per vase at the end of the experimental period.

After periods of 70 and 85 days, the vases were disassembled by washing with water and plants were dissected into leaves, stolons, roots and nodules. Nodules were manually removed, weighed, dried in an oven at 60 °C for 72 hours, weighed again to determine the dry weight and counted. The minimum, maximum and average temperatures were recorded in the period and showed average values of 17.5; 39.5; and 28.5 °C, respectively.

The results were submitted to variance analysis, considering the dose of N and the age × dose of N interaction as sources of variation in age. The interaction was deployed, or not, according to significance, and the effect of nitrogen was evaluated by regression analysis, using orthogonal polynomials, the sum of square decomposition of nitrogen in linear, quadratic and cubic orders. Ages were compared by the F test. The experiment adopted $\alpha = 0.05$.

Results and Discussion

The interaction between nitrogen doses and age was significant ($P<0.05$) for dry mass of green leaves and stolons. In 70 days, leaf dry matter responded linearly to the N doses, in which 4.5 g/vase was found at a N dose of 120 kg/ha, which corresponds to almost double the production in the absence of nitrogen (Table 1). For the age of 85 days, the response to N was quadratic, with maximum yield of dry mass of green leaves at 15.2 g/vase with a nitrogen dose of 80 kg/ha (Table 1). In the absence of nitrogen, the dry mass of the leaves in 85 days was 4.8 g/vase, a production three times lower than in the presence of N at 80 kg/ha. The dry mass of stolons increased linearly ($P<0.05$) between the ages of 70 and 85 days, when the dose of N of 120 kg/ha was responsible for the largest dry mass of stolons (3.1 and 14.9 g/vase, respectively), corresponding to 1.7 to 3.5 times the dry mass of stolons in the absence of nitrogen, respectively. This linear growth of stolons may be due to the priority of the plant in the horizontal growth for soil cover and subsequent development of erect leaves and secondary stolons (Ramos et al., 2010). Nitrogen increased the biomass of the forage peanut, which, according to Nabinger (2001), is due to the increase in carbon fixation, and nitrogen is a controller of different processes of growth and development of plants.

There was a sharp increase between 70 and 85 days, when there was a significant increase in dry mass of leaves and stolons, reaching triple and quadruple values, respectively. This response may have occured, since the stolons used for planting had only one node and the leaves were removed, so the initial growth of roots and leaves depended exclusively on the reserve of stolons. From the first formed leaves, the photosynthetic activity began to contribute in the development of the plant, being intensified when the plant had a larger number of leaves. The formation and development of leaves are important for plant growth, since the leaves are essential for photosynthesis, which, in turn, is necessary for the formation of new tissue (Lemaire & Chapman, 1996). Thus, between 70 and 85 days, photosynthetic activity may have been crucial to growth acceleration, contributing to the partition of biomass, justifying the enormous difference in the accumulated mass at 85 days, in the plant as a whole.

The dry mass of green leaves and stolons influenced the leaf/stolon ratio, so that, due to the linear response of the dry mass of stolons in response to the nitrogen fertilization ($P<0.05$), there was a decrease by 25% of this ratio because of age (Table 1). It can be observed that nitrogen increased the production of the aerial part of the forage peanut, probably in response to higher photosynthetic efficiency of leaves and elongation of stolons, which decreased the leaf/stolon ratio. This reduction is characteristic, since these plants have a prostrated growth and only one leaf at

Table 1 - Effect of nitrogen (N) on dry mass of green leaves, dry mass of stolons, leaf:stolon ratio, dry mass of root and shoot:root ratio of *Arachis pintoi* cv. Belmonte at 70 and 85 days after planting

Age (days)	kg of N/ha				Mean	CV (%)	P value		
	0	40	80	120			Linear	Quadratic	Cubic
					Dry mass of green leaf (g/vase)				
70[1]	2.4a	3.2b	3.7b	4.5b	3.5b	31.5	0.015	0.768	0.688
85[2]	4.6a	13.1a	15.1a	13.5a	11.6a	11.9	0.000	0.000	0.565
Mean	3.5	8.2	9.4	9.0					
					Dry mass of stolon (g/vase)				
70[3]	1.5a	2.5b	2.7b	2.9b	2.4b	19.4	0.001	0.764	0.931
85[4]	3.5a	8.9a	11.5a	14.4a	9.6a	27.4	0.000	0.299	0.585
Mean	2.5	5.7	7.1	8.6					
					Leaf/stolon ratio				
70	1.6	1.4	1.4	1.6	1.5a				
85	1.3	1.6	1.3	1.0	1.3b				
Mean[5]	1.5	1.5	1.4	1.3		18.3	0.001	0.736	0.739
					Dry mass of root (g/vase)				
70	2.2	3.2	2.6	2.6	2.7b				
85	3.6	6.3	6.4	5.0	5.3a				
Mean[6]	2.9	4.8	4.5	3.8		20.7	0.076	0.000	0.207
					Shoot/root ratio				
70	1.8	1.7	2.5	2.9	2.2b				
85	2.8	3.6	4.2	5.5	4.0a				
Mean[7]	2.3	2.6	3.4	4.2		25.5	0.000	0.377	0.862

[1] $\hat{Y} = 2.427 + 0.0172 \times N$ ($r^2 = 0.99$)

[2] $\hat{Y} = 4.768 + 0.0259 \times N - 0.0016 \times N^2$ ($r^2 = 0.99$)

[3] $\hat{Y} = 1.751 + 0.0108 \times N$ ($r^2 = 0.85$)

[4] $\hat{Y} = 4.275 + 0.0883 \times N$ ($r^2 = 0.97$)

[5] $\hat{Y} = 1.497 - 0.0017 \times N$ ($r^2 = 0.81$)

[6] $\hat{Y} = 2.995 + 0.0542 \times N - 0.0004 \times N^2$ ($r^2 = 0.93$)

[7] $\hat{Y} = 2.166 + 0.0159 \times N$ ($r^2 = 0.97$)

Means followed by different letters in the column differ ($P<0.05$) by the F test.

CV - coefficient of variation.

each node. The plants that received no nitrogen showed the highest leaf/stolon ratio (1.5:1); however, the dry mass of leaves was significantly reduced in relation to the presence of nitrogen, whereas the reduction in this ratio to 85 days, was most evident with N at 120 kg/ha, characterizing the largest production of stolons in that dose.

The dry mass of root responded quadratically (P<0.05) to nitrogen, with a positive effect also on the ages (P<0.05), which resulted, in general, in an increase of 2 times the dry mass of root at 85 days compared with 70 days of age (Table 1). At 85 days, the maximum value was 4.8 g/vase with N at 80 kg/ha, which corresponds to 1.6 times dry mass of root in the absence of nitrogen.

The shoot/root ratio was influenced (P<0.05) linearly by N rates and by age (Table 1), with 85 days to an increase in the shoot/root ratio of 1.8 times compared with 70 days. This increase was more significant for the N dose of 120 kg/ha, which reached a ratio of approximately 4:1, which also showed a higher dry mass of stolons with linear response to nitrogen. These data demonstrate the contribution of nitrogen, especially to the growth of shoots during the establishment of seedlings of forage peanut.

There was no statistical difference (P<0.05) in the dry mass and number of nodules according to age and nitrogen in the roots of forage peanut (Table 2). This response was due to the high coefficient of variation found for these variables. This implies that further studies should be conducted to evaluate the nodulation of *Arachis pintoi* in the presence of N, since the nutrient can affect the formation of nodules. Moreover, lower doses of this nutrient can contribute to the acceleration of plant establishment without significantly harming nodulation. This was evinced by Mendes et al. (2008), evaluating the interference of nitrogen fertilization on the dry mass of nodules on soybean roots; nodule damage was only verified when N was applied at 200 kg/ha.

The number of green leaves/vase showed a linear increase (P<0.05) compared with N doses (Table 3), with approximately 261 leaves/vase at a N dose of 120 kg/ha, corresponding to the number of green leaves two times in the absence of nitrogen. The age of 85 days showed an increase of 3.7 times in this parameter, in relation to the age of 70 days. Thus, as discussed above for the dry mass, the appearance of the first leaves started photosynthetic contribution to the development of the plant. With the increased number of leaves, photosynthesis gradually increased, and this probably promoted also a greater yield of leaves after 70 days. The number of leaves present in a plant is related to the potential for carbon assimilation and biomass accumulation, an important characteristic for the evaluation and management of forage plants, being a qualitative attribute of the pasture (Pompeu et al., 2010).

Nitrogen fertilization helped in biomass accumulation the forage peanut, reducing the time of its establishment, for assisting in the process of growth and development of the plant, especially production of leaf and stolons.

Nitrogen had a quadratic effect (P<0.05) on the leaf appearance rate, with no effect of age, and the highest rate (approximately 0.2 leaves/stolon.day) was found, according to the regression equation, at the nitrogen dose of 80 kg/ha, which corresponds to an increase of 1.3 times in the leaf appearance rate in the absence of nitrogen. Likewise, the phyllochron also had quadratic effect of N levels (P<0.05), also without effect of age, presenting a reduced number of days (4.6) at a N dose of 80 kg/ha (Table 3). The leaf appearance is directly related to dry mass production and better relation between carbon and nitrogen (Thomas, 1983; Santos et al., 2009). Furthermore, according Pompeu et al. (2010), N has an importance in reducing the time for the appearance of two consecutive leaves by increasing the production of new cells having a positive impact on the number of leaves per plant.

Table 2 - Effect of nitrogen (N) on the number and dry weight of nodules of *Arachis pintoi* cv. Belmonte at 70 and 85 days after planting

Age (days)	kg of N/ha				Mean	CV (%)	P value		
	0	40	80	120			Linear	Quadratic	Cubic
Number of nodules/vase									
70	7.3	90.3	3.50	40.3	35.3a				
85	220.8	291.0	35.3	41.0	147.0a				
Mean[1]	114.0	190.6	19.4	40.6		211.6	0.212	0.688	0.162
Dry mass of nodules (mg/vase)									
70	10.0	120.0	10.0	30.0	40.0a				
85	320.0	430.0	50.0	70.0	220.0a				
Mean[2]	170.0	270.0	30.0	50.0		205.1	0.155	0.652	0.145

[1] $\hat{Y} = 91.1$

[2] $\hat{Y} = 130.0$

Means followed by different letters in the column differ (P<0.05) by the F test.

CV - coefficient of variation.

The stolon elongation rate responded linearly to the ages and to N rates (P<0.05). The stolon elongation rate observed at 85 days of age was 2.6 times that observed in 70 days (Table 4), and 1.02 cm/day was found at a N dose of 120 kg/ha.

The final length of stolons responded to the ages and to N levels (P<0.05) quadratically. The maximum final length of stolons was estimated at 51.8 cm at a N dose of 100 kg/ha, corresponding to 1.6 times the final length of stolons observed in the absence of nitrogen. The age of 85 days had an increment of 1.5 times greater in length when compared with 70 days of growth.

The number of secondary stolons, counted from the main stolon, increased linearly with N fertilization (P<0.05). At the highest dose of N, over 10 secondary stolons were observed per main stolon in the 85 days, a value three times

Table 3 - Effect of nitrogen (N) on the number of green leaves per vase, leaf appearance rate and phyllochron of *Arachis pintoi* cv. Belmonte at 70 and 85 days after planting

Age (days)	kg of N/ha				Mean	CV (%)	P value		
	0	40	80	120			Linear	Quadratic	Cubic
				Total number of green leaves/vase					
70	54.8	92.0	62.0	109.5	79.6b				
85	188.0	313.3	359.0	312.8	293.3a				
Mean[1]	121.4	202.6	210.5	258.8		10.4	0.000	0.074	0.122
				Leaf appearance rate (leaves/day)					
70	0.15	0.19	0.19	0.19	0.18a				
85	0.15	0.19	0.19	0.21	0.19a				
Mean[2]	0.15	0.19	0.19	0.20		9.2	0.000	0.010	0.061
				Phyllochron (days/leaf)					
70	6.7	5.4	5.2	5.5	5.7a				
85	6.9	4.9	5.3	4.7	5.5a				
Mean[3]	6.8	5.2	5.2	5.1		10.7	0.000	0.001	0.080

[1] $\hat{Y} = 135.31 + 1.05 \times N$ ($r^2 = 0.91$)
[2] $\hat{Y} = 0.153 + 0.001 \times N - 0.000005 \times N^2$ ($r^2 = 0.91$)
[3] $\hat{Y} = 6.742 - 0.0413 \times N - 0.0002 \times N^2$ ($r^2 = 0.90$)
Means followed by different letters in the column differ (P<0.05) by the F test.
CV - coefficient of variation.

Table 4 - Effect of nitrogen (N) on the stolon elongation rate, final length of stolons, number of secondary stolons and number of stolons per vase of *Arachis pintoi* cv. Belmonte at 70 and 85 days after planting

Age (days)	kg of N/ha				Mean	CV (%)	P value		
	0	40	80	120			Linear	Quadratic	Cubic
				Stolon elongation rate (cm/day)					
70	0.4	0.5	0.5	0.5	0.5b				
85	1.1	1.3	1.4	1.4	1.3a				
Mean[1]	0.8	0.9	1.0	1.0		21.4	0.012	0.118	0.526
				Final length of stolons (cm)					
70	28.5	35.1	37.4	35.4	34.1b				
85	36.9	60.3	51.9	60.1	52.3a				
Mean[2]	32.7	47.7	44.7	47.8		15.7	0.000	0.026	0.238
				Number of secondary stolons					
70	2.5	4.3	6.8	7.3	5.2b				
85	3.3	6.8	9.5	10.8	7.6a				
Mean[3]	2.9	5.5	8.1	9.0		14.7	0.000	0.295	0.637
				Number of stolons/vase					
70	13.5	17.0	18.8	16.8	16.5b				
85	17.3	21.0	22.8	22.0	20.8a				
Mean[4]	15.4	19.0	20.8	19.8		14.7	0.004	0.016	0.776

[1] $\hat{Y} = 0.7902 + 0.0018 \times N$ ($r^2 = 0.79$)
[2] $\hat{Y} = 33.34 + 0.333 \times N - 0.0015 \times N^2$ ($r^2 = 0.95$)
[3] $\hat{Y} = 3.225 + 0.0525 \times N$ ($r^2 = 0.96$)
[4] $\hat{Y} = 15.0313 + 0.128 \times N - 0.0008 \times N^2$ ($r^2 = 0.99$)
Means followed by different letters in the column differ (P<0.05) by the F test.
CV - coefficient of variation.

greater than the one observed in the absence of nitrogen (Table 4). Also, there was effect from the age of the plant (P<0.05), with an average increase of 5.2 to 7.6 secondary stolons per main stolon. According to Valentim et al. (2003), the lateral growth of stolons is a feature of great influence on the speed of establishment of forage peanut, by determining the ability to colonize the area by plants. These authors, evaluating the establishment of accessions of forage peanut in the field, observed that cv. Belmonte showed 96% of soil cover with ten weeks after planting and that these plants are capable of emitting stolons and colonize an area with an average diameter between 174 and 204 cm in a period of 120 days of establishment.

The N doses influenced the number of stolons per vase quadratically (P<0.05), which also increased with age (P<0.05). There was an increase of 1.3 time in the number of stolons per vase for the age of 85 days compared with 70 days (Table 4). The highest number of stolons per vase (20 stolons/vase) was found at a N dose of 80 kg/ha.

It was found that nitrogen influenced both the production of leaves and the development of stolons, which, in general, contribute to the increase in photosynthesis and thus establishment of the plant. Whereas *Arachis pintoi* is a creeping plant with a prostrate growth (Jank et al., 2005), the increase in the number and length of stolons is fundamental for soil coverage. Furthermore, the appearance of the leaves occurs in the nodes, the growth of stolons and consequent larger number of nodes contribute to the greater number of leaves on the plant. These characteristics show the importance of nitrogen for the establishment of the forage peanut plants, since a higher speed of colonization may favor its use as an economically viable alternative for pasture systems.

Conclusions

For the establishment of *Arachis pintoi* cv. Belmonte, nitrogen is as an efficient tool, increasing the production of leaves and stolons and the speed of colonization of the area by this legume.

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