Research Article



Performance of the Cotton Variety BRS-336 Under Different Seed Densities

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Abstract | This research was done to measure the effect of spacing between rows and plants on the growth, development, and production of the cotton variety BRS-336. Twelve plant densities were used: 55 556, 37 037, 27 778, 50 000, 33 333, 25 000, 45 455, 30 303, 22 727, 41 667, 27 778 and 20 833 pl ha⁻¹, with single row planting arrangements. A randomized complete block design, in factorial arrangement (A x B), with four replications was used. The variables recorded were daily height increase (cm/day) in budding stage, plant height (cm), stem diameter (mm), crop canopy closure (days), internode length (cm), leaf greenness index (SPAD), number of cotton bolls opening/plant, number of open cotton bolls/plant, number of unopened cotton bolls/plant and yield of raw cotton (kg ha⁻¹). The results obtained in the research indicate that spacing between plants (m) was significantly influential in most of the agronomic and productive variables, compared to the spacing between rows, while the interaction was not significant. The plant spacing of 0.2 m showed the best plant development in the growth stage. The spacing of 0.2 m between plants recorded the lowest values in the productive traits, whereas spacing with 0.3 m and 0.4 m reported the best behavior. The spacing of 1.1 m between rows and 0.3 m between plants showed the best Marginal Rate of Return for cultivated cotton with the variety "BRS-336". The combination of the studied factors registered higher values in agronomic and productive variables when compared to the control treatment (conventional spacing used by farmers).

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Introduction

Cotton, Gossypium hirsutum L. (Malvaceae), is a crop of cultural, economic, and biological relevance (Ulloa *et al.*, 2006). It is considered the main natural fiber cultivated in the world, due to its wide use in the manufacture of fabrics and clothing (Dunne *et al.*, 2016). It is highly demanded by the



textile industry which uses fiber, as well as the food industry, that takes advantage of the high oil and protein content of the seed as a source of protein for animals (Brubaker *et al.*, 1999). The cotton is planted in more than 65 countries, many of which base their economies on this crop, approximately 32 million hectares are estimated in 2018, highlighting countries such as India, China, United States, Pakistan, Brazil, and Turkey, which contribute to 80% of the world production (FAO, 2022).

It is estimated that world cotton production exceeds 21 million metric tons. Based on the above considerations ICAC (2018), production in China was expected to increase by 1% (5.94 million tons) becoming the main producing country worldwide in 2018/19.

In Ecuador, cotton production had a great peak in the agricultural sector; however, it was declining in the nineties of the last century (Sotelo-Proaño *et al.*, 2022). According to statistics, the area went from 36000 ha in 1974 to 1800 ha in 2016, with an approximate production of 5000 MT at a production cost of 1,425 USD ha⁻¹, being the local demand of 20,000 MT per year of fiber (National Institute of Agricultural Research, 2018), a deficit that is covered with the import of cotton fiber from USA and Asia (FAO, 2017). In this regard, in 2017, 14,853 MT were imported from the USA and Asia.

There are several limitations to cotton production in the country, mainly the lack of high-yielding seed varieties, inadequate crop management, and low international prices (Cañarte-Bermúdez et al., 2020), in addition to environmental factors and phytosanitary problems. Within the technical management of the cotton crop in the country, adequate plant density by the cotton producer is not observed, which has an impact on the productivity of this fiber, becoming an important limiting factor in production. In this regard, it is known that, mainly, population density plays an important role during the crop cycle, to the point that its cultural tasks (increasing use of fertilizers, irrigation, herbicides), are planned according to the variety and population density of this crop (Menéndez-Natera, 2006). In this sense, Palomo-Gil et al. (2000) indicated that early cotton varieties sown in narrow rows (0.70 m) showed a higher yield potential than late varieties.

Several studies demonstrate the importance of studying the correct population density, including that one by Johnson *et al.* (1973), who determined that planting in narrower rows than the traditional ones, allows capturing a greater amount of solar radiation at an early stage of the crop cycle. Veramendi-Hidalgo and Lam-Vargas (2011), mention that cotton planting spacing can range from 0.90 m to 1.5 m between rows and 0.20 m to 0.60 m between plants.

However, from these aforementioned results, Gaytán-Mascorro *et al.* (2004), found that the reduction of the distance between rows and the increase in population density did not have a significant impact on cotton yield, but did cause precocity (crop closure, opening of buds, harvest, among others), without affecting production and quality.

This contrast of criteria and results regarding the adequate plant population density in the cotton crop, and considering the particular environmental conditions that are present in Manabí, Ecuador, justify the realization of this kind of study, which seeks to provide an alternative solution to the cotton sector regarding a definition of the adequate plant density of the cotton crop, which allows the producer to establish an adequate planting area, increasing the productivity of this important crop of Ecuadorian family agriculture and thus contribute to promote its reactivation.

With this background, the need for this research arises to evaluate the effect of spacing between rows and between plants on the growth, development, and productivity of the cotton variety "BRS-336", introduced by INIAP from EMBRAPA-Brazil. In this sense, it is necessary, within the framework of the adaptability of this variety in our country, to generate technologies for the benefit of the country's cotton producers

Materials and Methods

Location

The present research was carried out during the rainy season of 2020, from February to August, in the Teodomira farm of the Portoviejo Experimental Station of the INIAP, located in the Lodana parish of the Santa Ana canton, province of Manabi, at the geographical coordinates 01°09'51" S and 80°23'24" W, at an altitude of 60 meters above sea level; with the

following edaphoclimatic characteristics (temperature 26.4 °C; precipitation 851.57 mm; relative humidity 81%; annual heliophany 1604 hours of sunshine), flat topography and clay loam soil.

Development of the research

Seed of the cotton variety BRS-336 was used, introduced by INIAP-Ecuador from EMBRAPA-Brazil in November 2018; which has the following characteristics: pilosity in leaves and branches, medium-long fiber length, medium size, reaching 1.15 to 1.25 m in height (using growth regulators). At altitudes close to 700 meters above sea level, the emergence of the first flower occurs 60 to 65 days after the emergence (dde) of the plants and the opening of the first cotton boll occurs between 110 and 120 dde. Harvesting takes place from 170 to 180 dde. It reports a yield of 3,851 kg ha⁻¹ of raw cotton, and 1,527 kg ha⁻¹ of fiber cotton, with a fiber percentage of 38-39.5%. This material reports resistance to important cotton diseases and nematodes (Morello et al., 2012).

Two factors were studied: A. Spacing between rows (0.9, 1.0, 1.1, and 1.2 m) and B. Spacing between plants (0.2, 0.3, and 0.4 m), in addition to a control with a spacing of $0.8 \times 0.2 \text{ m}$. The combination of the factors under study resulted in the following treatments:

Treat-	Nomencla-	Spaci	Population	
ment	ture	Between rows	Between plants	(pl ha-1)
1	L1P1	0.9	0.2	55,556
2	L1P2	0.9	0.3	37,037
3	L1P3	0.9	0.4	27,778
4	L2P1	1.0	0.2	50,000
5	L2P2	1.0	0.3	33,333
6	L2P3	1.0	0.4	25,000
7	L3P1	1.1	0.2	45,455
8	L3P2	1.1	0.3	30,303
9	L3P3	1.1	0.4	22,727
10	L4P1	1.2	0.2	41,667
11	L4P2	1.2	0.3	27,778
12	L4P3	1.2	0.4	20,833

Statistical analysis

The experiment was carried out with a randomized complete block design, in factorial arrangement (A

x B), with four replications. Before subjecting the data to the analysis of variance, the assumptions of normality were tested using the Shapiro-Wilk test and homogeneity of variances with Bartlett's test. To test the effect of the treatments under study, comparisons of the means of the treatments were performed through Tukey's test (P<0.05). The statistical software R Studio version 3.6 (R Studio Team, 2019) was used for the analysis.

Experiment management

Soil preparation was carried out in a mechanized way, and the respective soil analysis was performed. Sowing was done manually, placing three seeds/site, using the spacings according to the treatments. Fifteen days after sowing (das), thinning was carried out, leaving one plant per site. The seed was treated with thiodicarb + imidacloprid, 25 mL /kg of seed. For weed control, the pre-emergent herbicide (pendimethalin 4 L ha⁻¹) + a contact post emergence herbicide (paraquat 4 L ha⁻¹) was applied, and the insecticide chlorpyrifos (1 L ha⁻¹) was added to control pests present in the soil.

Subsequently, after 20 das, the herbicide haloxyfopmethyl (0.6 L ha⁻¹) was applied post-emergent, being necessary to carry out two complementary manual weeding until the crop canopy closure. Pests were kept at low incidence, with only two phytosanitary controls at 40 das with lambda thiamethoxam+cyhalothrin (1 mL/L water) and at 86 das with abamectin (1.5 mL/L water), for the control of thrips, whitefly, and spider mites. Two applications of growth regulator (Mepiquat chloride) were made at 50 das, using a dose of 150 mL ha⁻¹ in 600 L ha⁻¹ of water and a second application at 84 das with a dose of 750 mL ha⁻¹ in 750 L ha⁻¹ of water. For fertilization, nitrogen was considered a missing element, so two applications were made at 15 and 45 das, using the urea + YaraMila® mixture. Finally, the harvesting was carried out in a dry environment, gradually at maturity, collecting mature cotton bolls at 133 and 169 das.

Data collection

Daily height increase (cm/day) in the budding stage was recorded between 29 to 35 days. Plant height (cm) was evaluated at 35 and 49 das, recording the height from the soil surface to the apex of each of the five plants. Stem diameter (mm) was registered at 52, 92 and 132 das, the stem diameter of the marked plants was determined, at a height of 10 cm from the surface of the soil, using a digital vernier caliper. The days to canopy closure were evaluated in each plot, considering for this purpose when the neighboring branches of the planting rows intertwined above 75%, thus covering the ground between the rows. The Internode length (cm) was registered at 92 das, the length of the five terminal internodes of each marked plant was measured.

The leaf greenness index (SPAD) was carried out at 120 das, recording the SPAD values in the five labeled plants. For this, the Minolta SPAD 502 $plus^{TM}$ chlorophyll meter determiner was used, the data was recorded in the upper third of the plant, in leaves exposed to light. These measurements were made between 11:00 am to 14:00 pm.

The number of open cotton bolls/plant was counted at 132 das. The number of productive branches was counted in the marked plants, considering those with at least one open mature cotton bolls suitable for harvesting. The number of cotton bolls per plant was counted. In each of the two harvesting passes, the weight of raw cotton in the useful plot was recorded, which was then accumulated in total kg/plot and from this, transformed to yield kg ha⁻¹. An economic analysis of the treatments was performed by calculating the partial budget, using the CIMMYT methodology with the calculation of the net benefit, variable costs, and marginal rate of return (CYMMYT, 1988).

Results and Discussion

According to the ANOVA, there was only a significant response in the daily height increase (cm/ day) in budding stage to factor B, spacing between plants (P<0.05). This was not the case for factor A, spacing between rows (m) nor for their interactions. The plants had a significant growth differentiated by the effect of the spacing between plants, in the bud formation stage (29 to 35 das); obtaining the greatest increase in height at a separation of 0.20 m between plants. The greater the plant spacing (0.4 m), the lower the increase in plant height (Table 1). For the variable plant height (cm), the analysis of variance determined significant statistical differences (P<0.05) for the spacing between plants (m), at 35 and 49 das (Table 1). While for the spacing between rows (m) and the interaction between the factors, no significant differences were recorded in the evaluations carried out. According to Tukey's separation of means test, at 35 and 49 das, the spacing lower than 0.2 m between plants stood out significantly, with the highest height (28.99 and 61.95 cm, respectively). While, in the greatest spacing between plants (0.4 m), plant height was lower (27.14 and 55.73 cm, respectively). These results suggest that, by decreasing the plant spacing and increasing plant density, plant growth is stimulated.

Table 1: Daily height increase (cm/day) in budding stage, plant height (cm), and stem diameter (mm) in the population density study of the cotton variety BRS-336. Teodomira, Santa Ana.

Factor	Daily height increase (cm/	Plant height (cm)		Stem diameter (mm)		
	day) in budding stage	i iant neight (em)		stem diameter (min)		
	29 to 35 das	35 das	49 das	52 das	92 das	132 das
Spacing between rows (m)	P>0.05	P>0.05	P>0.05	P>0.05	P<0.01	P>0.05
0.9 m	1.22	28.13	57.42	11.31	13.66 a	14.67
1.0 m	1.24	28.18	58.98	11.78	12.65 ab	15.36
1.1 m	1.2	27.73	58.28	11.33	11.33 b	15.58
1.2 m	1.22	27.78	57.85	11.56	11.57 b	15.87
Spacing between plants (m)	P<0.05	P<0.05	P<0.05	P<0.05	P>0.05	P<0.01
0.2 m	1.33 a	28.99 a	61.95 a	10.93 b	12.16	14.04 b
0.3 m	1.20 ab	27.73 ab	56.73 ab	11.55 ab	12.23	15.74 a
0.4 m	1.13 b	27.14 b	55.73 b	12.00 a	12.51	16.32 a
Mean	1.22	27.95	58.13	11.49	12.3	15.37
CV (%)	14.65	7.19	10.57	9.37	12.82	12.21

Means followed by the same letter did not differ statistically from each other according to Tukey's test (P<0.05). das = days after sowing

Table 2: Effect of spacing	between rows and space	ing between plants on	n physiological and agronomic	parameters
considered in the population	e density study of the cotte	on variety "BRS-336".	. Teodomira, Santa Ana.	

Factor	-	Internode length (cm) -92 das	Leaf green- ness index - 120 das	Number of open cotton bolls / plant - 132 das	Number of productive branches/plant	1	Yield of raw cotton (kg ha ⁻¹)
Spacing between rows	P<0.01	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
0.9 m	86.92 b	2.43	53.53	11.28	9.48	23.53	4992.67
1.0 m	87.58 b	2.62	53.01	14.23	10.97	25.95	4830.17
1.1 m	94.33 ab	2.48	54.08	13.08	10.1	28.6	5319.92
1.2 m	99.58 a	2.3	52.84	14.37	11.35	27.7	4934.67
Spacing between plants	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.01	P>0.05
0.2 m	88.31	2.67 a	51.92 b	9.91 c	9.26 b	21.59 b	5254.50
0.3 m	91.75	2.48 ab	52.93 ab	13.06 b	10.26 ab	27.55 a	5057.75
0.4 m	96.25	2.22 b	55.24 a	16.75 a	11.90 a	30.20 a	4745.81
Mean	92.1	2.46	53.36	13.24	10.48	26.45	5019.35
CV (%)	10.6	13.89	5.66	20.91	23.67	26.47	12.93

Means followed by the same letter did not differ statistically from each other according to Tukey's test (P<0.05). das = days after sowing.

These results are contradictory to those reported by Sierra *et al.* (2010), who indicate that, at low plant densities (20,000; 25,000 and 22,222 pl ha⁻¹), the plants showed the greatest average heights, which was also found by Braga-Meza and Rabery-Caceres (2004), when they stated that the final height of cotton plants increases with decreasing density. These same authors mention that, for every 20 centimeters that are given to the spacing between rows from 30 cm, the plants presented an increase in height of 4 cm up to 90 cm distance between rows.

In stem diameter (mm), the analysis of variance recorded highly significant differences (P<0.01), in factor A, spacing between rows, at 92 das. According to Tukey, the shortest distance between rows of 0.9 m was found to have the largest stem diameter (13.66 mm), while as the spacing between rows increased, the stem diameter decreased progressively, reaching a significantly smaller diameter at the greatest distance (1.2 m). In the case of factor B, spacing between plants (m), ANOVA presented significant differences (P<0.05) at 52 das and highly significant (P<0.01) at 132 das. It was observed on both dates that the highest values of stem diameter were reached at the greatest spacing between plants (0.4 m), followed by the 0.3 m spacing (Table 1).

The results of this research differ from those found by Quintana *et al.* (2013), who mention that, for the spacing between plants, the highest value is observed with a spacing of 20 cm between plants and 100 cm between rows (9.2 mm) in cotton NuOpal (BGRR) variety.

At the crop canopy closure, the analysis of variance established high statistical differences (P<0.01) for spacing between rows (m), while for spacing between plants and the interaction between factors, no significant statistical differences were observed (P>0.05) (Table 2). When the Tukey's separation of means test was performed, it was determined that, with the narrowest distances of 0.9 and 1.0 m between planting rows, the shortest time in days for the closure of the canopy was achieved, differing statistically from the rest of the treatments (Table 2). These results indicate that cotton responds positively to canopy closure as a function of the spacing between rows. In terms of spacing between plants, although no significant statistical differences were found, it was observed that reducing the spacing between plants (0.2 m) also reduces the time required to close the canopy.

These results coincide with was what found by Santacruz and Salas (2008), who state that with the shortest distance between rows (0.50 m), the closure of the canopy occurred faster, that is, after 45 days; likewise, Riar *et al.* (2013) also determined that the percentage of closure was higher in narrow rows of 38 cm.

In the variable internode length (cm), the analysis of variance only established significant differences (P<0.05) for Factor A, spacing between plants (m), at 92 das. According to Tukey, the shortest distance

between plants (0.2 m) was significant, with the longest internode length (2.67 cm), which decreased as the spacing between plants increased (Table 2).

In the SPAD, the analysis of variance determined significant statistical differences (P < 0.05) for the distance between plants (m), at 120 das (Table 2). The plot with the largest plant spacing (0.4 m) had the best photosynthetic pigment index (55.24), while at the smallest plant spacing (0.2 m), the SPAD values was significantly lower (Table 2). This result suggest that plants in closer space compete for sunlight, lowering their capabilty for chlorophyll production.

In the number of open cotton bolls/plant, the analysis of variance recorded significant statistical differences at 132 das (P<0.05) for plant spacing (m) (Table 2). The highest number of open bolls was recorded with the greatest plant spacing of 0.4 m (16.75 open bolls) (Table 2). In this regard, Quintana *et al.* (2013) also studied the response of this variable to spacing, reporting that, with the treatment of 20 cm between plants, a linear and positive trend was observed, indicating that for every 10 cm increase in the distance between rows there is an increase of 1.2 open bolls. The more access to sunlight in plants with more space could explain this result.

In relation to the variable total number of productive branches per plant, counted before the harvest, the analysis of variance recorded statistical differences (P<0.05), in the spacing between plants (m). The treatments with the greatest spacing between plants stood out again, significantly (Table 2). These results are inconsistent with the findings of Quintana *et al.* (2013), who mentioned that the number of fruiting branches per plant showed a significant effect on the spacing between rows, observing a decrease of 0.6 fruiting branches for every 10 cm increase in the spacing between rows. In addition, they found in their study that the greatest number of fruiting branches was obtained with the spatial arrangement of 10 and 20 cm between plants and 100 cm between rows.

In the total number of unopened cotton bolls/plant, the analysis of variance indicated highly significant differences (P<0.01) for factor spacing between plants (m) (Table 2). According to Tukey's test, the treatment with greater distance between plants, 0.4 and 0.3 m, recorded 30.20 and 27.55 accumulated cotton bolls, respectively. Despite not having reported statistical significance in the spacing between rows, we observed a tendency to increase the number of cotton bolls/plant in the widest distances (1.1 and 1.2 m) (Table 2). These results are supported by Quintana et al. (2013), who found that, in treatments with a spacing between plants of 10 cm, it was observed that the number of cotton bolls per plant increased linearly and positively with the increase in the spacing between rows, at a rate of 0.8 bolls for every 10 cm of spacing between rows.

In the total yield of raw cotton (kg ha⁻¹) no significant differences were reported (P>0.05). In the factor spacing between rows, the yield ranged between 4831 kg ha⁻¹ (1.0 m) to 5320 kg ha⁻¹ (1.1 m), while in the factor spacing between plants, the total cotton yield ranged between 4745.81 kg ha⁻¹ (0.4 m) to 5254.50 kg ha⁻¹ (0.2 m) (Table 2). Finally, according to the Marginal Analysis, it was determined that the best population density for cotton crop production under the conditions of Lodana, Santa Ana, was 30303 pl ha⁻¹ (1.1 x 0.3 m), with which the best Marginal Rate of Return TRM (547.47%) was obtained, which far exceeds the required rate (100%) (Table 3). These results indicate that by increasing the spacing between rows and decreasing the spacing between plants, a higher yield in kg ha⁻¹ was obtained, which is in agreement with Santacruz and Salas (2008), who mention that decreasing the spacing between plants (0.20 m) increases the yield of raw cotton.

Table 3: Marginal analysis of non-dominated treatments of the study: Performance of the cotton variety "BRS-336" under different seed densities. Teodomira, Santa Ana.

Spacing between		NB	VC	MRBN	MRVC	MRR	RRR
Rows (m)	Plants (m)	(USD./ha)	(USD./ha)	(USD./ha)	(USD./ha)	(%)	
0.9	0.4	1846.69	385.67*	93.45	48.59	192.32	
1.1	0.3	2085.55	429.30*	238.86	43.63	547.47	100%
1.1	0.4	1753.24	337.08*	111.64	25.1	444.78	
1.2	0.4	1641.6	311.98*				

NB= net benefit; VC= variable Cost; MRNB = marginal revenue of net benefit; MRVC= marginal revenue of variable cost; MRR= marginal rate of return; RRR = required rate of return.



However, these same authors mention that with regard to the spacing between rows, they obtained the best results in narrow rows of 0.50 m, coinciding with what is cited by Ibarra-Zamudio *et al.* (2011), who also mention that, with the lowest density and best spatial distribution (furrows 0.52 m), the DP 402 variety was the one that expressed the highest yields in all treatments.

Conclusions and Recommendations

The largest spacing between plants reduces high in the plant and increases the stem diameter in the cotton variety BRS-336. The spacing of 0.2 m between plants recorded the lowest values in the productive traits, whereas spacing with 0.3 m and 0.4 m reported the best behavior. The spacing of 1.1 m between rows and 0.3 m between plants showed the best Marginal Rate of Return for cultivated cotton with the variety BRS-336.

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Novelty Statement

In Ecuador the cotton variety "BRS-336", cultivated with the spacing of 0.3 m between plants and 1.1 m the spacing of between rows allow to obtain the best profit.

Author's Contribution

Gilmar Jesús Cañarte-Cañarte, Luis Fernando Díaz-Toral and Carlos Eddy Alvarado-Zamora: Conducted the experiment and the data collection.

Ernesto Gonzalo Cañarte-Bermúdez: Conceived the study idea, designed and supervised the experiment, and helped in the writing and reviewing the manuscript. **Fernando David Sánchez-Mora:** Contributed during writing up and editing of the manuscript. **José Bernardo Navarrete-Cedeño:** Helped in the conducted the experiment and did data analysis and wrote-up of manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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