Research Article

Row spacing and seed physiological quality of *Crotalaria* species[1]

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ABSTRACT

Crotalaria crops have important uses, such as in green manuring, nematode control, biological nitrogen fixation and sugarcane reform in Savanna areas. Due to its strategic importance, knowledge about crotalaria seed production technology is a relevant factor to ensure the availability of high physiological quality seeds. This study aimed to assess the effect of two row spacings (20 and 40 cm) on the germination and vigor of *Crotalaria ochroleuca* and *Crotalaria spectabilis* seeds produced in two crop seasons (2018 and 2019). The experimental design was randomized blocks, with four replications, in a 2 x 2 factorial scheme (spacing x crop season), for each species. The climatic conditions during seed production significantly influenced the physiological quality of the crotalaria seeds. The 2018 harvest, with more abundant rainfall and milder temperatures during flowering, provided more vigorous seeds for both species. The 20 cm spacing was more efficient to produce *C. ochroleuca* seeds with high physiological performance. Notwithstanding, *C. spectabilis* seeds showed a higher physiological quality when produced under 40 cm spacing than when produced under denser cultivation.

KEYWORDS: *Crotalaria ochroleuca*, *Crotalaria spectabilis*, seed vigor.

RESUMO

Espaçamento entre linhas e qualidade fisiológica de sementes de espécies de *Croatalaria*

Crotalárias são culturas utilizadas principalmente para a adubação verde, controle de nematoides, fixação biológica de nitrogênio e reforma de canaviais em áreas de Cerrado. Devido à sua importância estratégica, o conhecimento sobre a tecnologia de produção de sementes de crotalária é um fator relevante para garantir a disponibilidade de sementes com elevada qualidade fisiológica. Objetivou-se avaliar o efeito de dois espaçamentos de semeadura (20 e 40 cm) na germinação e vigor de sementes de *Crotalaria ochroleuca* e *Crotalaria spectabilis* produzidas em dois anos agrícolas (2018 e 2019). O experimento foi conduzido em blocos casualizados, com quatro repetições, em esquema fatorial 2 x 2 (espaçamento x safra), para cada espécie. As condições climáticas durante a produção de sementes influenciaram significativamente na qualidade fisiológica das sementes de crotalárias. A safra de 2018, com volume de chuvas mais abundante e temperaturas mais amenas durante o florescimento, proporcionou sementes mais vigorosas para ambas as espécies. O espaçamento de 20 cm foi mais eficiente para produzir sementes de *C. ochroleuca* com elevado desempenho fisiológico; porém, as sementes de *C. spectabilis* produzidas sob espaçamento de 40 cm apresentaram qualidade fisiológica mais elevada que as produzidas sob cultivo mais adensado.

PALAVRAS-CHAVE: *Crotalaria ochroleuca*, *Crotalaria spectabilis*, vigor de sementes.

INTRODUCTION

Researchers recommend using plants of the *Crotalaria* genus, especially *Crotalaria ochroleuca* and *Crotalaria spectabilis*, for green manuring, due to its high capacity of soil cover and biomass production (Berriel et al. 2020). Among other benefits of using plants of this genus is the phytonematode control (Cruz et al. 2020). These plants promote biological nitrogen fixation and adapt to the edaphoclimatic conditions of the Cerrado (Brazilian Savanna) (Reis et al. 2017).

The advantages of using *Crotalaria* species have increased the demand for seeds in recent years. However, due to the traditional interest in biomass production, its seed production technology is still incipient. In this context, researchers must develop efficient procedures for the production, commercialization and use of high-quality seed lots.

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The conditions of the cultivation environment and distribution of plants in the area may influence the production and seed quality. Therefore, the optimization of sowing spacing has become the focus of the main discussions in modern agriculture. This is because it enables a high yield from the use of increasingly smaller spaces. However, this technique requires increasing knowledge to obtain a final product with the highest possible quality (Raimondi et al. 2017). Smaller spacings in the same population enhance the spatial distribution of plants in the area, improving the solar radiation use efficiency. This is because it allows reducing row plant density. According to Ventimiglia et al. (1999), this conditioning improves the yield potential and actual grain yield, what justifies the increase in yield obtained by authors such as Bullock et al. (1998). Similarly, different spacings may influence seed quality.

Recommendations for sowing crotalaria include using spacings of 40 and 50 cm, which are traditional in the cultivation of other legumes such as soybean and bean, and would thus facilitate the use of the same machinery for sowing green manures (Amabile et al. 2000). However, the relationship between row spacing and seed production depends on the characteristics of each legume.

In pigeon pea, a 30-cm row spacing increased the seed production and a 50-cm row spacing increased the plant height and phytomass (Bertolin et al. 2008). Moreover, spacings of 50 and 100 cm provided the highest number and mass of velvet bean and lablab seeds (Monteiro et al. 2019a). Notwithstanding, Monteiro et al. (2019b) observed that spacings of 50, 75 and 100 cm between plants did not significantly influence the morphometry of velvet bean, jack bean and lablab seeds.

In a study with crotalaria, Garcia & Staut (2018) showed that the use of reduced spacing increased the phytomass production in crotalaria grown off-season, in addition to being an interesting strategy for weed control. The reduced spacing also improved plant arrangement, reducing intraspecific competition of plants for luminosity.

Regarding the effects of row spacing on the physiological quality of crotalaria seeds, studies are still incipient. Thus, this study aimed to assess the effect of row spacing on the physiological potential of *Crotalaria spectabilis* and *Crotalaria ochroleuca* seeds, in two crop seasons.

MATERIAL AND METHODS

Crotalaria ochroleuca and *Crotalaria spectabilis* seeds were produced under no-tillage, in succession to soybean, in the 2018 and 2019 crop seasons, in an experimental unit of the Embrapa Agropecuária Oeste (22º13’16’’S, 54º48’20’’W and 430 m of altitude), in Dourados, Mato Grosso do Sul state, Brazil. The soil of the experimental area, classified as Latossolo Vermelho Distroférrico (Santos et al. 2018) or clayey Rhodic Ferralsol (FAO 2006), had the following chemical characteristics: pH ($CaCl_2$): 5.1; P (Melich 1): 37.5 mg dm^{-3}; organic matter: 29.6 g kg^{-1}; H + Al: 5.8 cmol$_c$ dm^{-3}; K: 0.63 cmol$_c$ dm^{-3}; Ca: 4.0 cmol$_c$ dm^{-3}; Mg: 1.2 cmol$_c$ dm^{-3}; sum of bases: 5.83 cmol$_c$ dm^{-3}; cation exchange capacity: 11.7 cmol$_c$ dm^{-3}; base saturation: 50 %. The sowing of crotalaria plants did not include soil chemical correction or application of fertilizers.

The experimental design was randomized blocks, with four replications. The treatments consisted of two crop spacings (0.20 and 0.40 m) and the two crop seasons. Direct sowing was carried out mechanically, using a Semeato SHM 1517 seeder, on February 23, 2018 and June 3, 2019, with seed rates of 8 and 15 kg ha^{-1}, respectively, for both species. Each experimental plot was 4 m wide by 15 m long, totaling 60 m^2. After excluding the borders of each experimental unit, the sample was obtained in a useful area of 21 m^2 (1.5 m wide by 14 m long). Figure 1 shows the climatic data during the seed production seasons.

Irrigation was performed at the time of sowing, at a depth equivalent to 15 mm, with the aim of suppling water for seedling emergence, which occurred after four days. For the phytosanitary management of the area, an insecticide application was carried out in both seasons, with the active ingredient flubendiamide, at a concentration of 480 g L^{-1}, in the amount of 25 mL ha^{-1}, with the aim to control caterpillars in the reproductive phase. Weed control was carried out with manual weeding and disease control was not necessary. *C. ochroleuca* and *C. spectabilis* plants started to flower, respectively, at 61 and 51 days after emergence in 2018, and at 61 and 49 days after emergence in 2019.

The physiological maturity point of seeds was visually identified from the presence of more than 80 % of mature pods in the plants. At this stage, *C. spectabilis* pods are brown, while *C. ochroleuca*

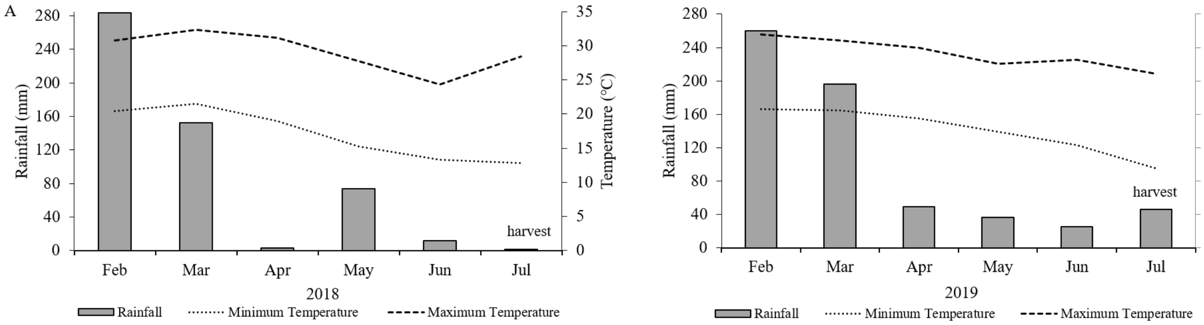

Figure 1. Temperature and monthly rainfall accumulated during the seed production of *Crotalaria ochroleuca* and *Crotalaria spectabilis* in 2018 (A) and 2019 (B) (Dourados, Mato Grosso do Sul state, Brazil). Source: Fietz et al. (2013).

pods are dark gray in color; and the seeds of both are loose inside the pods.

C. ochroleuca seeds were harvested mechanically at 141 and 130 days after emergence (DAE) and *C. spectabilis* seeds at 139 and 127 DAE, in 2018 and 2019, respectively. After harvesting, non-standard seeds (i.e., nonuniform and immature seeds) were discarded, in order to form homogeneous and uniform batches for each treatment.

The seed lots were sent to the laboratory and then subjected to the following tests and determinations:

Water content: determined by the gravimetric oven method (105 ºC; 24 h) (Brasil 2009). The *C. spectabilis* seeds had a water content of 9.7 % (wet basis) and the *C. ochroleuca* seeds a water content of 10.9 % (wet basis);

Germination: the test was conducted with four subsamples of 50 seeds, which were placed on Germitest® paper rolls previously moistened with water equivalent to 2.5 times the dry paper weight. The rolls remained in a B.O.D. chamber at a temperature of 20 ºC in the presence of white light for 16 hours, and at a temperature of 30 ºC in the absence of light for 8 hours (Brasil 2009). The counting was performed at 10 days after sowing (Brasil 2009) and the results expressed as percentage of normal seedlings;

Accelerated aging: the seeds were placed on a stainless steel screen inside plastic boxes with 40 mL of water at the bottom and taken to the B.O.D. at 41 ºC, for 24 h (Marcos Filho 1999). Then, four subsamples of 50 seeds were placed on paper rolls, according to the germination test methodology, and remained in the B.O.D. at a temperature of 20-30 ºC (Brasil 2009), for 4 days. The results were expressed as percentage of normal seedlings (Brasil 2009);

Cold test: four subsamples of 50 seeds were placed on paper rolls moistened with water equivalent to 2.5 times the dry paper weight. The rolls were kept in the B.O.D. at 10 ºC, for 5 days (Barros et al. 1999). After this period, the seeds underwent the germination test (Brasil 2009), with evaluations carried out at 4 days after sowing, computing the percentages of normal seedlings;

Seedling length: the test was conducted with four subsamples of 20 seeds each, positioned on the upper third of Germitest® paper moistened with water equivalent to 2.5 times the dry paper weight. The paper rolls remained in the B.O.D. at 20-30 ºC (Brasil 2009), for a period of 4 days. The shoot length considered the portion between the first protophyll and the root insertion area. The root length was determined by the measurement from the insertion to the tip of the primary root, with the aid of a millimeter ruler, and the results expressed in centimeters;

Seedling dry matter: normal seedlings from the length test were sectioned to separate shoots and roots, being then placed in paper bags and kept in an oven with forced air circulation at 41 ºC, until constant weight. Subsequently, the seedling dry matter was determined on a precision scale and the results expressed in grams per seedling;

Seedling emergence: the test was performed in cell trays filled with Dystroferric Red Latosol, in four subsamples of 50 seeds. Irrigation was performed whenever necessary. The evaluation was carried out at 15 days after sowing, computing the percentage of emerged seedlings (Nakagawa 1999);

Electrical conductivity: four subsamples of 50 seeds were previously weighed, placed in recipients with 75 mL of distilled water, and kept in the B.O.D. for 24 hours, at a temperature of 20-30 ºC (Vieira & Krzyzanowski 1999). After this period, reading was performed with a MS Tecnopon mCA 150 conductivity meter and the results expressed in µS^{-1} cm^{-1} g^{-1} (Vieira & Krzyzanowski 1999).

For each species, the results were subjected to analysis of variance, with the statistical software Sisvar (Ferreira 2019), and the means compared by the Tukey test at 5 % of probability, in a 2 x 2 factorial scheme (spacing x crop season).

RESULTS AND DISCUSSION

Regarding the significance of the interaction between treatments on seed germination, the row spacing did not influence the crops under study. However, when comparing the crop seasons, the conditions in 2018 were more favorable to the production of higher-quality seeds in both spacings (Table 1).

It is worth noting that the climatic conditions of the crops were different, especially in the critical stages of seed production. During flowering, the average maximum temperature increased by 3 degrees Celsius in 2019, in relation to 2018 (Figure 1). According to Kappes et al. (2012), the quality of crotalaria seeds may be negatively affected under certain climatic conditions, especially high temperatures during the reproductive stage of the crop.

In addition, the average rainfall in the seed harvest period of 2019 was approximately 40 mm higher than that of 2018, for the same period (Figure 1). Water excess during the final maturation stage, when the seeds are still arranged on the mother plant but not physiologically dependent on it, negatively affects physiological quality by accelerating seed deterioration (Ellis & Yadav 2016, Ellis 2019).

Similar to germination, in the absence of interaction with crop spacing, the seed production time was significant for accelerated aging, cold test, shoot length, root length, shoot dry matter, root dry matter, field emergence and electrical conductivity. The seed vigor attributes produced in 2018 were higher than those of seeds from the 2019 harvest (Table 2).

Crotalaria plants show rusticity, fast growth and good adaptation to high temperatures and drought (Leal et al. 2012). However, studies that mention these advantages have as a main objective growing phytomass as green manure. For seed production, the results indicate that the climatic conditions determined by increased rainfall at the final phase of the cycle significantly influence the physiological quality of *C. ochroleuca* seeds. For this reason, when the crop is intended for seed production, sowing should be carried out between March and April. In addition to providing better climatic conditions for harvest, this would allow obtaining lower plants and easier harvesting (Dourado et al. 2001).

The isolated effect of crop spacing was significant for seedling emergence, root length, shoot dry matter and cold test (Table 3). The use of the 20 cm spacing enabled the production of more

Table 1. Germination (%) of *Crotalaria ochroleuca* seeds produced under two sowing spacings in two crop seasons.

Crop season	Spacing (cm)	
	20	40
2018	76 Aa[1]	79 Aa
2019	67 Ba	69 Ba

[1] Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other by the Tukey test at 5 % of probability.

Table 3. Results of seedling emergence (SE; %), root length (RL; cm), shoot dry matter (SDM; g) and cold test (CT; %) in *Crotalaria ochroleuca* seeds produced under two sowing spacings.

Spacing (cm)	SE	RL	SDM	CT
20	89 a[1]	4.75 a	0.05 a	71 b
40	82 b	4.24 b	0.04 b	75 a

[1] Means followed by different letters in the column differ from each other by the Tukey test at 5 % of probability.

Table 2. Accelerated aging (AA; %), cold test (CT; %), shoot length (SL; cm), root length (RL; cm), shoot dry matter (SDM; g), root dry matter mass (RDM; g), seedling emergence (SE; %) and electrical conductivity (EC; µS cm^{-1} g^{-1}) of *Crotalaria ochroleuca* seeds produced in the 2018 and 2019 crop seasons.

Crop season	AA	CT	SL	RL	SDM	RDM	SE	EC
2018	77 a[1]	79 a	6.52 a	5.86 a	0.07 a	0.03 a	95 a	30.60 b
2019	52 b	67 b	2.82 b	3.13 b	0.03 b	0.01 b	76 b	35.35 a

[1] Means followed by different letters in the column differ from each other by the Tukey test at 5 % of probability.

vigorous seeds, in relation to the 40 cm spacing, except for the cold test (Table 3). In a study on the production of *C. juncea* seeds, Eiras & Coelho (2012) did not observe a significant effect of crop density on seed number and 1,000-seed weight. On the other hand, when using row spacings of 20 and 40 cm in a study with *C. juncea*, *C. ochroleuca* and *C. spectabilis*, Garcia & Staut (2018) observed a higher phytomass production with the smallest spacing. The authors mentioned it as an interesting strategy in weed control, which also reduces intraspecific competition of plants for luminosity due to the better arrangement of plants in smaller spaces.

Reducing row spacing favors nutrient absorption and use by plants, with positive effects on corn (Fumagalli et al. 2017) and soybean (Rocha et al. 2018) yield. In sowings with larger spacings, the absence of soil cover at the initial stages of the crop may lead to its exposure, and, together with light incidence, favor the establishment of weeds that can compete with the crop of interest (Lima et al. 2014).

The denser sowing of *C. ochroleuca* accelerated the row closure, suppressing weeds and thus ensuring a greater availability of nutrients to the main crop, what may have favored the production of high quality seeds (Table 3). The self-shading reported by Amabile et al. (2000) in dense crops was favorable for *C. ochroleuca*, which is a photoperiod-sensitive species characterized as a short-day plant. As seed production begins at flowering, the duration and conditions relevant to this stage are decisive in seed production quality.

According to the conditions of the present study, *C. ochroleuca* seeds produced in 2018 had greater germination and vigor than those from 2019 (Tables 1 and 2). Considering that water availability was adequate for the establishment of the population in the two seasons, the differences in the physiological quality of seeds may be due to the climatic conditions during seed harvest, especially the occurrence of high rainfall in the 2019 harvest, in relation to the first harvest (Figure 1).

Seed vigor progressively increases during seed development until the point of maximum quality, which coincides with the end of dry matter accumulation and, therefore, of the seed filling period (Finch-Savage & Bassel 2016). From then on, seed vigor begins to decline if the environmental conditions to which the seeds are subjected are unfavorable until harvest. High water contents can intensify seed respiration, accelerating deterioration and decreasing the physiological quality of seeds (Marcos Filho 2015).

Although recommendations for sowing crotalaria include using spacings of 40 and 50 cm for obtaining green phytomass, the results of the present study indicate that the denser spacing of 20 cm favored obtaining seeds with higher vigor (Table 3). Also noteworthy was the influence of meteorological conditions, especially rainfall at the time of harvest (Tables 1 and 2).

Seed germination and vigor of *Crotalaria spectabilis*, evaluated by means of cold tests, seedling shoot length and electrical conductivity, were higher in 2018 than in 2019. The isolated effect of spacing was significant only for root dry matter, indicating the positive effect of the 40 cm spacing, in relation to the 20 cm spacing (Table 4).

When sowing was carried out at 20 cm spacing, the production in 2018 provided seeds with greater vigor than in 2019. Similar results occurred when using the largest spacing, without, however, an effect between the years for the evaluations of seedling growth and dry matter (Table 5). The accelerated aging and seedling performance tests showed that, under the climatic conditions of the study, using the 40 cm spacing provided *C. spectabilis* seeds with a higher physiological performance (Tables 4 and 5).

The results show that the seed performance may also be determined by the genotype. For *C. ochroleuca* seeds, the spacing of 20 cm between plants favored the production of high-quality seeds. However, the 40 cm spacing was more favorable for the production of high-quality seeds of *C. spectabilis*.

Table 4. Results of germination (G; %), cold test (CT; %), shoot length (SL; cm), electrical conductivity (EC; µS cm^{-1} g^{-1}) and root dry matter (RDM; g) of *Crotalaria spectabilis* seeds produced under two sowing spacings in two crop seasons.

Crop season	G	CT	SL	EC
2018	74 a[1]	78 a	6.68 a	19.71 b
2019	71 b	50 b	3.71 b	20.26 a

Spacing (cm)	RDM
20	0.02 b
40	0.04 a

[1] Means followed by different letters in the column differ from each other by the Tukey test at 5 % of probability.

Table 5. Accelerated aging (AA; %), root length (RL; cm), shoot dry matter (SDM; g) and seedling emergence (SE; %) of *Crotalaria spectabilis* seeds produced under two sowing spacings (cm) and in two crop seasons.

Crop season	AA		RL		SDM		SE	
	20	40	20	40	20	40	20	40
2018	64 Ab[1]	73 Aa	2.10 Aa	2.35 Aa	0.06 Aa	0.07 Aa	94 Aa	82 Ab
2019	40 Ba	40 Ba	0.55 Bb	2.57 Aa	0.02 Bb	0.06 Aa	57 Ba	54 Ba

[1] Means followed by different uppercase letters in the column and lowercase letters in the row do not differ from each other by the Tukey test at 5 % of probability.

Management practices and the cultivation environment to which the plants are subjected influence physiological adaptations that reflect seed quality. Thus, for each genotype, it is important to check the climatic variations during production and the adequate adjustment of crop spacing, in order to obtain high-quality seeds.

The results are useful for the technology production of crotalaria seeds and indicate that spacings of 20 and 40 cm may be adopted to obtain high-quality seeds of *C. ochroleuca* and *C. spectabilis*, respectively, when climatic conditions do not pose challenges in establishing the seed production field.

CONCLUSIONS

1. The 20 cm spacing between plants favors the production of *Crotalaria ochroleuca* seeds with a high physiological performance through accelerated aging, seedling emergence and seedling performance tests. In turn, *C. spectabilis* seeds have a higher physiological quality through accelerated aging and seedling performance tests when produced under 40 cm spacing than when produced under denser cultivation;
2. Climatic conditions during seed production influence the physiological performance of crotalaria seeds. The reduction of rainfall during harvest is a determining factor in obtaining high quality seeds of *C. ochroleuca* and *C. spectabilis*.

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