Time of harvest and storability of Crotalaria juncea L. seeds¹

Época de colheita e armazenabilidade de sementes de Crotalaria juncea L.

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ABSTRACT - Although used as green manure, one of the challenges the cultivation of *Crotalaria juncea* is the low availability of quality seeds in the market. The harvest at the right time contributes to the achievement of better quality seeds. The objectives of this study were to evaluate the storability and characterize the physical and physiological changes of the seeds harvested at different times. The statistical design was completely randomized with three replications. Treatments consisted of four harvest seasons and two storage periods (0 to 8 months). The samples were collected when the plants reached 40, 60, 80 and 100% of dried pods. The following characteristics were evaluated: time for drying, moisture content, dry matter accumulation, physical purity, yield, uniformity testing, seed coat coloring, germination, germination first count test, percentage of hard seeds, speed index and percentage of seedling emergence. Data from ripening stages were submitted to regression analysis. The effect of storage within each time it was analyzed by the joint analysis of variance of the results and the Tukey test (p≤0.05). The determination of the sampling point based on the percentage of dried beans is efficiently. The physiological maturity of the seeds is achieved when the plants have about 86.1% of dry beans, with the Crotalaria juncea seeds harvesting is performed at the point where the plants have between 80 and 100% of dried pods. The storage for eight months reduces dormancy up to 75.4%.

Key words: Green manure. Storage. Dormancy. Physiological maturity.

RESUMO - Apesar de utilizada como adubo verde, uma das dificuldades do cultivo da *Crotalaria juncea* é a baixa disponibilidade de sementes de qualidade no mercado. A colheita no momento certo contribui para a obtenção de sementes de melhor qualidade. Os objetivos deste trabalho foram avaliar a armazenabilidade e caracterizar as alterações físicas e fisiológicas das sementes colhidas em diferentes épocas. O delineamento estatístico utilizado foi inteiramente casualizado, com três repetições. Os tratamentos consistiram em quatro épocas de colheita e dois tempos de armazenamento (0 e 8 meses). As colheitas foram realizadas quando as plantas atingiram 40, 60, 80 e 100% de vagens secas. As seguintes características foram avaliadas: tempo para secagem, grau de umidade, acúmulo de matéria seca, pureza física, rendimento, teste de uniformidade, coloração do tegumento, germinação, primeira contagem do teste de germinação, porcentagem de sementes duras (impermeáveis à água), índice de velocidade e porcentagem de emergência de plântulas. Os dados referentes aos estádios de maturação foram submetidos à análise de regressão. O efeito do armazenamento, dentro de cada época, foi estudado pela análise de variância conjunta dos resultados e pelo teste de Tukey (p≤0,05). A determinação do ponto de colheita baseada na porcentagem de vagens secas é eficiente. A maturidade fisiológica das sementes é atingida quando as plantas apresentam aproximadamente 86,1% de vagens secas, devendo a colheita de sementes de *Crotalaria juncea* ser realizada no ponto em que as plantas apresentarem entre 80 e 100% de vagens secas. O armazenamento, por oito meses, reduz a dormência em até 75,4%.

Palavras-chave: Adubo verde. Armazenamento. Dormência. Maturidade fisiológica.

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INTRODUCTION

A reduction in the productive capacity of the soils due to fertility loss has been reported in agriculture. Green manuring is among the technologies used to reverse this scenario. In this technique, plants are grown and incorporated or left on the soil. *Crotalaria juncea* L. (Fabaceae) is one of the most widespread species in the tropics for this purpose (PACHECO; SILVA-LÓPEZ, 2010).

Crotalaria stands out because it has a fast growth, dry matter production potential, and is efficient in the biological fixation of nitrogen and the cycling of nutrients in the soil (CRUSCIOL; SORATTO, 2009). It is used for the control of nematodes (ROSA; WESTERICH; WILCKEN, 2015) and in weed suppression by the allelopathic effect (MIYAZAWA *et al.*, 2010).

One of the obstacles to the cultivation of *C. juncea* is the low availability of quality seeds in the market. As it is used for green manure, farmers do not have the habit of cultivating it to obtain direct profitability by the commercialization of seeds (KAPPES *et al.*, 2012). However, this situation tends to be reversed, mainly due to the continuous increase of the use of this species in Brazilian agriculture and, consequently, the increasing demand for seeds. For the economic viability of green manuring, it is essential to use quality seeds, as this is reflected in the uniform and satisfactory establishment of the plants. This characteristic is relevant in rotation systems or consortium.

Harvesting the seeds at the ideal time minimizes the effects of deterioration that are caused by their longer permanence in the field. It also prevents early harvesting, reducing quantity of immature seeds in the lot and losses due to mechanical injuries. The ideal harvest point should be based on the simple, fast, and consistent identification of plant and/or fruit morphological characteristics.

Thus, this article was developed with the objective of determining the ideal harvest time of *Crotalaria juncea* L., based on the percentage of dry pods, and of evaluating the storability of the seeds harvested at separate times.

MATERIALS AND METHODS

The research was carried out in Viçosa-MG (20°45'14" south and 42°52'53" west, at 650 m altitude). According to the Köppen climatic classification, the regional climate is of Cwb type, which is characterized as tropical at altitude, with dry winter and mild summer, with an average temperature of the warmer month below 22 °C.

Manual sowing was performed in furrows 5 cm deep, spaced 0.50 m apart. Forty seeds per linear meter were distributed in an area of 100 m². The water requirement was supplied through sprinkler irrigation.

After the seeds were harvested, the other procedures were conducted in the laboratory or in a greenhouse. The statistical design was completely randomized, with three replications. The treatments consisted of four harvesting times (40, 60, 80 and 100% dry pods) and two storage times (0 and 8 months), totaling 24 plots.

Harvests were performed when the plants reached 40, 60, 80, and 100% dry pods (DP). We considered as dry pods the ones that were yellow inside with free seed in the interior, producing a characteristic sound of rattle when shaken (PACHECO; SILVA-LÓPEZ, 2010). Flowering, characterized when 50% of the plants had at least one flower in anthesis, occurred at 71 days after sowing. The harvests corresponded, respectively, to 133, 140, 148, and 155 days after anthesis.

Three samples of 100 seeds were used to determine the degree of humidity at harvest, using the oven method at 105 ± 3 °C for 24 hours (BRASIL, 2009). Concurrently with the determination of the water content of the seeds, after drying at 105 ± 3 °C, for 24 h, the accumulation of dry matter in the seeds was determined, and the results were expressed in mg seed-1 (LOPES; QUEIROZ; MOREIRA, 2005).

The seeds were submitted to shaded drying in an environment with natural air circulation. While drying, the air temperature was 22.3 ± 4.3 °C and the relative humidity of the air was $65.4 \pm 11.7\%$. Both climatic elements were monitored by means of a digital thermohygrometer. The drying process was maintained until the seeds reached 10% moisture. The time elapsed between the beginning and the end of drying was computed.

Impurities lighter than the seeds were discarded in a South Dakota model blower with 6 mm aperture for 30 seconds. The other impurities were removed by hand. At the end of the uniformity test, as described below, the seeds that passed through the 3.0 mm sieve were also considered as impurities. The portion of pure seeds was weighed, and the result was expressed as a percentage. The yield (kg ha⁻¹) was determined, and the averages were corrected to 13% moisture.

In each experimental unit, a sample was taken to verify the immediate effects of the treatments. The remaining portion was kept stored for another eight months in a controlled environment (temperature of 20 °C and relative air humidity around 50%). Before storage, the evaluated characteristics were:

- a) Uniformity test (sieve retention): conducted in duplicates of 100 g of seeds and by manual sieve classification, with circular sieves in the dimensions of 5.0, 4.5, 4.0, 3.5, and 3.0 mm in diameter. The portion of pure seeds was weighed, and the result was expressed as a percentage.
- b) Staining and texture of the seeds: duplicates of 100 g of each experimental unit were used. The seeds were separated into three groups, according to the physical appearance of the integument, namely: i) Stage I: greenish color, with a wrinkled appearance; (ii) Stage II: greenish coloration, but well formed, with smooth integument and; (iii) Stage III: dark brown, with smooth integument. In each group, the number of seeds was counted, expressing the results in percentage.

The following characteristics were evaluated before and after storage:

- c) Germination: quadruplicates of 50 seeds, treated with fungicide (1.5 g of the active ingredient N-trichloromethylthio-4-cyclohexene-1.2-dicarboximide per kilo of seeds), were placed to germinate on the substrate between germitest paper, roll, moistened to 2.5 times the mass of the dry paper. The test was conducted in a BOD type chamber, with an alternating temperature of 20-30 °C and photoperiod of 8 hours of daily light. The evaluations were carried out at 4th, 10th and, due to the presence of hard seeds, the seeds remained for another seven days after the test installation, following recommendations by Brasil (2009). The results were obtained by the sum of the three evaluations and expressed as a percentage of normal seedlings (BRASIL, 2009).
- d) First germination count: performed together with the germination test. The percentage of normal seedlings was recorded on the fourth day after sowing.
- e) Percentage of hard seeds: the seeds that were hard on the 17^{th} day of the germination test were computed.
- f) Germination with seeds after scarification: the seeds that remained without absorbing water (hard) at the end of the germination test had gone through scarification in the region opposite the hypocotyl-radicle axis, with the aid of an 80-grade sandpaper. The seeds were then submitted to the germination test again for another 10 days (BRASIL, 2009). The results were added to those obtained by the germination test (conducted before scarification) (NAKAGAWA *et al.*, 2007).
- g) Emergency velocity index (EVI): the samples collected for the study before storage were evaluated under field conditions, aiming to enhance the expression of seed vigor. The soil was prepared with two gradations. After being treated with fungicide as described in the

germination test, quadruplicates of 50 seeds, from each experimental unit, were seeded, equidistantly, in grooves approximately 5 cm deep. Daily readings were taken, and the emerged seedlings were counted, considering those with an angle greater than 90° between the stem and the cotyledonary axis. The evaluations were carried out until the thirteenth day after sowing, where stabilization was observed in the number of emergent seedlings. The EVI was determined through the equation proposed by Maguire (1962).

The seeds used for the study after storage were seeded in trays containing sand and the test was conducted in a greenhouse. This was necessary due to the low temperatures observed in the municipality of Viçosa-MG at the time of implantation of this test, which would impair the emergence in the field since *C. juncea* is sensitive to cold (BURLE *et al.*, 2006). Except for the substrate and the site of execution, conduction and evaluations were performed in the same manner as in the assay with the non-stored seeds.

h) Seedling emergence: in both trials, before and after storage, at the end of the EVI test, i.e., at the 13 th day after sowing, the normal seedlings were counted, expressing the result as a percentage of the emergence of plants.

The data referring to harvest times were submitted to regression analysis. The results concerning the physiological quality of the seeds were submitted to joint variance analysis, except for those referring to EVI and emergence, due to differences in methodologies. To compare the means, the Tukey test (p ≤ 0.05) was used.

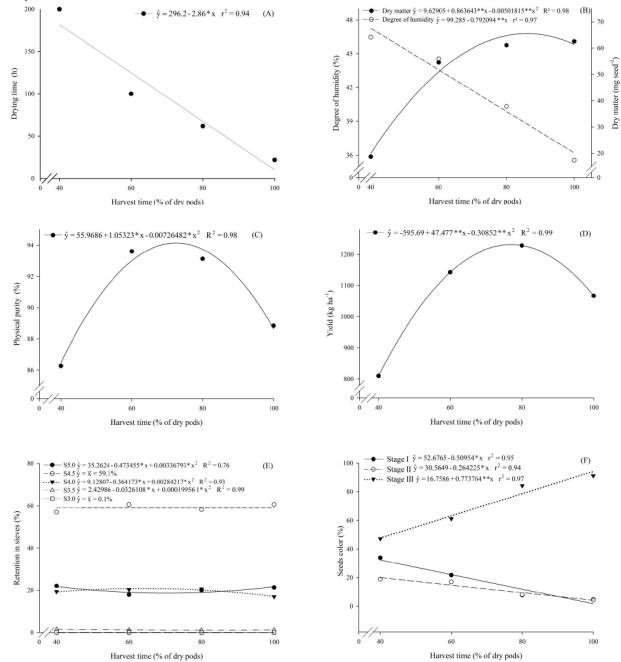
RESULTS AND DISCUSSION

The results concerning the time required to dry the seeds showed a linear behavior (Figure 1A). The time required to dry the seeds of the first harvest was almost 10 times higher than that required for drying the seeds of the last harvest. This difference is due to the different degrees of humidity observed in each harvest season (Figure 1B).

Seed development was characterized by the accumulation of dry matter, and their results were adjusted to a quadratic model (Figure 1B). The deposition of dry matter increased rapidly from the first to the second harvest. This means that, at this point, histodifferentiation and morphogenesis were still occurring, concomitantly with the accumulation of reserves (BEWLEY *et al.*, 2013).

After harvesting, the shortest drying time is important to maintain seed quality. The respiratory intensity in this phase remains high, which leads to high

Figure 1 - Drying time (A), degree of humidity and accumulation of dry matter (B), physical purity (C), yield (D), retention in sieves (E), and color (F) of *Crotalaria juncea* seeds due to harvest season. E: In the equations, S means sieve, and the subsequent number indicates the diameter of the sieve



degradation rates of reserves substances and the release of large amounts of calories, which will no longer be used to nourish the embryonic axis, besides serving as an energy source for microorganisms or insects (FRANKE; TORRES; LOPES, 2008).

**, * significant, respectively, at the 1 and 5% level by the "t" test

The maximum deposition of dry matter, estimated based on regression, occurred when the plants reached 86.1%

DP, at which time 46.8 mg seed⁻¹ was observed. This point is set in the physiological maturity of the seeds, and may or may not coincide with the maximum physiological potential.

In *C. juncea*, Mozambani, Sader and Pinto (1993) verified the maximum deposition of 49.3 mg seed⁻¹ of dry matter. Therefore, it was very close to the one found in this work.

Data on the moisture content of the seeds showed linear behavior (Figure 1B). In the first harvest, a high degree of humidity (64.2%) was observed. The high water content in the initial stages of maturation is necessary for cell expansion and to allow the transfer of metabolites from the plant to the seeds (ANGELOVICI et al., 2010). When the maximum accumulation of dry matter occurred, the moisture content of the seeds was 31.1% (Figure 1B). Mozambani, Sader and Pinto (1993) found different moisture levels at this point (44%). On the other hand, Lopes, Queiroz and Moreira (2005) noticed that the seed moisture at physiological maturity is between 28 and 39%, therefore, close to the one found in this article. Seeds of Mucuna aterrima Holland have also been reported different values, due to the non-uniformity of flowering and maturation of the pods (NAKAGAWA et al., 2007), which is also observed in C. juncea.

Data on physical purity revealed quadratic behavior (Figure 1C). At the 40% DP harvest, the lowest physical purity was observed, estimated at 86.5%. The impurities were composed of badly shaped seeds and those that passed through the sieving screen with a diameter of 3.0 mm. The maximum physical purity of the seeds, 94.1%, was reached when the harvest was estimated at 72.5% DP.

The percentage of pure seeds decreases in the last harvest, mainly due to the higher number of seeds severely attacked by fungi. Days before the last harvest there was an increase in temperature and rainfall, which may have favored the proliferation of these pathogens. This has also been reported in soybean (ALBRECHT *et al.*, 2009).

Early harvesting results in a decrease in production due to incomplete seed development (SMIDERLE; PEREIRA, 2008). The greater permanence of the seeds of the last harvest in the field may also have favored the loss of seed mass. The relative humidity of the air, the temperature and the action of insects and microorganisms contribute to the acceleration of the respiratory process and the consequent oxidation of the reserve compounds, with a reduction in seed mass. (BEWLEY *et al.*, 2013).

The data for sieve screens with a diameter of 3.0 and 4.5 mm did not fit the pre-defined mathematical models. However, the data referring to 3.5, 4.0, and 5.0 mm sieves showed quadratic behavior (Figure 1E). Although the data from these sieves conform to a mathematical model, the difference between the means is minimal. Thus, in general, seed uniformity was not significantly influenced by the harvest season. Generally, the seeds of Fabaceae grow rapidly, reaching maximum size in a brief period, in relation to the total duration of the maturation period (GURJÃO *et al.*, 2006). Therefore, the seeds harvested with 40% DP already had the maximum size.

The yield of the seeds throughout the harvesting times revealed a quadratic behavior (Figure 1D). Based on the regression curve, the maximum yield (1230.8 kg ha⁻¹) was reached when the plants reached about 76.9% DP. The lower yields of the first and last crops are related to the greater elimination of unsuitable seeds for commercialization by the physical purity analysis (Figure 1C).

Data on the percentage of seeds according to the color and integument texture revealed linear behavior (Figure 1F). The relative number of mature seeds (stage III) increased from 47% in the first crop to 91% in the last crop. On the other hand, the percentages of seeds in stages I and II were reduced throughout the harvesting times. Pascualides and Ateca (2013) similarly reported mixtures of colors in the seed lots of *C. juncea*. In the *Crotalaria* genus, polymorphism in the color of the integument is related to the physical dormancy, the soaking patterns during germination (PASCUALIDES; PLANCHUELO, 2007), and vigor (PASCUALIDES; ATECA, 2013). In *Mucuna aterrima* (NAKAGAWA *et al.*, 2007) and in soybean (MERTZ *et al.*, 2009), the coloration is also directly related to the physical dormancy of the seed.

As for the percentage of hard seeds, there is a significant interaction between harvesting and storage (Table 1), indicating that the percentage of hard seeds harvested at separate times is affected differently by storage.

Throughout the harvest, hard seed data, before storage, presented quadratic behavior (Figure 2A). The maximum percentage of hard seeds (16%) was estimated at 70% DP. At that time, the maximum accumulation of dry matter in the seeds had not yet occurred. This was also observed in *Mucuna aterrima* (NAKAGAWA *et al.*, 2007).

The development intensity of integument impermeability depends on the stage of seed maturation when drying is started (BASKIN; BASKIN, 2014). In *Mucuna aterrima*, the harvest of more immature seeds, submitted to drying, become dormant (NAKAGAWA *et al.*, 2007), as observed in this article.

Seeds that remain in the field after physiological maturity, as occurred in those from the 100% DP crop, are subject to the alternation of temperature and relative humidity. This predisposes the occurrence of injuries to the integument, due to the expansions and contractions after a series of events of water absorption and loss by the seeds. As the integument is not perfectly elastic, some ruptures can occur, which favors the breakage of seed dormancy (SMÝKAL *et al.*, 2014).

From the regression study, there was a decreasing linear behavior of the hard seed data after storage (Figure

Table 1 - Summary of the analysis of hard seed (HS) variance, germination (G), germination after scarification (GAS), and first count (FC) of the germination test for *Crotalaria juncea* seeds, harvested at separate times, before and after storage

Sources of variation	Degrees of freedom -	Middle square				
		HS	G	GAS	FC	
Harvest time (HT)	3	53.23***	1278.33***	1224.07***	774.89***	
Storage (S)	1	119.60***	284.40**	$3.12^{\rm ns}$	3599.49***	
HT x S	3	34.17***	17.82 ^{ns}	7.89^{ns}	90.15**	
Residue	16	4.60	19.40	18.41	12.96	
CV (%)		22.50	6.42	5.54	6.69	

ns, not significant; ***, **, significant, respectively, at the 0.1 and 1% level by the F test

2A). After storage, the dormancy intensity of Croletaria seeds remains higher in the seeds harvested earlier than in those harvested later. After drying, the physical dormancy occurs due to the contraction of the cell walls of the palisade layer (BASKIN; BASKIN, 2014), present in the crotalaria integument (PASCUALIDES; PLANCHUELO, 2007). This contraction must have been higher in the seeds harvested at 40% DP, due to the higher moisture content observed at harvest. At harvesting with 100% DP, the seeds were already in the process of natural drying, in a slower way.

Moreover, the level of abscisic acid (ABA), the hormone responsible for the promotion of dormancy during seed maturation, is higher in the first half of seed development, declining only at the later stages of maturation (BLÖCHL *et al.*, 2005). The higher persistence of dormancy in the seeds of the first harvest after storage may have been caused by ABA since the seeds were still in the process of maturation at harvest time.

There was no effect of storage on the percentage of hard seeds in the harvests with 40 and 60% DP (Table 2). However, for the 80 and 100% DP harvests, storage reduced dormancy by 75.4 and 51.1%, respectively. The integument of the crotalaria seeds has exotesta formed by macroesclereids (PASCUALIDES; PLANCHUELO, 2007), whose cells have thick walls strongly joined together. This favors the conservation of seed viability for a longer period. However, during storage, it is common to observe the reduction of dormancy, due to the occurrence of ruptures in the integument. The reduction of moisture during storage favors this phenomenon (SOUZA *et al.*, 2012).

Regarding germination, irrespective of scarification, there was no significant interaction between harvesting and storage (Table 1). This means that the percentage of germination of the seeds harvested at separate times is not affected differently by storage.

Regression analysis showed linear and increasing behavior in the germination data, before and after storage (Figure 2B). The same behavior was observed with the seeds after scarification (Figure 2C) and with the results of the vigor tests (Figure 2D-F).

In the literature, there is controversy regarding the moment when the maximum physiological quality occurs in relation to the maximum accumulation of dry matter in *C. juncea*. According to Mozambani, Sader and Pinto (1993), the maximum accumulation of dry matter occurs after the point where the maximum germination percentage and the maximum vigor are observed. According to Lopes, Queiroz and Moreira (2005), the maximum accumulation of dry matter occurs slightly before the highest physiological quality. In this article, the maximum accumulation of dry matter (Figure 1B) occurred before the maximum germination percentage and maximum vigor, confirming the information of Lopes, Queiroz and Moreira (2005).

Seed production is associated with dry matter accumulation and yield, with attention focused on physiological maturity. At this point, it was postulated to occur also the maximum germination and vigor and that, from this point forward, the seeds begin to lose quality. However, it is now known that seed vigor and longevity may continue to increase even after physiological maturity (BELEY *et al.*, 2013).

In soybean, Zanakis, Ellis and Summerfield (1994) found that several components of seed quality, including desiccation tolerance, vigor, and longevity, were developed after physiological maturity and before harvest; a period in which seed moisture is still declining. The final adjustments of the organization of the membrane system, the synthesis of certain enzymes, and adjustments in the structure and metabolism of orthodox seed development occurs in Phase IV of the maturation process (ANGELOVICI *et al.*, 2010). This may also explain the higher germination values of the

seeds harvested in the 100% DP season, since they remained in the field after physiological maturity (Figure 1B), not being subjected to water loss as drastically as with seeds from the harvest with 40% DP.

Premature desiccation affects the synthesis of proteins as well as of enzymes essential for germination. The seed cell membranes system remains disorganized during most of the plant matter transfer period (BEWLEY

Figure 2 - Hard seed (A), germination (B), germination after scarification (C), first germination test count (D), emergency velocity index (E), and emergency of *Crotalaria juncea* seeds due to the harvest time and before and after storage. BS: Before storage. AS: After storage

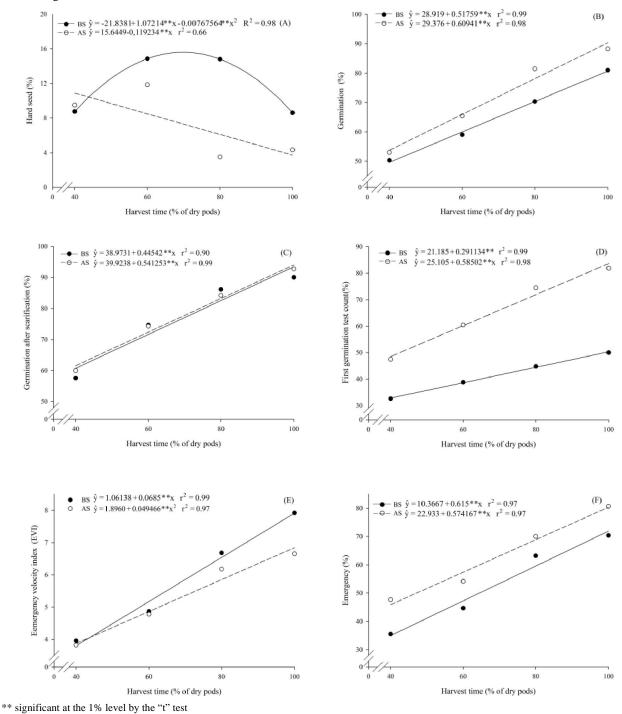


Table 2 - Hard seeds (HS), first counting of the germination test (FC), germination (G), and germination after scarification (GAS) of *Crotalaria junce* a seeds before and after storage, at different harvesting times

Variables	Storage	40% DP	60% DP	80% DP	100% DP
SD (%)	Before	9.5 a	15.5 a	14.2 a	8.8 a
	After	8.6 a	11.9 a	3.5 b	4.3 b
FC (%)	Before	32.6 b	38.8 b	44.8 b	50.0 b
	After	47.5 a	60.5 a	74.5 a	81.8 a
G (%)	Before	50.3 a	59.0 a	70.3 b	81.0 a
	After	53.0 a	65.4 a	81.5 a	88.3 a
GAS (%)	Before	57.5 a	74.3 a	84.2 a	90.0 a
	After	60.0 a	74.7 a	86.2 a	92.8 a

Within each variable, averages followed by the same letter in the column do not differ statistically from each other according to the Tukey test (p>0.05)

et al., 2013). Thus, in an early harvest, as is the case of the harvest with 40% VS, a higher rate of dead seeds may occur, resulting in a decrease in the germination percentage. When there is a marked reduction of the water content, the structures of nucleic acids, proteins, and lipids can be altered due to the weakening of hydrophilic and hydrophobic interactions (JUSTO et al., 2007). Legumes only tolerate rapid desiccation in the final phase of the process of matter accumulation (CORBINEAU et al., 2000), which in the case of crotalaria occurs between 80 and 100% DP harvests.

For commercialization, the *C. juncea* seeds must present at least 60% of germination (BRASIL, 2008). In this research, the seeds from the first harvest, after scarification, presented average germination of 58.8%, which would make commercialization unfeasible. From the second harvest season, the seeds could already be commercialized, since they presented 74.5% of germination. Thus, it is possible to raise the minimum percentage of germination for commercialization according to the Ministry of Livestock and Food Supply, making available in the market seeds endowed with better physiological quality.

The maintenance of seed viability after storage may be due to the anatomical composition of the integument. As already discussed, the macrosclerid layer of the integument favors the conservation of seed viability. This is because metabolic processes such as respiration and deterioration are mainly contained by the water content remaining low and constant (BRANCALION *et al.*, 2010). This phenomenon has been reported in certain unimproved soybean strains (MERTZ *et al.*, 2009).

Regarding vigor, after analyzing the results of the first germination test count, all harvests had higher averages after storage (Table 2). The greater dormancy of the seeds before storage, as well as its reduction during the germination test, due to the action of the substrate water and to the temperature alternation (20 and 30 °C), influence these results, and not the vigor itself. Thus, the first-count test should be used with caution to evaluate the vigor of *Crotalaria juncea* seeds, as was also demonstrated by Nakagawa *et al.* (2007), in seeds of *Mucuna aterrima*.

CONCLUSIONS

- 1. Determination of the seed harvesting point of *Crotalaria juncea* based on the percentage of dry pods is efficient;
- 2. Harvesting of *Crotalaria juncea* seeds should be carried out at the point where the plants have between 80 and 100% dry pods;
- 3. Storage for eight months reduces physical dormancy and, consequently, increases germination in *Crotalaria juncea* seeds.

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