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Late harvest and chemical treatment of cowpea seeds

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Abstract
Reduced quality of cowpea due to late harvest and therefore pathogen incidence may influence the results of the germination test, whenever losses are associated with the agents adhered to seed coat; in that context, chemical treatment of seeds during the evaluations may reflect more accurately physiological quality of the samples, which in turn may assure the emergence results of healthy seedlings and establishment of appropriate plant populations in the field. This study had the objective of evaluating the effects of fungicide treatment on minimizing physiological quality losses of cowpea seeds due to late harvest in germination and seedling development tests. The experimental design was the completely randomized with four replications. Treatments consisted of cowpea, cultivar BRS Guariba, harvested at different times (0, 3, 6, 9, 12 and 15 days after physiological maturity) and treated or not with fungicide carboxim+thiram for seed quality tests. Seeds were initially characterized by seed moisture and mass. Afterwards, samples were evaluated by tests of germination percentage, germination first count, seedling length and dry matter. Data was submitted to variance analysis and means were analyzed as a 2 x 6 factorial. Means from chemical treatment and harvest times were compared by the Tukey test and regression analysis ($p \leq 0.05$), respectively, with subsequent unfolding of any significant interactions. Delayed harvest of cowpea seeds sharply reduces germination and vigor; fungicide treatment before performing physiological tests enables to establish that seed quality losses can be in part caused by pathogen vehiculation on the seed coat.
Keywords: *Vigna unguiculata*; fungicide; physiological maturity.

Atraso na colheita e tratamento químico de sementes de feijão-caupi

Resumo
A diminuição da qualidade de sementes de feijão-caupi decorrente do atraso da colheita e, consequentemente, incidência de patógenos pode influenciar nos resultados do teste de germinação, quando as referidas reduções estão associadas aos patógenos aderidos ao tegumento; nesse sentido, o tratamento químico das sementes para as avaliações pode refletir, de modo mais preciso, a real qualidade fisiológica das amostras, que, por sua vez, assegura os resultados de emergência de plântulas sadias e o estabelecimento de estande adequado de plantas no campo. Este trabalho teve como objetivo avaliar os efeitos do tratamento fungicida para minimizar as reduções na qualidade fisiológica das sementes do feijão-caupi em decorrência do atraso da colheita nos testes de germinação e desenvolvimento de plântulas. O delineamento experimental foi o inteiramente casualizado, com quatro repetições. Os tratamentos corresponderam às sementes de feijão-caupi, cultivar BRS Guariba, colhidas em diferentes épocas (0, 3, 6, 9, 12 e 15 dias após a maturidade fisiológica) e tratadas ou não com o fungicida carboxim+thiram para avaliação da qualidade fisiológica. As sementes produzidas foram inicialmente caracterizadas por avaliações de teor de água e massa. Em seguida, procedeu-se as análises de germinação, primeira contagem de germinação, comprimento e massa seca de plântulas. Os dados foram submetidos à análise de variância e as médias analisadas em arranjo fatorial 2 x 6. As médias referentes ao tratamento químico e às épocas de colheita foram comparadas pelo teste de Tukey e análise de regressão ($p \leq 0,05$), respectivamente, realizando-se o desdobramento quando da significância da interação entre os tratamentos. O atraso na colheita das sementes de feijão-caupi acarreta redução da germinação e do vigor;

o tratamento das sementes com fungicida anteriormente às análises de qualidade fisiológica possibilita atribuir, em parte, a redução da qualidade aos patógenos associados ao tegumento.
Palavras-chave: *Vigna unguiculata*; fungicida; maturidade fisiológica.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is a legume species mostly produced in tropical and subtropical regions worldwide, especially in African countries, but also relevant to Brazilian agriculture (FILGUEIRAS *et al.*, 2009). In Brazil, North and Northeast regions benefit from both the economic and social role of cowpea in supporting rural communities (CRAVO *et al.*, 2009). In the Central-West region, it has been expanded and grown at large scale from 2006 on (FREIRE FILHO *et al.*, 2017).

Many species can be successfully included in rotation and integrated systems aiming to improve soil quality and production as a whole. In this context, and despite being traditionally associated to small farms, new breeding technologies and intense research have drawn attention to the possibility of expanding cowpea to larger new areas, where it can be cultivated in offseason or as a main crop. According to Rocha *et al.* (2017), the development of new varieties, with early and uniform maturation cycles, enabled mechanized crop systems and breeding for high grain quality. However, intense research and technology are still required to enhance cowpea yield and decrease losses (PEREIRA *et al.*, 2020).

Grain yield is influenced by many factors, such as disease, pests, weeds, mineral nutrition, environmental conditions and seed density. Among those practices, the establishment of appropriate plant populations and development, as a consequence of seedling emergence in the field, can influence production (HAMPTON, 2002). In this scenario, the use of high-quality seeds is fundamental to obtain uniform plant distribution and final population as means to obtain high grain yield and expressing maximum genetic potential (MONDO *et al.*, 2016; SHAIBU; IBRAHIM, 2016).

Seed quality is nothing more than a reflection of all attributes built up along the maturation process, such as germination and vigor. In this context, it is essential do guarantee that seeds have accumulated maximum dry matter assimilates from the mother plant before harvest. In some species, identifying that cited moment is rather difficult (MARCOS-FILHO, 2015), especially in indeterminate growth habit species such as cowpea. Uneven maturation is often a major obstacle once it is common to have plants that do not show senescence signs and additional stay green traits, with dry pods, green pods and flower buds in the same plant (MENEZES JUNIOR *et al.*, 2017).

The phenological scale suggested by Campos *et al.* (2000) for cowpea does not describe physiological maturity with precision, but defines the two last reproductive stages to be R4 (maturity of 50% of the pods) and R5 (maturity of 90% of the pods). Moura *et al.* (2012) also presented a scale for cowpea, being R5 (pods with fully developed grains) and R6 (maturity of 50% of the pods) the final development stages. Nogueira *et al.* (2014) found that cowpea seeds cv. BRS Guariba can be harvested between 14 and 18 days after anthesis without damage to their vigor.

Theoretically, harvesting seeds at the physiological maturity stage would be ideal, with extremely low deterioration levels (MARCOS-FILHO, 2015). Nevertheless, high moisture content impairs mechanical harvest and seeds have to remain in the field longer enough to dry. During that period, quality losses due to natural aging would be insignificant whenever weather environmental conditions are favorable. Weather adversities, however, mainly high temperatures and relative humidity, can significantly reduce seed sanitary and physiological quality. Extended exposure under natural environmental conditions may increase the amount of seeds infected by pathogens or damaged by pests (MIGUEL, 2003).

Seeds may be considered the main vehicle for pathogen dissemination and survival. That way, providing chemically treated seeds for crop sowing turns out to be essential in cowpea, being virtually mandatory for controlling diseases, as well as reducing fungicide spraying during plant cycle and environment contamination (MACHADO, 2012). Seed borne diseases are very important because of the introduction of new pathogens, quantitative and

qualitative crop losses and permanent contamination of soil (ORA *et al.*, 2011).

Fungicidal seed treatment is not to be applied on low-quality cowpea in order to increase germination rates, but it can do so if that was a result of pathogenic contamination. As late harvest contributes to seed exposure to unfavorable conditions in the field, it is highly probable those carry a great number of pathogens, both externally and systemically. That way, in a given situation, there could be an improvement in quality, as observed by Matos *et al.* (2013). The authors found improved physiological quality of corn seeds treated with fungicides as a result of the lower incidence of *Fusarium verticillioides*. For that matter, chemically treating the seeds for the germination tests may provide better conditions for seeds to be properly evaluated for their quality, reflecting emergence potential in the field in a more accurate way.

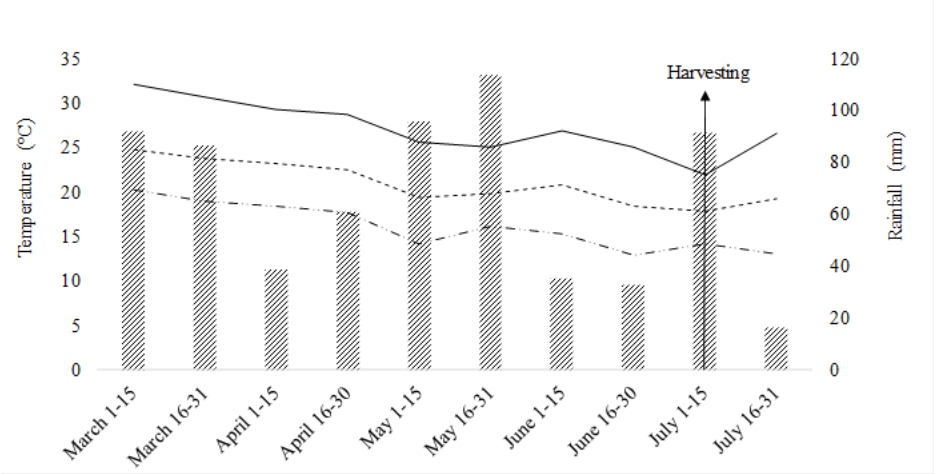
Determining the right time to harvest species with continuous flowering and grain filling such as cowpea can be difficult, once early harvest may be an obstacle to obtain maximum yield; on the other hand, late harvest may decrease seed quality due to deterioration processes that take place in the field. In this context, this study had the objective of evaluating the effects of fungicide treatment on minimizing physiological quality losses of cowpea seeds due to late harvest in germination and seedling development tests.

Material and Methods

Seed production

The study was carried out in Dourados, MS, Brazil (54°48'23" W, 22º13'18" S; 430 m asl). Soil in the experimental area is a Rhodic Ferralsol (SANTOS *et al.*, 2018) and climate is a tropical monsoon (ALVARES *et al.*, 2013), with average temperature and rainfall of 22.7 °C and 1,428 mm, respectively, according to Köppen-Geiger's classification. Figure 1 shows climate data recorded in the area during the experiments.

Figure 1. Rainfall (columns), maximum, average and minimum temperatures (lines) during cowpea production.



The experimental design was a complete randomized block with four field replications. Treatments consisted of different harvest times after physiological maturity and later seed treatment with fungicide, before quality tests. Each replication was a experimental unit with five 5-m plant rows, and harvests took place within the three central rows, except 1 m in each border.

Cowpea BRS Guariba was sown in a 0.45-m row spacing aiming a population of 250,000 plants hectare^{-1} (FREIRE FILHO *et al.*, 2008), over desiccated soybean residues, in off-season. Fertilization consisted of 280 kg ha^{-1} of 4-14-8 NPK formula applied at sowing, plus 30 kg ha^{-1} of side dressing nitrogen applied as urea 30 days later.

Field treatments consisted of different harvest times, i.e. 0, 3, 6, 9, 12 and 15 days after physiological maturity, or R5 (CAMPOS *et al.*, 2000), when plants showed 90% of mature pods. The first and last harvests took place in June, 30th and July, 15th, respectively, completing 95 and 110-day cycles at those dates. Meira *et al.* (2017) stated the influence of the environment during cowpea growth and the prevalence of phenotypic

variation over genotype. At harvest, plants were cut close to soil surface and seeds were threshed manually.

Seed quality evaluation

Right after each harvest, water content was assessed according to Brasil (2009). Later, after natural drying, seeds were stored in paper bags under environmental conditions for the evaluations of physical and physiological quality. Moisture content was assessed once more, as well as seed mass (BRASIL, 2009). Water content was determined using two subsamples of 20 seeds per field replication dried in oven at 105 ± 3 °C for 24 h. Weight of 100 seeds was determined on eight subsamples of 100 seeds per field replication.

Seed quality from each harvest was assessed with and without fungicide treatment. Seeds were placed into plastic bags with carboxim+thiram (Vitavax-Thiram® 200SC), at the dose of 300 mL 100 kg^{-1}, and mixed until being completely coated. Both samples, treated and untreated seeds, were evaluated by physiological quality tests. Germination percentage and first count were assessed using four subsamples of 50 seeds per field replication, being distributed on paper towels moistened with water equivalent to 2.5 the weight of the dry paper. Paper rolls were kept at 25 °C for 8 days in a Bio-Oxygen Demand incubator, in absence of light, with a previous count on the 5^{th} (BRASIL, 2009). Abnormal seedlings consisted of either damaged, deformed and deteriorated ones, including the ones resulted from primary infection, which compromised their normal development (BRASIL, 2009). For seeding length, four subsamples of 10 seeds per field replication were sown over a straight line drawn on paper towels moistened with water equivalent to 2.5 times the weight of the dry paper. Rolls were placed in plastic bags and left to germinate in a Bio-Oxygen Demand incubator, in absence of light, in the upright position at 25 °C for 5 days (NAKAGAWA, 2020). Primary root, hypocotyl and total seedling length were measured separately. Means were calculated by dividing the sum of all values by the total number of seeds per subsample (VANZOLINI *et al.*, 2007). At last, normal seedlings from the seedling growth test were placed in paper bags and dried at 80 °C for 24 h for seedling dry matter assessment. Seedling dry weight was calculated based on the number of normal seedlings dried (NAKAGAWA, 2020).

Data was submitted to analysis of variance, analyzed as a 2 x 6 factorial (with/without fungicide treatment x harvesting times). Treatment conditions were compared by the Tukey test ($p \leq 0.05$) and harvest times by regression analysis, with subsequent unfolding of any significant interactions. Pearson´s linear correlation test was applied to germination data.

Results and Discussion

Cowpea seed moisture was uniform regardless any harvesting times before physiological assessment, differently from that observed after each harvest (Table 1).

Table 1. Moisture (%) after harvest and before physiological tests and mass (g) of cowpea seeds previously to chemical treatment

Harvest times (days after maturity)	Moisture after harvest	Moisture before tests	Mass of 100 seeds
	---------- % ----------		g
0	35,2	7,4	14,82 ab[1]
3	45,2	7,5	14,93 ab
6	36,2	7,5	15,00 ab
9	32,0	7,5	14,99 ab
12	41,8	7,1	15,07 a
15	46,4	7,2	14,75 b
F_{calc}	-	-	2,67*
LSD	-	-	0,30
C.V. (%)	-	-	2,80

[1]Means followed by different letters in the column differ significantly from each other. * significant at 5% probability level.

Moisture content at each harvest was variable as expected whenever seeds experience a close interaction with environmental conditions in the field. Although water content is naturally reduced as harvest delays (OLIVEIRA; MORAIS, 2019), air temperature and relative humidity play an important role in establishing hygroscopic equilibrium of seeds. In this context, it was observed intense rainfall within the first half of July (Figure 1), by the time seeds were harvested. In another perspective, uneven cowpea maturation contributes to moisture oscillations, once seeds are found under different development stages (MENEZES JUNIOR *et al.*, 2017). According to the results, moisture was not compatible with mechanical harvest even after a 15-day period after physiological maturity.

Seed moisture before physiological assessment was uniform and low due to hygroscopic equilibrium with the environment. Those minor differences were within limits established by Marcos-Filho (2015), of up to 4%.

Harvest times significantly affected seed mass, but showed minor variations within treatments. Once deterioration processes advanced naturally and get accelerated under climate adversities (CARVALHO; NAKAGAWA, 2012), it is expected to observe differences in seed weight considering the dynamics of accumulating reserves. Upon studying indeterminate growth habit soybean, Toledo *et al.* (2014) found seed mass to increase with time, up to 9 days after physiological maturity and decrease thereafter, similarly to the results in this study. Dry matter accumulation in seeds is reported to increase slowly during the beginning of the filling stage, becoming more rapid and constant and reaching a maximum level at physiological maturity (CARVALHO; NAKAGAWA, 2012).

Seed germination and vigor, evaluated by the first count germination test, as well as percentage of non-germinated seeds, were affected independently by both harvest times and fungicide treatment, as opposed to the interaction between treatments to influence abnormal seedlings (Table 2). Germination percentage and first count were lower when seeds were not treated with fungicide previously to the test, as opposed to the number of non-germinated seeds.

Table 2. Germination aspects of cowpea seeds harvested at 0, 3, 6, 9, 12 and 15 days after physiological maturity, with and without fungicide treatment.

Source of variation	Germination percentage	Germination first count	Abnormal seedlings	Non-germinated seeds
	---------- % ----------			
Harvest time (days after physiological maturity)				
0	86	84	10	3
3	55	53	26	19
6	58	56	26	16
9	42	39	33	26
12	39	39	32	29
15	12	12	32	56
Fungicide treatment				
With	52 a[1]	50 a	24	23 a
Without	46 b	44 b	28	26 b
Harvest times (H)	**	**	-	**
Fungicide treatment (F)	**	**	-	*
Interaction H x F	ns	ns	**	ns
C.V. (%)	22.94	24.15	32.14	31.87

[1]Means followed by different letters in the column comparing fungicide treatments differ significantly from each other. * and ** significant at 5 and 1% probability level, respectively; ns: not significant.

Oliveira *et al.* (2015) also found fungicide treatment to increase germination compared to a control, being tiametoxam, fipronil and piraclostrobina+tiofanato metílico, as well as imidacloprido+tiodicarbe, beneficial to cowpea seeds. Gomes *et al.* (2009), working with

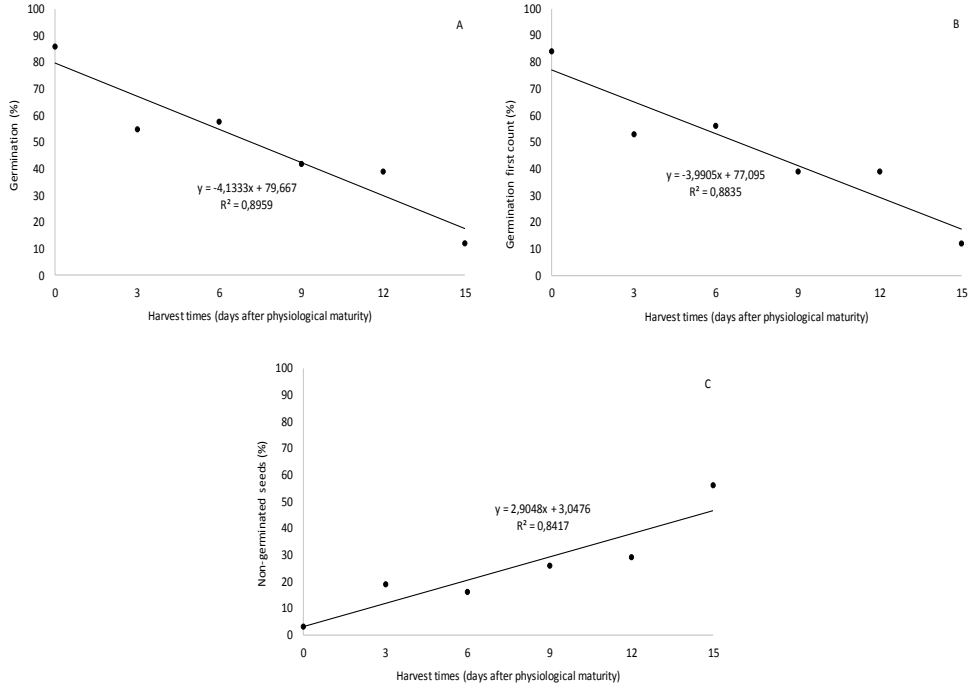
soybeans, found that high vigor seeds do not require fungicide treatment once they show rapid germination and emergence, which avoids any possible contamination of the substrate. In this study, however, there was no interaction between harvest times and chemical treatment, even though delayed harvest provided seeds with lower quality. That cited relationship between seed quality and fungicide use was only observed for the percentage of abnormal seedlings.

Both germination final percentage and first count were linearly decreased by delayed harvest, as opposed to the increasing number of non-germinated seeds as time passed (Figure 2). Significant negative correlation was found between germination and abnormal seedlings (-0.86*) as well as germination and non-germinated seeds (-0.98**). That way, it was clearly observed that delayed harvest decreases seed germination as a consequence of those increased parameters. Limiting environmental conditions, especially temperature and relative humidity, may considerably reduce seed quality while harvest is not yet performed. Late harvest may cause seed moisture to vary and damage seed coat, consequently intensifying deterioration processes and making way for pathogenic infection (ZITO *et al.*, 1995).

Among all morphological and physiological changes since fertilization to seed total independence from the mother-plant, a set of preparatory phases for the germination process, characterized for the synthesis and accumulation of nutrient reserves (MARCOS-FILHO, 2015) can be reflected on final seed attributes. Information on physiological maturity and ideal harvest time of cowpea seeds are still scarce, especially for the cv. BRS-Guariba, being essential to determine the stages associated to the lowest deterioration levels. In addition, different cultivars may develop differently in each region, as the effects of the environment strongly influence plant cycle. Oliveira and Morais (2019) found cycle of 82 days for Guariba in the State of Bahia, shorter than observed in this study, mainly due to the cooler temperatures in Mato Grosso do Sul in offseason.

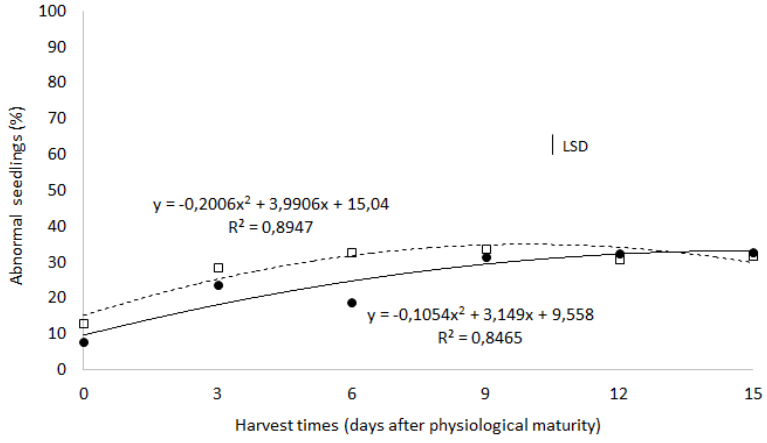
Once cowpea presents deep heterogeneous maturation, the task of identifying the right moment to harvest high quality seeds is impaired by natural characteristics of this species. As maturation progresses there can concomitantly be observed both accumulation and aging process in the same plant. Nogueira *et al.* (2014) studied different times for cowpea harvesting and established that the higher germination rates are observed between the 14^{th} and 18^{th} day after anthesis; in that same period, it was also observed the highest values for the first count of germination, root length, shoot length and seedling dry mass. In indetermined-growth habit soybean, Toledo *et al.* (2014) observed percentage germination and first count germination to be increased by delaying harvest until the 9^{th} day after physiological maturity with later decrease; however, the authors initiated seed harvest early, based on phenological stages of determined-growth types.

Figure 2. Germination (A), germination first count (B) and non-germinated (C) cowpea seeds harvested at 0, 3, 6, 9, 12 and 15 days after physiological maturity.



The effects of harvest delay usually observed on seed quality are translated into low germination rates and increased seedling abnormalities (SMIDERLE; CÍCERO, 1998). The percentage of abnormal seedlings was increased as harvest was postponed (Figure 3), regardless any use of fungicide. However, in another perspective, treated seeds produced less abnormal seedlings. While treated seeds showed increasing rates of abnormalities until the latest harvest, that was only observed for non-treated seeds sampled up to the 9th day after maturity. Considering the use of fungicide, and according to these results, chemical treatment did not influence the number of abnormal seedlings whenever harvest took place from the 9th day. According to Netto and Faiad (1995), the association with micro-organisms and seeds sometimes does not suppress germination, but causes abnormalities as well as lesions on seedlings. That appears to be related to seed vigor at some level. Gomes *et al.* (2009) stated that soybean seeds with mean and high vigor treated with fungicide show less contaminated seeds compared to low-vigor non-treated seeds.

Figure 3. Abnormal seedlings in the germination test produced by cowpea seeds harvested at 0, 3, 6, 9, 12 and 15 days after physiological maturity, with (●) and without (□) fungicide treatment



Cowpea seed vigor was interactively affected by both harvest time and use of chemical treatment, considering physiological performance in seedling development tests (Table 3). It is relevant to mention that abnormal seedlings were not measured and that seedling length was obtained by dividing the sum of all measures by the number of seeds sown on the paper (VANZOLINI *et al.*, 2007). That may include an indirect effect of germination rate over the results, which is important considering that germination percentage was decreased right after three days after the first harvest (Figure 2).

Table 3. Developmental aspects of seedlings produced by cowpea seeds harvested at 0, 3, 6, 9, 12 and 15 days after physiological maturity, with and without fungicide treatment

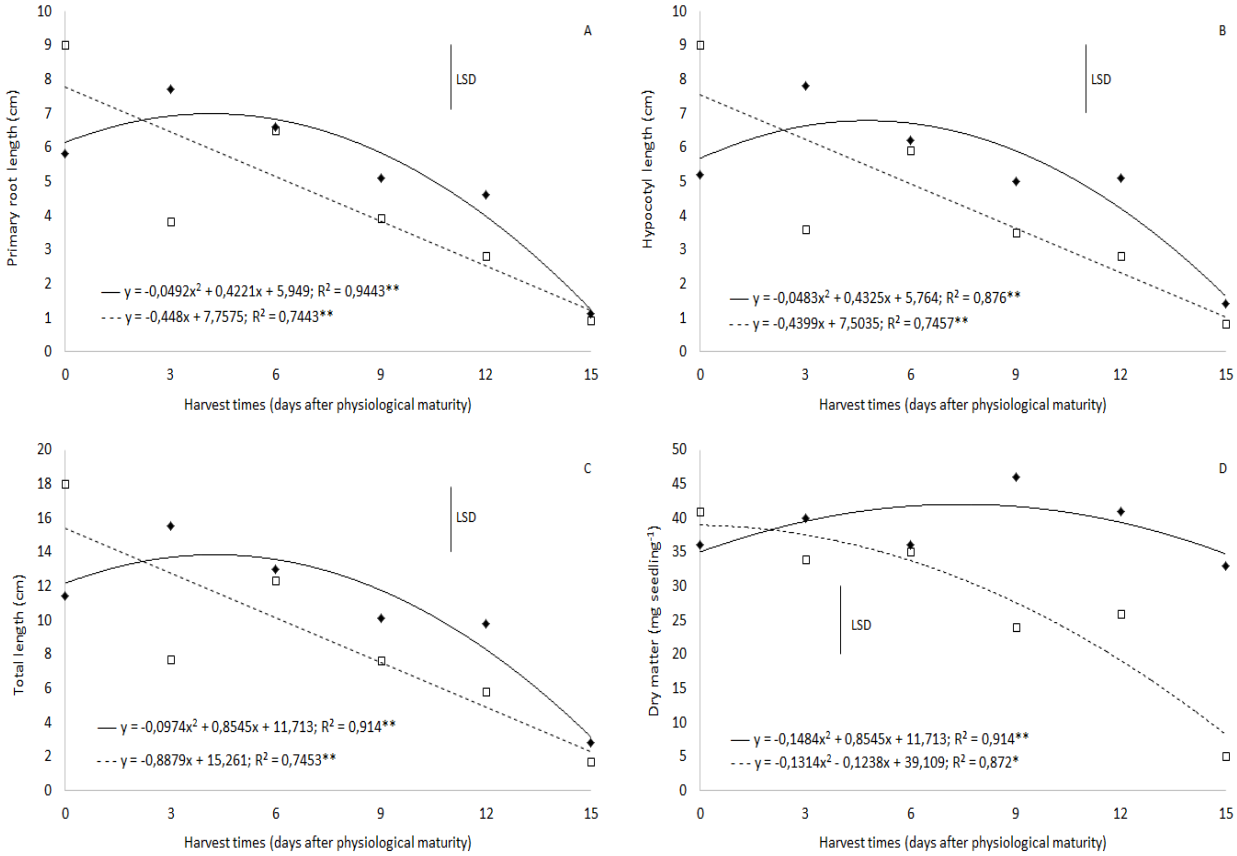
Source of variation	Primary root length	Hypocotyl length	Seedling length	Dry matter
	---------- cm ----------		mg seedling^{-1}	
Harvest time (days after physiological maturity)				
0	7.42	7.10	14.49	39.18
3	5.60	5.58	11.15	36.81
6	6.46	6.08	12.61	36.58
9	4.43	4.20	8.60	34.16
12	3.61	3.73	7.33	32.73
15	0.96	0.92	1.88	17.22
Fungicide treatment				
With	5.06	5.03	10.08	38.22
Without	4.44	4.18	8.60	27.34
Harvest times (H)	**	**	**	**
Fungicide treatment (F)	ns	*	*	**
Interaction H x F	**	**	**	**
C.V. (%)	57.35	54.05	54.45	44.71

* and ** significant at 5 and 1% probability level, respectively; ns: not significant

Seedling length and dry matter reduced as harvest was delayed, but sharp decrease was mostly observed whenever seeds were not chemically treated (Figure 4). According to Carvalho and Nakagawa (2012), it is expected that the longer the seeds remain in the field the intensified the deterioration processes occur, reflecting in dry matter accumulation. Besides, the pathogens adhered to seed coat was partially controlled by fungicide treatment before the test, showing that reduced vigor observed was also a result of sanitary conditions of the seedlot, not exclusively physiological losses.

Fungicide treatment attenuated deleterious effects of late harvest once there is often a high number of seeds infected by pathogens in the field (MIGUEL, 2003), thus reducing vigor. It is worth highlighting that chemical treatment does not improve seed physiological quality but enhances sanitary aspects. That may assure other seed attributes to be preserved and guarantee germination and vigor of infected seeds.

Figure 4. Length (primary root – A, hypocotyl – B, total – C) and dry matter (D) of cowpea seedlings produced by seeds harvested at 0, 3, 6, 9, 12 and 15 days after physiological maturity, with (●) and without (□) fungicide treatment.



Conclusions

Delayed harvest of cowpea seeds sharply reduces germination and vigor; fungicide treatment before performing physiological tests enables to establish that, in part, seed quality losses can be caused by pathogen vehiculation on the seed coat.

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