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Maturation and germination of *Vernonanthura discolor* seeds

Maturação e germinação de sementes de *Vernonanthura discolor*

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ABSTRACT

Vernonanthura discolor is a native species with potential for reclamation of degraded lands, and the manufacture of wood panels. This study has aimed to evaluate the process of maturation and germination of *Vernonanthura discolor* seeds, determining the harvest time, and conducting the germination test. A periodic monitoring of the reproductive development of sources of *Vernonanthura discolor* was conducted, located in the municipality of Bocaiúva do Sul, PR, Brazil; and starting from the anthesis, weekly collections of seeds were performed to evaluate the percentage of dry matter, germinating power, and vigor, in order to identify the physiological maturity of the seeds. In the germination test, two combinations of four temperatures (20, 30, 20-30 and 30 °C), and two light regimes were tested, seeking to define the best methodology and evaluation dates of the test. It was concluded that the seeds physiological maturity *Vernonanthura discolor* reached 45 days after the anthesis, when the maximum values of dry weight, germination and vigor are achieved. Germination test may be conducted on blotting paper at 20 or 25 °C with a supply of light, or at 25 °C in the dark, the first count being on the 13th and the last one on the 29th day after seeding.

Index terms: Physiological quality; dry matter; seed harvest.

RESUMO

Vassourão-preto é uma espécie nativa com potencial para a recuperação de áreas degradadas e confecção de painéis de madeira. No trabalho, objetivou-se avaliar o processo de maturação e germinação de sementes de vassourão-preto, definindo o ponto de colheita e a condução do teste de germinação. Realizou-se acompanhamento periódico do desenvolvimento reprodutivo de matrizes de vassourão-preto, localizadas no município de Bocaiúva do Sul, PR, Brasil, sendo que, a partir da antese foram realizadas coletas semanais das sementes, avaliando-se a porcentagem de matéria seca, o poder germinativo e o vigor, a fim de identificar o ponto de maturidade fisiológica das sementes. No teste de germinação, foram testadas combinações de quatro temperaturas (20, 30, 20-30 e 30 °C) e dois regimes de luz, procurando-se definir a melhor metodologia e as datas de avaliação do teste. Conclui-se que a maturidade fisiológica das sementes é atingida, aos 45 dias após a antese, quando os máximos valores de massa seca, poder germinativo e vigor são alcançados; o teste de germinação pode ser conduzido sobre papel mata-borrão, a 20 ou 25 °C com fornecimento de luz, ou a 25 °C no escuro, sendo a primeira contagem aos 13 e a última aos 29 dias após a semeadura.

Termos para indexação: Qualidade fisiológica; matéria seca; coleta de sementes.

INTRODUCTION

Vernonanthura discolor (Spreng.) H.Rob., a native species of the Asteraceae family, occurs naturally in the Araucaria Forest, being characteristic of secondary vegetation, and common in clearings and in coppices (Siminski; Fantini, 2011). It is a pioneer plant that grows rapidly and tolerates low temperatures, considered a great choice for mixed plantings on permanent preservation areas in order to prepare the environment for the development of climax species (Empresa Brasileira de Pesquisa Agropecuária-Embrapa, 2010).

Although known commercial plantations of this species do not exist, its rapid growth, coupled with the potential for use in industrialized wood panels such as MDF (medium-density fiberboard) makes *V. discolor* an alternative to the planting of exotic species for this purpose (Siminski; Fantini, 2011).

According to Basso et al. (2012), the cultivation of forests in Brazil is mainly focused on exotic species of the Pinus and Eucalyptus genus; however, with the increasing demand for timber in the country, it is necessary to diversify the cultivated species. Thus, the search for

natives, such as *V. discolor*, with potential for cultivation to meet the national timber industry sector, is justified.

The production of seeds of forest species should be based on two basic points: obtaining seeds with sufficient genetic representation of the population from which they were harvested (Gemaque; Davide; Faria, 2002), which is essential when the aim is the reconstruction of natural forests (Gois et al., 2014), and knowledge of the factors that affect the physiological quality of the seeds (Gemaque; Davide; Faria, 2002). Regarding the physiological quality, studies must involve the determination of: the point of collection of fruits and seeds, the most suitable method for its processing, the definition of ideal conditions for germination, and seed storage (Pereira; Cuquel; Panobianco, 2010).

The identification of the process of seed ripening is important because it allows the understanding of the behavior of the species as for their breeding, thus allowing to estimate the establishment and proper harvest time (Alves et al., 2005). Success in determining the point of harvest depends on the identification of safe and practical parameters, such as changes in morphological characteristics and dehiscence or fruit drop.

Furthermore, the collection of seeds at the appropriate time favors the obtainment of seeds of good quality, a characteristic that is essential to the propagation and production of seedlings. Thus, the importance of defining protocols for the test of germination of seeds is also highlighted, which is the main parameter to determine their quality (Martins; Machado; Nakagawa, 2008) since the species displays various performances in different temperatures and substrates, which are the basic components of germination tests (Andrade et al., 2006; Mondo et al., 2008).

Because of the importance of conserving native species, and the scarcity of information on the technology of *V. discolor* seeds, this study aimed at evaluating the process of maturation and germination of these seeds.

MATERIAL AND METHODS

Field of study

The experiments were conducted from July to December of 2013 using materials collected from sources of *V. discolor* (Figure 1A) located in the municipality of Bocaiúva do Sul, Paraná, Brazil (25°, 15.139' S and 49° 06.096' W). The climate of the region is classified according to Köppen-Geiger as Cfb, i.e., temperate climate with average temperatures of 18 °C and 22 °C in the coldest and hottest months of the year, respectively; with cool summers and without a defined dry season (Caviglione et al., 2000).

Selection and characterization of sources

20 sources of *V. discolor* were randomly selected, and each was identified, measured, and mapped by the georeferencing system. Sources had an average diameter at breast height (DBH) of 19.94 cm, with maximum variation of 37.50 cm and minimum of 9.50 cm; and an average record of total height (base of the trunk to the end of the top) of 9.42 m, ranging from 6.92 to 13.50 m.

Study of the process of seed maturation

Monitoring of the reproductive development of the trees began in July 2013, performing biweekly collections of branches (Figure 1B and 1C) using the trimmer and posterior observation in a stereoscopic microscope of: inflorescences, flowers and achenes (seeds). From the second half of September, when it was found that 50% of inflorescences (Figure 1E) were found in anthesis (Figure 1D), the interval between the observations was reduced to one or two weekly inspections in the field, and at least one branch of each marked tree was collected. Seed collection began 10 days after the anthesis and lasted up to 55 days when its total dispersal occurred, totaling eight collections. To study the process of seed maturation, the percentage of dry matter, seed germination and seed vigor were evaluated.

In determining the percentage of dry matter of the seeds, 20 infructescences were highlighted for each branch collected from marked trees on each of the eight samples taken after anthesis, and then the seeds were withdrawn. After manual homogenization (Brasil, 2009), four replicates of 200 seeds (achenes + papus) (Figure 1F) were weighed (fresh weight) on an analytical balance (0.001 g), packed in paper bags (Kraft type), and then kept in an oven with forced circulation at 65 °C, until it obtained a constant weight, thus achieving dried mass.

The evaluation of the germination power of the seeds was performed in the last six collections, i.e., starting from 34 days after anthesis, when it was observed through a stereoscopic microscope that the seeds had their structures formed and had begun a natural dispersal.

Study of the germination test

Initially, the seeds taken from infructescences were lightly rubbed on a circular sieve with 1.8 and 1.6 mm

diameter for the removal of papus (structure that assists the dispersion), processed with a blower (brand Elo's) to remove most impurities, and then manually homogenized (Brasil, 2009).

The installation of the germination test was performed with seeds collected at 34, 41, 45, 48, 52 and 55 days after anthesis, with four repetitions of 50 intact seeds (visually selected) (Figure 1G) seeded in transparent plastic boxes (11.0 x 11.0 x 3.5 cm) on two sheets of blotting paper, and moistened with a water quantity equivalent to 2.5 times the mass of the dry substrate (Brasil, 2009). The tests were conducted with temperatures of 20, 25, 30 and 20-30 °C, with and without the provision of constant light. Counts were done periodically, featuring normal and abnormal seedlings, based on the observation of the first normal seedling, until germination became constant, when the germination percentage was determined and the first and last test scores were defined.

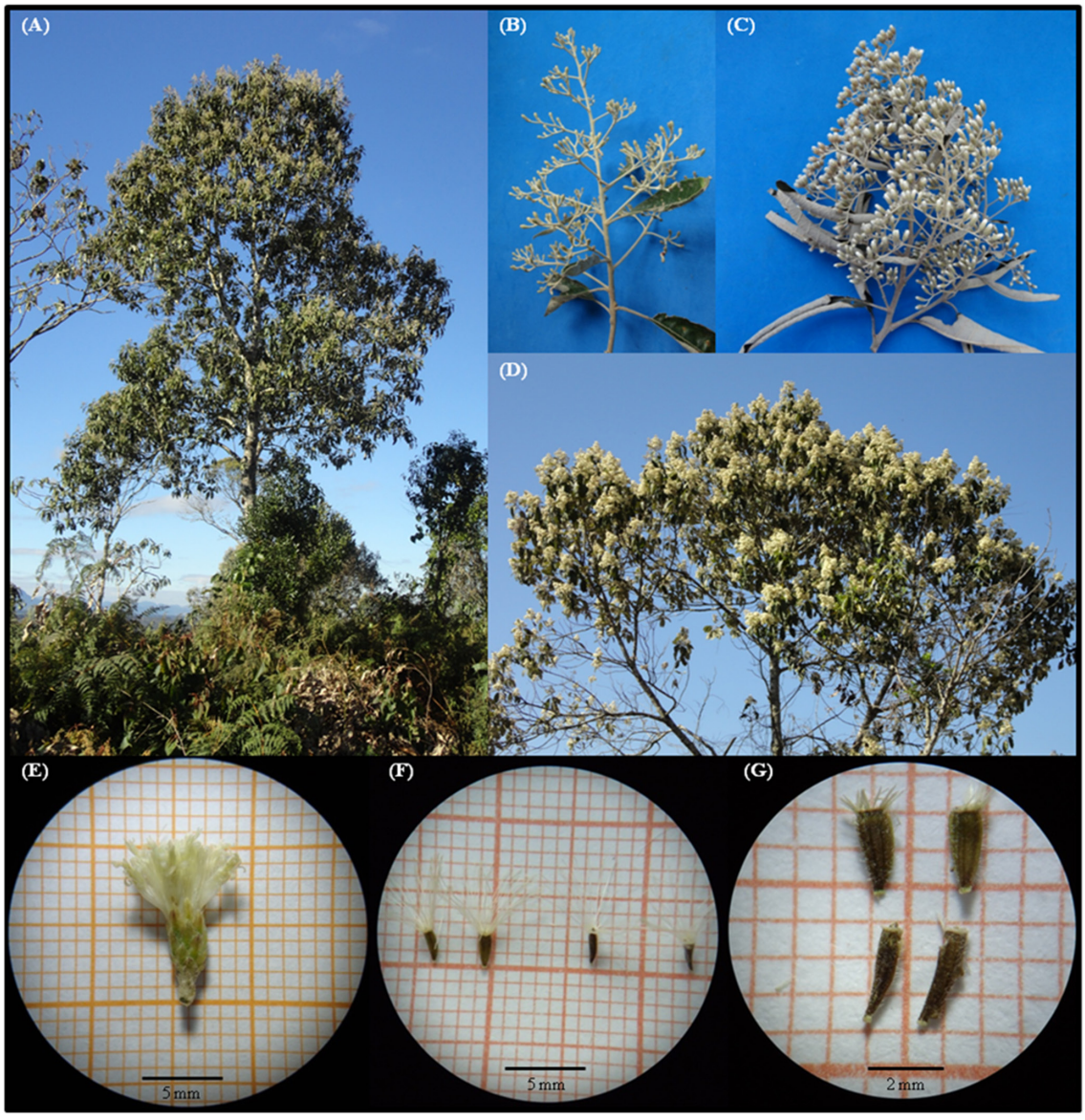

Figure 1: Details of the *V. discolor* species*:* (A) tree; (B) branch on the 65th day before anthesis; (C) branches on the 20th day before anthesis; (D) tree with more than 50% of the inflorescences in anthesis; (E) inflorescence; (F) dispersion unit (achene + papus); (G) achenes.

Evaluation of the strength of the seeds

The strength was determined according to the germination speed index – GSI (Maguire, 1962), and the first count test, which were conducted along with the germination test for each treatment tested.

Data analysis

The experimental design was randomized, with four repetitions, in a 4 x 2 factorial arrangement for the germination test (four temperatures and two light regimes) and the obtained data were submitted to an analysis of variance, and the means were compared by the Tukey's test ($p \leq 0.01$). The data of the study were analyzed according to the regression model, with adjustment of better trend for the curve model.

RESULTS AND DISCUSSION

The increment of dry matter in seeds of *V. discolor* is shown in Figure 2, where a significant effect of quadratic order for the variable is found, in which it is possible to observe that the maximum accumulation occurred 45 days after anthesis, obtaining 91.6% of dry mass. At this point, the seeds were physiologically disconnected from the parent plant and began to act as independent individuals, i.e., according to classical concepts in the literature, reaching physiological maturity (Marcos-Filho, 2005). After reaching physiological maturity, the seeds showed a decrease in dry mass.

As Carvalho and Nakagawa (2012) have pointed out, the accumulation of dry matter of seeds in formation initially happens slowly, followed by rapid and constant accumulation, until a maximum is reached and maintained for some time, and may in the end suffer a small decrease. The period of maintenance of high levels of dry matter depends directly on the influence of the environment, because less favorable conditions of relative humidity, temperature and action of insects and microorganisms contribute to the acceleration of the respiratory process, resulting in oxidation of the reserve of substances (Marcos-Filho, 2005).

In the case of *V. discolor*, there was a drop in dry matter after a relatively intense physiological maturity; such behavior may be associated with the size of the seed, around 3.0 mm, which increases the contact surface exposed to the environmental changes, thereby affecting the process of respiration and consumption of reserves. Another hypothesis is that, because it is a native forest species, there is heterogeneity in the tree as to the formation of seeds, which causes a peak production of mature seeds and after this time, the natural seed dispersal quickly begins, remaining on the tree only those that are still developing, shriveled and/or malformed, i.e., with reduced values of dry matter.

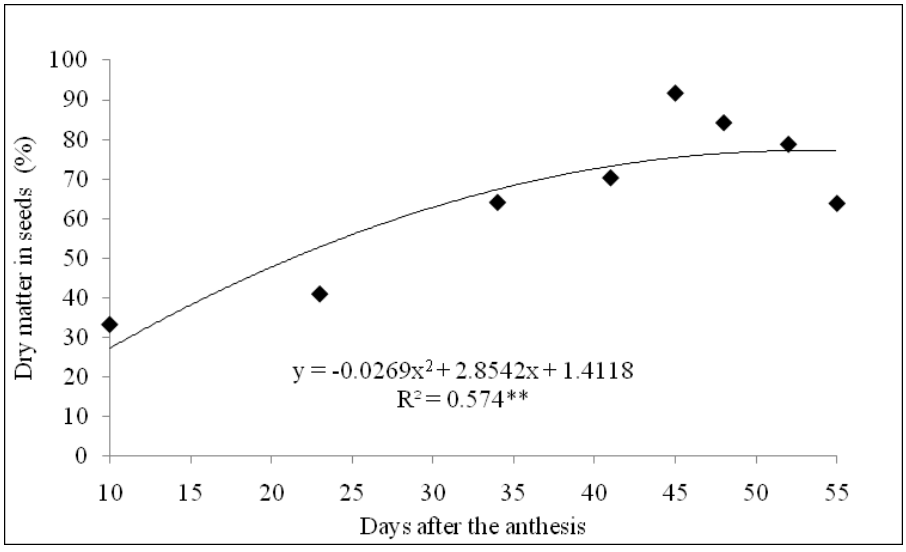

Figure 2: Dry matter accumulation in *V. discolor* seeds during the maturation process.
** Significant at 1% of probability by the F test.

The determination of dry matter to identify the point of physiological maturity was also effective for seeds of various species, such as pink-ipê (Gemaque; Davide; Faria, 2002), quaresmeira (Lopes; Dias; Pareira, 2005), thrush (Alves et al., 2005), Surinam cherry (Avila et al., 2009), and Indian coral tree (Matheus; Lopes; Corrêa, 2011).

The visual characteristics of branches and seeds of *V. discolor* at physiological maturity, i.e., 45 days after anthesis, are shown in Figure 3. At this time, the seeds were ready for natural scatter, which occurs through the wind, also called anemocori (Almeida et al., 2008). Once reaching physiological maturity, the natural dispersal of seeds of *V. discolor* is very fast, and after 10 days there were no more seeds to collect from the tree.

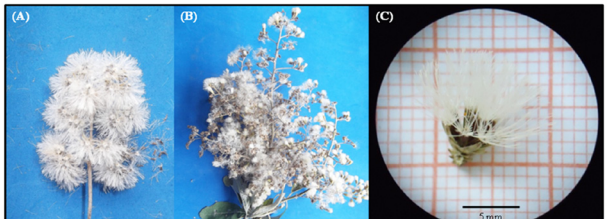

Figure 3: Characteristics of branches and seeds of *V. discolor* on Physiological Maturity: (A) branch with 100% of the seeds ready for dispersion, (B) branch with 50% of seeds already dispersed; (C) detail of the seed at the point of physiological maturity.

Once the point of physiological maturity was defined, we were able to study the germination test for seeds of *V. discolor* more consistently, using them fully ripe. In Table 1 are the germination percentages obtained from the combination of four temperatures and two light regimes, and the variance analysis showed that the factors are not independent. We found that when constant light was provided, there was no statistical difference between the temperatures tested, i.e., *V. discolor* seeds germinate in a wide temperature range (20-30 °C). However, in the absence of light, only the temperature of 25 °C showed the same behavior as in the presence of light; at other temperatures, especially higher ones (20-30 and 30 °C), there was a drastic reduction in the germination percentage.

Table 1: Average data of germination of *V. discolor* seeds, testing done in a light regime and incubation temperatures, on paper substrate.

	Germination	
Temperature	with light	without light
	.....%.....	
20 °C	57aA	42aB
25 °C	50aA	47aA
30 °C	41aA	1cB
20-30 °C	45aA	22bB
C.V. (%)	23.51	

Means followed by the same lowercase and uppercase letter on the same line, do not differ statistically according to the Tukey's test ($p \leq 0.01$).

The verification that it is possible to use more than one methodology for the test of germination of *V. discolor* seeds can be explained by the characteristic of the species, considered a pioneer, i.e., one of the first plant species to establish themselves in the community (forest); its natural germination occurs with or without exposure to solar radiation; since the dispersion unit is small, when deposited in the soil it may or may not be shaded by other developing plant species.

In the routine analysis of seed laboratories, the germination test conducted without the need of artificial light facilitates the evaluations, since they can be conducted in less sophisticated equipment. However, when possible, illumination during testing is recommended in order to promote the involvement of the essential structures of the plant seedlings, facilitating the evaluation, and reducing the attack of microorganisms (Brasil, 2013).

The percentages of normal seedlings per day of assessment on the total of germinated seeds of *V. discolor*, at temperatures of 20 °C with supply of light and 25 °C with and without light regime, are presented in Figure 4. We found synchronism in the germination of the seeds in these incubation conditions, and 82-95% of normal seedlings were germinated 13 days after installation of the test and the formation of seedlings from the thirty-first day was not observed, i.e., for *V. discolor* the first and last count of the germination test can be performed on the 13th and 29th days after sowing.

According to Borghetti and Ferreira (2004), the timing of seed germination is lower under extreme temperatures and tends to be higher the closer the incubation temperature is to the optimum range of germination. Assuming that the optimum temperature for germination is the result of the physiological adaptation of seeds to local environmental conditions, or cultivation of the species, there may be a direct relationship between this temperature and the biome in which the seeds were produced (Brancalion; Novembre; Rodrigues, 2010).

This relationship was observed in seeds of *V. discolor*, since the region where the species naturally occurs, the Araucaria Forest (Siminski; Fantini, 2011), presents temperate climate with average temperatures of 18 °C and 22 °C in the coldest and hottest months of the year, respectively (Caviglione et al., 2000). Furthermore, our results agree with those reported by Brancalion, Novembre and Rodrigues (2010), who, studying the ecological and applied aspects of temperature on the germination of Brazilian tree species, concluded that it is possible to indicate that the test of germination of seeds with species of the Atlantic Forest biome is conducted through the use of constant temperature 25 °C.

The use of a blotter substrate was effective for the germination test, taking into account the size of the seed and the need to test the supply of light. Analyzing the Instructions for Analysis of Seeds of Forest Species (Brasil, 2013), we found that the average amount of time used for the germination test of the cited forest species is around 30 days, similar to that observed in the seeds of *V. discolor*.

Normal intact seedlings of *V. discolor* can be seen in Figure 5A, in which are the essential well developed, complete, proportionate and healthy structures (Brasil, 2013), i.e., long and slender taproot and coated by numerous absorbents, with a tapered end, straight hypocotyls and two green and leaf-like cotyledons in opposite positions. In abnormal seedlings found in the study of the germination test we observed an undeveloped primary root (Figure 5B and 5C), cotyledons missing (Figure 5C and 5D), and / or stunted hypocotyls (Figure 5D).

Relating the dry matter accumulation with the percentage of germination and seed vigor during the maturation process of *V. discolor* (Figure 6), there were significant effects of quadratic, cubic and fourth order degrees for the variables dry matter, first count and germination, and GSI, respectively. We can prove that the maximum physiological seed quality was achieved 45 days after anthesis (physiological maturity), since at this point the seeds showed the maximum dry matter accumulation, germination and vigor.

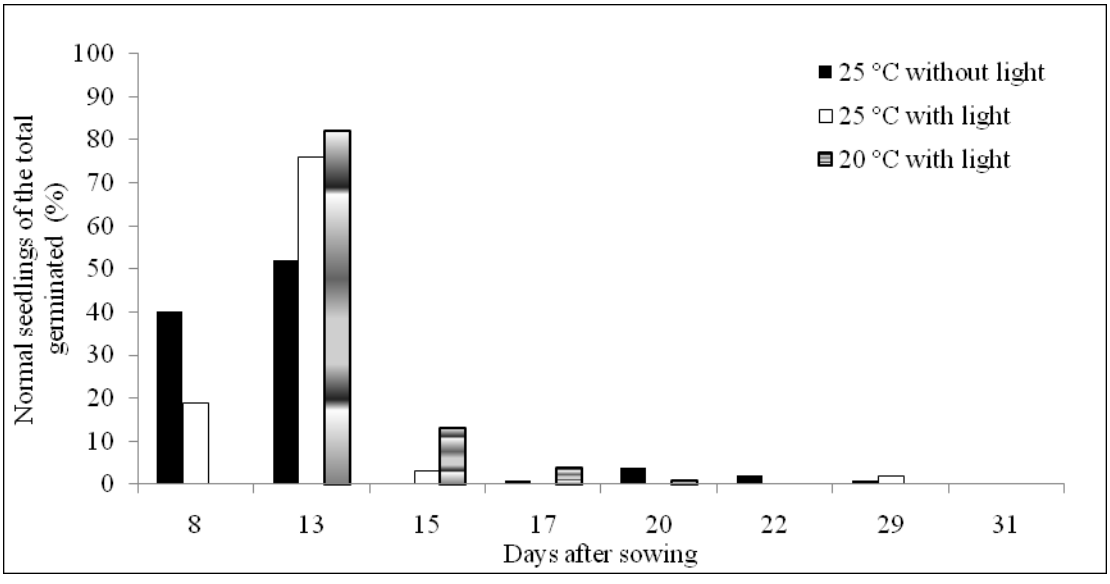

Figure 4: Percentage of normal seedlings per day of assessment on the total of germinated of *V. discolor* seeds at 20 °C and 25 °C with and without the provision of light.

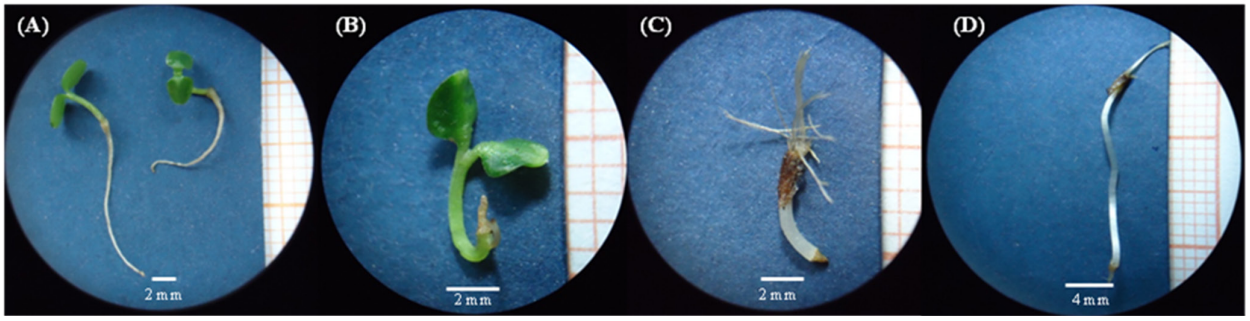

Figure 5: Normal seedlings (A) and abnormal seedlings (B, C, D) of *V. discolor.*

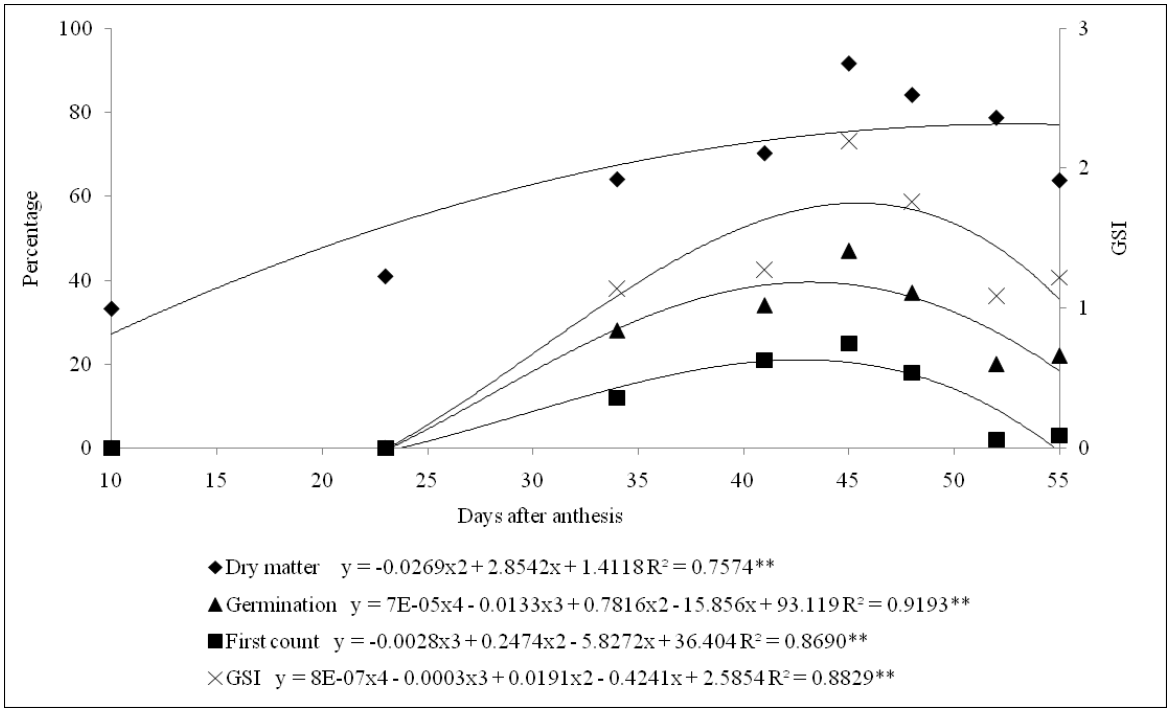

Figure 6: Accumulation of dry matter, germination and vigor (germination speed index - GSI) of *V. discolor* seeds during the maturation process.

** Significant at 1% of probability by the F test.

The process of seed maturation is composed of morphological, physiological and functional changes such as increased size, variations in water content, physiological quality (germination and vigor) and dry matter accumulation, occurring from the fertilization of the ovum until the time when the seeds are ripe (Carvalho; Nakagawa, 2012). The seed reaches physiological maturity when it ceases the transfer of dry matter from the parent plant, and has high, if not maximum, levels of germination and vigor (Marcos-Filho, 2005).

The identification of these parameters that characterize the physiological maturity of seeds was used in several species, such as pink-ipe (Gemaque; Davide; Faria, 2002), quaresmeira (Lopes; Dias; Pareira, 2005), thrush (Alves et al., 2005), red oak (Lopes and Soares, 2006), Brazil wood (Aguiar et al., 2007), Surinam cherry (Avila et al., 2009), canafístula (Nakagawa et al., 2010), Indian coral tree (Matheus; Lopes; Corrêa, 2011), and Starburr (Duarte et al., 2012).

CONCLUSIONS

The physiological maturity of seeds of *Vernonanthura discolor* is reached 45 days after anthesis, when maximum values of dry weight, germination power and vigor are achieved. The germination test for seeds of *V. discolor* can be conducted on blotting paper, at 20 or 25 °C with supply of light, or at 25 °C in the dark, being the first count conducted on the 13th day and the last on the 29th day of the setup of the test.

ACKNOWLEDGEMENTS

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