

Aspects of *Peltophorum dubium* Sprengel (Taubert) seeds in an aerial seed bank¹

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ABSTRACT – Knowledge concerning natural regeneration strategies is important due to the effect of natural regeneration on the local ecosystem. The aim of this study was to examine the ecophysiological aspects of the *Peltophorum dubium* aerial seed bank. Fruits were harvested monthly, before and after seed maturation, from selected trees in the municipality of Lavras, MG, Brazil, for determination of moisture content, acquisition of tolerance to desiccation, seed viability, maintenance of dormancy, and mean germination time. Physiological maturity of the species was attained in June, when low moisture content, maximum dry matter, and high germination percentage were observed. We found that there was no change in dormancy, in germination percentage, or in the protein profile of seeds during the period they remained in the aerial seed bank. The *P. dubium* species creates an aerial seed bank of short serotiny, with fruits remaining attached to the tree for 9 months.

Index terms: serotiny, seed longevity, seed vigor, *angico-amarelo*

Comportamento de sementes de *Peltophorum dubium* Sprengel (Taubert) em banco de sementes aéreo

RESUMO - O conhecimento sobre estratégias de regeneração natural é importante devido a sua influência na ecossistema local. Objetivou-se estudar o comportamento do banco de sementes aéreo de *P. dubium*. Os frutos foram colhidos mensalmente antes e após a maturação das sementes em matrizes localizadas no município de Lavras – MG. Mensalmente, foram determinados o conteúdo de água, aquisição da tolerância a dessecação, viabilidade das sementes, manutenção da dormência e tempo médio de germinação. Verificou-se que a maturidade fisiológica da espécie ocorreu no mês de junho, quando foi observado baixo conteúdo de água, máxima matéria seca, e alta porcentagem de germinação. Verificou-se que não houve alteração na dormência, porcentagem de germinação e no perfil proteico de sementes durante o período em que permanecem no banco de sementes aéreo. A espécie *P. dubium* forma banco de sementes aéreo de curta serotinosidade, com frutos permanecendo presos à planta por 9 meses.

Termos de indexação: serotinosidade, longevidade, vigor, *angico-amarelo*.

Introduction

Native forests make use of diverse regeneration strategies for the purpose of maintaining resilience after being disturbed, ensuring the continuity of ecophysiological processes (Tonello and Teixeira Filho, 2012). Thus, knowledge of strategies of forest species becomes important to understand processes such as colonization, which is fundamental for planning and restoration of forest ecosystems (Albuquerque et al., 2010; Guimarães et al., 2014).

Among the best-known plant establishment strategies are vegetative reproduction, seed rain, and soil seed and seedling bank (Braga et al., 2015; Durigan et al., 2011; Miranda Neto

et al., 2010). However, there are other species that have little-known regeneration strategies, such as delay in seed dispersal. These species maintain viable seeds attached to the mother plant for at least three months after reaching physiological maturity, forming the aerial seed bank (Baskin and Baskin, 2014). The length of time seeds remain in the aerial seed bank depends on the species, as was observed in species from the northern hemisphere (Bastida et al., 2010; Teste et al., 2011).

Species that form aerial seeds banks are called “serotinous” species, which fall into two classifications: species with short serotiny, when seed dispersal is delayed for up to one year, and those with long serotiny, which maintain seeds in the aerial seed bank for periods exceeding one year. In addition,

¹Submitted on 08/30/2016. Accepted for publication on 12/15/2016.

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the serotiny of the species is associated with regions subject to fire, dry climate, and low rainfall (Lamont et al., 1991; Wenk and Falster, 2015).

The species *P. dubium* is an arboreal Fabaceae popularly known as *canafistula* and *angico-amarelo*, considered as a rapid growth pioneer species, with a great deal of potential for reforestation (Bertolini et al., 2015). With only one field guide report (Lorenzi, 2002), it was observed that the fruit of this species can remain attached to the plant; however, without any study of seed quality. Thus, characterization of seed quality and the length of time fruit remains attached to the mother plant can indicate that the species is serotinous, which would provide added ecological value to the species *P. dubium* since serotiny increases the recruitment of individuals to the environment (Gao et al., 2014). In Brazil, however, the only species described as forming an aerial seed bank is *Parkia pendula* (Oliveira et al., 2006).

Proteins are the main reserve components of the seeds of various leguminous plants, such that there is a tendency for these reserves to be used for maintaining seed vigor (Wang et al., 2015a; Wang et al., 2015b; Wu et al., 2011) up to their dispersal; a reduction in protein content can occur as of that point (Kalemba and Pukacka, 2008). Nevertheless, the literature has not reported if this type of response occurs in serotinous seeds.

Consequently, the present study was carried out with the objective of characterizing the aerial seed bank of *P. dubium* through analysis of possible changes in the physiological quality of seeds.

Materials and Methods

Four *P. dubium* trees were selected for the monthly collections between April 2014 and February 2015 on a private property in the rural area of the municipality of Lavras, MG, Brazil, at the geographic coordinates 21° 15' 54"S and 45° 01' 30"W. The criteria for selection of the trees were uniformity of fruit maturation, verified by the coloring of the fruit (dark brown) and beginning of its natural dispersal (Aquino et al., 2006), absence of fruit from the previous harvest, abundance of fruit for monthly collections made up to the end of the dispersal period, minimum distance of 50 meters between the trees, and plant health appearance (absence of fungi and insects).

After each collect, the seed were manually processed, removing the seeds from the fruit, and they were immediately used in physiological evaluations.

Physiological maturity of the fruit was identified at four stages of maturity: April, March, June and July. The fruit in April was green, but in the following months had a

brown color (Aquino et al., 2006). Analyses of germination, moisture content, and tolerance to desiccation were performed monthly. Soon after determination of the point of physiological maturity, the aerial seed bank was characterized and the ecophysiological aspects were monitored through germination and physical dormancy of the seeds; moisture content (Brasil, 2009) and mean germination time (MGT) were also monitored (Labouriau, 1983).

The *P. dubium* seeds were germinated in a roll of paper, with four replications of 25 seeds per sampling point. As of May, when the hard seed coat appeared, before each test, physical seed dormancy was broken, which consisted of cutting the seed coat (approximately 1 mm) with a blade on the side opposite the micropyle.

After dormancy was broken, the seeds were immersed in a 1% sodium hypochlorite solution for three minutes, and immediately after, they were washed in running water for one minute. The experiment was conducted in a B.O.D. germination chamber with four white fluorescent lights regulated at 25 °C and with a 12-hour photoperiod. The seeds were evaluated daily up to the 7th day. Radicle protrusion (≥ 1.0 mm) was considered as the germination criterion.

Seeds collected during development in the months of April and May were used to study tolerance to desiccation. To do so, soon after collection, the seeds were dried in a controlled environment (40% RH and 20 °C) for 72 hours. After this period, the germination test was carried out, using four replications of 25 seeds (Silva et al., 2012). Seeds that were not dried were used as a control.

In analysis of possible change in physical dormancy of the seeds in the aerial seed bank, the collected seeds were placed to germinate after treatment with sodium hypochlorite under the same conditions described above (in a roll of paper, 25 °C, and with 12-h photoperiod), without, however, performing any treatment for breaking dormancy. As a control for this analysis, seeds that went through the treatment for breaking dormancy (cutting the seed coat on the side opposite the micropyle with a blade) were used.

For determination of moisture content, soon after the fruit was collected and processed, the fresh weight of the seeds was obtained on an analytical balance (0.0001 g). They were then placed in a laboratory oven at 105 °C for 24 hours and once more weighed to obtain dry matter and determine moisture content (Brasil, 2009). Four replications of five seeds were used, according to the methodology adapted from Aquino et al. (2006). Beginning in April, when the hard seed coat appeared, before determination of moisture content, the seeds were cut in half before being placed in the laboratory oven.

In each one of the sampling points analyzed, a 50 seed

sample was instantly frozen in liquid nitrogen and stored in an ultrafreezer at $-80\text{ }^{\circ}\text{C}$, and these samples were used for analysis of changes in the protein profile of *P. dubium* seeds. For extraction, 100 milligrams of whole seeds were macerated in 1 mL of extraction buffer (500 mM of Tris HCl pH 7.5, 5 mM of NaCl, 5 mM of MgCl_2 , 0.001 M of protease inhibitor [SIGMA FAST™ PROTEASE] and 1 μL of β -mercaptoethanol). The samples were centrifuged for 30 minutes at 13.200 g at $4\text{ }^{\circ}\text{C}$. After that, the supernatant was divided into two subsamples. The first (total proteins) was kept on ice, while the second (heat-resistant proteins) was kept in a water bath for 15 minutes at $85\text{ }^{\circ}\text{C}$. After that, both subsamples were centrifuged at 13.200 g for 30 minutes at $4\text{ }^{\circ}\text{C}$ and the supernatant was collected in 100 μL aliquots and stored in a freezer at $20\text{ }^{\circ}\text{C}$. The extracts were quantified by the Bradford (1976) method, and the values were used as a basis for electrophoresis.

Electrophoresis was performed in discontinuous polyacrylamide gel (1 mm) at the concentration of 12.5% (separating gel) and 6% (stacking gel). For the aliquots of total proteins, the volume of extract needed to obtain 40 μg of proteins was calculated. For the aliquots of heat-resistant proteins, the same volume used for the respective aliquot of total proteins was used. The quantity of 25 μL of loading buffer was added to these samples, and they were heated at $95\text{ }^{\circ}\text{C}$ for five minutes before beginning electrophoresis.

Electrophoresis was carried out in vertical unit SE600 (Hoefer) at 160 V for 7 hours at $15\text{ }^{\circ}\text{C}$. After running, the gel was fixed in a solution containing 40% methanol and 7% acetic acid for 30 minutes and stained for 72 hours in solution with 0.08% (w/v) of Coomassie Blue G-250 containing 1.6% (v/v) orthophosphoric acid and 12% (w/v) ammonium sulfate. After that, the gels were decolorized in 0.26% (w/v) solution of Trizma base pH 6.5, with final washing in 40% (v/v) methanol solution for one minute. The gels were kept in ultrapure water for 72 hours, at which time they were stored at $5\text{ }^{\circ}\text{C}$ in acetic acid solution (5%). The gels were scanned in the high resolution Immage Scan III scanner using the LabScan 6.0 software.

In data analysis, the rainfall and mean monthly temperatures of all the collection periods were obtained from the weather station 1 km from the area of the experiment. The climate data were used as additional information to assist in understanding and discussion of the results.

To study seed germination during physiological maturity, a completely randomized design was used, with four stages of maturity as treatments and four replications of 25 seeds, corresponding to collections made in April, May, June and July 2014. The data regarding germination percentage were subjected to regression analysis using the SISVAR (Ferreira, 2010) software.

For analysis of tolerance to desiccation, the results of the means of the treatments in the months of April and May were subjected to Student's t-test at 5% significance. For analysis of seed dormancy, germination data from the aerial seed bank were used; the experiment was set up in a factorial arrangement with 2 dormancy treatments (with and without breaking dormancy) and 9 collection periods, with four replications of 25 seeds. The data on germination percentage, MGT, and percentage of dormant seeds in the aerial seed bank were subjected to Shapiro-Wilk normality testing. The germination, MGT, and dormant seed data not normalized were transformed to $\log(x)$; after normalization of the data, analysis of variance was carried out, and the means were subjected to regression analysis. The data were subjected to analysis of variance (ANOVA) and the means of the treatments were subjected to regression analysis at 5% probability with the aid of the SISVAR (Ferreira, 2010) statistical program.

Results and Discussion

The moisture content of the seeds decreased from the first to the second collection (April and May) from 66 to 13% and remained stable as of that point (Figure 1A). The results of analysis of variance indicated that there was the effect of collection times of the fruit on viability of the *P. dubium* seeds during their formation, which is observed upon comparing germination from collection in April 2014 (7%) with collection in May, when mean germination was greater than 90% (Figure 1A).

In relation to tolerance to desiccation, it was observed that after drying the seeds, germination was 10% (April); however, in the second collection, germination after drying was 99%, indicating that as of the second collection (May), the seeds had already acquired tolerance to desiccation (Figure 1B). Thus, associating the data of seed physiological quality, it was considered in this study that seeds achieved physiological maturity in June, when the fruit had a dark brown color, and, from that time on, studies of the aerial seed bank were carried out.

During formation of *P. dubium* seeds, the difference in moisture content observed between the first two stages of maturity (Figure 1A) may have been due to the immaturity of the seeds, since the variation observed, which reduced over time, is typical of orthodox seeds before physiological maturity (Nakada et al., 2011; Ricci et al., 2013). Tolerance to desiccation is acquired during development and allows reduction in moisture content to occur without damage to seeds (Bewley et al., 2013; Garnczarska et al., 2009). In *P.*

dubium seeds, it was found that acquisition of tolerance to desiccation occurred between April and May, which coincided with the increase in maximum germinability (Figure 1B). It addition, it is commonly observed that acquisition of tolerance to desiccation occurs before the seeds achieve their maximum germinability, as a manner of preparation for dispersal (Angelovici et al., 2010; Gojlo et al., 2015).

Analyzing the data on moisture content, seed viability, and tolerance to desiccation, it can be concluded that physiological maturity occurred in June. Furthermore, other authors, such as Aquino et al. (2006) upon studying the physiological maturity of *P. dubium*, also found that the physiological maturity of the species under study occurs in June.

Analyzing the results of germination of *P. dubium* seeds in

the aerial seed bank (June 2014 to February 2015), no significant effect ($F = 0.05$; $p \leq 0.05$) was found of the collection times of fruit on the viability of *P. dubium* seeds. Collected seeds showed viability higher than 90% (Figure 2A).

No significant effect ($F = 0.05$; $p \leq 0.05$) was observed of the collection times on dormancy of *P. dubium* seeds; that is, there was no natural breaking of seed dormancy during the period within the aerial seed bank. The mean percentage of dormant seeds during the period of study was greater than 84% (Figure 2A).

In regard to moisture content of the seeds over the period they remained in the aerial seed bank, there were small variations between June 2014, at 7.7%, and February 2015, at 8.1% (Figure 2A), maintaining practically stable values

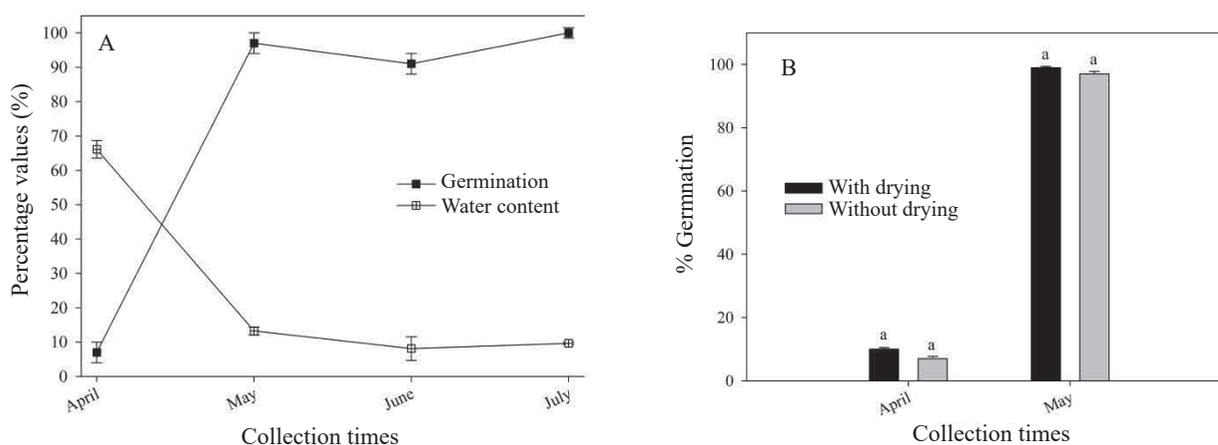


Figure 1. A - Germination (%) and moisture content during *P. dubium* seed formation from April 2014 to July 2014. B - Acquisition of tolerance to desiccation during development of *P. dubium* seeds. The bars represent standard deviation from the mean.

*Means followed by the same letter in each collection time do not differ among themselves by Student's t-test at 5% probability.

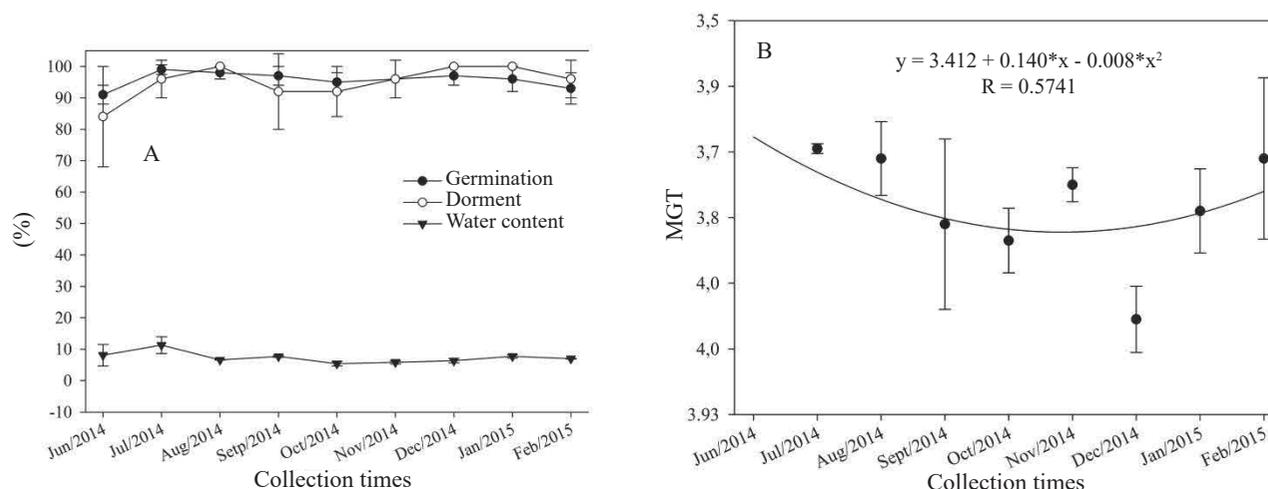


Figure 2. A - Germination (%), dormant seeds (%), moisture content of *P. dubium* seeds in the aerial seed bank. B - MGT of *P. dubium* seeds in the aerial seed bank. The bars represent the standard deviation from the mean.

throughout the experimental period.

There was a significant effect for mean germination time of the aerial seed bank ($F = 0.05$; $p \leq 0.05$), with the longest MGT registered in July 2014, at four days; in contrast, the shortest MGT was registered in December 2014, at three days and a half (Figure 2B).

It was observed that the seeds maintained viability throughout the period they remained in the aerial seed bank. Most of the fruits promptly disperse all their seeds upon reaching maturity so that the seeds encounter favorable conditions to germination or otherwise are incorporated in the soil seed bank (Long et al., 2015). Generally, after reaching physiological maturity, seeds are subject to environmental variations (moisture and temperature) and begin a natural deterioration process, culminating in loss in vigor (Baskin and Baskin, 2014).

According to observations made in this study, the species *P. dubium* forms an aerial seed bank, with fruits remaining attached to the plant for a period of up to nine months, which may be classified as short serotiny (Lamont et al., 1991).

In Brazil, the only species described as forming an aerial seed bank was *Parkia pendula*, which maintains fruits attached to the plants for up to six months (Oliveira et al., 2006). It is known that there are few studies related to aerial seed banks in Brazil; however, *P. dubium* is the first native species of the semideciduous seasonal forest to be described with this type of activity.

Studies related to aerial seed banks in general report the absence of seed dormancy throughout the period of seed dispersal (Kim et al., 2009; Baskin and Baskin, 2014). Nevertheless, this was not observed in *P. dubium*, for which no change in physical dormancy of the seeds was observed over their time within the aerial seed bank (Figure 2A).

When dormant seeds are exposed to environmental variations such as oscillations in temperature and moisture, dormancy may be broken (Jha et al., 2015; Newton et al., 2015) moist temperate woodland species *Galanthus nivalis* and *Narcissus pseudonarcissus* are complex and poorly understood. Temperature, light and desiccation were investigated to elucidate their role in the germination ecophysiology of these species. The effect of different seasonal temperatures, seasonal durations, temperature fluctuations, the presence of light during different seasons and intermittent drying (during the summer period). The lack of variations in physical dormancy of *P. dubium* seeds during the period in the seed bank may indicate that these environmental variations are insufficient to change the physical dormancy of *P. dubium*, and may ensure that germination be delayed until there are favorable conditions for germination and seedling

establishment (Dalling et al., 1998). This contributes to the formation of the soil seed bank, with continuous inputs of seeds during the period in which seeds are being dispersed in the aerial seed bank.

The climate variations observed during the period of study indicate the influence of variations of temperature and rainfall on the physiological quality of the seeds over the time they remain attached to the mother plant. Within the period of April 2014 to February 2015, the lowest rainfall amount was in June 2014 (3.7 mm); in contrast, the greatest amount was in November 2014 (249.4 mm) (Figure 3). This may suggest that *P. dubium* has the ability to adapt and establish itself in environments with contrasting conditions without harm to the physiological quality of its seeds (Dürr et al., 2015).

The MGT indicated that, apart from the effect of climate variations on the aerial seed bank, there was a reduction in MGT up to the seventh month (December 2014) after characterization of the aerial seed bank. For some authors (Mavi et al., 2010; Soltani and Baskin, 2015), the MGT may be correlated with field emergence, in which the shorter the MGT, the greater the chances of seedling establishment. Thus, reduction in the MGT of the *P. dubium* species showed that although maturity occurred in June, the quality of the seeds evaluated by this variable increased; it is noteworthy that the shortest value of MGT occurred in the rainiest period (Figure 2B).

Throughout the period of seed formation and time in the aerial seed bank (March 2014 to February 2015), the protein profile exhibited small changes related to the accumulation of total and heat-resistant proteins. High intensity bands

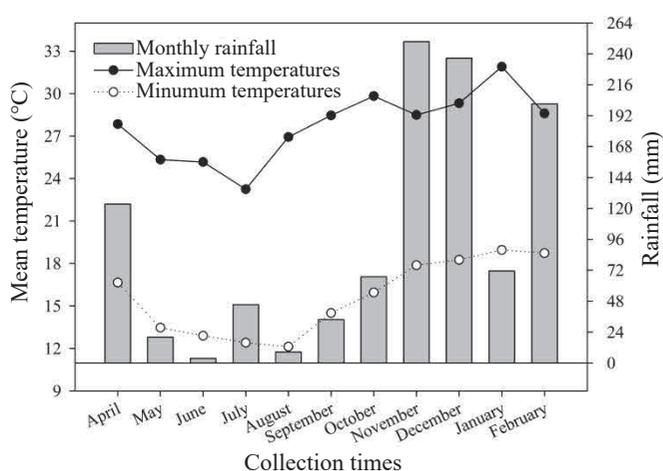


Figure 3. Climatological variations (maximum and minimum temperatures and rainfall) in the period from April 2014 to February 2015. Source: data obtained from the climatological station of UFLA.

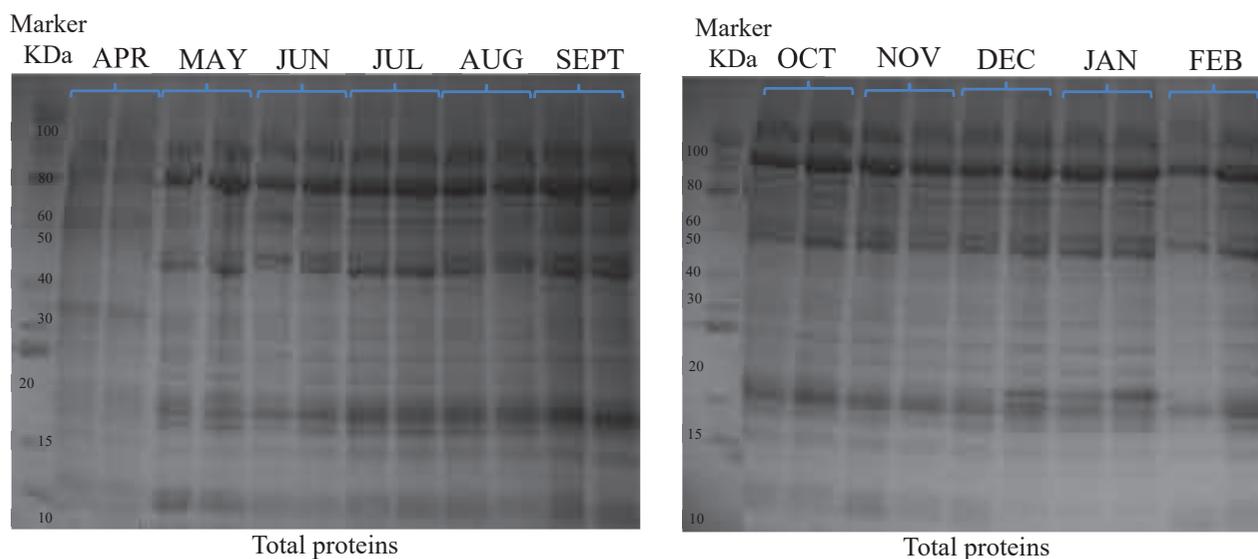


Figure 4. Electrophoretic pattern of total proteins extracted from *P. dubium* from April 2014 to February 2015. Intensity of the bands represents concentration of total proteins in their respective molecular weight (KDa).

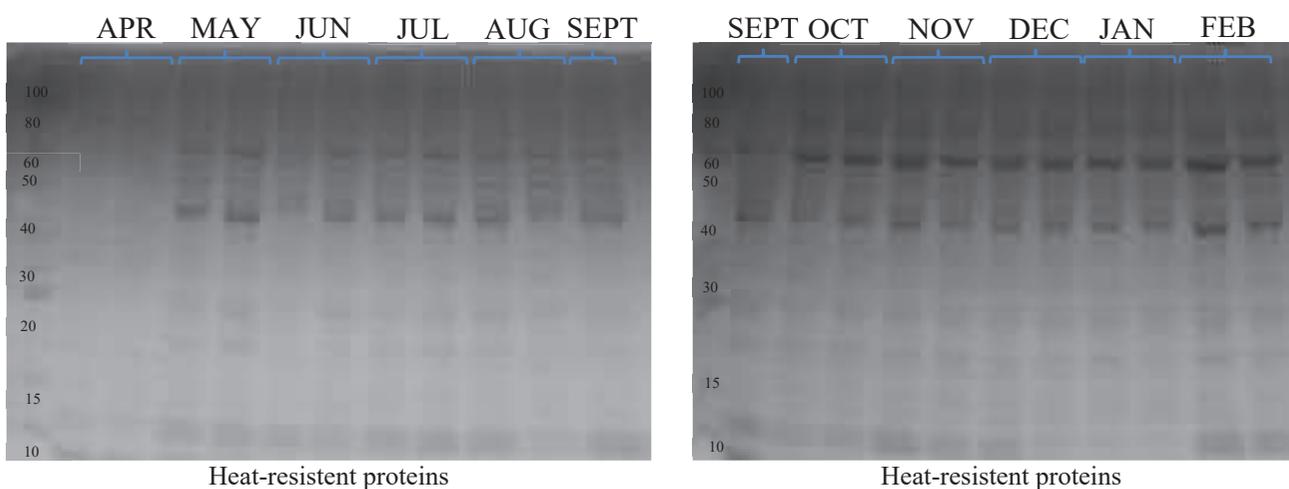


Figure 5. Electrophoretic pattern of heat-resistant proteins extracted from *P. dubium* from April 2014 to February 2015. Intensity of the bands represents concentration of heat-resistant proteins in their respective molecular weight (KDa).

were observed throughout the gel (10-100 KDa) when total proteins are analyzed (Figure 4); however, for the heat-resistant proteins, greater concentrations were observed between 40 and 80 KDa (Figure 5). During the period of seed maturation (between the collections of April and May 2014), the accumulation of total proteins and heat-resistant proteins is observed, shown by less visible bands in the gel in this period, and, as already observed by physiological data, the seeds had not yet reached physiological maturity, which occurred in June 2014.

Metabolic events that occur during maturation have the purpose of preparing the seed for its germination and, consequently, development of the seedling (Thomas, 1993;

Kalemba and Pukacka, 2008). The increase in the concentration of proteins and other compounds during development and their maintenance after physiological maturity is important, especially when dealing with seeds that remain for a long period apart from the mother plant, as in serotinous species (Santini and Martorell, 2013). Maintenance of protein content is associated with low metabolic activity, as a result of reduction in moisture content during development and by the low activity of proteases, and by maintenance of the apparatus that impedes damage caused by reactive oxygen species and free radicals (Murthy et al., 2003).

As the main components of seed reserves of various leguminous plants, proteins are used for maintenance of seed

vigor (Wang et al., 2015a; Wang et al., 2015b; Wu et al., 2011) until their dispersal, as of which time, reduction in protein content may occur (Kalemba and Pukacka, 2008). However, such a response cannot be observed in this study, which would indicate low metabolic activity and, consequently, maintenance of the protein concentration in the aerial seed bank. Another indication of low metabolic activity and low consumption of reserves would be the maintenance of seed vigor after formation of the aerial seed bank up to the last period of evaluation in February 2015.

Conclusions

P. dubium is a species that forms an aerial seed bank of short serotiny.

During the period that fruits remain in the aerial seed bank, there are no changes in the physiological quality of the seeds of the species *P. dubium*.

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