



Radiographic image analysis of *Anacardium othonianum* Rizz (anacardiaceae) achenes subjected to desiccation

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ABSTRACT. Studies evaluating the internal morphology and seed quality of native species are essential for successful conservation programs. Our aim was to verify the efficiency of X-ray imagery in evaluating cashew-tree-of-the-cerrado (*Anacardium othonianum* Rizz.) achene viability after desiccation. The achenes were collected at 12% water content (w.b.) and dried in silica gel until they reached 10, 8, 6, and 4% (w.b.). The fruit morphology and the quality of the seeds were evaluated by X-ray test together with vigor, electrical conductivity and emergence tests. Achenes with different water contents were exposed to an X-ray machine at 18 kV for 11 s and were thereafter submitted to emergence tests. The images were analyzed, and the achenes were classified based on internal morphology as completely full, malformed, or empty. These results were compared to those from the emergence tests. The statistical design was a complete randomized factorial (5 x 3). Desiccation to 4% (w.b.) did not damage or modify the internal structures. X-ray was efficient in evaluating the internal morphology and detecting achene quality, making it possible to remove empty and abnormal fruit and form vigorous seed lots, reducing the cost of storage and bedding plant production for this native species.

Keywords: X-Ray, fruit tree, cerrado, desiccation, internal morphology.

Análise de imagens radiográficas em aquênios de *Anacardium othonianum* Rizz (anacardiaceae) submetidos à secagem

RESUMO. Estudos referentes à avaliação da morfologia interna e da qualidade de sementes de espécies nativas são de fundamental importância para o sucesso dos programas de conservação. Objetivou-se com este estudo verificar a eficiência do teste com raios X na avaliação da viabilidade de aquênios de caju-de-árvore-do-cerrado (*Anacardium othonianum* Rizz.) submetidos à secagem. Para tal, aquênios de *A. othonianum* Rizz foram coletados com 12% (b.u.) e submetidos à secagem em sílica gel até a obtenção dos teores de água de 10, 8, 6 e 4% (b.u.). Os frutos foram avaliados quanto à morfologia interna e qualidade física pelo teste de raios X, e o vigor das sementes pelos testes de germinação, condutividade elétrica e emergência de plântulas. Para a obtenção das imagens radiográficas, aquênios com diferentes teores de água foram expostos à radiação em um aparelho de raios X digital "Faxitron HP", MX-20 à intensidade de 18 kV por 11 segundos, e posteriormente foram submetidos ao teste de emergência. As imagens radiográficas foram analisadas e os aquênios classificados de acordo com a morfologia interna em três categorias: Cheios, mal formados e vazios. O delineamento estatístico utilizado foi o inteiramente casualizado em esquema fatorial (5 x 3). A secagem dos frutos até 4% (b.u.) não danificou ou alterou as estruturas internas dos aquênios. O teste de raios X é eficiente na avaliação da morfologia interna e na detecção da qualidade de aquênios de *A. othonianum* Rizz, possibilitando a separação de aquênios vazios ou com anormalidades embrionárias e permite a formação de lotes de sementes mais vigorosas, reduzindo os custos tanto de armazenamento quanto os programas de produção de mudas desta espécie nativa.

Palavras-chave: raios X, frutos, cerrado, secagem, morfologia interna.

Introduction

Anacardium othonianum Rizz, commonly known as the cashew-tree-of-the-cerrado, cajuzinho, or cajú, is a native species from the Brazilian Cerrado belonging to the Anacardiaceae family. It is 1 well known

and appreciated in the Cerrado of the Central Brazilian Plateau, inhabiting the "campo sujo" and the "cerradão" areas (Mendonça et al., 1998). It is the main cashew of economic importance in this region and its name pays homage to Dr. Othon Xavier de Brito

Machado, the first botanist to describe the cashew tree species from the Cerrado (Rizzini, 1969).

This species differs from all of the other species of cashew found in the Cerrado domain due to its high stature, reaching up to 4 m in height and 3–4 m in diameter in the canopy and because it predominantly spreads via seeds. The flowers of the cashew trees are hermaphroditic and unisexual, although the males appear at the beginning of flowering, and the hermaphroditic flowers appear near the end. The fruit is an achene, whose peduncle develops into a pseudo-fruit that can be 2–4 cm in length and 2–3 cm in diameter, with a weight of 5–12 g and coloration ranging from yellowish to reddish (Ferreira, 1973; Paula & Heringer, 1978). The combination of the fruit (capsule) and the pseudo-fruit constitutes the “double fruit” characteristic of the genus. The achenes also vary in size and shape between individuals in the same population (Bessa, Silva, Moreira, Teodoro, & Soares, 2013). It is an economically and socially important species to the local population, being a pseudo-fruit consumed *in natura* or as juice, liqueurs, ice cream, or sweets (Agostini-Costa, Faria, Nave, & Vieira, 2006). In addition, the achenes are also roasted to extract the nuts, which have high nutritional and energetic value and are rich in proteins, lipids, calcium, iron, and zinc. As such, this fruit is of great interest in the food industry (Sousa, Fernandes, Alves, Freitas, & Naves, 2011).

The native forest species undergo high rates of predation, insect attack, empty fruit, and deficiency in the formation of the embryo, all of which compromise the performance of the seed and the establishment of seedlings in the field (Gomes, Martins, Martins, & Junior, 2014). Studies on the quality of the native forest seeds represent only 0.1% of the species found in the Rules for Seed Testing (Brasil, 2009). Thus, it is necessary to promote studies that can provide information on the physical and physiological quality of seeds. Recovery programs for degraded areas place a large demand for seedlings of native species, leading to an increased number of studies and the need for the development of efficient and rapid methods that evaluate the viability of forest seeds (Matos, Martins, & Martins, 2009). Thus, the analysis of images, mainly obtained through X-rays, has been successfully utilized to elucidate various aspects of the behavior and morphology of native forest seeds. This technique consists of radiographing seeds with the goal of assessing the internal morphology and

connecting that to seed performance (Gomes Junior, 2010; Silva, Freitas, Cícero, Marcos Filho, & Nascimento, 2014).

The X-ray test was developed by Simak and Gustafsson (1953) as a method to evaluate the quality of seeds of some conifers and was identified in the 1980s, by the International Seed Testing Association (ISTA), as a means to detect empty, full, and mechanically damaged seeds or those infested by insects. It is a rapid and non-destructive testing method, which allows the evaluation of the internal morphology and the physical quality of the seeds, as well as the identification of damaged and empty seeds. This technique has been successfully employed in quality control programs because it allows for the selection of undamaged seeds to form lots with high physiological quality and vigor, which is favorable to storage success and the conservation processes of seeds in germplasm banks. It is a highly precise method where the seeds can be examined individually in amplified images, enabling, in detail, the identification of the location and extensiveness of damaged or altered structures. In addition, being a non-destructive method, the seeds under analysis can be subjected to physiological tests (germination and vigor), thus allowing the creation of associations between the damage or changes observed and the subsequent damage caused to the physiological potential of the seeds (Carvalho, Carvalho, & Davide, 2009; Cicero, 2010; Masetto, Davide, Silva, & Faria, 2007).

Given the economic and conservation interests of this Cerrado tree species, it is necessary to intensify the research on the assessment of the quality of the seeds. Thus, the X-ray evaluation of the internal morphology of seeds is a promising technique, allowing the enhancement of the quality of the seed lots by knowing their physical and physiological attributes. The radiographic imaging analysis technique has been used with success in several native forest species, including the seeds of *Eugenia pleurantha* (Masetto et al., 2007), *Cecropia pachystachya* Trec. (Pupim, Novembre, Carvalho, & Cicero, 2008), *Cedrela fissilis* (Masetto, Faria, & Queiroz, 2008), *Tabebuia heptaphylla* (Amaral, Martins, Forti, Cicero, & Marcos Filho, 2011), *Xylopia aromatica* (Socolowski, Cicero, & Vieira, 2011), and *Syagrus romanzoffiana* (Cham) Glassman (Sturião, Landgraf, & Rosa, 2012), and in the morphological characterization of embryos of *Tecoma stans* L. JUSS. ex KUNTH (Socolowski & Cicero, 2008), the measurement of internal free areas in seeds of *Acca sellowiana* (Silva, Sarmiento, Silveira, Silva, & Cicero, 2013), and the viewing of the internal morphology of seeds of *Terminalia argentea* (Gomes et al., 2014).

Given the scarcity of information on the internal morphology and the effects of desiccation on achenes of species in the genus *Anacardium*, the objective of this study was to verify the efficiency of X-ray testing in evaluating the morphology and viability of *A. othonianum* achenes subjected to desiccation.

Material and methods

The current study was developed in the Seed Laboratory of the Federal Institute at Rio Verde Campus (Goiás State) and in the Seed Analysis Laboratory of the Department of Agriculture at the Federal University at Lavras, Minas Gerais State, Brazil.

Collecting and Processing:

Ripe fruits were collected at the Poçoões Farm (Fazenda Poçoões) in the municipality of Diorama, Goiás (16°05'41.06"SL, 51°12'58.88"WL). The fruit were collected in a grassland area where there were five grown trees. The achenes were manually separated from the pseudo-fruit, immersed in a solution of 5% sodium hypochlorite (a commercial solution with 2.5% active chlorine) for 3 min. Thereafter, they were washed with distilled water and placed into plastic trays covered with paper towels where they remained until the excess superficial water was removed.

Desiccating until the desired water contents were achieved: The initial water content of the achenes was determined by the oven method at $105 \pm 3^\circ\text{C}$ for 24h (Brasil, 2009 adapted), using four subsamples of 10 achenes, and the results were expressed in percentage (% w.b.). After determining the initial water content, the achenes were desiccated using silica gel until the designated water contents were achieved. The achenes, in hermetically closed plastic containers, were placed in direct contact with a uniform layer of silica gel at $25 \pm 4^\circ\text{C}$. The mass of the achenes was verified periodically using a precision scale until the mass of water content reflected 10, 8, 6, and 4% w.b. Water loss in achenes was determined using the following formula (Sacandé et al., 2004):

$$Pf = Pi \cdot \left(\frac{100 - TAi}{100 - T Af} \right)$$

In which Pf is the final mass of the sample (g); Pi is the initial mass of the sample (g); Tai is the initial water content of achenes (% w.b.); T Af is the level of desiccation (% w.b.).

Soon after desiccation levels were reached, the achenes had their internal morphology evaluated by X-ray, and their physiological quality and seed vigor estimated.

X-ray test and the analysis of radiographic images of the achenes: Achenes with different water contents were packed in airtight cartons and sent to the Seed Analysis Laboratory (LAS) at the Federal University of Lavras (UFLA). Every treatment had ten replicates of 20 achenes radiographed to maintain water content. The achenes were arranged in individual cavities in Styrofoam panels and radiographed using the 'Faxitron HP,' model MX20. The radiation intensity automatically adjusted by the equipment was 18 kV, and the exposure period was 11 s. Digital radiographic images were stored in the hard drive of a Core 2 Duo computer (4 GB of RAM memory, 500 GB hard disk). After image analysis, the achenes were classified into three categories, based on internal structures: Full achenes with all the internal cavity filled by the cotyledons and embryo axis without damage; Malformed achenes with some abnormality in the morphology of the cotyledons or embryonic axis; and Empty achenes with the internal cavity fully translucent or having less than 50% of the tissues essential for germination.

After radiographic image analysis and the classification of achenes into three classes, the emergence test was conducted in the LAS at the Goiano Federal Institute - Campus Rio Verde, Goiás State. For the emergence test, the achenes were sown in plastic trays at a 3 cm depth in a washed sand substratum. The substratum was irrigated three times every day, and the trays were maintained in a greenhouse with an average temperature of $28.5 \pm 5^\circ\text{C}$ and relative humidity of $68 \pm 8.5\%$ during the evaluation period. The final evaluation occurred 30 days after the sowing time. The results of the X-ray test were interpreted by comparing the classes with the results from the emergence test. The achenes of *A. othonianum* Rizz had an intense fungi infestation during germination on the substrate-type "germitest" paper, which could affect the interpretation of the results. Because of this, we decide to grow the achenes from the X-ray test in washed sand.

The experiment was conducted in a complete randomized design using a (5 × 3) factorial with five water contents and three classes from the X-ray. The data were submitted to an analysis of variance (ANOVA) with 5% probability and polynomial regression. All analyses were performed with SISVAR statistical software, version 4.6.

Evaluation of the seed quality and vigor: The following seed quality and vigor tests were compared to the X-ray images to verify the efficiency of the radiographic images. Germination test: The achenes were sown on paper rolls using three sheets of “germitest” paper. The substrate was moistened with distilled water until the moistened paper was 2.5-fold the dry weight of the paper (Brasil, 2009). Four replicates of 25 achenes were assessed. The rolls were maintained in the germinator at a temperature of 30°C for 30 days. Daily evaluations were performed until complete stabilization in order to calculate the germination index (IG) based on the Maguire (1962) recommendation. The electrical conductivity test: The electrical conductivity test had four subsamples of 15 achenes for every treatment. The mass of the achenes was determined by an electronic scale with an accuracy of two decimal places. The achenes were placed in plastic cups containing 75 mL of deionized water and soaked for a period of 24h in the biochemical oxygen demand (BOD) chamber-like germinator regulated at 25°C. Next, the electric conductivity of the soaking water was determined by the Technal digital conductivity meter, model TEC-4 MP, and the results were expressed in $\mu\text{S cm}^{-1} \text{g}^{-1}$, based on the methods described by Vieira, and Krzyzanowski (1999). Emergence test: The emergence test was performed in plastic containers containing washed sand as the substratum. Four replicates of 25 achenes assessed with X-ray were sowed at a 3 cm depth. Daily counts and seedling emergence above 1.5 cm determined the seedling emergence index (IE). Forty-five days after sowing, the percentage of normal seedlings were counted (seedlings with all the essential structures and without any damage).

Statistical design: The experimental design was a complete randomized design, with four replicates. These data were submitted to ANOVA and polynomial regression. All analyses were carried out using the SISVAR statistical software, version 4.6. The polynomial regressions were adjusted by the software Sigma Plot®10.0.

Results and discussion

X-ray test and the analysis of radiographic images of the achenes

Water content is an important factor when implementing the X-ray test because it can directly affect the quality and clarity of the radiographic images. However, in this study, there were no differences in sharpness or quality of the achenes images with the different water contents. This can

be explained by the low initial water content of the achenes (12% w.b.), which favored sharp images. For example, for sweet pepper seeds, a minimum of 12% water content was necessary for sharper radiographic images (Gagliardi & Marcos-Filho, 2011).

The exposure of the achenes to 18 kV radiation for 11 s allowed the visualization of the internal morphology of the achenes of *A. othonianum* Rizz. and the identification of the integument, cotyledons, and the embryonic axis (Figure 1).

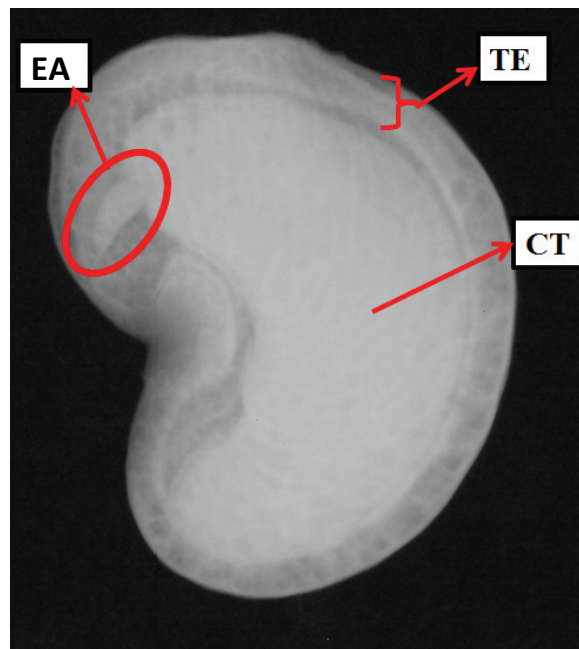


Figure 1. Radiographic image of an achene of *Anacardium othonianum* Rizz, highlighting the embryonic axis (EA), tegument (TE), and the cotyledons (CT).

Through radiographic image analysis, it was possible to evaluate the internal morphology of achenes and establish three classes: filled achenes (fully formed), malformed achenes (or damaged), and empty achenes (Figure 2).

For malformed achenes, it was possible to identify different points of malformation, such as damage to the cotyledons (Figure 2B) and damage to the cotyledons and in the region of the embryonic axis (Figure 2C).

In the ANOVA, for the percentage of achenes of *A. othonianum* Rizz. with different levels of water and in each category of the X-ray test, significance differences were detected among the categories, but no significant differences were detected among water content values or with the interaction between the factors (Table 1).

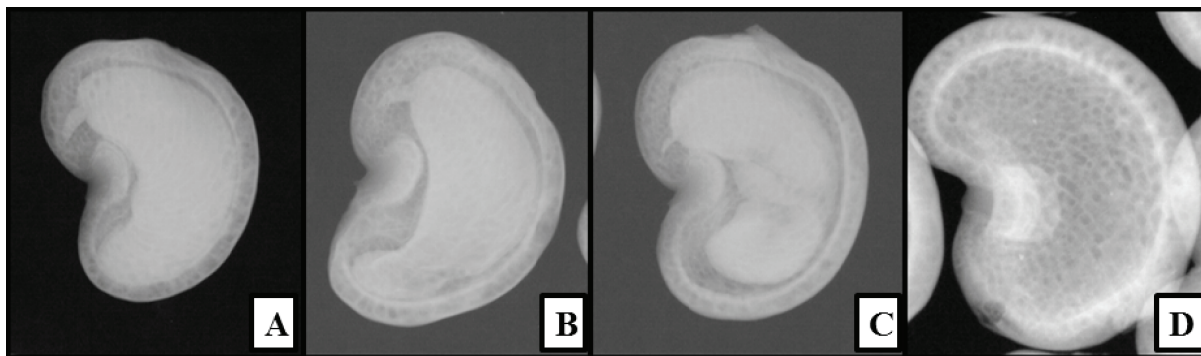


Figure 2. Achenes of *Anacardium othonianum* Rizz. classified as full (A), malformed (B - C), and empty (D) using the radiographic image analysis.

Table 1. Analysis of variance for the percentage of achenes of *Anacardium othonianum* Rizz. with different contents of water in each class of the X-ray test, considering the factors, levels and classes, as well as their interactions.

| Source of variation | GL | SQ | QM | F | Sig |
|---------------------|----|--------------|-------------|------------------------|--------|
| Water content | 4 | 524.80042 | 131.20010 | 1.0153 ^{ns} | 0.4097 |
| Classes | 2 | 113270.81033 | 56635.40517 | 438.2966 ^{**} | 0.000 |
| Content X Classes | 8 | 1073.41883 | 134.17735 | 1.0384 ^{ns} | 0.4223 |
| Residuals | 45 | 5814.76885 | 129.21709 | - | - |

**p < 0.01; ^{ns}Non significant (p ≥ 0.05).

With respect to classes, regardless of the water content of the achenes, over 93% were classified as full and fully formed, demonstrating the high vigor of the batch of achenes evaluated (Table 2).

Table 2. Classification of the achenes of *Anacardium othonianum* Rizz. with different contents of water based on radiographic image analysis.

| Classes of X-ray test | Percentage of achenes |
|-----------------------|-----------------------|
| Full | 93.30 a |
| Malformed | 1.11 b |
| Empty | 1.16 b |

*Means followed by the same letter in the column did not differ significantly by 5% using the Tukey test.

Full achenes were classified as the highest percentage of normal seedlings (97.97%) observed in 12% water content (w.b.), but decreased in response to a reduction of water content (Figure 3).

Achenes that were malformed or empty did not form normal seedlings. This result demonstrates the negative effect of the presence of achenes these classes have on a seed lot, significantly reducing the strength and potential of the analyzed batch.

Achenes classified as malformed or damaged had 100% abnormal seedlings for all water contents (Figure 4).

For full achenes, there was an increase in the percentage of abnormal seedlings in response to a reduction of water content, with a higher percentage of abnormal seedlings (5.16%) observed at the 4% water content (w.b.). Despite this fact, the dry silica gel of the 4% water content (w.b.) did not cause damage to the internal structures of the achenes and did not affect their physiological performance. Additionally, germination was not recorded for empty achenes.

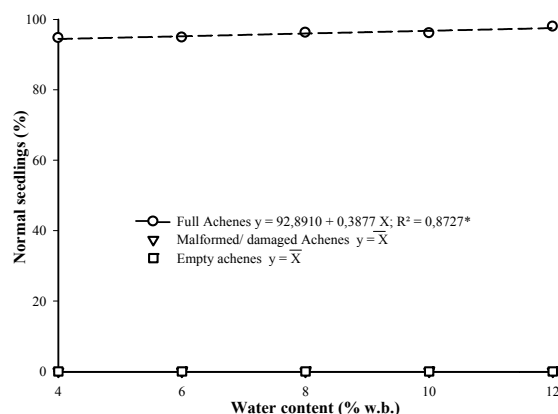


Figure 3. Percentage of normal seedlings (%) of *Anacardium othonianum* Rizz. with different levels of water (% w.b.) and classified based on radiographic image analysis. *p < 0.05.

While radiographic image analysis identified structural damage and mechanical cracking promoted by drying, even achenes with 4% water content (w.b.), demonstrated that the drying of the achenes on silica gel did not alter its internal morphology and did not reduce the embryo volume.

The X-ray test was efficient in evaluating the effects of desiccation on the diaspores of *Cryptocarya aschersoniana* Mez., demonstrating that an increase in the distance between the endocarp and the seed occurs as water content is reduced, reaching 0.65 mm when the water content of the seeds was reduced to less than 26%. With X-ray images it was observed that a reduction in the volume occupied in the embryonic cavity by the embryo occurs with a reduction in the water content in seeds of *Euterpe edulis* Martius (Cursi & Cicero, 2014). In addition, the radiographic image analysis was efficient in identifying cracks in rice seeds (cultivars IRGA 417 and IRGA 420) subjected to artificial desiccation, and it was possible to correlate the cracks with the occurrence of normal and abnormal seedlings in germination tests (Menezes, Cicero, Villela, & Bortolotto, 2012).

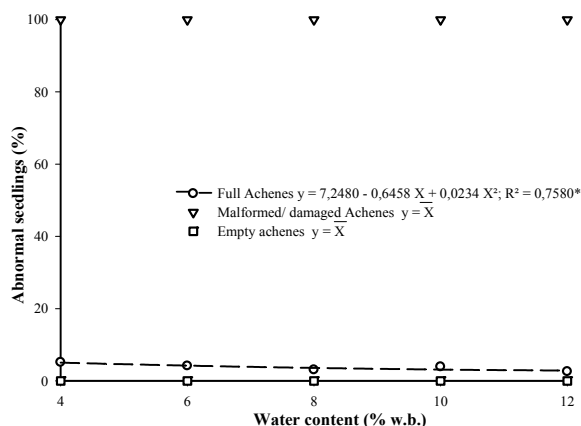


Figure 4. Percentage of abnormal seedlings (%) of *Anacardium othonianum* Rizz. with different levels of water (% w.b.) and classified based on radiographic image analysis. * $p < 0.05$.

In general, regardless of the analyzed water content, more than 93% of the achenes were classified as filled and exhibited no damage, resulting in a mean of 96% normal seedlings at the end of the emergence test (Figure 5).

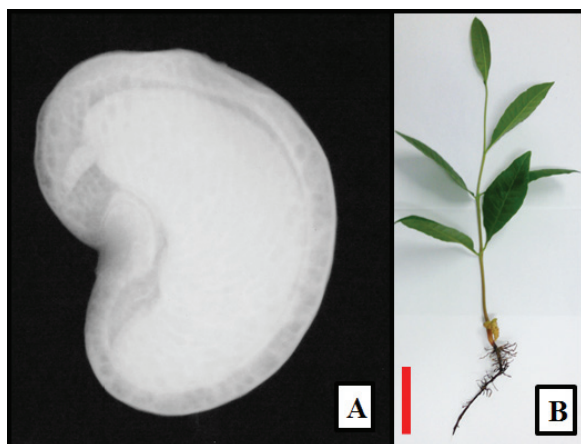


Figure 5. Achene of *Anacardium othonianum* Rizz. classified by image analysis as full and without any internal damage (A) and normal seedlings at the end of the emergence test (B). Bar: 2 cm.

Achenes classified as malformed, regardless of the water content, damage, or malformation, gave rise to 100% abnormal seedlings at the end of the emergence test (Figure 6).

Damage to the structure of the cotyledons was more common than damage to the embryonic axis. Damage by insect attack was not noted and larvae were not found in the interior of the achenes. For achenes classified as empty, there was no emergence, resulting in 100% dead or ungerminated achenes at the end of the emergence test (Figure 7).

The presence of damaged or empty seeds compromises the quality of seed lots and adversely affects the establishment of seedlings in the field.

Thus, radiographic image analysis was efficient in evaluating the internal morphology and the quality of achenes of *A. othonianum* Rizz., thus allowing the identification of damaged or empty achenes. In addition, results demonstrated that achenes classified under these categories by X-ray imaging should be removed from the seed lots intended for storage or production of seedlings, as they will not produce normal seedlings.

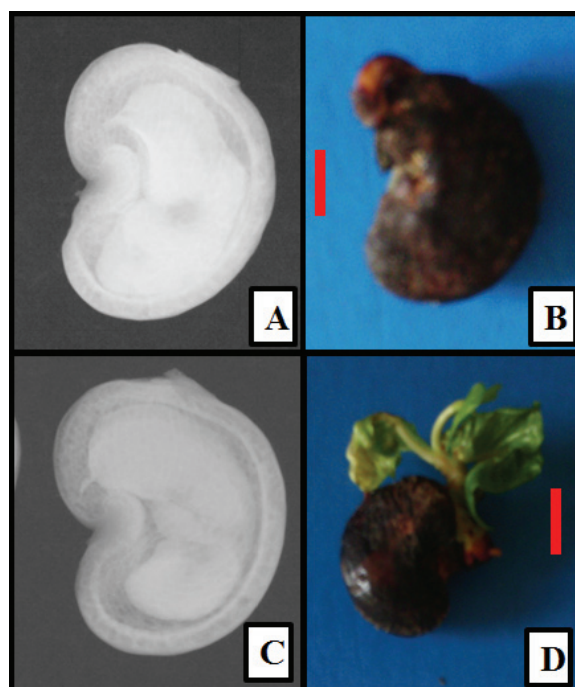


Figure 6. Radiographic image of achenes of *Anacardium othonianum* Rizz with malformations (A and C) and their respective abnormal seedlings at the end of the emergence test (B and D). A, achenes with 4% (w.b.); C, achenes with 12% (w.b.). Bar: 1 cm.

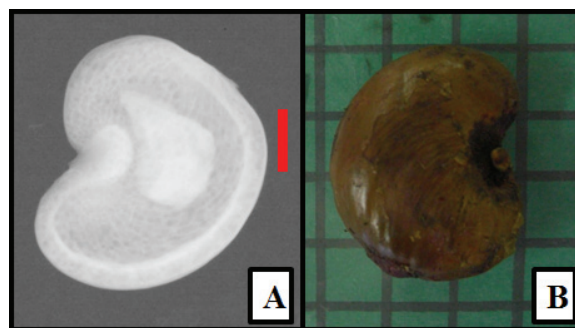


Figure 7. Radiographic image of an empty achene of *Anacardium othonianum* Rizz (A) and the respective image of a dead achene at the end of the emergence test (B). Bar: 1 cm.

It has been shown that X-ray imaging allows the improvement of the physical and physiological quality of seed lots by removing internally damaged, poorly formed, or empty seeds, as was observed for

Cecropia pachystachya Trec. seeds (Pupim et al., 2008), seeds of arboreal Lauraceae species (Carvalho et al., 2009), *Tabebuia heptaphylla* (Amaral et al., 2011), and *Terminalia argentea* (Gomes et al., 2014). It also allows for the identification of damaged or abnormal embryos and their removal from work samples, as observed in *Lithraea molleoides* (Machado & Cicero, 2003) and *Malpighia emarginata* D.C. (Nassif & Cicero, 2006). Finally X-ray imaging allows for the observation of structural changes during desiccation, as was observed for *Quercus rubra* L. seeds (Goodman, Jacobs, & Karrfalt, 2005), and the identification of damaged seeds caused by insect infestation, as observed in seeds of *Eugenia pleurantha* (Masetto et al., 2007) and diaspores of *Cryptocarya aschersoniana* Mez (Muxfeldt, Faria, Tonetti, & Silva, 2012).

The data obtained by the X-ray test reaffirmed the importance of radiographic image analysis to obtain more vigorous seed lots, allowing the separation of damaged or empty seeds or dispersion units, and enabling the use of high-quality seeds in conservation programs, *in situ* or *ex situ*.

Evaluation of the quality and vigor of the achenes

The percentage of germination was 100% for achenes with 12% (w.b.) initial water content and for the achenes with 10% (w.b.) water content. At 8%, there was a reduction in germination due to water loss in the achenes, decreasing to 85% germination for achenes with 4% (w.b.) water content (Figure 8).

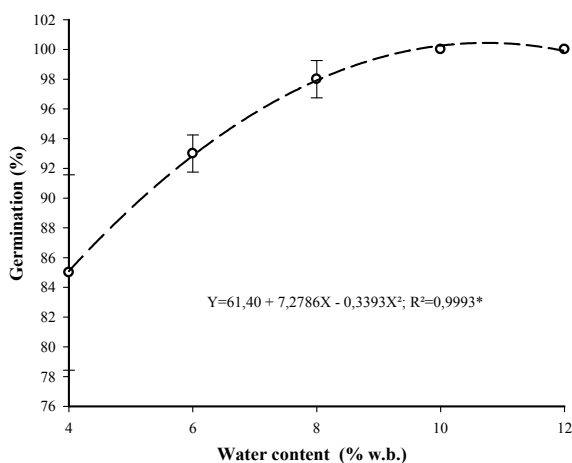


Figure 8. Germination percentage of achenes of *Anacardium othonianum* Rizz. with different water content. * $p < 0.05$. Bar: standard error of mean.

Despite the reduction observed in the percentage of germination, the germination value achieved for

achenes with ultra-low water content (4% w.b.) was still considered satisfactory. This result is consistent with the expectations of conventional seeds and highlights the high quality of the lot of achenes evaluated.

The germination speed index (IVG) followed the same pattern that was observed for germination percentage. The highest value was observed for achenes with 12% (w.b.) initial water content, with 2.04 achenes germinated per day of the evaluation. This value decreased as the water content of the achenes was reduced (Figure 9).

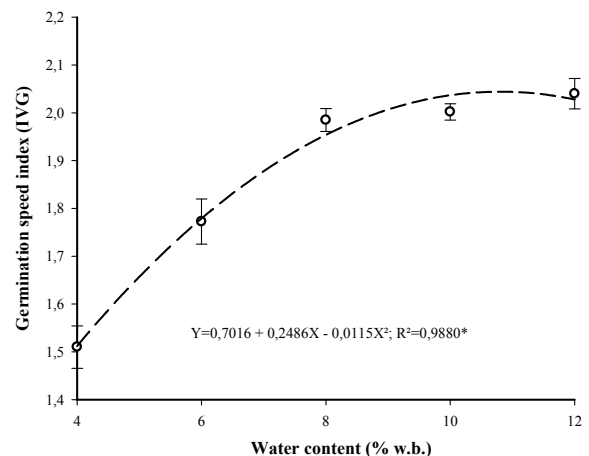


Figure 9. Germination speed index (IVG) of achenes of *Anacardium othonianum* Rizz. with different water content. * $p < 0.05$. Bar: standard error of mean.

There was a reduction in the electrical conductivity of the soaking water in response to the desiccation of achenes of *A. othonianum* Rizz. (Figure 10).

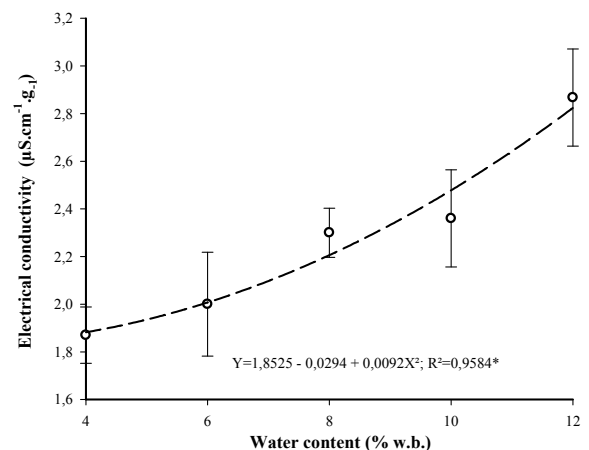


Figure 10. Electrical conductivity of soaking water of achenes of *Anacardium othonianum* Rizz. with different water contents. * $p < 0.05$. Bar: standard error of mean.

A similar result was found by Silva, Sales, Silva, and Ferreira (2013), who observed a reduction in the

release of exudates in achenes of *A. othonianum* Rizz subjected to desiccation in silica gel up to 4% (w.b.) water content. The disruption of the membrane system resulted in the loss of their semi-permeability and led to the leaching of essential metabolic components, marking the beginning of the process of seed deterioration (Golovina, Van, & Hoekstra, 2010; Santos, Menezes, & Villela, 2005). However, in conventional seeds, genes that express mechanisms of protection or repair of membranes are activated in response to the reduction in water content, preventing the occurrence of damage and contributing to increased tolerance to desiccation (Goyal, Walton, & Tunnacliffe, 2005; Illing, Denby, Collett, Shen, & Farrant, 2005).

Achenes with 12% (w.b.) initial water content reached 99.75% emergence. The desiccation of the achenes led to a reduction in the emergence percentage. However, even with a water content of only 4% (w.b.), 89% emergence was achieved (Figure 11).

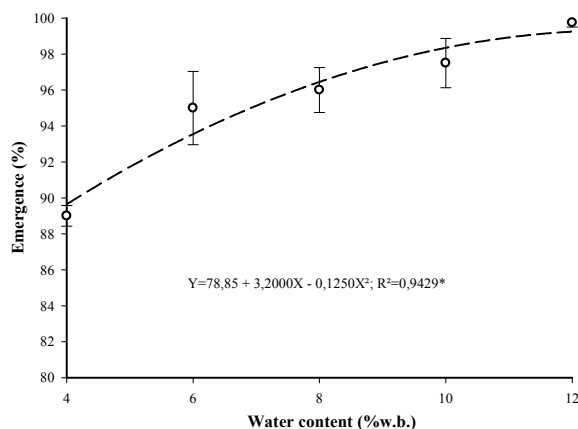


Figure 11. Percentage of the emergence of seedlings from achenes of *Anacardium othonianum* Rizz. with different water content.* $p < 0.05$. Bar: standard error of mean.

The emergence speed index (IVE) also underwent a reduction as achene water content decreased (Figure 12).

Despite the reduction observed in the germination and emergence of seedlings, the results obtained through the quality and vigor tests confirmed the pattern that was observed with the radiographic image analysis, which indicated that desiccation of achenes up to a water content of 4% (w.b.) did not cause damage or affect the formation of normal seedlings. In addition, the reduction in the percentage of germination and emergence observed by the quality and vigor tests was likely caused by the presence of malformed and empty achenes in the samples evaluated. It was not directly related to the reduction in water content because

achenes classified as full with 4% (w.b.) water content resulted in 94.74% normal seedlings.

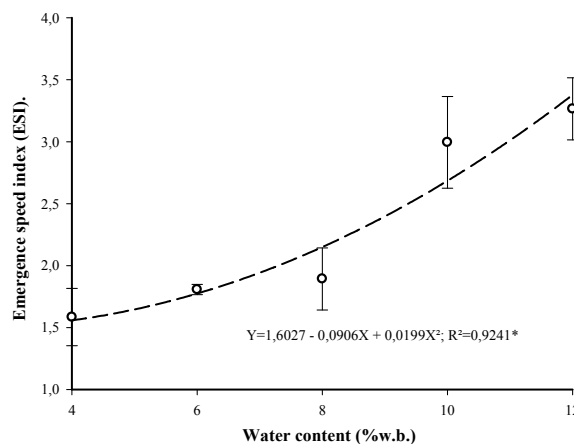


Figure 12. Emergence Speed index (IVE) of seedlings from achenes of *Anacardium othonianum* Rizz. with different water contents.* $p < 0.05$. Bar: standard error of mean.

Based on the results of this research, we can state that the X-ray test is an efficient tool for the evaluation of internal morphology and the quality of achenes of *A. othonianum* Rizz and can be adopted as a method of analysis in the conservation programs for this species.

Conclusion

Desiccation in silica gel up to a water content of 4% (w.b.) did not damage the internal structures of achenes, and no reductions were observed in the volume occupied by the embryo and the physiological performance of achenes subjected to desiccation.

The X-ray test was efficient in the evaluation of the internal morphology and the detection of the quality of achenes of *A. othonianum* Rizz, thus enabling the removal of empty achenes and those with embryonic abnormalities. This allowed for the creation of more vigorous lots, which will reduce storage costs and enhance the production programs for this native species.

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