Evaluating Seed Quality of *Amburana cearensis* Using X-Ray Scanning

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ABSTRACT

Legume seeds are commonly damaged by mechanical trauma invisible to the human eye, making other testing methods such as X-ray scanning useful for injury evaluation. The present study used X-ray and germination testing to detect alterations in the internal morphology of *Amburana cearensis* seeds and to relate their effects to seed quality. *Amburana cearensis* seed lots were collected from 8 locations. Four replicates of 25 seeds were used from each seed lot. Following radiographic analysis, individually identified seeds were placed between moist paper towels and incubated for 12 d at 30 °C and a 12-h photoperiod, and their germination parameters evaluated. It was possible to use X-rays to identify the different types of internal tissues of *A. cearensis* seeds, as well as detect the presence of morphological and physical damage. Radiographic analysis proved to be an effective method of monitoring *A. cearensis* seed lot quality. This non-destructive analysis allowed for the prediction of seedling performance and could ensure high-quality seed standards for use in precision agriculture.

INTRODUCTION

Amburana cearensis (Allemão) A.C. Smith is an arboreal forest species that can grow up to 10 m tall in the Caatinga (dryland) regions of Brazil (Corrêa, 1978; Lorenzi, 2002), and up to 20 m in forest zones (Lorenzi, 2002). The seeds and bark of this species are commonly used in folk medicine as emmenagogues, anti-spasmodic and antirheumatic agents, as well as in the treatment of respiratory afflictions such as bronchitis, colds, and flu (Braga, 1976; Matos et al., 1992; Lorenzi and Matos, 2002). The wood of *A. cearensis*, popularly known as "cerejeira", is valued for its durability and locally used in carpentry for making furniture, paneling, counter tops, sculptures, cooperage (Lorenzi, 2002).

Harvested seeds of forest species are often damaged by insects, fungi and other means (Battisti et al., 2000; Machado and Cicero, 2002), so rapid and precise identification of problems associated with low germination is essential in seed testing. Available methodologies include X-ray scanning, recommended by many researchers as a promising technique for efficiently evaluating seed quality (Bino et al., 1993; Craviotto et al., 2002; Machado and Cícero, 2003; Oliveira et al., 2003; Oliveira et al., 2004; Nassif and Cícero, 2006; Masetto et al., 2008; Pupim et al., 2008; Carvalho et al., 2009; Melo et al., 2009). X-ray

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testing is a simple, rapid, and non-destructive method (Machado and Cicero, 2003) that does not compromise seed viability, since only low radiation doses are utilized (Bino et al., 1993), making additional testing of the same seed possible. Based on these parameters, X-ray testing was recommended by ISTA (1996) and presented in a separate chapter in the Brazilian *Rules for seed analysis* used to determine seed quality (Brasil, 2009).

Interpretations of X-ray analyses and comparisons with results of parallel germination tests allow researchers to relate the integrity of the seeds' vital parts to physiological potential (Pinto et al., 2009). For the forest species *Lithraea molleoides* (Vell.) Engl. (aroeira-branca), morphologically abnormal embryos yielded abnormal seedlings or seeds that did not germinate (Machado and Cicero, 2003), and embryos from *Peltophorum dubium* (Spreng.) Taub. (canafístula) seeds with more than 50% of their surface area damaged did not germinate at all (Oliveira et al., 2003). Other workers demonstrated that totally formed *Cecropia pachystachya* Trécul (embaúba) seeds used in germination tests gave rise to 67% normal seedlings, while all of the partially formed seeds resulted in abnormal seedlings or simply did not germinate at all (Pupim et al., 2008). Likewise, seeds of forest species of Lauraceae with visibly damaged embryos or malformed cotyledon tissues were non-viable in germination tests (Carvalho et al., 2009).

A detailed knowledge of internal seed morphology of a given species allows for reliable seed quality evaluation using vigor tests, such as the tetrazolium test. For little-known species, as are many forest trees, characterization of internal seed morphology by X-ray testing will allow an evaluation of seedling normality and germination success (Gomes-Junior, 2010), establishing relationships between observed internal damage and loss of seed quality (Dell' Aquila, 2009).

X-ray techniques can be very useful in evaluating the internal morphology of seeds, but test methodologies must necessarily be adapted to the species under study. As such, the present research was carried out to evaluate the possibility of using X-ray testing to identify damaged or abnormal *A. cearensis* seeds, and to relate the effects of these anomalies on germination success.

MATERIALS AND METHODS

The study was carried out at the Imaging Analysis and Seed Analysis Laboratories of the Crop Science Department, College of Agriculture "Luiz de Queiroz," University of São Paulo, Piracicaba, SP, Brazil. Eight seed lots of *A. cearensis* were used from eight different locations (Table 1). The collections were made in September of 2010 and the experiments run in January of 2011, between which times the seeds were held in a refrigerator in sealed aluminum foil packages, following the recommendations of Guedes et al. (2010b).

Seed moisture content (FW basis) was determined using four subsamples of 25 seeds from each lot, dried in an oven at 105 ± 3 °C for 24 h (Brasil, 2009).

X-ray testing

X-ray testing was carried out with four replications of 25 seeds from each lot. To obtain the radiographs, seeds were placed on a strip of transparent double-

TABLE 1. Location of collection sites (municipalities) of different seed lots of *Amburana cearensis* used for X-ray and germination tests.

Lot	Municipality of origin – State	Geographic Coordinates
L_1	Serra Negra – Rio Grande do Norte	S 06° 40′13.9″/ W 37° 25′55.8″
L_2	Serra Negra – Rio Grande do Norte	S 06° 39′08.2″/ W 37° 29′2.2″
L_3	Serra Negra – Rio Grande do Norte	S 06° 39′28.5″/ W 37° 26′45.8″
L_4	Santa Gertrudes – Paraíba	S 06° 56′34.8″/ W 37° 24′01.5″
L_5	São Bentinho – Paraíba	S 06° 51′48.2″/ W 37° 44′07.6″
L_6	Patos – Paraíba	S 06° 58′55.9″/ W 37° 19′50.4″
L_7	Malta – Paraíba	S 06° 55′00.1″/ W 37° 28′46.7″
L_8	Aparecida – Paraíba	S 06° 54′40.9″/ W 37° 49′77.6″

faced adhesive tape and fixed onto one side of a 24 × 18 cm transparent, 2 mm thick acrylic plate. Seeds were then radiographed at a distance of 28.6 cm from the radiation source (Faxitron digital X-Ray, model MX-20DC12). Radiographs were used to determine the number of well-formed or damaged seeds, and the causes of observed damage. According to their observed internal morphology, seeds were classified as either full seeds (internal structures formed and totally filling the seed cavity), malformed seeds (only rudimentary internal seed structures), or seeds damaged by insects (presence of larvae). After the seeds, which were individually identified, were radiographed, they were evaluated for germination. Seeds were sown onto paper towels moistened with distilled water (3.5 times the dry weight of the paper) and maintained at 30 °C, under a 12-h photoperiod (Guedes et al., 2010a).

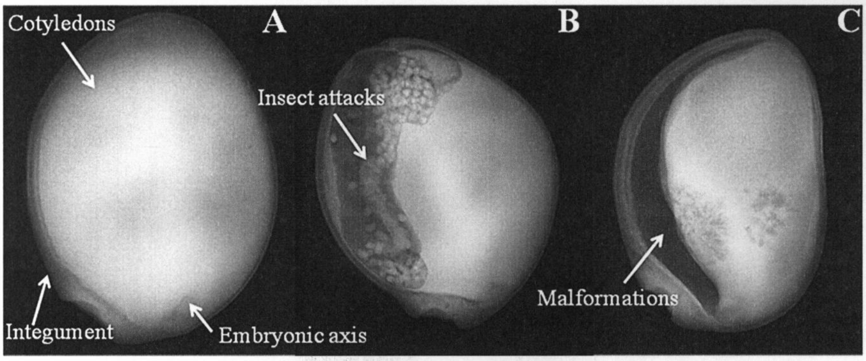
Seeds were evaluated 12 d after sowing and classified as either normal seedlings with a developed primary root and epicotyl, abnormal seedlings with poorly developed primary root and epicotyl, or ungerminated seed with no visible signs of development. Interpretation of the results was through comparing radiographic images of the seeds with results of the corresponding germination tests. The chi-square test ($\alpha \leq 0.05$), using SAS (SAS Institute, Inc., Cary, NC), was used to evaluate relationships among X-ray categories and germination results.

RESULTS AND DISCUSSION

Prior to the X-ray and germination tests, moisture content of the eight *A. cearensis* seed lots varied from 7–8%, which allowed for an adequate visualization of their internal structures. Seed moisture content can markedly influence the quality of X-ray images. Simak (1991) noted that moisture content of seeds affected their optical density, and that the less water they contained, the greater would be their optical density, allowing for better differentiation of their internal structures on radiograph images. Research undertaken with *Cedrela fissilis* Vell. likewise demonstrated acceptable results by X-ray imaging when seeds had water contents of 9% (Masetto et al., 2008).

It was possible to visualize the internal structures of seeds in most cases under the conditions of the test, including the integument, in clear and sharp images.

FIGURE 1. Radiographic images of *Amburana cearensis* seeds classified as full (A), attacked by insects (B), and malformed (C).



Sometimes, however, dark areas were observed in some parts of the seed images that could be confused with damaged areas, but these were identified as areas where the integument was wrinkled and less dense, allowing more X-ray penetration. Authors such as Simak (1991) and Bino et al. (1993) also reported that dark areas in radiographic images corresponded to areas of less dense seed tissue with greater X-ray penetration, while lighter areas represented denser tissues.

X-ray testing was able to identify the essential parts of *A. cearensis* seeds (Fig. 1), and characterize internal damage detrimental to their physiological potential. A significant relationship ($p \leq 0.01$) was found between the occurrence of damaged seeds and the percentages of abnormal seedlings and dead seeds (Table 2). Although seed lots did not differ, full seeds combined over all lots resulted in approximately 94% normal seedlings, seeds with insect damage resulted in 34% dead seeds and 8% abnormal seedlings, and malformed seeds resulted in 63% dead seeds and 16% abnormal seedlings (Table 2).

The positive associations between radiographic seed images and seedlings (or non-viable seeds) at the end of the germination test indicated that the majority of seeds from all lots classified as full resulted in normal seedlings (Table 2; Fig. 2, A1 and A2), or, in lesser numbers, abnormal seedlings (Fig. 2, B1 and B2). Lots L_1, L_2 and L_3 had the highest germination (physiological potential) while lot L_8 had the lowest physiological potential with the highest percentages of abnormal seedlings and dead seeds, consistent with the higher numbers of malformed seeds and those attacked by insects (Table 2).

As in the present work, X-ray testing was efficient in detecting differences in the physiological potential of *Eremanthus erythropappus* (DC.) MacLeish seeds of different origins, with low germination percentages attributed to the large number of empty achenes and malformed embryos (Feitosa et al., 2009). The authors concluded that X-ray techniques aided in evaluating the physical quality of seeds.

Malformed embryos, especially cotyledonary tissues, impeded the normal development of seedlings (Table 2). Malformations were observed using X-ray testing of seeds of other forest species such as *L. molleoides* (Machado and Cícero, 2003) and *P. dubium* (Oliveira et al., 2003), and indicated their lack of viability.

TABLE 2. Percentage of full, insect damaged and malformed *Amburana cearensis* seeds, as determined by X-ray scanning, with corresponding percentages of normal seedlings (NS), abnormal seedlings (AS) and dead seeds (DS) determined by germination tests, from eight different seed lots.

	Lot											
	L_1†			L_2			L_3			L_4		
X-ray category	NS	AS	DS	NS	AS	DS	NS	AS	DS	NS	AS	DS
	(%)											
Full	97	0	0	90	1	2	85	2	3	80	6	2
Insect damaged	1	0	1	4	0	1	4	0	2	3	1	1
Malformed	0	1	0	1	0	1	1	0	3	2	1	4
Total	98	1	1	95	1	4	90	2	8	85	8	7
χ^2	149.5**			15.2**			32.4**			33.9**		

	Lot											
	L_5			L_6			L_7			L_8		
X-ray category	NS	AS	DS	NS	AS	DS	NS	AS	DS	NS	AS	DS
	(%)											
Full	83	2	4	79	5	3	78	5	3	76	6	0
Attacked by insects	2	2	3	3	0	4	5	1	2	7	0	3
Malformed	2	0	2	2	0	4	0	2	4	0	2	6
Total	87	4	9	84	5	11	83	8	9	83	8	9
χ^2	33.6**			39.4**			38.9**			62.5**		

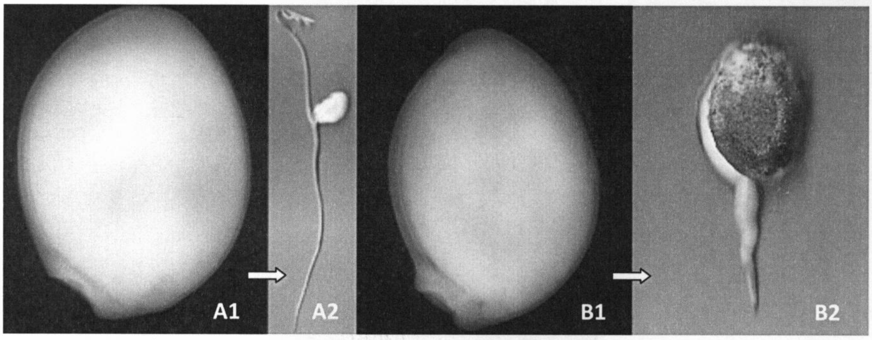
	Overall means		
	NS	AS	DS
	(%)		
Full	93.8	3.8	2.4
Attacked by insects	58.0	8.0	34.0
Malformed	21.0	15.8	63.2

†L_1, L_2 and L_3: seeds lots from Serra Negra – Rio Grande do Norte);
L_4, L_5, L_6, L_7 and L_8: seeds lots from Santa Gertrudes, São Bentinho, Patos, Malta and Aparecida, respectively, Paraíba State.
**Significant at the 5% probability level.

Therefore, discarding such seeds will improve a lot's germination percentage. Radiographic analyses of *C. fissilis* seeds detected malformed seeds (30%), empty seeds (13%), and full seeds (35.25%), the latter giving rise to mostly normal seedlings in germination tests, although there were also some dead seeds in this group. Masetto et al. (2008) reported that *C. fissilis* seeds classified as malformed gave rise to some abnormal seedlings, with the majority being dead.

It should be noted, however, that a small number of full seeds in all seed lots except L_1 resulted in abnormal seedlings or dead seeds based on the germination test (Table 2). These types of results are to be expected in germination tests, as X-ray images indicate if embryonic tissues are present or not, but do not establish any direct relationship with the physiological processes of those seeds. Even morphologically perfect seeds, as identified by X-ray testing, may

FIGURE 2. Radiographic (X-ray) and light images of *Amburana cearensis* seeds. Seeds that were full and showed no damage (A1 and B1) gave rise to both normal (A2) and abnormal seedlings (B2), but in different proportions.



be dormant, dead, or give rise to abnormal seedlings. Observed variability in seedling quality may be due to unfavorable germination conditions, the presence of seeds with latent infections, seeds that had died of natural causes and were in advanced stages of deterioration, or that had been inadequately stored (Van der Burg et al., 1994). Tests performed with *Malpighia emarginata* DC. demonstrated that seeds with otherwise morphologically normal embryos (as detected by X-rays) did not always yield normal seedlings. This may have been due to unfavorable conditions for germination, the presence of seeds with latent infections, seeds that had died of natural causes, or that had been inadequately stored (Swaminathan and Kamra, 1961; Van der Burg et al., 1994).

Damage produced by insect attacks, and affecting about 50% of the area of the embryo, was often a misleading symptom of the loss of physiological potential. Most of these seeds produced normal seedlings with visible damage only to their cotyledons (Table 2; Fig. 3, A1 and A2). On the other hand, seeds with insect damage affecting more than 50% of the seed, especially when the damage

FIGURE 3. Radiographic (X-ray) images of two *Amburana cearensis* seeds with damage due to insect attacks (A1 and B1), with one resulting in a normal seedling with visible damage only to the cotyledons (A2), and the other resulting in a dead seed (B2).

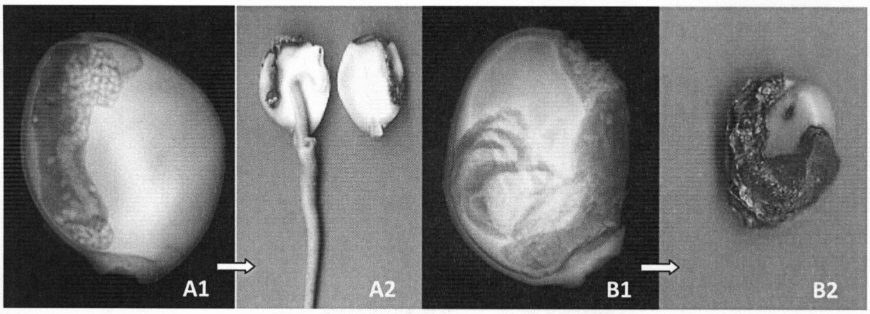
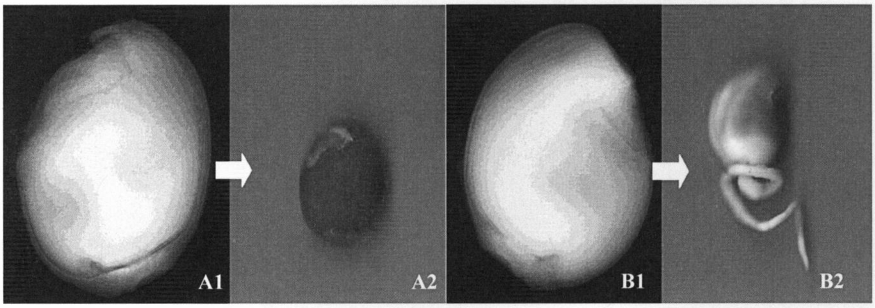


FIGURE 4. Radiographic (X-ray) and light images of two *Amburana cearensis* seeds, with X-ray images revealing malformations (A1 and B2), with one resulting in a non-germinating seed (A2) and the other in an abnormal seedling (B2).



occurred in vital areas such as the embryonic axis, rarely did germinate (Fig. 3, B1 and B2). As such, seed lots of *A. cearensis* in which damage to more than 50% of the total embryo is apparent using radiographs should be discarded, to guarantee the overall physical quality and physiological potential of the lot.

The presence of insects inside seeds of many forest species cannot be detected with the naked eye, but is often easily visualized using radiographic analysis, as was reported for *P. dubium* (Oliveira et al., 2003), *Platypodium elegans* Vogel (Souza et al., 2008), and *Nectandra* spp. (Carvalho et al., 2009). The presence of insects was also found to directly affect the germination potential of these species.

Amburana cearensis seeds that were classified as malformed did not usually germinate (Fig. 4, A1 and A2), although some yielded abnormal seedlings (Fig. 4, B1 and B2). These findings agree with the view that the internal morphology of a seed as visualized by X-ray imaging can indicate its potential viability (Oliveira et al., 2003). Although X-ray testing is not specific for viability monitoring, it can detect morphological deficiencies that indicate potentially unviable seeds (Simak et al., 1989). Marcos-Filho (1994) reported that tests revealing morphological aspects or physical characteristics of seeds were predictive of their vigor. As such, the results of the present research indicated that the classification of *A. cearensis* seeds based on radiographs was efficient in determining their viability, and that this technique is useful for identifying malformed units or those attacked by insects and that should be culled, thus contributing to optimizing germination and seedling yields.

In conclusion, X-ray testing was efficient for evaluating the internal morphology of *A. cearensis* seeds and allowed the assessment of the extent of internal damage. Insect damage and malformations, as detected by radiographs, will affect seed germination and reduce the physiological potential of seed lots of *A. cearensis*.

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