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Use of X-ray to evaluate damage caused by weevils in cowpea seeds

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ABSTRACT

In Brazil, the cowpea [*Vigna unguiculata* (L.) Walp], is important in the Northeast Region, where it is typically grown on family farms. The importance of the damage caused to the seed quality of this species by stored pests has been described in various studies. Using X-ray, it is possible to see the internal seed structures and identify possible changes and damage. The objective of this study was to identify the damage caused by the weevil [*Callosobruchus maculatus* (Fabr.)] by analyzing X-ray and evaluate its relationship to the physiological quality of the cowpea seed. Three cultivars were used (IPA-206, BRS-Pajeu and BRS-Potengi) and two lines (L 281.005 and L ESP 10). The samples were exposed to X-ray and germination test to determine the cause-effect relationship between weevil damage and seed germination. X-ray images were evaluated to determine damage severity and location in the seed. Seed damage classified as severe, located in the embryonic axis or in the cotyledons, resulted in abnormal seedlings or dead seeds. The X-ray test, therefore, is efficient for evaluating weevil damage in cowpea seeds and the damage caused to be associated with any resulting adverse germination effects.

Keywords: *Vigna unguiculata*, *Callosobruchus maculatus*, image analysis, internal damage.

RESUMO

Utilização de raios X na avaliação de danos causados por caruncho em sementes de feijão-caupi

No Brasil, o feijão-caupi [*Vigna unguiculata* (L.) Walp], tem destaque na Região Nordeste, sendo uma cultura típica da agricultura familiar. A importância dos danos causados por pragas de armazenamento em sementes da referida espécie, em relação à sua qualidade, tem sido evidenciada em vários trabalhos. Através de imagens de raios X é possível visualizar as estruturas internas da semente, identificando possíveis alterações e danificações. Dessa forma, esse trabalho teve o objetivo de identificar os danos causados por caruncho (*Callosobruchus maculatus*) e sua relação com a qualidade fisiológica das sementes de feijão-caupi, por intermédio da análise de raios X. Foram utilizadas três cultivares (IPA-206, BRS-Pajeu e BRS-Potengi) e duas linhagens (L 281.005 e L ESP 10). As amostras foram submetidas ao teste de raios X e ao teste de germinação, a fim de determinar a relação de causa e efeito entre os danos provocados pelo caruncho e a germinação das sementes. Nas avaliações das imagens de raios X foi considerada a severidade e a localização dos danos na semente. Para os danos classificados como severos, localizados no eixo embrionário e, ou nos cotilédones, as sementes originaram plântulas anormais ou as sementes estavam mortas. Portanto, o teste de raios X é eficiente para a avaliação de danos causados por caruncho em sementes de feijão-caupi, permitindo relacionar os eventuais danos com os prejuízos causados à germinação.

Palavras-chave: *Vigna unguiculata*, *Callosobruchus maculatus*, análise de imagens, danos internos.

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The cowpea [*Vigna unguiculata* (L.) Walp] is one of the most adapted, versatile and nutritious legumes among the cultivated species. It is grown on 11 million hectares in tropical and subtropical regions of Africa, Asia and the Americas (Singh *et al*., 2002). In Brazil, the cowpea is grown in the Northeast Region and is a typical crop of family agriculture (Freire Filho *et al*., 2005), being the principal subsistence crop in the semi-arid region (Silva, 2005).

The importance of the damage caused by the weevil, *Callosobruchus maculatus* (Fabr., 1775) (Coleoptera: Bruchidae), to the quality of cowpea seeds has been described by various researchers (Tanzubil, 1991; Quintinela *et al*., 1991; Gallo *et al*., 2002; Silva *et al*., 2005; Pereira *et al*., 2008). This damage is a consequence of the colonization of the interior of the seeds by the larvae, and causes weight loss, commercial devaluation, a reduction in nutritive value and product hygiene by the presence of feces, eggs and insects, and also reduced seed germination (Gallo *et al*., 2002).

This weevil species is considered the most important pest of cowpea during storage and can cause losses of up to 60% (Tanzubil, 1991). The adult beetle is about 3 mm long, with brownish marks on the elytra, which form a "X" at rest and it survives for around 5 to 8 days. The female lays an average of 80 eggs on the seed surfaces (Quintinela *et al*., 1991) and emerging larvae penetrate the seeds, feeding on the internal contents. Inside the seeds, the larvae turn into pupae and, after emergence, the adults break through the tissues and start the biological cycle once more (Silva *et al*., 2005).

The low energy X-ray test for determining the physical quality of seeds is recommended by ISTA (1996).

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This technique was first introduced into Sweden in the 1950s, to evaluate the seed quality of forest species (Simak & Gustafsson, 1953). According to Bino *et al*. (1993), when the X-ray passes through a seed, the radiation is absorbed to various degrees, depending on seed thickness, density, composition and the radiation wave length, thus creating a permanent image on radiographic film. It is a quick, non-destructive method for detecting well-formed and badly-formed seeds with mechanical damage and insect attack (ISTA, 1996) producing a permanent image on the radiographic film.

With this X-ray image, it is possible to observe the internal seed structures, identifying possible changes, especially in the embryonic axis (ISTA, 1995). According to Cicero & Banzatto Junior (2003), the use of X-ray image analysis to evaluate seed quality is promising; it constitutes a precise method where the internal seed structures can be examined from enlarged images to show details of the damaged area, its location and extent. Since this method is non-destructive, the seed can be submitted to physiological tests and a cause and effect relationship can be established.

The technique of image analysis with X-ray has been used to evaluate the physiological potential of *Cucumis melo* seeds (Kamra, 1966; Obando-Flor, 2003), *Pinus sylvestris* L. (Simak, 1991), *Solanum lycopersicum* (Liu *et al*., 1993), *Peltophorum dubium* (Oliveira *et al*., 2003) and *Cecropia pachystachya* (Pupim *et al*., 2008). This technique also detects damage from humidity (Pinto *et al*., 2007) and mechanical damage to soybean seeds (Obando-Flor, 2003) and corn (Cicero *et al*., 1998) and also damage by stinkbugs in common bean seeds (Forti *et al*., 2008).

Both the physical damage caused by insects, and also other types of damage, can be quickly identified by X-ray with promising results (Simak, 1980).

The objective of this study was to identify the damage caused by weevils and its relationship with the physiological quality of cowpea seeds, by the X-ray analysis technique.

MATERIAL AND METHODS

The research was done at the Seed Analysis Laboratory of the Crop Science Department, of São Paulo University in Piracicaba, São Paulo State, on the March-June period of 2009.

The seeds from three cowpea cultivars were used (IPA-206 (IPA), BRS-Pajeu and BRS-Potengi (Embrapa)) and two lines (L ESP 10 (UFRPE/IPA) and L 281.005 (IPA)). The seeds were obtained from the institutions who owned the genotypes and they had been kept in cold and dry storage for a year.

To detect internal damage, the seeds were exposed to X-ray, using four replications of 50 randomly chosen seeds for each of the three cultivars and two lines, respectively, totaling 200 X-rayed seeds per genotype. The seeds were placed in individual wells in a 100-well acrylic plate, kept in place with a transparent adhesive tape and identified according to their position on the plate. The X-ray was obtained by placing the acrylic plate with the seeds directly on an X-ray film (Kodak MIN-R EV 2000), measuring 18 x 24 cm, at a distance of 57 cm from the X-ray emission source (FAXITRON X-Ray, model MX-20), using an intensity of 25 kV and a 40-second exposure.

The films were developed in a processor (Hope X-Ray, model 319 Micromax) and then the X-ray images were scanned (Umax, model Power Look 1100) and transferred to a computer. Image analyses permitted the enlargement and evaluation of each X-rayed seed.

The germination test was done after the X-ray procedure, according to the Rules for Seed Analysis (RSA) (Brasil, 2009). For the germination test, the seeds (in groups of 10) were distributed equidistantly on the upper third of the substrate over a paper towel "Germitest". The paper sheets were moistened with a quantity of water equivalent to 2.5 times their weight. The paper rolls were then placed in a germinator and kept at 25°C. Five days after starting the test, the seedlings (normal and abnormal) and the dead seeds were photographed with a digital camera (Nikon, model D1) attached to a computer. Later, the images of the obtained seedlings (normal and abnormal) and dead seeds were compared, side-by-side, with the X-ray images so that any weevil damage could be associated with any changes in seed germination.

Damage was classified according to criteria described by Cicero *et al*., (1998) with some modifications. Each seed was given two rankings, the first referring to damage to the embryonic axis and the second, to any cotyledon damage. When there was no damage to the embryonic axis or to the cotyledons, that is, no damage was observed, a ranking of 1.1 was given to the X-ray image of the seed. Light damage is that observed in both the embryonic axis and the cotyledons, but which does not affect normal seedling formation; thus, a 2.2 was given to cases where the damage was classified as not being severe, either for the embryonic axis or for the cotyledons. On the other hand, damage was classified as severe when it was greater on the embryonic axis or cotyledons and interfered with seed germination, stopping normal seedling formation; damage to the cotyledons, next to the embryonic axis region, was also generally considered as severe, since it affects nutrient translocation to the embryonic axis during germination. Therefore, this last situation was given a ranking of 3.3, signifying severe damage to the embryonic axis and cotyledons.

The experimental design was completely random, with 5 treatments (three cultivars and two lines) and 4 replications of 50 seeds each. Analysis of variance was done using the F test and the Tukey test to compare means at a 5% probability level.

RESULTS AND DISCUSSION

The simultaneous examination of the internal seed images (X-ray), seedlings and dead seeds after the germination test permitted a diagnosis for each case, resulting in the establishment of a cause and an effect, as was done in common beans by Mondo *et al*. (2009).

Table 1 shows mean values in percentages for damage intensity detected by X-ray, caused by weevils to the embryonic axis region and, or the cotyledons, in the three cultivars and two lines analyzed.

Table 1. Mean values (%) for seeds with different damage rankings given for weevil damage to the embryonic axis and cotyledons in the 5 lots evaluated by X-ray (valores médios (%) de sementes com diferentes notas atribuídas aos danos causados pelo caruncho. no eixo embrionário e nos cotilédones. nos cinco lotes avaliados por meio de raios X). Piracicaba, USP/ESALQ, 2009.

Damage site	Genotypes	Damage ranking[1]		
		1	2	3
Embryonic axis	L ESP 10	100.0 a[2]	0.0 b	0.0 b
	IPA-206	63.5 b	10.5 a	26.0 a
	L 281.005	95.0 a	0.5 b	4.5 b
	BRS-Pajeu	98.5 a	0.0 b	1.5 b
	BRS-Potengi	94.5 a	1.0 b	4.5 b
CV (%)		4.82	39.81	40.48
Cotyledons	L ESP 10	100.0 a	0.0 c	0.0 c
	IPA-206	21.5 d	29.5 a	49.0 a
	L 281.005	84.0 c	9.5 b	6.5 b
	BRS-Pajeu	95.5 b	3.0 bc	1.5 c
	BRS-Potengi	88.8 bc	4.5 bc	7.0 b
CV (%)		5.71	46.20	13.38

[1]Damage rankings 1= Undamaged; 2= Light damage; 3= Severe damage; [2]In the column, for each site of damage, means followed by the same letter do not differ among themselves based on the Tukey test, at the 5% probability level (valores de dano 1= não observado; 2= dano não severo; 3= dano severo; [2]Na coluna, para cada valor de dano, médias seguidas da mesma letra não diferem entre si pelo teste de Tukey, a 5% de probabilidade).

Table 2. Values (%) for normal seedlings (NS), abnormal seedlings (AS) and dead seeds (DS), obtained in the germination test and respective rankings given to X-ray images for weevil damage (valores (%) de plântulas normais (NS), de plântulas anormais (AS) e de sementes mortas (DS) obtidos no teste de germinação e respectivas notas atribuídas às imagens de raios X, para danos por caruncho). Piracicaba, USP/ESALQ, 2009.

Genotypes		Damage ranking*								
		1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3
L ESP 10	NS	95.5	0	0	0	0	0	0	0	0
	AS	4.5	0	0	0	0	0	0	0	0
	DS	0	0	0	0	0	0	0	0	0
IPA-206	NS	8.0	12.0	5.5	0.5	1.5	2.0	0.5	0	0.5
	AS	11.5	10.5	12.5	0	2.5	4.0	0	2.5	18.5
	DS	1.0	0.5	2.0	0	0	0	0	0	4.0
L 281.005	NS	71.5	8.5	1.5	0	0.5	0	0	0	2.0
	AS	11.5	0	0.5	0	0	0	0	0	2.0
	DS	1.0	0.5	0	0	0	0	0	0	0.5
BRS-Pajeu	NS	94.0	3.0	0	0	0	0	0	0	0.5
	AS	1.5	0	0	0	0	0	0	0	0.5
	DS	0	0	0	0	0	0	0	0	0.5
BRS-Potengi	NS	78.0	2.5	2.5	0	0.5	0	0	0.5	2.0
	AS	8.0	1.0	0	0	0	0.5	0	0	1.5
	DS	2.5	0	0	0	0	0	0	0	0.5

*1= undamaged, 2= light damage, 3= severe damage. The first algarism of the ranking refers to the embryonic axis and the second algarism to the cotyledons (1= danos não observados, 2= danos não severos, 3= danos severos. O primeiro algarismo da nota refere-se ao eixo embrionário e o segundo aos cotilédones).

Seeds of cultivar IPA-206 showed more damage to the embryonic axis and cotyledons, which was ranked as severe (3.3), compared to the other genotypes. However, the occurrence of light damage was less for the BRS-Pajeu and BRS-Potengi cultivars and the L ESP 10 and L 281.005 lines, with these four genotypes showing higher mean number of seeds with no damage either to the embryonic axis or to the cotyledons.

The percentages of normal and abnormal seedlings and dead seeds observed in the germination test, for seeds which have weevil damage and were evaluated by X-ray, are shown in Table 2. The results demonstrate that those seeds which suffered no damage to the embryonic axis or cotyledons (Figure 1A) mostly produced normal seedlings (Figure 1B) after the evaluation of germination on the day stipulated by the RSA for the first count. This can be seen for the cultivars BRS-Pajeu and BRS-Potengi and the lines L ESP 10 and L 281.005 (Table 2). However, some seeds where no damage was observed (ranking 1.1) produced abnormal seedlings; according to Burg *et al*. (1994), this type of problem may be due to infection by microorganisms, physiological deterioration and, or tissue death.

For damage ranked as severe, both to the cotyledons and embryonic axis, for seeds from the five genotypes, there was a direct relationship between damage and the number of abnormal seedlings and dead seeds, considering the first count of the germination test. The number of dead seeds in the five genotypes was low, with the highest percentage (7.5%) for IPA-206.

Seeds with weevil damage are shown in Figures 1C, 2A, 2C and 2E. Figure 1C, shows a seed with severe damage only to the cotyledons (ranking 1.3), resulting in an abnormal seedling (Figure 1D). According to Cicero *et al*. (1998), damages to cotyledons, which restrict nutrient translocation to the embryonic axis, or which directly affect the embryonic axis, adversely affect seed germination.

Figure 2A shows a seed with light damage to the embryonic axis and

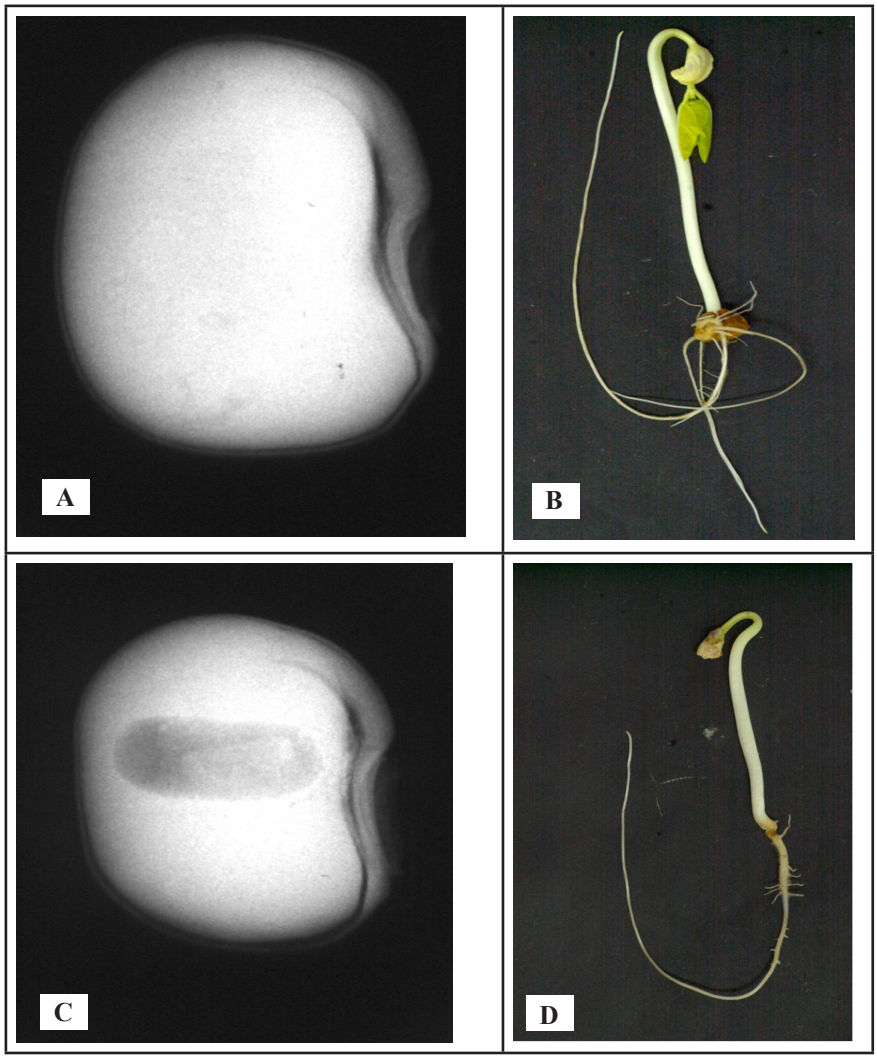

Figure 1. Image of a seed of the L ESP 10 line obtained from X-ray, undamaged by weevils (A), ranking 1.1, resulting in a normal seedling (B). Image of the seed of cultivar IPA-206 obtained from X-ray, with weevil damage (C), with ranking 1.3, resulting in an abnormal seedling (D) (imagem de semente da linhagem L ESP 10 obtida por meio do teste de raios X, sem dano por caruncho (A), nota 1.1, originando uma plântula normal (B). Imagem de semente da cultivar IPA-206 obtida por meio do teste de raios X, com dano por caruncho (C), nota 1.3, originando uma plântula anormal (D)). Piracicaba, USP/ESALQ, 2009.

severe damage to the cotyledonal region, ranked as 2.3 and gave rise to an abnormal seedling (Figure 2B).

Figure 2C shows a seed classified as 3.2, which has severe damage on the embryonic region and light damage on the cotyledonal region, resulting in an abnormal seedling (Figure 2D).

Figure 2E presents a seed with severe damage to the cotyledons and the embryonic axis, classified as 3.3, which produced a dead seed on the germination test (Figure 2F). Based on an analysis of the seeds of the three cultivars and two lines, it was seen that severe damage to the embryonic axis and cotyledons resulted in dead seeds or abnormal seedlings. The same result was also verified in common bean seeds (Forti *et al*., 2008) and in soybean seeds (Pinto *et al*., 2009), where stinkbug damage ranked as severe, that is, occurring to the embryonic axis and, or the cotyledons, resulted in abnormal seedlings or dead seeds.

The results show, therefore, that X-ray are efficient for evaluating weevil damage in cowpea seeds, and permit this damage to be associated with harmful effects caused at germination.

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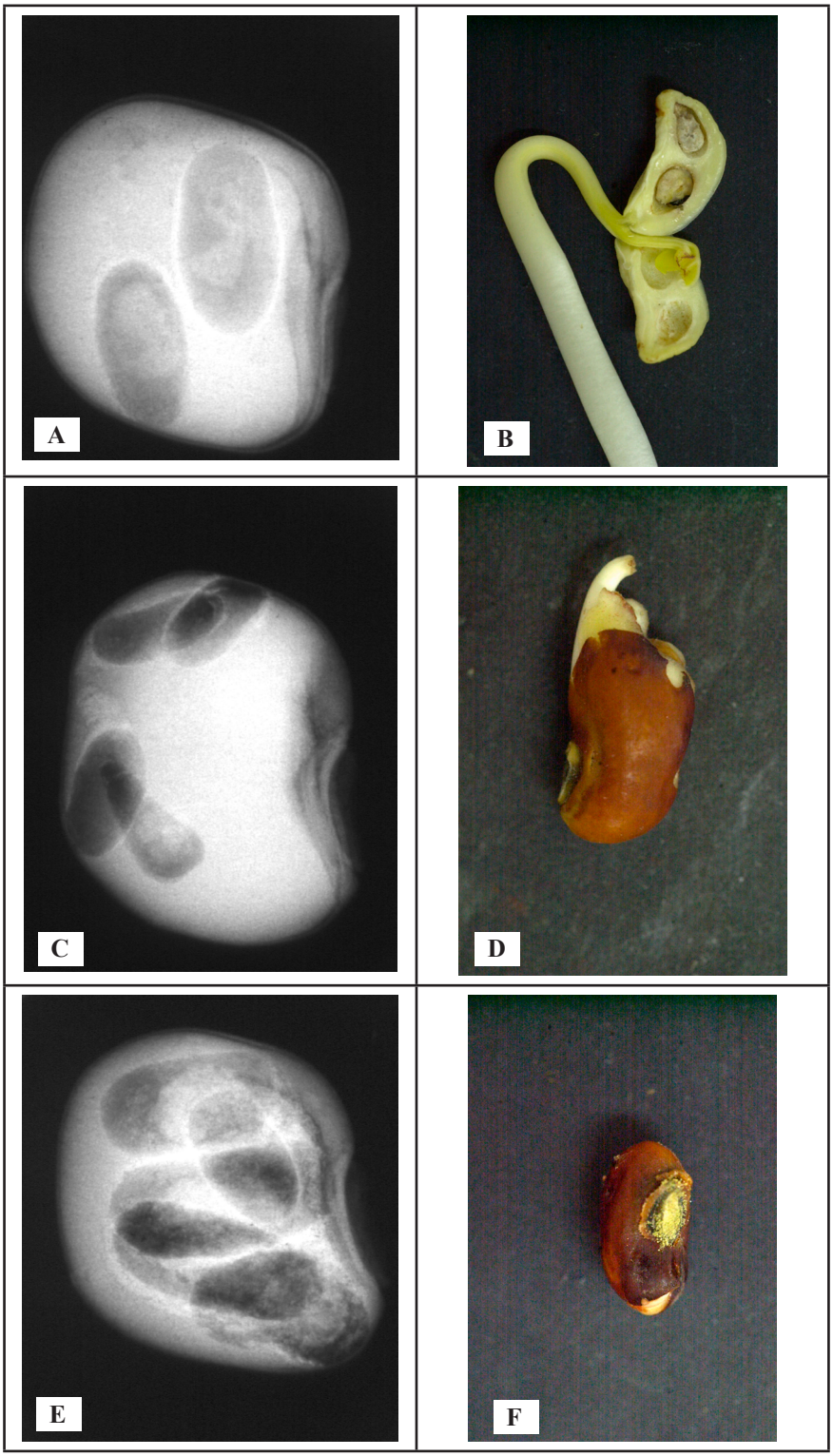

Figure 2. Image of a seed of cultivar IPA-206 obtained from X-ray, with weevil damage (A), with ranking 2.3, resulting in an abnormal seedling (B). Image of a seed of cultivar BRS-Pajeu obtained from X-ray, with weevil damage (C), with ranking 3.2, resulting in an abnormal seedling (D). Image of the seed of cultivar IPA-206 obtained from X-ray, with severe weevil damage (E), with ranking 3.3, resulting in a dead seed (F) (imagem de semente da cultivar IPA-206 obtida por meio do teste de raios X, com dano por caruncho (A), nota 2.3, originando uma plântula anormal (B). Imagem de semente da cultivar BRS-Pajeu obtida por meio do teste de raios X, com dano por caruncho (C), nota 3.2, originando uma plântula anormal (D). Imagem de semente da cultivar IPA-206 obtida por meio do teste de raios X, com dano severo por caruncho (E), nota 3.3, originando semente morta (F)). Piracicaba, USP/ESALQ, 2009.

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