

Effect of Size Classification on Physical and Physiological Quality Aimed at Pelletizing Seeds of Eucalyptus Species

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Abstract

The aim of this work was to verify the effect of size classification on the physical and physiological quality of *Eucalyptus* spp. seeds to optimize the selection for covering. The seeds were separated in sieves with six size classes: 850, 710, 600, 500, 425 μm , and a non-perforated bottom ($< 425 \mu\text{m}$). Were evaluated the sieve retention, purity, weight of a thousand seeds, germination, and first count of germination. Sieve classification is effective for the separation of materials according to the best physical and physiological qualities. *E. grandis* seeds from the 710 μm sieve, *E. robusta* from the 850 μm sieve, and *E. urophylla* from the 710 and 850 μm sieves can be selected for covering, as they present maximum physical and physiological quality. For the three species, the smallest materials, retained in the 500, 425, and bottom $<425\mu\text{m}$ sieves, must be discarded due to low purity and germination potential.

Keywords: Germination, pelleting, sieves, purity, vigor.

1. INTRODUCTION AND OBJECTIVES

Marketed eucalyptus seed lots are a mixture of pure seeds with inert material, such as unfertilized eggs and crop remains (Affonso et al., 2018; Brasil, 2013). Due to the small size and similar physical characteristics of these materials, it is difficult to obtain seed lots with high purity by processing only (Santos, 2016). Those lots have a different seeds size, it can vary according to the nutritional and water supply, as well as the seasonality of production, mainly in forest species such as eucalyptus and also by the point of physiological maturity (Hoppe & Brun, 2004).

For species such as *E. grandis* W. Hill ex Maiden and *E. urophylla* S.T. Blake (Aguiar et al., 1979), the classification of seeds by sieves made it possible to obtain larger seed lots that germinated faster, although the final germination percentage was not affected by the size. However, there are reports that the size of *E. grandis* seeds might affect seedling germination and survival in nurseries, as was found by Naidu & Jones (2007). These studies show us only the physiological aspects, without analyzing the physical aspects of the seed lots, namely size class.

Physical quality, as defined by the evaluation of seed weight and purity, is critical because, in a eucalyptus seed lot, depending on the species, the amount of impurities can be higher than that of fertile seeds (Silva et al., 1994). The same authors observed that, for *E. maculata*, larger seeds, between 1.680 μm and 2.000 μm , showed higher quality than smaller ones in terms of physical purity, the weight of a thousand seeds, vigor, and germination, but we do not have any records of similar studies on *Eucalyptus robusta*, *E. urophylla*, or *E. grandis*.

Eucalyptus seeds with high purity, vigor, and germination require mechanized, precision sowing techniques, such as the pelleting process, in which the seeds are covered with an inert material to increase seed size and planting ease (Walker et al., 2011; Santos, 2016). In small and medium-sized nurseries, where seeds are used for seedling production, pelleting is important for species with very small seeds, such as *E. grandis* (Walker et al., 2011).

There is a demand for covered seeds to facilitate the sowing process and ensure the acquisition of lots with high purity. Considering that the use of sieves can increase the purity and increase the physical and physiological quality of the lots, the objective of this work to verify the effect of size

classification on the physical and physiological quality of *Eucalyptus* spp. seeds to optimize the selection for covering.

2. MATERIALS AND METHODS

The seeds of *E. grandis*, *E. robusta*, and *E. urophylla* were harvested in seed production areas (APS) located in Anhembi-SP, Brazil, and processed by passing through a fan, sieving machine, gravitational table, and air column by the Seeds Sector at the Forest Research and Studies Institute (IPEF) to obtain marketed lots.

Representative samples of the lots, containing approximately 200 g per species, were homogenized, and sent to the Seed Analysis Laboratory of the Faculdade de Ciências Agrárias e Veterinárias – UNESP, Campus Jaboticabal-SP. In this study, these samples are referred to as marketed seeds because they are a mixture of real seeds with unfertilized eggs and are sold to nurseries for seedling production. The seeds were subjected to the following tests and evaluations.

Retention in sieves. For each eucalyptus species, two marketed seed repetitions of 100 g were used. The seeds were placed on the upper sieve of a set of six sieves, placed one on top of the other, in decreasing order of size, from the top to the base. Under the overlapping sieves, a non-perforated bottom was fitted, and this set was shaken for 3 min. In this way, the marketed seeds were separated into six size classes: 850, 710, 600, 500, 425 μm , and less than 425 μm .

The marketed seeds retained by each sieve, which had passed through the sieve immediately above, were separated, weighed, and their percentage calculated. This test was carried out following the methodology prescribed in the instructions for forest species analysis (Brasil, 2013) but adapted due to the greater number of sieves. The results presented are the average of the percentages of two repetitions in whole numbers (Brasil, 2013).

Physical purity. This feature was determined using two 1g subsamples, which were weighed on a precision scale (0.0001 g) followed by manual separation performed under a magnifying glass (10x) to obtain pure seeds, with the results expressed as a percentage (Brasil, 2013). The weight of the subsamples was greater than that recommended by the instructions for forest species analysis and was used to ensure greater representation of the components present in the lot and to obtain the appropriate number of seeds to carry out all the proposed tests and determinations.

Weight of one thousand seeds. These were weighed on an analytical scale, with eight repetitions of 100 pure seeds per treatment (Brasil, 2013).

Germination test. Conducted with four replications, but with two sowing methods: i) 100 pure seeds; ii) 0.1 g of marketed seeds (with impurities), the latter followed the recommendations of Brasil (2013). The samples were sown on two sheets of blotting paper moistened with distilled water in the amount of $2 \times$ the mass of the paper, in transparent plastic boxes ($11.0 \times 11.0 \times 3.5$ cm) kept at a temperature of 20 °C for *E. robusta* and 25 °C for *E. urophylla* and *E. grandis*. The normal seedling count was carried out at five and 14 days after sowing (Brasil, 2013). The results obtained from the sowing of pure seeds were presented as a percentage and those based on the mass of marketed seeds, in normal seedlings present in 0.1 g of sample.

Test of vigor by first germination count. The germination test was performed using the same methodology but with the evaluation of normal seedlings on the 17^o day after sowing (Brasil, 2013; Martins *et al.*, 2014).

The experimental design used was completely randomized, with four repetitions and six treatments (classes of seed size), for each eucalyptus species. Only the results of the sieve retention test and purity were not subjected to statistical analysis, following the recommendation of the instructions for forest species analysis (Brasil, 2013). The data were not transformed because they met the assumptions of normality and homogeneity tests, were subjected to analysis of variance, and when significant, the means were compared by the Tukey test ($P < 0.05$).

3. RESULTS AND DISCUSSION

The evaluation of the sieve retention test allowed us to verify that the percentage participation of a determined size of seeds in the lot depends on the species. For *E. grandis* and *E. robusta*, there was a higher concentration of material in the 500 and 425 μm sieves. These, together, retained 76 and 74% of the marketed seeds of *E. grandis* and *E. robusta*, respectively (Figure 1A and B).

For *E. urophylla*, there was a better distribution of the material between the sieves, with greater proportions retained in the following sieves 425 μm (27%), 500 μm (21%), bottom < 425 μm (19%), and 850 μm (17%) (Figure 1C).

We observed in the literature that the seed size of different species of eucalyptus is very variable. Silva *et al.* (1994) verified in *E. maculata* lots the predominance of larger seeds, between 1680 and 2000 μm , although some seeds were found in smaller sieves, up to 1190 μm . Therefore, the seed size of *E. maculata* would be greater than double the seeds of the three species evaluated in the present study, which was between 425 and 850 μm (Figure 1).

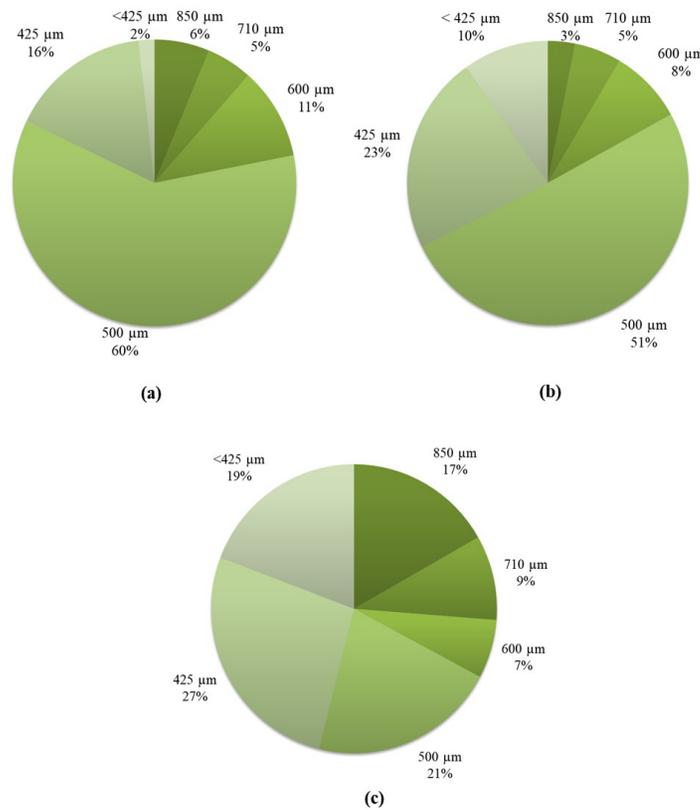


Figure 1. Weight percentage distribution of seed size classes of A) *Eucalyptus grandis*; B) *E. robusta* and C) *E. urophylla* after sieve retention test.

For *E. grandis*, the fractions of marketed seeds from the 500 and 425 µm sieves were found in a higher percentage in the lots, with a low purity of 13.9% and 5.1%, respectively (Figure 2A). For this species, the fraction of seeds retained

in the non-perforated bottom (< 425 µm) also showed a low purity percentage (5%), although the latter contributed to the smallest fraction of the sample, only 2% of the lot weight (Figure 1A and 2A).

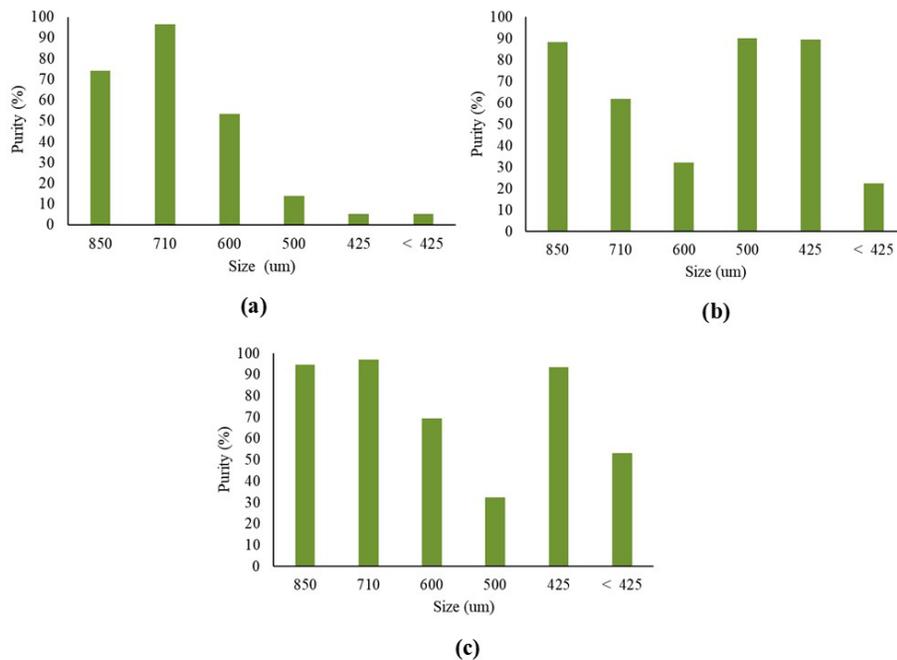


Figure 2. Purity (%) for the different size classes of A) *Eucalyptus grandis*; B) *E. robusta* and C) *E. urophylla*.

It is, therefore, apparent that the amount of impurities can be excessive in marketed seed lots of some eucalyptus species, depending on the sieve used in the processing, and this is in agreement with reports by Silva et al. (1994). In this case, the use of sieves in processing could be adopted for the separation and disposal of impurities, as has been done for small seeds such as tobacco (Caldeira et al., 2016), guinea grass (Melo et al., 2016), and turnip (Nery et al., 2009), to obtain lots with higher physical and physiological quality.

For *E. grandis* seeds, it was found that the percentage of purity increased with the sieve size of the classification since the fractions retained in the 600 sieves or bigger (710 and 850 µm) were 54, 97, and 74%, respectively (Figure 2A). However, these fractions with higher percentages of true seeds represented a small portion of the lot, since together they contributed 22% of the lot's initial weight (Figure 1).

The fraction of *E. grandis* seeds with the highest purity (97%) was retained in the 710 µm sieve. This fraction was the only one that could be submitted for recovering because, for this procedure, the recommendation is to use seeds with a purity greater than 95 % to avoid the risk of recovering inert materials as if they were pure seeds (Almeida & Rocha, 2008).

To achieve maximum physical purity and vigor in pelleted seeds of *Eucalyptus* spp., it is necessary to eliminate sterile, empty, very small, weak, and impure seeds (Santos, 2016).

Regarding the physiological quality, *E. grandis* seeds with sizes between 600 and 850µm showed maximum vigor and germination. It was found that the seeds retained in the 600, 710, and 850µm sieves had bigger germination values of 86%, 91%, and 95%, respectively (Table 1). The results of this work corroborate those observed by Naidu & Jones (2007) in which the highest germinative performance of *E. grandis* was observed in the largest seeds when compared to the smallest ones.

Table 1. Germination (G) and vigor by the test of the first count (V) in percentage (%) and by mass in 0.1 g of the sample and weight of one thousand seeds of *Eucalyptus grandis* classified by size in the sieves with round sieves of 850, 710, 600, 500, 425 µm, and bottom (less than 425 µm).

Size (µm)	V	G	V	G	Weight of a thousand seeds (mg)
	%				
	in 0.1g				
850	93a	95a	134b	142b	460a
710	76a	91a	204a	217a	330b
600	82a	86a	193a	210a	190c
500	24b	28b	35c	62c	90d
425	3b	6c	5cd	12d	40e
< 425	1b	2c	1d	5d	10f
C.V.%	27,9	11,8	14,1	14,8	3,34
F	42,234**	210,377**	192,322**	141,535**	3264,85**
d.m.s.	28,9	13,6	30,3	3,6	1,40

**Significant at 1% probability by F test. Means followed by the same letter in the column do not differ from each other by the Tukey test (p<0.05).

In the germination evaluation by weight of the sample, the maximum germination value and vigor were verified only for the seeds retained in the sieves between 600 and 710 µm. Using this methodology, recommended by the instructions for forest species analysis (Brasil, 2013), the sample obtained from the 850 µm sieve did not present a maximum germination result from sowing according to the number of seeds. This result was because sowing based on weight is influenced by impurities present in the sample and the seed size, affecting the results.

Thus, the methodology based on the number of pure seeds would be more reliable for assessing the effect of classification on germination and vigor, although it is the most challenging and consumes more time for the laboratory analyst.

E. grandis seeds with sizes equal to or less than 500 µm showed germination equal to or less than 28% (Table 1). According to Lúcio et al. (2011), these samples will be low quality because they present values lower than 46.4% of germination. Thus, considering the percentage of pure seeds, *E. grandis* seed fractions retained in 500 µm smaller sieves should be discarded since only 0% to 4% of the sample weight consists of seeds and goes on to germinate (Figure 2A).

For *E. robusta*, the fractions that predominated the lot composition, from sieves 425 and 500 µm, presented values of 90% and 90%, respectively, which reflects the high physical quality of these classes of seeds (Figure 1B and 2B). For *E. grandis*, although the seed samples from the same sieves predominated in the lot, in the other hand, the purity values were much lower, 5 and 14%, respectively (Figure 1A and 2A). Therefore, it was found that the physical quality of the marketed seed fractions retained in a certain sieve depended on the eucalyptus species. For seeds of this genus, physical purity depends on the number of true seeds present in percentage by weight of the sample (Silva et al., 1994; Brasil, 2009).

Although the fractions of marketed seeds of *E. robusta* retained in the 425 and 500 µm sieves had a maximum percentage of pure seeds, these showed minimum germination percentages of 0% and 3%, respectively (Table 2).

The seeds retained in the non-perforated bottom (< 425 µm) also did not germinate, although this fraction represented only 10% of the original lot weight (Figure 1B and 2B).

For *E. robusta*, a third sample with a maximum purity of 88% was verified, and this was the one retained in the 850 µm sieve. It also showed maximum germination of 94%, statistically similar to the germination of the fraction obtained in the 710 µm sieve (97%). However, the fraction retained in this last sieve was of inferior physical quality, verified by the purity of 62%, which can be considered an intermediate value of purity in comparison to the samples obtained in the other sieves (Table 2).

To increase the purity of the seeds of the 710 μm sieve, the transfer could be carried out by other processing machines, such as the gravitational table. This procedure has been shown to be efficient in increasing the purity of many small seeds, such as mung bean and guinea grass (Araújo et al., 2011; Melo et al., 2016). However, the fraction of *E. robusta* seeds retained in the 710 μm sieve represented only 5% of the original lot.

Table 2. Germination (G) and vigor by the test of the first count (V) in percentage (%) and by mass in 0.1 g of the sample and weight of a thousand seeds of *Eucalyptus robusta* classified by size in the sieves with round sieves of 850, 710, 600, 500, 425 μm , and bottom (less than 425 μm).

Size (μm)	V	G	V	G	Weight of a thousand seeds (mg)
	%		in 0.1g		
850	85a	94a	167a	183a	550a
710	85a	97a	147b	155b	400b
600	42b	48b	64c	75c	260c
500	2c	3c	6d	10d	200d
425	0c	0c	2d	3d	110e
< 425	0c	0c	1d	1d	80f
C.V.%	14,6	9,8	10,5	7,3	4,95
F	253,720**	546,828**	501,429**	967,804**	734,5**
d.m.s.	11,7	8,81	15,2	11,7	3,02

**Significant at 1% probability by F test. Means followed by the same letter in the column do not differ from each other by the Tukey test ($p < 0.05$).

The fraction of *E. robusta* seeds retained in the 850 μm sieve, although it had the lowest percentage participation in the lot, of only 3% (Figure 1B) would be the one indicated for the recovery process, as it presented maximum quality in terms of purity, germination, and vigor by the first count test, with values of 88%, 94%, and 85%, respectively (Figure 2B and Table 2).

Compared to the values established by Lúcio et al. (2011) as a reference for the germination of *E. robusta* seeds, the samples from sieves 710 and 850 μm can be considered to be of a very high standard, as they showed values above 94% of germination (Table 2). On the other hand, the seeds of the 600 μm sieve would be a medium standard, with 48% germination.

The fraction of seeds retained in the 600 μm sieve represented 8% of the total weight of marketed seeds and showed 32% purity (Figure 1B and 2B). Therefore, this fraction and that retained in the non-perforated bottom (< 425 μm) presented the worst results in terms of purity (Figure 2B). As for the percentage share, both fractions of marketed seeds could be considered intermediate in comparison to the others, with participation between 8 and 10% of the lot (Figure 1B).

In the case of a fraction less than 425 μm in size retained in the non-perforated bottom, the low purity combined with null germination (Figure 1B, 2B and Table 2) allows us to infer that this material should be discarded after sieve classification.

On the other hand, those retained in the 600 μm sieve showed germination and vigor of 48% and 42%, respectively, which were intermediate values in comparison to the seeds selected by other sieves. Therefore, due to the presence of viable seeds, the marketed seeds of this sieve could be sold and sown separately from others of larger size and quality to avoid competition. This procedure is recommended for *Eucalyptus* spp. of high genetic value (Silva et al., 1994; Araújo et al., 2011).

Considering the physical and physiological quality of *E. urophylla* seeds, it was found that the best fractions of marketed seeds were those retained in the largest sieves, 850 and 710 μm , as they presented maximum percentages of purity, germination, and first count, with values from 95 to 97%, 92 to 93%, and 51 to 54%, respectively (Figure 1C and Table 3). These added fractions represent 26% of the lot (Figure 2C) and should be covered because of their purity, above 95% (Almeida & Rocha, 2008), in addition to their maximum vigor and germination.

Table 3. Germination (G) and vigor by the test of the first count (V) in percentage (%) and by mass in 0.1 g of the sample and weight of a thousand seeds of *Eucalyptus urophylla* classified by size in round sieves of 850, 710, 600, 500, 425 μm , and bottom (less than 425 μm).

Size (μm)	V	G	V	G	Weight of a thousand seeds (mg)
	%		in 0.1g		
850	51a	93a	252a	307a	550a
710	54a	92a	193b	263b	320b
600	37b	82b	121c	161c	230c
500	8c	21c	67d	110d	100d
425	0c	1d	3e	17e	90d
< 425	0c	0d	1e	5e	30e
C.V.%	15,2	6,6	14,3	10,1	4,31
F	177,723**	816,800**	181,293**	292,661**	1474,58**
d.m.s.	8,5	7,2	38,1	32,7	2,20

**Significant at 1% probability by f test. Means followed by the same letter in the column do not differ from each other by the Tukey test ($p < 0.05$).

For *E. urophylla*, the sample of marketed seeds obtained in the 600 μm intermediate sieve could be classified as having intermediate physical and physiological quality, as it presented 69% purity, 82% germination, and vigor evaluated by the first 37% count test (Figure 2C and Table 3). These values were statistically lower than those seen in the samples from the 850 and 710 μm sieves and higher than those seen in samples from smaller sieves.

As with the *E. robusta* seeds of intermediate quality (710 μm), it is recommended that the transfer of *E. urophylla* seeds from the 600 μm sieve could most efficiently be carried out by machines such as the gravitational table to increase the purity of the lot as this would be the main quality problem of this fraction of seeds (Araújo et al., 2011; Melo et al., 2016). However, it must be considered that the economic viability

of this procedure depends on the size of the original lot and the marketed value of the seed, as this fraction of seeds had the lowest percentage share in the lot, 7% (Figure 1C).

Eucalyptus urophylla seeds retained in 500 μm , 425 μm , and non-perforated bottom ($< 425 \mu\text{m}$) sieves showed germination of 21%, 1%, and 0%, respectively (Table 3). It is noteworthy that the sample of *E. urophylla* seeds retained in the 425 μm sieve showed high purity, with 93% of true and pure seeds in the composition. However, only 1% of these cells were viable and able to germinate (Figure 1C, 2C and Table 3).

It is noteworthy that the sample of *E. urophylla* seeds retained in the 425 μm sieve showed high purity, with 93% of true and pure seeds in the composition. However, only 1% of these cells were viable and able to germinate (Figure 1C, 2C and Table 3). These small and low weight seeds, as seen in Table 3, probably have low germination rates because they are low in reserves and malformed. These features can result from the harvesting of immature fruits or the incidence of pests and diseases (Martins et al., 2008; Pessoa et. al, 2010; Neto et al., 2015).

The weights of the seeds of *E. grandis*, *E. robusta*, and *E. urophylla* increased with their size, as verified by the weight of a thousand seeds (Table 1, 2 and 3) and were, respectively, between 46 and 1mg, 55 and 8mg, 55 and 3mg, depending on the seed size. The greater weight of larger seeds was also reported for *E. maculata* by Silva et al. (1994).

The classification of *E. grandis*, *E. robusta*, and *E. urophylla* seed lots by size made it possible to obtain larger, heavier seeds with better germination rates and vigor (Table 1, 2 and 3). Aguiar et al. (1979) in a similar study with seeds of *E. grandis* and *E. urophylla* found that the size of the seed did not influence the germination percentage of these seeds, but the speed of germination. However, these results differ from those obtained in the present study, which can be attributed to the germination test method, which was performed with different volumes of samples depending on the size of the seed.

For the three species studied, the results of germination using the sowing prescribed in the instructions for forest species analysis (Brasil, 2013) (based on the weight of marketed *Eucalyptus* seeds and not on the number of pure seeds) was influenced not only by the germination capacity of seeds of different sizes but also by the purity of the sample (Table 1, 2 and 3, Figure 1 and 2). When comparing the two sowing methods for each species and sizes of sieves, it was found that the quality of germination was influenced by the method adopted (Table 1, 2 and 3).

Thus, for *E. grandis* the highest levels of germination and vigor evaluated in 0.1 g of the sample were verified for the marketed seed samples (with impurities) retained in the 600 to 710 μm sieves, partially contradicting the

results of germination and vigor in percentage, in which the seeds of these sieves did not differ from those of the 850 μm sieve (Table 1).

For *E. robusta* and *E. urophylla* seeds, the highest germination and vigor in 0.1 g of the sample was verified only for the 850 μm sieve sample, partially contradicting the results of the test by number of seeds, in percentage, in which the seeds of these sieves did not differ in physiological quality from those of the 710 μm sieve (Table 2 and 3).

In addition, it should be considered that in weight sampling, samples from smaller sieves should have a greater number of seeds in 0.1 g of material than those retained in larger sieves (Aguiar et al., 1979). Therefore, sowing methodology based on the mass of the sample of marketed seeds can lead to misunderstandings of results when comparing lots with different characteristics in terms of seed size or purity percentage.

4. CONCLUSIONS

The use of classification sieves is efficient for the separation of materials of high physical and physiological quality and could help us to obtain the best material for covering. *Eucalyptus grandis* seeds of size 710 μm , *E. robusta* of 850 μm , and *E. urophylla* of 710 and 850 μm can be indicated for recovery, as they present maximum physical and physiological quality. For the three species, the smallest material, retained in 500, 425, and $< 425 \mu\text{m}$ sieves, must be discarded due to low purity and germination levels.

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