DRYING AND STORAGE OF *Eugenia involucrata* DC. SEEDS

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ABSTRACT: The ...gical quality of seeds of native species is important to produce healthy saplings and therefore guar... success of programs to recover disturbed vegetation. This reinforces the necessity for investigating t... logical quality of those seeds. To evaluate the effects of different drying rates on the germination, m... ontent and storability of *Eugenia involucrata* diaspores, mature fruits collected at Mogi Guaçu, SP, ... their epi- and mesocarps removed by washing and were dried at 30, 40 or 50ºC until their water c... as reduced from 57% (fresh diaspores) to 13% (final drying), totaling six drying levels. In a secon... ent, diaspores had their moisture content reduced from 57% to 49%, at 30ºC, totaling six drying ...0h, 1h, 2h, 3h, 4h and 5h), and were kept for 180 days in plastic bags under cold storage. The drying rate had no effect on tolerance to desiccation by *E. involucrata* diaspores; water contents lower than 51% decreased both germinability and storability. Diaspores can be stored for up to 180 days as long as their water content is reduced to 53% and they are kept inside plastic bags under cold storage.
Key words: germination, drying rate, recalcitrant seed

SECAGEM E ARMAZENAMENTO DE SEMENTES DE *Eugenia involucrata* DC.

RESUMO: O uso de sementes de espécies nativas de alta qualidade é fundamental nos programas de recomposição vegetal, o que fortalece a necessidade de se investigar o potencial fisiológico das mesmas. Esta pesquisa objetivou avaliar os efeitos da velocidade de secagem dos diásporos de *Eugenia involucrata* sobre a sua germinação e vigor, bem como as relações entre teor de água e capacidade de armazenamento. Foram colhidos frutos maduros em pomar instalado em Mogi Guaçu, SP (22º15-16'S, 47º8-12'W), que tiveram seu epicarpo e mesocarpo removidos por lavagem. A seguir, os diásporos (semente + endocarpo) foram submetidos a secagem controlada a 30, 40 e 50ºC, com reduções progressivas do teor de água inicial de 57% para até 13%, obtendo-se seis níveis de secagem em cada temperatura. Em um segundo experimento, a secagem foi realizada a 30ºC por 0h (controle), 1h, 2h, 3h, 4h e 5h, tendo atingido, neste último período, 49% de água. Neste experimento, os diásporos foram avaliados quanto à germinação até 180 dias de armazenamento em sacos plásticos em câmara fria (8 ± 2ºC). A velocidade de secagem não alterou a sensibilidade dos diásporos à dessecação. A redução da umidade para valores inferiores a 51% prejudica a capacidade germinativa e o potencial de armazenamento. A redução do teor de água para 53% permite conservação dos diásporos de *E. involucrata* por até 180 dias, sob condições de câmara fria e em embalagens plásticas.
Palavras-chave: germinação, velocidade de secagem, semente recalcitrante

INTRODUCTION

Native tree species are closely linked to Brazil's history and economical development. The country has quite a diversified tree flora, but has been submitted to devastation: in addition, a lack of technical direction and ecological awareness in exploring forest resources, has already caused irreparable damage to environmental interactions (Raven, 1996; Lorenzi, 1992). Interest on plant recovery has been on the rise during the past decades, increasing the demand for the use of native species seeds with potential for reforestation, provided they are good quality as well. There is, therefore, an increasing need for investigations on the physiological potential of seeds of native species, enabling their utilization in reforestation programs (Araújo Neto, 1997).

The initial quality of the collected seeds must be preserved as much as possible before seeding (Carneiro & Aguiar, 1993). Preserving the physiological potential of seeds is one of the most important stages in the process of reforestation of degraded areas, since it allows plant species to be used in seasons and places diverse from their origin. It could also be very important for the formation of germplasm banks, submitted to preservation techniques efficient only for orthodox seeds (Silva et al., 1993).

The ideal storage conditions for orthodox seeds (low temperature and water content lower than 12-15%) are frequently lethal for recalcitrant seeds (Ellis, 1984; Neves, 1994, Barbedo & Marcos Filho, 1998). Handling these seeds is difficult since they do not tolerate storage temperatures below 0°C and, depending on the species,

they will not withstand reductions in water content to values below 12-31% (Roberts, 1973; Silva et al., 1993; Bilia et al., 1999); no conclusive recommendation exists to date for their conservation.

Seeds of some species of the family Myrtaceae present recalcitrant behavior. Research projects dealing with seeds from this family are important in recovery programs for degraded areas, since there are fruit species which restore not only the flora, but also the fauna, by attracting birds and other animals.

Eugenia involucrata DC, popularly known as cerejeira, cerejeira-do-mato, cerejeira-da-terra and cerejeira-do-rio-grande, is both rugged and ornamental. In addition to its ecological uses, it can be utilized as an urban tree, and for making preserves, jams and brandies (Sanchotene, 1989; Lorenzi, 1992). Information on the behavior of seeds of this species is scarce. Silva et al. (1993) suggest that the diaspores should be submitted to shade drying techniques after the fruit has been pulped. However, Barbedo et al. (1998) submitted cerejeira diaspores to shade drying and verified that the fruit presented sensitivity to desiccation, and only non-dehydrated diaspores maintained their viability. After 120 days under storage, diaspores that had not been submitted to drying still germinated at a proportion of 78%, while those submitted to drying did not attain 30% germination.

Since drying rate is a factor considered to influence the behavior of seeds that are sensitive to desiccation, this study was conducted to evaluate the effects of temperature and drying periods on the post-harvest behavior of cerejeira diaspores, as well as their effects on conservation during storage.

MATERIAL AND METHODS

Two experiments were conducted, collecting mature "cherries" (drupes) from several matrix trees, at Mogi Guaçu, SP, Brazil (22°22'20"'S, 46º56'32"W). In both experiments, fruits were washed in the day after collectin, in running water over a sieve, to remove the juicy epicarp and mesocarp, yielding the diaspores consisting of seeds with the coriaceous endocarp. Diaspores were maintained on filter paper for 12 hours, to eliminate surface humidity resulting from washing.

Drying rate

To evaluate the effects of the drying rate on *E. involucrata* diaspore sensitivity to desiccation, as well as to evaluate the limit at which viability was maintained as a function of the water content attained by the diaspores, after sampling, diaspores were submitted to controlled drying at 30°C, 40°C and 50°C (± 2°C), in a forced air circulation. Six similar desiccation levels were obtained for each drying temperature by constantly monitoring the mass of the sample. A split-plot experiment was set up, with three plots (temperatures) and six subplots (drying levels), in a completely randomized design (n=4).

Weight loss monitoring of the samples during drying and the attempt to obtain diaspores with similar water contents, at the different temperatures, within each level, required intermitent dryings to be implemented (10 hours drying /14 hours rest). Therefore, the process would be interrupted during the nightime and restarted the following day. The effective drying time was considered as the sum of periods during which the diaspores remained under the action of each temperature.

Drying period and maintaining viability under storage

Based on values obtained in the first experiment, an attempt was made to specify the limit of viability loss for those diaspores, and to associate those limits to their capacity to maintain viability under storage.

After removing a control sample, i.e., diaspores not submitted to drying, the rest of the lot was submitted to controlled drying in a forced air circulation oven, at 30°C ± 2°C, for 1h, 2h, 3h, 4h, and 5h. Treatments consisted of drying times. At the end of each drying time, some diaspores were submitted to the germination test and others were stored under two distinct conditions, consisting in the storage treatments: a) controlled temperature (8°C ± 2°C), with diaspores placed inside perforated plastic bags, and b) room temperature (23°C ± 8°C), simulating the conditions of the region from which the fruits were harvested, with diaspores placed inside paper bags. This material was evaluated again regarding its physiological potential at 60, 120 and 180 days under storage, through germination tests and diaspore water content evaluation, under the same conditions described earlier.

In both experiments, the germination test was performed on a paper roll, with the germinator set to 30°C and a 12-hour photoperiod (Brazil, 1992), with four replicates of 25 seeds in the first experiment and of 20 seeds in the second experiment. Germination was evaluated 30 days after installation of the test (first germination count, utilized as a vigor criterion) and 90 days after installation (total germination). The germination evaluation was based on the presence of normal seedlings, showing a developed primary root and aerial part, without apparent damage. The water content determination was performed with four replicates of 20 seeds, by the oven method at 105°C ± 3°C/ 24 hours (Brasil, 1992). Results were expressed as percentages (wet basis).

All percentage data were transformed according to arc sen $(\%/100)^{0.5}$ (Steel & Torrie, 1980), to approximate the normal distribution. The analyses of variance ($P = 0.05$) were performed as split-plots (first experiment) and as a factorial design (second experiment). The initial characterizations of the materials were interpreted based on the means of the non-transformed data.

RESULTS AND DISCUSSION

Drying rate

The effective drying period and the water content attained by the diaspores, at each temperature and for each drying level, are presented in Table 1. The periods elapsed for the first two drying levels, at the three temperatures (30, 40 and 50ºC), demonstrate that, in spite of being intermitent, the water loss was much faster than when it was conducted at 25ºC, as obtained by Barbedo et al. (1998). To attain the water contents in these first two levels, the effective periods at 30, 40 and 50ºC, for the first level, were, respectively, 11h, 7h and 4h, and for the second level were 66h, 58h and 10h (Table 1), while at 25ºC it took 48 hours to attain the first level and 264 hours to attain the second (Barbedo et al., 1998).

The weight loss control in the lots during drying caused the variation in water content attained at the different temperatures to be controlled as well; it did not exceed 3% between temperatures, within each drying level (Table 1). Starting from diaspores with 60.7% water, diaspores were obtained containing 52.0±1.0%, 40.5±1.5%, 31.5±0.5%, 27.5±1.5%, 21.5±1.5% and 14.5±1.5% water, corresponding to the six drying levels, respectively.

Germination and vigor results (first germination count) for each drying treatment at the different temperatures are presented in Table 2. When compared to the undried control, germination = 92.5% with 80% of normal seedlings in the first count, all other drying levels, with the exception of level 1, presented a reduction in germination, proportional to the drying period. No germination was verified in levels 5 and 6, and were not included in the statistical analysis. *E. involucrata* diaspores completely lost their germinative capacity starting at water contents from 20% to 25%. Regarding the control, the first seed desiccation level delayed the onset of germination when drying was performed under 30°C and 50°C (Table 2), but it did not reduce its germinative capacity (87% to 93%). However, at this first level, the water content in the diaspores still remained very close to the values observed at the time they were collected. The second desiccation level, however, in addition to severely delaying the onset of germination, reduced the germinative capacity by more than 50% (Table 2).

The water content and germination values of diaspores submitted to the first two drying levels, at 30, 40 and 50°C, are very close to those obtained by Barbedo et al. (1998) after drying at 25°C for 48 and 264 hours, respectively. The main difference, however, was observed for the second drying level at 30°C which, in spite of exhibiting diaspores with the same water content (40%), presented 52% germination, at least 13% higher than the others. Results obtained in the present experiment, together with results observed by other authors, (Pritchard & Prendergast, 1986; Probert & Longley, 1989; Pritchard, 1991; Barbedo et al., 1998; Bilia et al., 1999) suggest that the critical water level must lie between 41% and 50%, and is probably closer to this latter value. This value is near to the figure obtained by Gentil & Ferreira (1999) for another species of the same genus, *E. stipitata* ssp. *sororia*; the authors established the critical level to sit between 47.1% and 58.8%. However, the critical water level may not be well defined, and for greater precision it is still necessary to evaluate the behavior of seeds under new drying conditions, especially with reference to drying rate and temperature, according to the statements made by Pammenter et al. (1998).

Table 1- Time effectively elapsed (T, h) and water content (WC, %) attained by *Eugenia involucrata* diaspores as a function of temperatures (30, 40 and 50°C) and drying levels (1 to 6).

Drying	Drying temperature (°C)					
	30		40		50	
levels	T	WC	T	WC	T	WC
	h	%	h	%	h	%
0	0	60.7	0	60.7	0	60.7
1	11	51.0	7	51.3	4	53.1
2	66	40.5	58	41.7	10	38.7
3	134	32.2	95	31.6	17	31.3
4	154	28.6	122	25.9	*	25.6
5	193	22.5	*	20.5	24	22.6
6	*	15.9	*	13.1	38	14.0

*not recorded

Table 2 - Germination (% of normal seedlings) and vigor (normal seedlings in the first germination count, at 30 days, %) of *Eugenia involucrata* diaspores as a function of temperatures (30, 40 and 50°C) and drying levels (1 to 6).

Drying	Drying temperature (°C)			Means
levels	30	40	50	
	Germination (%)			
0	92.5	92.5	92.5	92.5 a
1	87.2	88.4	93.1	89.6 a
2	52.0	38.9	34.9	41.9 b
3	20.6	13.6	5.1	13.1 c
4	2.3	1.0	0	1.1 d
Means	52.0	44.8	38.7	
C.V. (%)	14.51			
	First Count (%)			
0	80.0 aA	80.0 aA	80.0 aA	
1	30.5 bB	75.1 aA	65.0 bA	
2	2.5 cA	0 bB	2.3 cAB	
C.V. (%)	12.50			

Means followed by a common lower case letter in the columns and by a common upper case letter in the rows do not differ at 5% by Tukey test.

Drying period and maintaining viability under storage

Based on the results obtained in the first experiment, in which a reduction in germination and vigor was observed when the diaspores attained water values near 50%, in the second experiment an attempt was made to evaluate the effect of small reductions in diaspore water content, i.e., mild drying, within the range between 50% and 60% water, both on their initial behavior and on their conservation capacity under storage. Even though two storage conditions were evaluated, cold storage + plastic bag and natural conditions + paper bag, the analysis of variance did not include the last factor, because storage under natural conditions resulted in extremely impaired germination, right during the first storage period (Figure 1).

Germinative potential and seed germination velocity were reduced as the storage period progressed. In the first count, the germination of diaspores not submitted to storage was 35%; no germination was observed 60 days later (Figure 1). This same tendency was observed for total germination, with non-stored diaspores showing 64% and stored diaspores showing 4% germination after 60 days, and no germination from 120 days under storage.

Table 3 shows water contents attained by cerejeira diaspores, after each drying time at 30°C and germination and vigor means for *E. involucrata* seeds, as a function of drying and storage periods. Before storage, the behavior of diaspores submitted to reduction of water content to 49.2%, for five hours of drying, did not present changes in seed germination or vigor. However, after 120 days under storage, it was observed that drying for up to 3 hours (53.0% water) allowed the germinative capacity to be maintained at its best. The beneficial effect of small reductions in diaspore water content, especially up to 2 hours of drying (55.3% water), could be observed in the very first 60 days of storage, when vigor was evaluated.

Therefore, in general, reductions of 4% to 5% in the water content of recently-collected diaspores, as long as it remains above 53%, allow the quality of seeds to be maintained. However, confirming results obtained in the first experiment, it was again verified that drying to water content values below 51% does not seem to promote this beneficial action. Storage for up to 120 days,

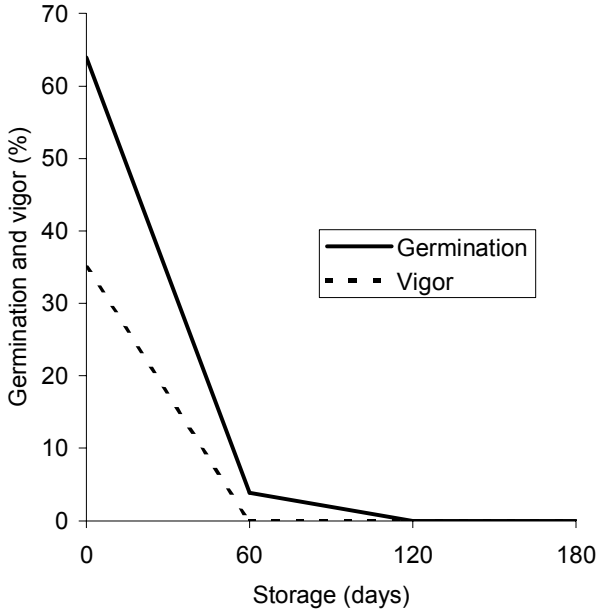

Figure 1 - Germination and vigor results (first count) for *Eugenia involucrata* diaspores submitted to natural storage, evaluated every 60 days.

Table 3 - Germination and vigor (first count) of *Eugenia involucrata* diaspores as a function of drying times (0 to 5) at 30°C + 2 and their corresponding water content, and of storage period under low temperature.

Storage	Drying time (h)					
(days)	0	1	2	3	4	5
	Water Content (%)					
	57.0	56.5	55.3	53.0	52.4	49.2
	Germination of normal seedlings (%)					
0	64 aA	61 aA	68 aA	61 aA	73 aA	65 aA
60	33 aA	44 abA	62 aA	54 aA	49 abA	45 abA
120	45 aAB	71 aA	44 abAB	65 aA	36 bB	25 bcB
180	3 bB	22 bAB	20 bAB	35 aA	26 bA	8 cAB
C.V. (%)	35.30					
	Vigor (% of normal seedlings in the first count)					
0	35.0 aA	24.6 aA	25.1 aA	20.2 aA	37.9 aA	21.5 aA
60	8.5 bA	10.6 aA	35.4 aA	11.3 aA	9.2 bA	8.5 abA
120	21.2 abA	21.0 aA	19.9 aA	21.0 aA	4.6 bAB	0 bB
C.V. (%)	45.92					

Means followed by a common lower case letter in the columns and by a common upper case letter in the rows do not differ at the 5% level by Tukey test.

in general, did not harm diaspore germinative capacity; at 180 days, there was a substantial reduction in germination. The negative effects of longer drying times (up to 5 hours, near 50% water) can be observed at 120 days under storage, both in vigor and in germinative capacity (table 3).

When compared to germination values obtained by Barbedo et al. (1998), the germination of stored *E. involucrata* diaspores indicates that there can be variations in behavior relative to their ability to maintain viability as a function of seed lot, because of the season in which the diaspores are produced depending on of their degree of ripening, or because other factors. In the present work, after 120 days, the best treatment was not for diaspores not submitted to drying, but for those that had their water content reduced to 56.5%, while the authors mentioned above found that the best treatment was for diaspores not submitted to drying (63.4% water). Despite of all, there are many similarities in the results of both reports. For the same storage period, the mentioned authors obtained 28% germination, with 2% normal seedlings in the first count, for diaspores with a water content of 51.4%. This water content corresponds, in this work, to treatments with 52.4% and 49.2% water, which presented 25-36% germination, with 0-4.6% normal seedlings in the first count.

Even though the potential for maintaining viability under storage for seeds from species of the genus *Eugenia* is considered small (Rizzini, 1970; Lorenzi, 1992; Bülow et al., 1994; Lughadha & Proença, 1996; Gentil & Ferreira, 1999), the results obtained in the present work, as well as those obtained by Barbedo et al. (1998), suggest that for *E. involucrata*, the ability to store diaspores is not as reduced as reported in the literature for other species in the same genus.

After 180 days, diaspores that entered storage with water content near 52-56%, presented 20% to 35% germination. It is also important to note that the seed lot utilized in this experiment, with initial germination of 64%, had initial quality lower than that obtained by Barbedo et al. (1998), which was 90% germination in the initial evaluation. Therefore, the possibility exists for extending the storage period.

Even when the drying rate is modified (by raising the temperature in the process), apparently *E. involucrata* diaspores do not withstand reductions in water content to values below 51%. Drying procedures at 30°C which would reduce the initial water content to values near 55% can be beneficial, especially with respect to maintaining viability during storage. However, the practice of not submitting the diaspores to drying after they are collected is also interesting, since such procedure ensures maintaining viability to up to 120-180 days, when they are kept under cold storage.

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