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Refrigerated storage of seeds of *Araucaria angustifolia* (Bert.) O. Kuntze over a period of 24 months

V. PIRIZ CARRILLO,[1] A. CHAVES,[1] H. FASSOLA[2] AND A. MUGRIDGE*[1]

[1] CIDCA: Centro de Investigación y Desarrollo en Criotecnología de Alimentos. CONICET- Facultad de Ciencias Exactas - UNLP. 47 y 116, (CP B1900AJJ) La Plata, Prov. Buenos Aires, Argentina (E-mail: amugridg@dalton.quimica.unlp.edu.ar)

[2] INTA: Instituto Nacional de Tecnología Agropecuaria, EEA Montecarlo, CC N°4 (CP N3384AKW), Montecarlo, Prov. Misiones, Argentina

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Summary

The objective of the present work was to preserve the germination capacity of *Araucaria angustifolia* (Bert.) O. Kuntze seeds over a period longer than 12 months by using refrigerated storage and modified atmospheres inside the package.

The refrigerated storage were conducted in cold stores set at 0, 4 and 10°C. The modified atmosphere was generated by using packagings made up of flexible plastic films: polyethylene (PE) and Ethyl Vynil Acetate (EVA) with different permeabilities to gases and water vapor. Determinations of weight loss, package atmosphere composition, moisture content, starch content and seed germination capacity were conducted on samples every two months. The results showed that weight losses increase during storage in all conditions tested with lowest values recorded for the storage at 0°C. In turn, the seed moisture content increased by about 10%, while starch concentration decreased but not enough to be statistically significant, though the decrease was more noticeable at 10°C.

Concerning the proportion of CO_2 found after the first 7 storage months, it was higher both at 4 and 10°C in packagings made of PE.

Germination capacities found were different, depending on the films used, refrigeration temperatures and storage times tested. Germination capacities were best kept during storage at 0°C.

Introduction

Seeds from some forestry species, especially those of tropical and subtropical trees, are considered as recalcitrant (King and Roberts, 1979; Côme, 2000). Some authors (Côme, 2000; Vazquez-Yanes, 1987) coincide when describing these seeds as generally large and fleshy, with high moisture content and unable to resist intense desiccation without losses in viability. Roberts (1973) describe them as those seeds intolerant to dehydration, whose moisture content cannot be reduced below 20-30% without causing damage and germination losses.

According to Vazquez-Yanez (1987), recalcitrant seeds require constant conditions of high moisture and temperature, leading to immediate germination.

* Author for correspondence

Wang (1988) has stated that there are occasions where this type of seed does not present dormancy, that they are a few weeks or months old when stored and that some are damaged during storage at low temperatures.

Côme (2000) has indicated that the storage of recalcitrant seeds is very difficult since they require a humid environment to avoid dehydration but these conditions increase risks of germination and microorganism growth. Therefore, temperature must be sufficiently low to prevent germination or reduce seedling development yet high enough to prevent cold-induced damage. This is because recalcitrant seeds are sensitive to low temperatures. According to Tompsett (1984), four species of the Araucaria genus cannot be safely stored with moisture contents below 25-40%. In the specific case of *Araucaria angustifolia* (Bert.) O. Kuntze, the critical level below which all germination capacity is lost, is about 38% (Eira *et al.,* 1994). This characteristic prevents the seed being conserved for long times.

Various studies were carried out to find some effective conservation methods for recalcitrant seeds. Prage (1964) did conserve *Araucaria angustifolia* (Bert.) O. Kuntze, keeping germination high over five months by using low temperatures (0-5°C), and glass and polyethylene containers for storage. In Argentina, conservation of *Araucaria angustifolia* (Bert.) O. Kuntze was achieved at 0°C, with samples packaged with EVA film. In such works, germination was maintained for 9 months (Chaves *et al.,* 1999) and 12 months (Piriz Carrillo *et al.,* 2000).

Improved methods would allow even longer conservation times of *Araucaria angustifolia* (Bert.) O. Kuntze seeds which would help overcome the seasonal character of seeds production and to possibilitate the availability of an adequate supply of seeds for those years where production is scarce both at field and tree nursery.

In this regard, application of low temperatures during the storage time of plants, the rate of metabolic processes is lowered. Besides, the use of flexible plastic bags for packaging prevents dehydration and allows the generation of a modified atmosphere inside the package that may assist product conservation. The composition of such atmosphere will be dictated by film gas permeability, surface available for gaseous exchange, amount of product packaged and product respiratory activity (Day, 1990).

The objective of this work was to study the influence of various modified atmospheres (arrived at by using plastic films with different permeabilities to gases and water vapor) and refrigerated storage temperatures on the conservation of the germination capacity of *Araucaria angustifolia* (Bert.) O. Kuntze seeds over a period longer than twelve months.

Materials and methods

Vegetal material

The seeds used were collected in the planted fields placed in San Antonio of Misiones, Argentina geographically situated at 26° 04' South Latitude and 53° 45' Longitude West of Greenwich, at an Altitude of 565 m over sea level. Seeds were transported by terrestrial means to our laboratory placed at La Plata, Province of Buenos Aires, Argentina.

The initial germination capacity was evaluated after collection to be 35%, as well as after arriving at the laboratory.

Conditioning

Seeds were brought from its collection site to the laboratory inside containers. These, on arrival, were placed in a cold store at 4°C for 24 h before conditioning the samples for the experiments. This cooling stage prevented moisture condensation on the seeds during packaging for the experiments.

Before packaging, seeds were treated using a Captan PM - water paste (2:1 w/v). Then an amount of seeds weighing 800 g (about 120 seeds), randomly sampled were placed in flexible plastic film packagings. Films used were: Ethyl Vinyl Acetate (EVA) and Polyethylene (PE), whose technical characteristics are shown in table 1.

Storage conditions

Seeds were kept in cold stores set at 0, 4 and 10°C for 24 months.

Table 1. Technical characteristics of the flexible plastic films used for seed packaging.

Characteristics	Films	
	PE	EVA
O2 permeability (cm^3/m^2/24 hs/atm/23°C)	3.000	3.732
CO2 permeability (cm^3/m^2/24 hs/atm/23°C)	10.000	17.012
Water vapor transmission (gr/m^2/24 hs/%/HR/30°C)	7,2	16
Thickness (μ)	50	15

Determinations

Weight loss

Weight losses were determined by differences between the initial weight of the bags containing the seeds and the corresponding weight after different periods of refrigerated storage. To this end, a balance accurate to ± 0.1 g was used, and the results were expressed in %.

Moisture content

Moisture content was determined on 10 seed samples taken from every testing condition. An oven set at 103°C was used until constant sample weight.

Starch content

The starch content present in the seed was determined by endosperm hydrolysis with hydrochloric acid 10% v/w to quantify resulting sugars by HPLC in a Water chromatograph fitted with refraction index detector. An Accubond amino 5 μ column was employed with acetonitrile-water (89:11) as running solvent.

Modified atmosphere composition

Concentrations of CO_2 in the atmospheres developed inside the different packages were periodically determined. The gaseous sample was taken using a syringe through the film, the orifice being immediately blocked with adhesive tape. A Shimadzu gas chromatograph fitted with CTR1 Alltech column was used, the results being expressed as CO_2 (%).

Spontaneous seeds sprouting during storage

Samples packaged with the different films used, selected at random, were taken after various storage periods to count those found in sprouted state, which, knowing the total number of seeds in the package, allowed the results to be expressed as percentage. The sanitary state of seeds was also observed.

Germination capacity

To determine the germination capacity, seeds samples were placed on wet sand, in plastic trays. Trays were introduced in a germination chamber set at 27°C, and periodically sprayed with a solution of 20 g Captan PM in 10 L of water. Seeds were kept under these conditions for a maximum of 60 days. A germinated seed was considered as that having a radicular emergence of 3 mm. Results were expressed as % germinated seeds.

The initial germination capacity was determined as soon as the seeds were received at the laboratory. To this end, a 40-seeds sample (formed by taking 10 at a time) was placed in the germination chamber.

Samples were taken every two storage months in each testing condition (storage temperature and packaging film) to determine germination capacity. Twenty seeds were used from each sample, which were divided in two subgroups of 10 seeds each, and placed in corresponding trays in the germination chamber.

In order to determine the germination capacity after each refrigerated storage period, those seeds already sprouted in the bags were not considered, as they would experience damage during sowing. Therefore, the data reported for germination capacity refer to unsprouted seeds taken from the different cold stores.

Experimental design

A 2 × 3 × 12 factorial design was used with the following factors: type of film, temperature and duration of the refrigerated storage, with two replications. The data collected was processed by Analysis of Variance (ANOVA), the means being compared with the LSD test at a significance level α=0.05. In all conditions tested, samples were taken on a bimonthly basis.

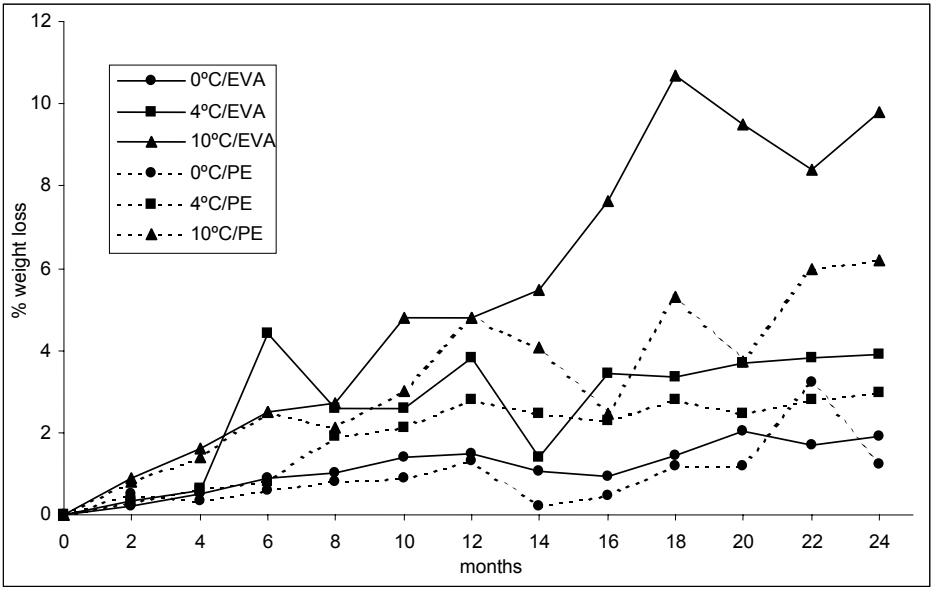

Figure 1. Evolution of weight loss of *Araucaria angustifolia* seeds stored at 0,4 and 10°C in the indicated conditions. LSD=2.34

Results

Weight losses

Figure 1 shows weight loss values found along storage. Weight losses increase linearly ($r=0.902$, $P<0.05$) with the progress of conservation time in all conditions. Besides, by comparing at the same storage temperature, it can be observed that weight losses were higher in seeds packaged with EVA films, especially after 16 storage months at 10°C ($P<0.05$).

Regardless of the films used for packaging, the lowest weight losses over storage time were found in seeds stored at 0°C (not greater than 2%), whereas the highest values were determined at 10°C (where, after 18 months of testing, they reached values of about 8%).

Moisture content

Seed moisture content was 44.6% (figure 2) at the beginning of the experiments, a value that, according to Eira *et al.* (1994), is 6.6% above the critical threshold for *Araucaria angustifolia* (Bert.) O. Kuntze. Moisture content increased by about 10%, on average, over the first 12 months of testing, to remain almost constant from then up to the end of storage (24 months), regardless of the film used and of conservation temperature.

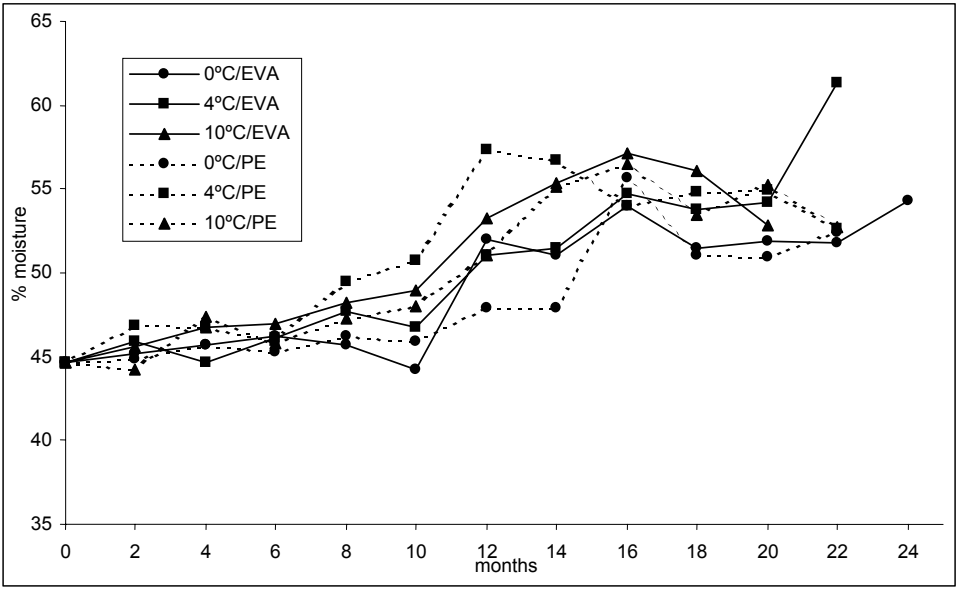

Figure 2. Evolution of moisture content of *Araucaria angustifolia* seeds stored in the indicated conditions. LSD=4.02

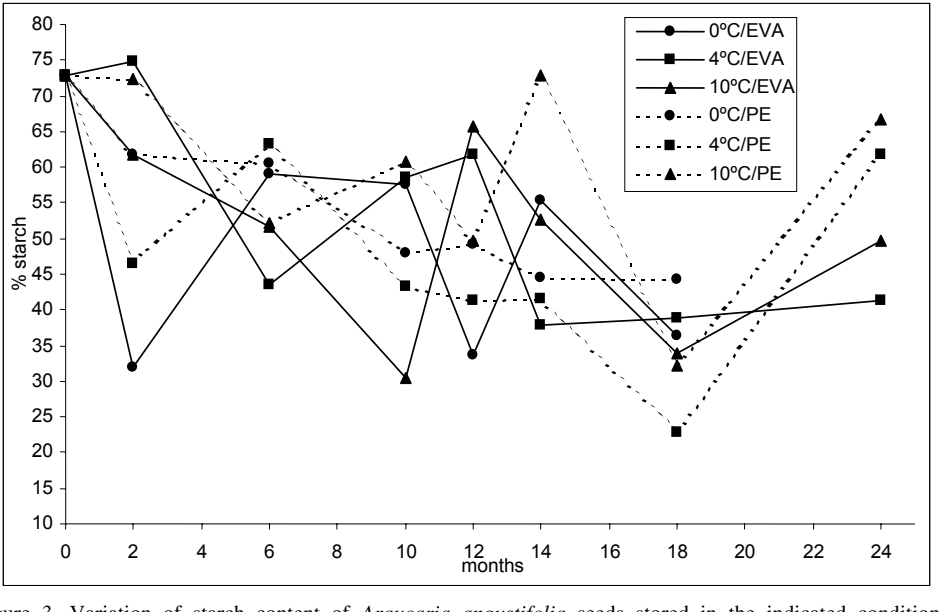

Figure 3. Variation of starch content of *Araucaria angustifolia* seeds stored in the indicated conditions. LSD=11.81

Starch content

On analyzing measured data on the variation of seed starch content (figure 3), the differences found owing to the type of film and storage temperature conditions were not significant ($P<0.05$).

Nevertheless, a decrease in starch content below its initial value of 72.9% was observed from a storage time of 2 months in all experimental conditions, the lower values being found in seeds packaged in EVA film and stored at 4 and 10°C ($P<0.05$, figure 3). At 0°C, the starch content decrease linearly ($r=0.902$, $P<0.05$) to a value of approximately 40%.

Composition of the modified atmosphere

Significant differences ($P<0.05$) were found after using the two films tested as packagings. Higher CO_2 values were measured in those packages made of PE (figure 4).

From the seventh month of storage, an increase of CO_2 was observed, especially in the bags made with PE and stored at 4 and 10°C (figure 4), where concentrations reached 8 and 12%, respectively. For each type of film used, the lowest CO_2 concentrations ($P<0.05$) were found in the packages stored at 0°C.

Spontaneous seeds sprouting during storage

From the second month of refrigerated storage, seed sprouting in variable degree of development was observed in some bags stored at 4 and 10°C. The proportion of sprouted seeds increased strongly with storage time (LSD time=6.98), regardless of the film used. This increase showed a lineal correlation with storage time (4ºC: $r=0.902$, $P<0.05$; 10°C: $r=0.918$, $P<0.05$). No sprouting was observed inside packages stored at 0°C.

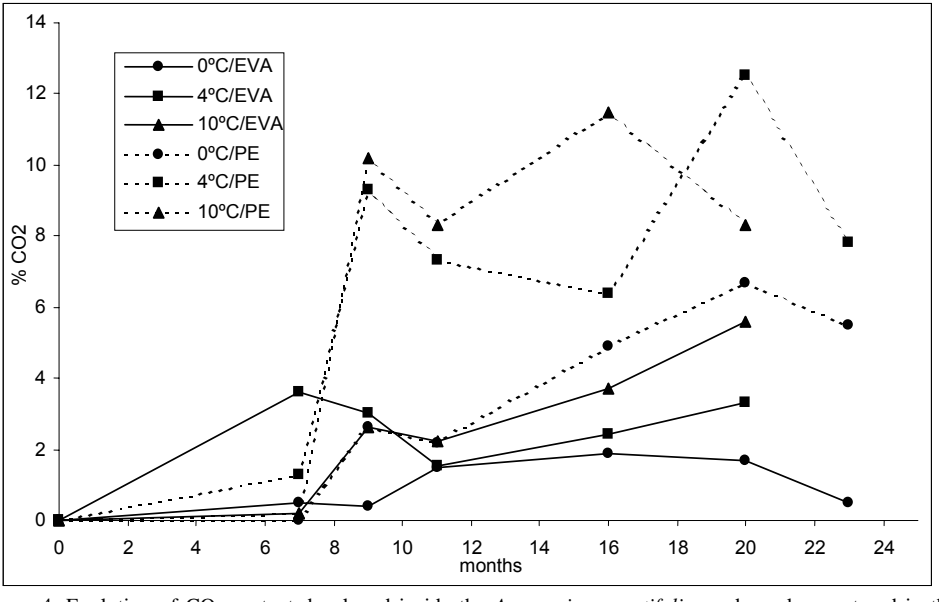

Figure 4. Evolution of CO_2 content developed inside the *Araucaria angustifolia* seeds packages stored in the indicated conditions. LSD=7.3

Figure 5 shows that the proportion of sprouted seeds was higher at 10 than at 4°C for both films.

Concerning the comparison at the same temperature and storage times, the proportion of germinated seeds inside packages of PE did not differ significantly (P>0.05) with the values found for EVA, though a tendency to find higher values in PE packages was observed.

Germination capacity

The initial germination capacity determined on sample arrival in the laboratory was 35%.

Table 2 show the average germination percentages obtained for the different films, temperatures and storage times tested.

In all experimental conditions, an increase in germination capacity was observed after two months of refrigerated storage (P<0.05), reaching a maximum between 4 and 6 months. That value varied between 80 and 95%, so that it was some 2.5 times above the initial value. At the month 8, germination capacity decreased (P<0.05) to almost the initial levels. After this time, germination kept decreasing in seeds stored at 4 and 10°C to reach zero at the end of storage. In contrast, the germination capacity of seeds stored at 0°C experienced a new increase (P<0.05) between 12 and 20 months, to reach values alike those measured between 4 and 6 months of testing. After 24 months, seeds stored at 0°C presented a germination capacity of about 40%.

Reverting to the packaging stored at 4 and 10°C, presence of molds was detected at the end of the refrigeration period.

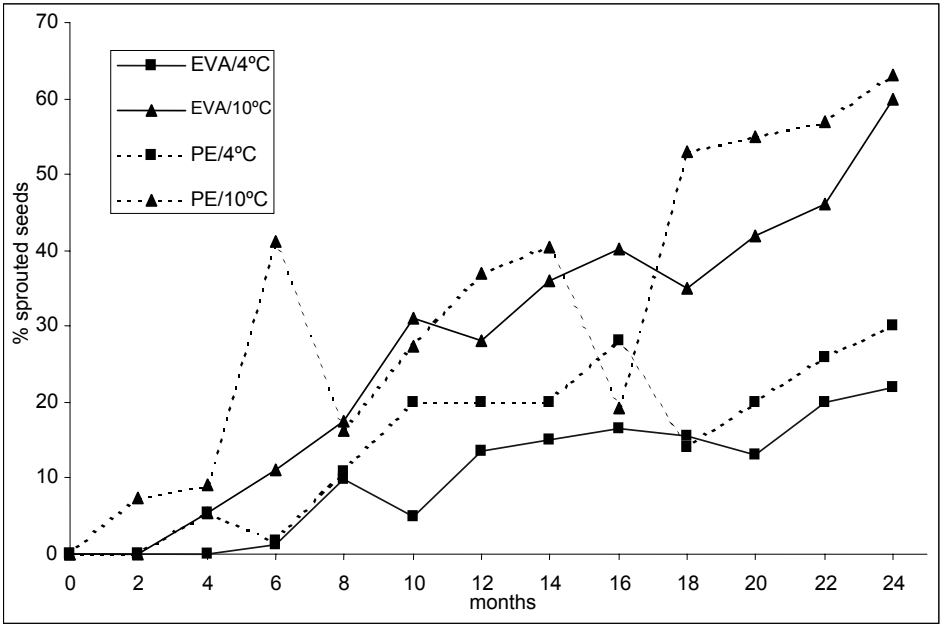

Figure 5. Changes in the sprouted seeds stored in the indicated conditions. LSD=17.10

Table 2. Change in germination capacity (expressed as percentage) of *Araucaria angustifolia* seeds packed in EVA or PE film and stored at 0, 4 or 10°C. LSD=25.0

	Film Eva			Film PE		
Months	0°C	4°C	10°C	0°C	4°C	10°C
0	35	35	35	35	35	35
4	90	90	77.5	77.5	82.5	60
6	95	80	60	90	82.5	60
8	20	30	55	5	35	37.5
12	72.5	40	30	57.5	27.5	32.5
18	85	15	17.5	72.5	12.5	0
24	50	0	0	55	0	0

Discussion

Plastic packaging constitute generally the best storage to preserve moisture (Bean *et al.,* 1984; Copeland *et al.,* 1995). In fact, an nonlinear increase of moisture content of seeds during refrigerated storage was detected in the present work. A mechanistic growth model was employed for the regression of values (moisture= a*(1-0.273*exp(-0.273*tiempo)), r=0.998, P<0.05). Tompsett (1982) has also observed moisture gains (on fresh weight basis) of 0.23% per day in *Araucaria hunsteinti* seeds stored in polyethylene bags sealed for 56 days and concluded that the additional water is originated by the respiration of the seeds reserve.

On the other hand, it is known that as far as seed storage is concerned, a high humidity inside the package reduces the conservation potential (Ellis *et al.,* 1982) by increasing possibilities of mold growth. Precisely, the presence of molds inside the package is likely to be responsible for the germination reduction to null values at 4 and 10°C after 22 months, whereas at 0°C, germination values kept higher to the initial percentage (in both films).

The gaseous composition changes with the progress of the experiment, according to the film permeability used for packaging, the storage temperature and the respiration of seeds and associated microflora. The maximum CO_2 percentage was 11% in seeds stored with PE film at 10°C. In contrast, owing to the permeability characteristics of EVA films, a more intense gaseous exchange was established with the environment, resulting in a lower CO_2 percentage inside the packages for all testing temperatures. At 0°C, an lineal increase in CO_2 percentage was found (r=0.893, P<0.05).

In all experimental conditions, germination capacity values were observed to increase above the initial value, especially between 4 and 6 months of refrigerated storage. Storage temperatures exerted a significant influence on seeds germination capacity. In seeds stored at 0°C for 6 months, germination capacity reached 95% when kept in EVA films while the

value for PE film was 90%. Aquila *et al.* (1984) have indicated that an initial dormancy may be present which is eliminated by short-term storage. In turn, Chaves *et al*. (1999) and Piriz Carrillo *et al.* (2000) have found an important increase of germination capacity respect to the initial value after 4 months of storage at low temperatures. After that, the germination begins to decrease. In seeds stored at 4 and 10°C the decrease continues up to the end of storage, whereas, in seeds refrigerated at 0°C, it reverts to a new increase of germination percentage, reaching 85% for EVA and 72.5% for PE films.

From the studies carried out in this work, one of the main causes arising as responsible for the decrease of germination capacity of seeds under refrigeration conditions at temperatures above 0°C is mold development. Although seeds were treated with a commercial fungicide before being packaged, its residual action was lost during the course of storage. The results of this work suggest that a temperature of 0°C and the use of EVA or PE film as material for packaging the seeds constitute appropriate conditions to store *Araucaria angustifolia* (Bert.) O. Kuntze for a 2-year period keeping a good germination capacity.

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