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INFLUENCE OF PHYSIOLOGICAL MATURITY AND  
OF THE INTERVAL BETWEEN HARVEST AND THE  
START OF THE STORAGE PROCESS ON THE VIA-  
BILITY OF THE SEEDS OF THE RUBBER TREE.

----- (Hevea spp)<sup>1</sup> -----

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SUMMARY

Various time intervals between rubber tree seed selection and the initiation of seed storage were observed in order to ascertain effects on preservation and viability. Harvested seeds were subjected to the following quality evaluations: physiological, maturity, moisture content, germination capacity, carbohydrates, lipides and crude protein. Concerning seed physiological maturity, it was verified that maximum values of dry seed - weight coincided with both percentages of germination and lowest seed moisture contents when seeds were harvested directly from capsules on the tree. In general, data indicated that a delay between seed collection and storage altered seed quality. Seeds stored immediately after harvesting maintained their viability, no matter the origin of the seed. Additionally, a significant variation was observed in total soluble carbohydrates, lipids and crude protein of seeds during storage, but not during the time between harvest and storage. Initial moisture content of stored seeds was, according to these results, the determining factor in seed preservation availability.

INTRODUCTION

Horticulture does not possess an adequate technology for conservation of seeds so as to assure a high germinative potential after several months of storage. This difficulty

occurs owing to the strong decrease in the germinative capacity of the seeds a few days after they are harvested in the rubber plantation, thus leading to serious setbacks on the implanting of the nurseries as it affects the cost, the size and the chances of implantation of the same.

The most deep-rooted causes of this rapid deterioration of viability in the seeds of this variety are not well known although, it has been pointed out that the level of moisture (18.23), oxidation of lipides (4) or even the presence of internal fungus (31) decisively influence its physiological quality. These factors are in turn affected by the degree of physiological maturity of the seed, an aspect of which has not yet been studied much, especially in reference to the storage of seeds of the rubber tree, taking into consideration that after the bursting of the seed pod on the trees, until they are harvested, may lie for varied stretches of time on the ground or may be temporarily stored, undergoing internal changes of a qualitative and/or quantitative nature which may seriously affect their initial good quality.

The present work aims at evaluating the effect of physiological maturity and of the length of time between the harvest and the beginning of the process of storage on the preservation of the germinative capacity of the seeds of the rubber tree.

### MATERIALS AND METHODS

The present research was carried out in 1981-82 at the National Centre for Research on the Rubber tree and Dende (sort of coconut) (CNPSP) with headquarters at Manaus-AM Brazil and belonging to the Brazilian Enterprise of Research in Husbandry (Agro pecuaria) (EMBRAPA).

The seeds used in this study are taken from a small rubber plantation (approx. 10,000 square meters - one hectare) centered about 15 km from the CNPSP, with a prominent genetic lack of uniformity and with trees of great height. The plantation showed decay followed by a heavy fall of leaves in the month of June 1981, with appearance of fresh foliage and florescence in the month of August of the same year, the latter lasting till approximately the second fortnight of the following month. In general florescence and fructification were not observed to be abundant specially on the trees in the centre of rubber plantation.

#### Physiological maturity

With a view to the establishment of the curve of physiological maturity on two trees which originated from the type of seed called "pi franco", five harvests of fruits were made per tree beginning on 28/10/81 and subsequently continued on 30/11/81, 28/12/81, 30/01/82 and 15/2/82. On each of these dates three samples of 45 seeds each were collected in order to determine the moisture content and the weight of the dry material, while for the tests of germination three samples of 20 seeds were collected.

The moisture content, moist basis and the dry material were evaluated together by using the method of steaming (estufa) at a constant weight of the samples. The germination tests were made for a period of 30 days in a seed bed open to the sky with a sulstrata of wet sawdust covered artificially with roof reeds. The seeds were considered germinated with the liberation of the plumule to a length of 0.5 cm through the protecting sheath.

#### Harvesting and conditions of storage

Soon after the fourth harvest on 30/01/82, a general harvest of the ripe fruit of the trees was made in a single day's operation. Approximately one week before the general harvest a collection had also been made of the seeds which had fallen naturally on the ground. After this the seeds extracted from the capsules as well as those taken from the ground were subjected to a solution of Benlate (soluble powder 0.3%) for 15 minutes. Then samples were taken for the initial determination of moisture content and germination. The sample for the determination of moisture content was made up of 45 seeds each time for three repetitions. In the case of germination also three repetitions were made but with 50 seeds each time. In the evaluation of moisture content and of germination these values should be understood, unless the contrary is pointed out.

With reference to the remaining seeds the latter were separated into two groups. The first group of these (on zero day) were immediately stored in plastic bags provided with

six small holes (23) containing 300 seeds per bag and kept in a room at a temperature of  $22 \pm 2^{\circ}\text{C}$  for 2, 4, 6, 8 and 10 months. A second group was kept in the open for 30 days in a room under uncontrolled environmental conditions. The seeds of the latter group were withdrawn at regular intervals of ten days to evaluate their moisture content, germination and to begin the process of storage under the conditions already described 10, 20 & 30 days having passed since the date of the general harvest. During the period of storage evaluations of moisture content and germination were made at intervals of 2 months upto 10 months simultaneously with the evaluation of moisture content and of germination determinations were also made of total soluble carbohydrates lipides and crude protein.

For the determination of carbohydrates three repetitions of 0.5 g of dried and ground seeds were made. The extraction was made with 25 ml of water, in erlenmeyer of 50 ml at temperature of the surrounding air, during six hours with revolving done by hand at an interval of 15 minutes. The extract was filtered and completed with water to make up a volume of 100 ml. From this volume an aliquot part of 10 ml was taken diluting it to make up a volume of 100 ml, out of which three samples of 2 ml were taken to be dosed by the method of antrona (2, 30) with the help of a spectrophotometer Bausch & Lomb Spectronic 20, read in the band of 623 n.

In the quantification of lipids three repetitions of one gram of dried and ground seeds were used, subjected to the extraction with ether of petroleum (p.e. 30 60°C) in Soxhlet systems during 3 hours. Once this period of time was completed, the extracts were evaporated in a steam rotar at low pressure and the residue calculated as a percentage (22).

As for the content of crude protein, the analysis of the three repetitions of one gram of dried and ground seeds were made by the Method semi-micro Kjeldahl (analysis made by the plant Laboratory of UEPAE/Manaus) taking 6.25 as a factor for conversion of nitrogen into protein.

#### Statistical analysis

The experimental outline used in the determination of physiological maturity of seeds was that of random blocks with 5 treatments (dates of harvesting) and 3 repetitions, including 6 treatments (months of storage) and 3 repetitions. The same outline was used to evaluate the germination during the storage process. For moisture content of lipides, soluble carbohydrates and crude protein, a graph with subdivided parts was used with three repetitions, the days being considered as divisions (0-10-20-30) and the months of storage as sub-divisions (2-4-6-8-10). The comparison of averages was made by the test of Tukey to the level of 5% probability and the percentage data was transferred to arc  $\sin \sqrt{x\%}$ .

### Results and discussion

Figures 1 and 2 show that in the developing seeds the maximum values of germination are situated among the highest values of weight of dry material and least values of moisture content, however, very near the first in tree A. A similar tendency has been verified by other authors (25) and is also in agreement with the initial hypothesis of the work, in the sense that the fruits that were ripe and ready to experience bursting of the seed pod (in the present case end of January and first fortnight of February 1982) already contained seeds in the phase of physiological maturity, that is, with greater weight of dry material and greater percentage of germination (24). With reference to this, it was evident that the values of the weight of dry material of the forest and fifth harvests (30/01-15/02/1982) were not different among themselves in Tree A as well as in Tree B. A similar fact occurred with moisture content (37%) in Tree A (Figure 1); however in Tree B, Fig.2, these last two points 43% and 38% were statistically different, notwithstanding, that the curve in that section had a tendency to stabilize, in spite of the seeds showing, still a high content of moisture content. High values of moisture content have also been found in the physiological maturity of *pterogynemiten*s (5) and *capsicum annum* (19) oscillating between 60-65% and 50% respectively.

It is suitable to mention that a high rate of germination (95%) was observed in the third harvest (28/12/81) of

Tree A (Fig.1) in spite of the values of moisture content and the weight of dry material of the seeds not being stabilized and in spite of dealing with fruits of a completely green epicarp. This result gives evidence that about 30 days before physiological maturity the seeds already show germinative capacity, so this could anticipate the installation of small seed plots or, in general motivate their use for experimental purposes. In Tree B (Fig.2) the greatest rate of germination occurred on the fourth day of the harvest, pointing out that there was lack of uniformity in the flowering and fructification between the trees.

Although the measurements of physiological maturity have been initiated only beginning from 60 days after the evidence of the start of flowering, the results obtained satisfy the necessity of knowledge regarding the moisture content and percentage of germination of the seeds near the period of fall and the beginning of storage. Technical difficulties for the determination of physiological maturity in annual species have been cited (20). In the rubber tree, the lack of uniformity in the flowering constitutes a technical difficulty for a more exact determination of the maturity referred to. In spite of this, the results presented in this study has the advantage of being similar to the condition in a native rubber plantation which is the source of the greater part of seeds used in the husbandry of this cultivation in the Amazon basin.

Seeds collected from the ground or directly from trees left exposed to open air for varying periods before the beginning of storage have shown evidence of a sharp fall in moisture content and germination (Fig.3). It has become clear that, with the passing of time, the initial moisture content of the seeds collected from the trees and from the ground, 39% and 36% respectively fell, and concomitantly the percentages of germination also. However, in the seeds from the ground the declines were more premature and may be attributed to the fact of having entered at zero time (date of the general harvest) with a lesser value of moisture content. The presence of free fatty acid may also be pointed out as another factor involved in the rapid fall of germination (12).

The loss of weight may have a practical application in the determination of germinative power (P.G.) of the seeds, for these become lighter when dehydrated. Seeds recently collected from trees (39% of moisture content) showed 200 units per kilo and P.G. of 92%, while 30 days after harvest that number reached 270, with zero percent germination.

Although the number of seeds per kilo may vary owing to the function of various factors, this parameter has potential of becoming a more practical alternative than the colour of almond (11), or the tests of tetrazolium (26) in the rapid evaluation of viability of the seed by the horticulturist or by technical assistance.

The percentage of germination and moisture content between two and 10 months of storage pertaining to the treatment zero days, are found in Fig.4 concerning the seeds collected from the trees, it was evident that there was no significant difference between the initial germination and the one which occurred at 2 months of storage, however, statistically it was higher than that of the remaining months.

The germination which occurred at 2 months did not significantly differ from the remaining months, although there was a pronounced fall in the germination between the fourth and sixth month, probably caused by the presence of fungi in the interior of bags (visual evidence).

Notwithstanding the existence of the evidence of the limited efficiency of Benlate in the conservation of seeds (17), the infection of fungi in the seeds used did not provoke serious damage. The presence of fungi in the interior of the seeds presents a challenge to research concerning what is related to proceedings and fungicides to be used aiming to reach the pathogen in the interior and not only externally (10). As for moisture content of the seeds of the trees, it was very uniform during the period of storage, around 40%, and similar to the seeds on the ground (fig.4). With regard to the seeds on the ground significant differences in germination were not verified nor were fungi evident to the naked eye in the ones that had not germinated. The high values of germination attained by both groups of seeds supports the

recommendation of the plastic bag as packing for the seeds of the rubber tree (23). This type of packing, undoubtedly, contributed to maintain the initial internal moisture content of the seeds and was a necessary condition, together with the temperature used to maintain a high level of germination for the period of 10 months storage, supposedly till the last start of the next rainy season, November-December, which is the occasion when, in the region seeds for the plantation do not yet exist.

In spite of the moisture content of the seeds stored immediately after the harvest not having suffered significant variations in the treatment of zero days (Fig.4) from the beginning of the eighth month of storage a percentage of germination of about 5% was evident in the interior of the bags, depending on its size, this percentage may become a serious obstacle to storage for a long period of time. Possibly, the germination of some seeds in the interior of the bags might be due to a distinct need of rehydration, breathing, energy charges, genetic variability, etc.

The effect of the period prior to the storage of the seeds of the trees or of the ground on the germination and moisture at 1, 4, 6, 8 and 10 months are shown in Fig.5A, B & C. A fall in germination is clear beginning from the initial day (date of the general harvest). In case of the seeds which remained in the open air for 30 days, this fall was more prominent and had negative effect on germination in the different months of storage, as with the moisture content of 15% no

germination was noticed beginning from the 30 days on word. For the seeds left in the open air for 10 days before storage moisture content values were observed to be in the band of 20 to 25% (Fig.5A), corresponding to a relatively low germination, 60% and 48% for seeds collected from the trees and from the ground, respectively. An abrupt fall in germination was noticed starting from the two months of storage, inclusive not necessitating any statistical comment on the comparison of averages.

Reflections on the germination, caused by a low initial humidity, 21% and 15% Fig.5B, are also clearly seen during the entire period of storage, thus showing once again, that the initial moisture content of the seeds is a crucial point in what concerns the maintenance of viability with a view to storage. In other words, the nearer the harvest and the beginning of the storage are to physiological maturity, the less damage can affect the germination during storage. In the meanwhile, an increase in the moisture content of seeds, at times very significant was evident (Fig.5A, 5B) beginning from its initial value at 10 and 20 days. Perhaps it was a question of hygroscopic balance with the external environment made possible by the presence of holes in the plastic bags used. Nevertheless, the fact that the moisture content of the seeds kept in the open air for 30 days remained invariable during storage, weakens such a hypotheses as the conditions were similar. On the other hand, there are references (17, 23) of internal variations in moisture content occurring and also of the increase of germinative power (23) which does

not seem to agree with the point of view that the physiological quality of seeds does not improve during storage.

References on the effect of natural dryness of the metabolical aspects of the seeds of the rubber tree have not been frequent, nevertheless, there has been information that the level of hydrocyanic acid (HCN) or of the toxicity lowers with the loss of water through the seeds (32). In the seeds of the rubber tree, the content HCN is related to the enzyme linamarase and the glicosidio cianogenico linamarina or the pH (slightly acid), of the inter-cellular medium (27).

In contrast with other seeds like those of the gramineas and of leguminous plants and the longevity of the seeds is short, suggesting the probability of intolerance of the protoplasmatic colloid of the embryo to suffer profound changes in their degree of viscosity or hydration in the post-harvest phase. These variations in the interior of the cell may be related with alterations in the integrity of the fine structure of the membranes and/or organelas; modification in the rate of synthesis or activity of molecules such as enzymes, co-factors, etc., and changes in the population of monosomes, polisomes and ribosomes joined to membranes (1, 7, 28). Hence at a level of moisture content of 15% germination is nil; which points out that the level of deterioration of the embryo was irreversible and that the hydration in the phase of soaking was incapable of recomposing or activating the processes compatible with germination.

In accordance with the figure 6, 7 & 8 in the four periods prior to storage (0, 10, 20 & 30 days) there was practically no variation between the seeds collected from the trees and from the ground with regard to the level of carbohydrates, lipides and crude protein, suggesting little or no effect on germination. In fact, the average values not transformed in 30 days into carbohydrates were 11% (ground) and 11.5% (tree) and into lipids they were 40% and 44% in the seeds from the ground and from the trees, respectively. With regard to crude protein in the seeds from the ground and the trees, they were, respectively, 19.3% and 19.0%: these percentages are high and explain the interest of the research for using them as new sources of food (27, 33).

Fig.6 shows a sharp tendency of decrease of total soluble carbohydrates during storage, with reference to their respective controls (0, 10, 20 and 30 days). The hardness of the integument of the seeds and the probable decrease of the concentration of oxygen in the interior of the bags suggest that only the need of anaerobic glicolise (fermentation), in its lactic or alcoholic unfolding, had been activated sufficiently. In the meanwhile, the last mentioned factor is not considered decisive in the reduction of longevity of the seeds stored of the tree Araucaria hunsteinii (29). With a basis of the previous reasoning, it is possible to suppose that the similarities in the content of the carbohydrates between the controls have been influenced by the conditions of non-confinement and

of the non-restricted atmosphere which surrounded the seeds during the period prior to its storage. Similarly, the differences in germination, for example, between zero and the other days may be attributed to the levels of moisture inside the seeds.

The high values of extract of ether found in the seeds used (Fig.7) suggest that the endosperm of these seeds is a great drain of carbohydrates for later conversion into fatty acids such as has been informed for *Ricinus Communis* (9). Hence, it is supposed that the endosperm is not only related to the synthesis of triglycerides but also with that of being an active web of reserve of the lipids to be transformed into carbohydrates later during germination, probably via the cycle of glioxolato (6, 8, 16). In the meantime, during the period of storage the high content of lipides may be acting in accumulation of triglycerides and/or fatty acids occurring from the hydrolysis of the former or simply from its synthesis starting from acetyl CoA to sacarose (14) but without any great unfolding later, for metabolic events linked with these compounds are activated during germination (21). Although the germination was nil during some months of storage and certain level of extract of ether lowered significantly, such a point of view shall not be interpreted so rigidly, for, in spite of the embryo being damaged, as postulated in the present case, the metabolic activity of the seed may continue for quite some time. This view point coincides with what was verified in

other cases (3), when respiratory activity was detected without there having been any germination. This hypothesis makes more understandable the tendency of increase of the level of crude protein during storage (Fig.8). Notwithstanding, questions related to the types of protein accumulated in proteic bodies either globulin and/or albumen (15) and subsequent insertion of the embryo in the unfolding of these molecules (13) open new perspectives of investigations in the seeds of the rubber tree.

### Conclusions

1. The seeds of the rubber tree present in the fruit ready for the bursting of the seed pod, are found, about six months after the first indication of flowering, at the right point of physiological maturity, that is, having the maximum value of dry material germination and least moisture content.
2. About 30 days prior to the bursting of ped of the fruit, the seeds of some trees already show high germinative capacity (95%) although they have not yet attained physiological maturity.
3. The moisture content with which the seeds fall on the ground oscillates at about 40% and as the seeds lose their initial moisture content, their germinative power begins to decrease
4. The number of seeds per unit of weight may become a practical proceeding to estimate the germinative process of one lot of seeds of rubber tree.

5. The reduction of the period between the fall of the seeds from the tree, harvest and the beginning of the storage is fundamental for the maintenance of their viability among storage.
6. The combined effect of the plastic bag (half-full), moisture content of the seeds nearing 40% and temperature of 22°C contribute effectively to the success of the storage dealing with seeds of the rubber tree that have not yet experienced the calamities of the area.
7. The contents of carbohydrates, lipids and crude protein were significantly changed by the prevailing environmental conditions, during the periods of zero, ten, twenty and thirty days prior to storage.
8. During storage variations in content of carbohydrates, lipids, and crude protein may occur including seeds without germinative capacity.
9. Evidence was not found to show that the content of carbohydrates, lipids and crude protein reduce the viability of the seed.

#### Summary

The effect of maturation and the interval of time between the harvesting of seeds of the rubber tree collected from the ground as well as directly from the capsules of trees and the

beginning of seed storage on the preservation of the germinative capacity of seeds was evaluated during a period of 2 to 10 months.

With this aim, the following parameters were evaluated, physiological maturity, moisture, germination, carbohydrates, lipids and crude protein. Concerning seed physiological maturity it was verified that the maximum values of dry seed weight coincided with the highest values of germination as well as with the lowest values in moisture when the seeds were harvested from capsules on the tree. Depending on the period of time prior to seed storage it was verified that the highest percentages of germination coincided with the seeds taken from the trees and from the ground when stored immediately after harvesting. Significant variations in the content of total soluble carbohydrates, lipids and crude protein were observed not during the period prior to storage but during the period of storage. The initial moisture content of the seeds for storage was the main determining factor in the preservation of viability.

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FIG 1

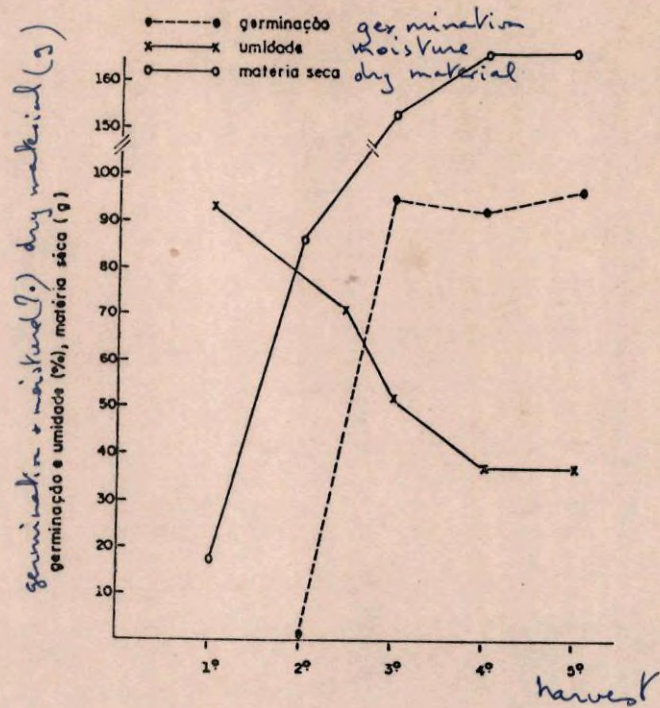


Fig 1. Variations in germination, dry material and moisture content in the seeds of the rubber tree in different harvests of Tree A.

FIG - 2

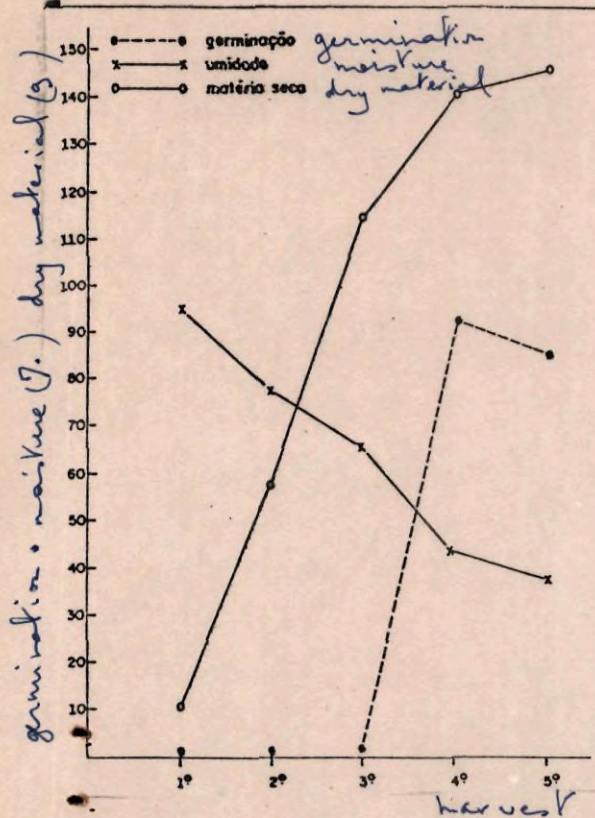


Fig 2. Variations in germination, dry material and moisture content in the seeds of the rubber tree in different harvests of Tree B.

FIG-3

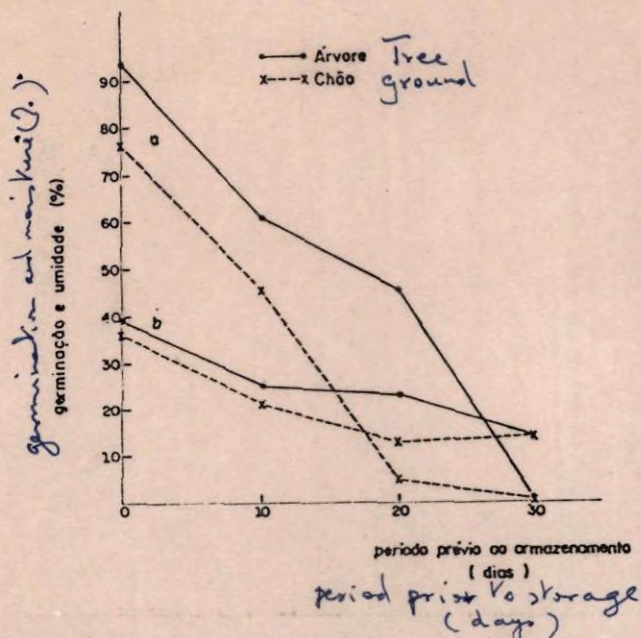


Fig 3. Germination and moisture content of the seeds of the rubber tree during the period prior to storage  
a: germination b: moisture content

FIG-4

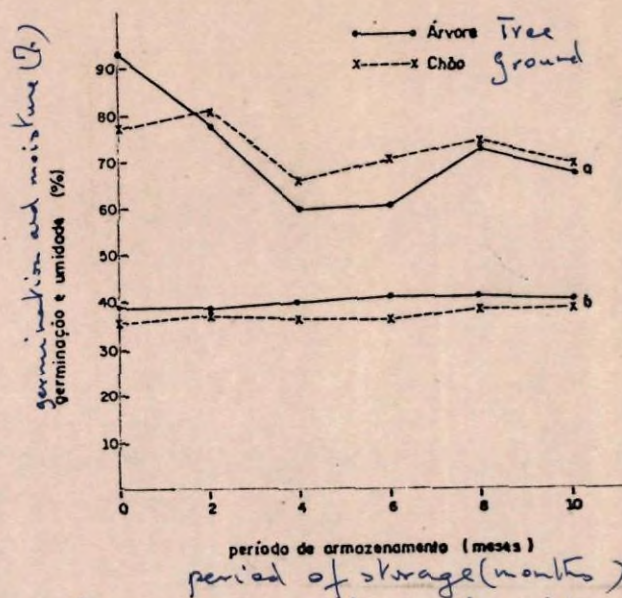
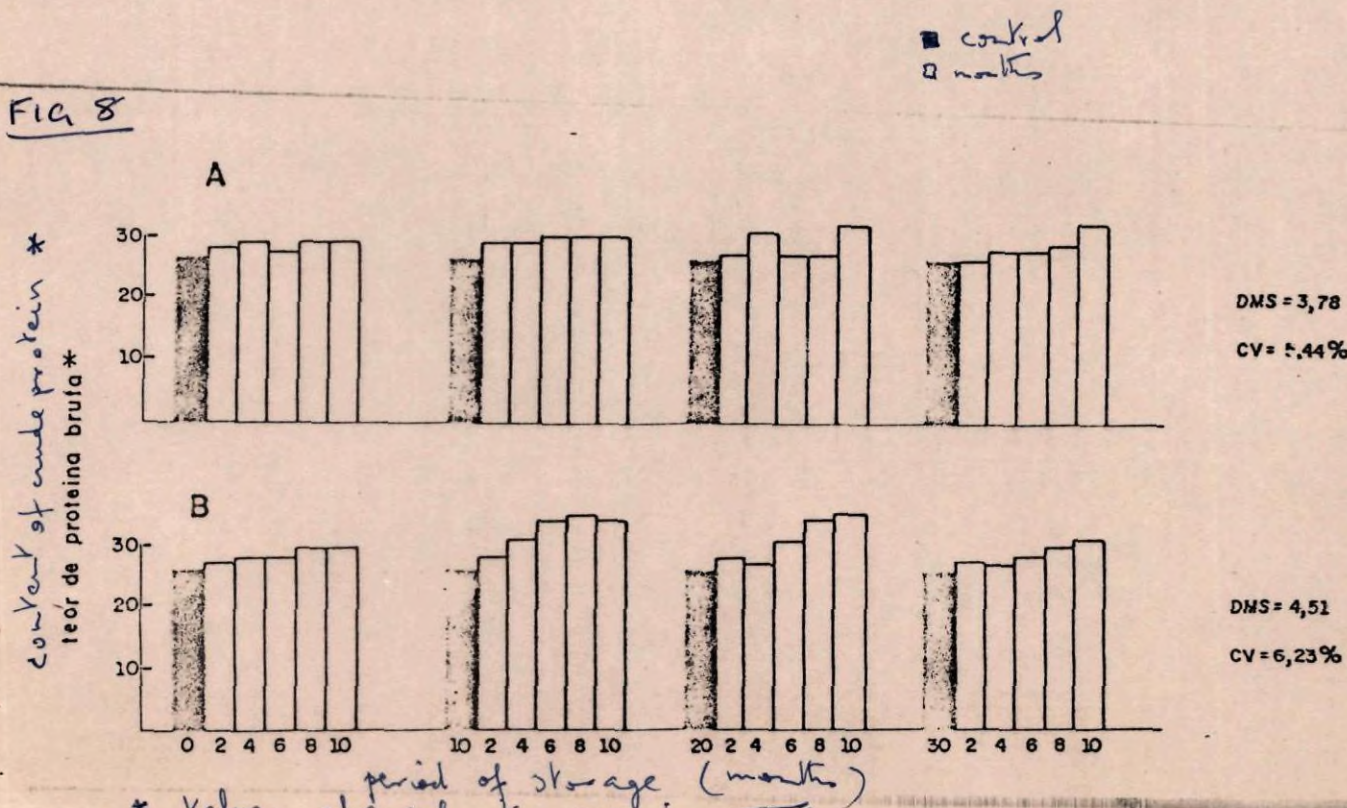


Fig 4. Germination and moisture content of the seeds of the rubber tree, stored immediately after the general harvest.  
a: germination, b: moisture content

31

31

FIG 8



\* Values changed to arcsin  $\sqrt{x/2}$

43

FIG-5

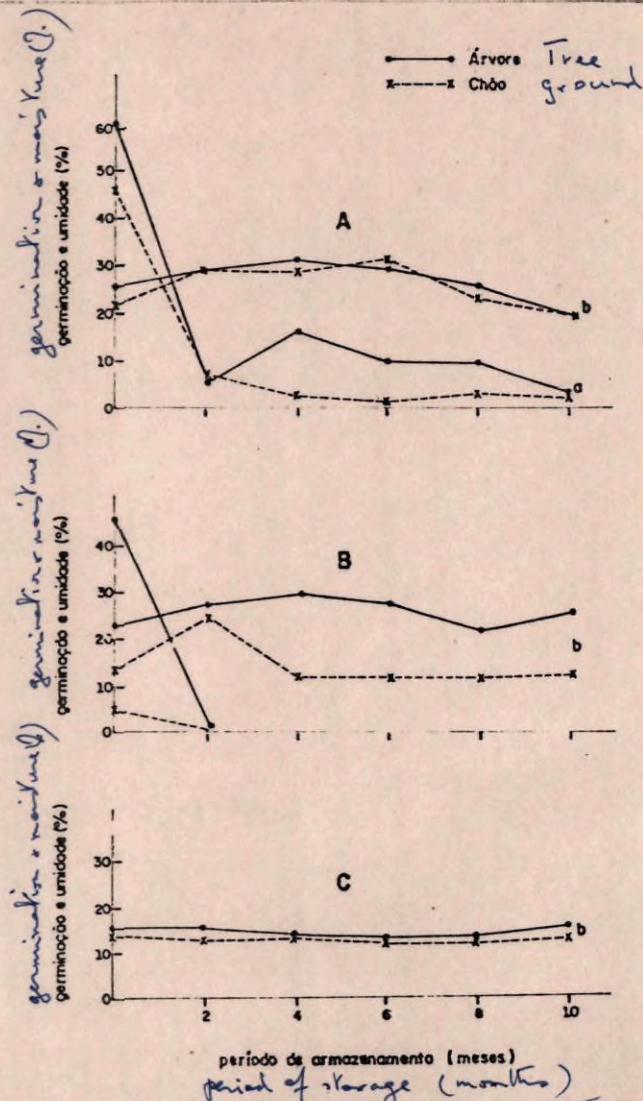


Fig 5 Effect of the period between the general harvest and the beginning of storage of the seeds of the rubber tree on germination (a) and moisture content (b) during storage.  
A: 15 days B: 20 days C: 30 days