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Controllable storage conditions increase survival and germination rates of *Quercus hintonii* acorns

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ABSTRACT

Quercus hintonii is an endangered species of red oak that grows in the province of Sierra Madre del Sur. It sheds acorns from August to September, when the mean monthly temperature is from 15-25°C. The acorn fruit can be considered a recalcitrant seed type, whose germination rate drops to 4.5% only 30 days after shedding. Considering how rapidly *Q. hintonii* acorns lose viability, it is of the utmost importance to find controlled conditions that allow the long-term preservation of viability and vigour. Herein, we propose the use of a saturated solution of magnesium chloride and calcium chloride, which provides a relative humidity of 33% upon hydration at 7°C for acorn storage. We found that the storage conditions for acorns of *Q. hintonii* are crucial for extending acorn survival time, preserving a higher germination rate and expanding the time needed for 50% germination, which varied between 115 and 183 days depending on the harvest. In addition, the time for germination ranged from 5 days to 14 days when the storage period was prolonged. Moreover, the vigour of seed obtained in 2004 was greater than that of the 2007 seeds, as measured by increase in survival, plant vigour, increased stem size and an increase in leaf number. The different environmental conditions during harvesting showed strong effects on seed germination and plant development, which likely resulted from moisture levels being high in 2007 compared with 2004. Thus, acorns harvested in 2004 experienced a drier environment, did not germinate in the field at that time and reached the end of the maturation phase; whereas in 2007 a greater number of acorns germinated in the field at the time of its collection. Therefore, the 2007 harvest conditions affected the time needed to equilibrate at 30% relative humidity, exposed the acorns to a greater time in the sensitivity window, deteriorated them further and diminished the germination index and seedling vigour.

KEYWORDS: acorns, germination, oak, *Quercus hintonii*, storage conditions, viability

ABBREVIATIONS

dDT, days of plant development; dST, days of storage time; FW, fresh weight; G½, time needed for 50% germination; P_{2004}, plants of 2004 harvest; P_{2007}, plants of 2007 harvest; 1°B, first bud; 2°B, second bud; 3°B, third bud

INTRODUCTION

Mexico contains tremendous diversity in oak trees; more than 125 species can be found [1-3], and nearly 70% of these are endemic. Some of these species grow in the south-western states of Mexico within the province of Sierra Madre del Sur in the sub-province of the Río Balsas Depression. This region experiences a warm and sub-humid climate with a notoriously dry season during the spring and abundant rain in summer. The predominant vegetation corresponds to caducifolious oak woodland mainly consisting of *Quercus hintonii*,

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Quercus magnoliifolia, *Quercus obtusata* and *Quercus peduncularis* [4]. The last three species correspond to white oaks, which are widely distributed throughout Mexico [5, 6]. However, *Quercus hintonii* Warburg is an endemic species of red oak that plays a dominant role in the woodland flora [7]. Moreover, its importance is highlighted by the fact that it has been included in the Red List of endangered species by the International Union for the Conservation of Nature.

Recalcitrant seeds are characterised by a post-harvest life span of the order of days to months, or, for some temperate species, perhaps one year or two, as long as such seeds will tolerate low (not sub-zero) temperatures [8, 9]. Oaks are propagated through a fruit specifically referred to as an acorn, which contains a single recalcitrant seed. Acorns of *Q. hintonii* stored under laboratory conditions lose viability within 30 days, and the germination rate drops to 4.5% [10].

Originally, the categorisation of seeds fell into two distinct groups according to their post-harvest or storage response: either orthodox or recalcitrant [11]. More recently, an intermediate seed group has been defined as seeds that are shed at a relatively high water concentration and can withstand considerable dehydration without attaining the degree of tolerance of an orthodox seed [12]. This definition favours an open-ended continuum of seed behaviours, subtended by extreme orthodoxy at one end of the spectrum and the highest degree of recalcitrance at the other [13-17]. Understanding the phenomenon of seed recalcitrance and consequently developing sound conservation practices for species producing such seed is of major scientific and practical importance [9].

There are marked differences in the degree of dehydration that recalcitrant seeds of individual species will tolerate, although the lowest water content for their survival depends on other parameters, especially on the rate at which water is lost. Recalcitrant seed of most of the tropical/subtropical species that have been investigated thus far have water concentrations at the high end of the spectrum ($\geq$1.5 g/g fresh weight) and the embryo are damaged after only slight dehydration, particularly if water loss is low [18]. Because recalcitrant seeds are not only hydrated but also metabolically active, their developmental status changes more or less rapidly, depending on the species, after they are shed [9].

The only way in which the vigour and viability of a recalcitrant seed can be maintained is to keep them at the lowest temperature at which they will survive and under conditions that do not permit excess water loss and minimise the seed-associated mycoflora [9]. However, establishing a protocol for a recalcitrance test may not be so straightforward if one considers the variable drying rates and different drying temperatures used. Test conditions reported in the literature are typically described in terms of relative humidity or temperature. Some of the reported temperatures are 2°C [19], 15 to 20°C [20, 21], 25°C [22] and 35°C [23]. Relative humidities are not always specified, but those reported range from 15% [20] to 50% [22]. Additional complications may be seed size [21], stage of seed development or maturity [21, 22, 24] and the chemical nature of the principal food reserves [20].

The little published information on either storage requirements for Mexican oak acorns or about their optimal storage time [2]. Considering how rapidly *Q. hintonii* acorns lose viability, it is of the utmost importance to find controlled conditions that allow the long-term preservation of viability and vigour, as this information will aid in future basic research studies and will also provide the basis for reintroduction and reforestation projects using this species.

MATERIALS AND METHODS

Study area

The study region is located in Mexico in Rincón de Ugarte, Tejupilco de Hidalgo, the 18° 56´ 27´´ north latitude and 100° 09´ 20.9´´ west longitude parallels, at 1500 m a.s.l. in the orographic system of the Sierra Madre del Sur province, in the Balsas Depression sub-province.

Acorn harvest and storage

In 2004 and 2007, mature *Q. hintonii* acorns were harvested from the soil near randomly distributed trees in fructification. The study was limited to these two harvest years because in the intervening years there was not an adequate amount of viable acorns. We confirmed that the harvested acorns

were mature by means of maturity indicators such as the colour of the pericarp and cotyledons, as well as the separation between the cupule and the acorn. Healthy and non-germinated fruits were selected from those that had been damaged using the water flotation method [6, 25].

Acorns from 15 parent trees were mixed for each harvest. A sample of 1000 acorns was stored. Approximately 1 kg of acorns (~400 acorns/container) was stored in 2 L hermetic plastic containers. The interior of the containers was lined with absorbent paper towels. In order to maintain constant relative humidity, a wide-mouth glass jar containing a saturated solution of magnesium chloride and calcium chloride was placed inside each plastic container, which upon hydration and provided that the solution remains saturated at 7°C, yields a relative humidity of 33% [26]. These conditions were maintained for a maximum of 256 days.

Germination and plant development

Samples of 50 acorns stored under the aforementioned controlled temperature and relative humidity conditions were taken after 48, 158, 181, 216 and 253 days of storage time (dST) for the 2004 harvest; whereas samples for the 2007 harvest were taken after 21, 63, 92, 126, and 169 dST.

The fresh weight (FW) for each acorn was determined for each sample. The pericarp was scarified using sand paper, and it was superficially disinfected with 3% sodium hypochlorite for 10 minutes and then rinsed three times with distilled, sterile water. The germination phase was carried out in 40 mL lidded glass jars, whose interior was lined with moist absorbent paper (under sterile conditions). An acorn was placed in each jar and was incubated in a germination apparatus (LabLine Instrument) at 27°C with a light/dark cycle of 12/12 h. Daily observations were carried out over a 21 day period of plant development (dDT) to determine the germination time and the morphometric parameters of the seedling such as root length, FW, bud quantity and length, and leaf number.

RESULTS

Our results show that acorns from the 2004 harvest have an initial FW of 2.36 ± 0.071 g. Seeds germinated after 5 days; thus, the germination capacity was maintained from the moment of harvesting until 216 dST; and the germination capacity was extended from 5 to 14 days for acorns at 253 dST. The acorn germinating capacity was 85.29%, and it decreased as the storage time increased (Fig. 1A). After 183 dST, the germination capacity was halved (G½), a value obtained through an exponential regression analysis (not shown).

Acorns harvested in 2007 had an average FW of 2.60 ± 0.079 g and a maximum germination value of 86% after 63 dST, a slightly higher value compared with the 2004 harvest, but the difference is not statistically significant. However, the germination capacity decreases with greater losses when the dST exceeds 169 days (Fig. 1B). Seeds germinate at an interval between 5 to 14 days after imbibition is started, and shows a G½ of 115 dDS.

Survival rates decrease as acorn storage time increase. At 21 dDT, the plants from the 2004 harvest had survival rates higher than 90% for 48 dST, which then decreased from 72 to 68% for 158 to 181 dST, and for the longer storage times (216 to 253 dST) the number of initial surviving plants decreased to approximately 60% and finally fell to 26% for 253 dST (Fig. 2A).

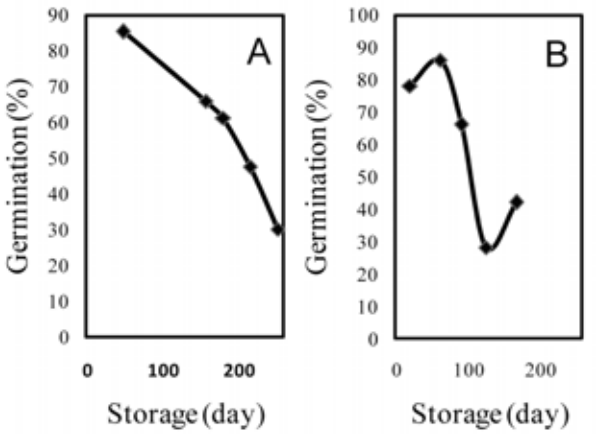

Fig. 1. Storage effect on acorn germination. Acorns was stored at 7°C and 33% relative humidity. A: 2004 harvest. B: 2007 harvest. Data show the average of a 50 acorn sample for each storage period time. These data represent the mean from a sampling of 50 acorns.

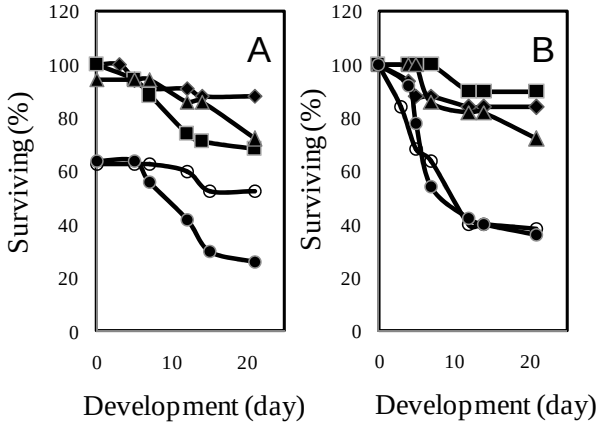

Fig 2. Survival. The graph shows the survival of plants (produced by acorns stored under controlled temperature and relative humidity) within a developmental period of 21 days. A; 2004 harvest: ♦, 48 dST; ■, 158 dST; ▲, 181 dST; ○, 216 dST; •, 253 dST. B; 2007 harvest: ♦, 21 dST; ■, 63 dST; ▲, 92 dST; ○, 126 dST; •, 169 dST. An initial sample of 50 plants was used for each period of storage time.

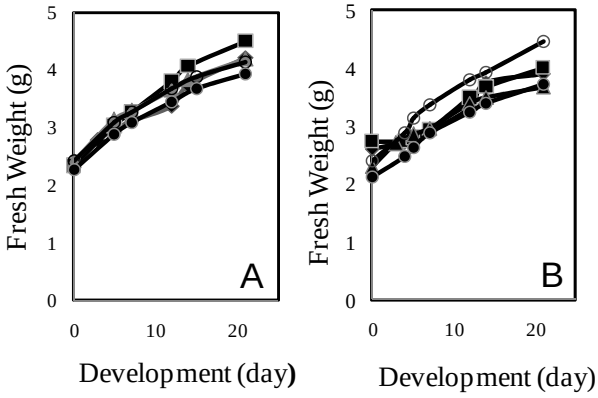

Fig. 3. Plant fresh weight. The change in fresh weight of acorns and seedling of surviving plants from Fig. 2, during the 21-day developmental period, for each storage period of time under controlled conditions, is shown. A; 2004 harvest: ♦, 48 dST; ■, 158 dST; ▲, 181 dST; ○, 216 dST; •, 253 dST. B; 2007 harvest: ♦, 21 dST; ■, 63 dST; ▲, 92 dST; ○, 126 dST; •, 169 dST. The data represent the mean values for healthy acorns or plants from Fig. 2. For simplification, only the mean values are shown without standard error values.

In contrast, the survival of plants from the 2007 harvest decreased with shorter storage times. They remained in good condition with survival rates of 90% and 84% at 21 and 63 dST and then decreased to 72% for plants grown from acorns at 92 dST, and the minimum survival value of 36% was reached when the storage times were from 126 to 169 days (Fig. 2B).

The FW of the plants harvested in 2004 (P_{2004}) at 21 days of growth was similar over the different storage times (Fig. 3A), the relationship between the plant and acorn fresh weight is 1.7 at 21 dDT. The plot of the plants harvested in 2007 (P_{2007}) shows some dispersion in this parameter, and this value is greater between prolonged storage times (126 vs. 169 dST). The relationship between the plant and acorn fresh weight was 1.8 at 21 dDT (Fig. 3B).

No significant differences in root length were found between harvests (Fig. 4A). The plants from the 2004 harvest with a short to moderate storage time (48-181 dST) had an average root length of 4.48 ± 2.37 cm after 14 days of development, and this parameter decreased to 2.3 ± 2.04 cm with storage times of 216 to 253 dST (Fig. 4B). After a developmental period of 21 days, the root length reached an average of 8.62 ± 3.80 cm, but there was no significant difference among treatments (Table 1). The plants from the 2007 harvest showed slightly lower values than those for the 2004 harvest (Fig. 4A). A dispersion in root length values can be observed for each day of development in relation to different storage times (Fig. 4C), with a lower average length value for 92 and 126 dSD (Fig. 4C, Table 1).

At 12 dDT, 91% of the P_{2004} show initial growth of what will be the principal stem that generates the first bud (Fig. 5A); the size of the first bud (1°B) is different for each plant depending on storage time. The smaller buds were obtained for the plants generated by acorns of short storage time (21 dST) and long storage time (253 dST), and these showed an average length of 3.23 ± 0.92 cm, whereas buds corresponding to the three intermediate storage times showed similar lengths with an average of 5.77 ± 0.88 cm (Fig. 5B). Thus, the average size of the bud at 21 dDT for all the storage times is 4.76 ± 0.90 cm. A fraction of the plants form a second bud (2°B) with a maximum at 158 dST (52% of the plants; Fig. 5A). The ratio between the size of 1°B and 2°B varies

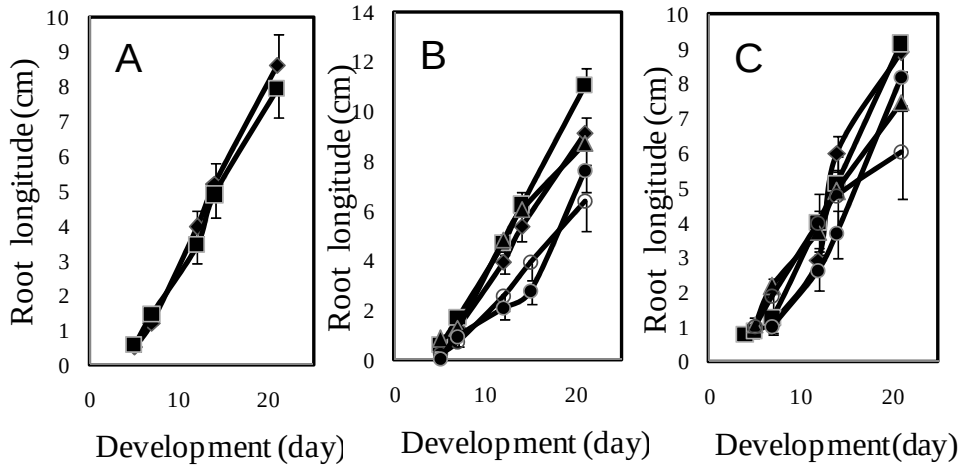

Fig. 4. Root size of seedling and plants obtained from stored acorns. A; Average root size after 21 days of growth derived from acorns under controlled storage conditions: ♦, 2004 harvest; ■, 2007 harvest. B; Root size comparison of plants derived from different storage times of acorns harvested in 2004: ♦, 48 dST; ■, 158 dST; ▲, 181 dST; ○, 216 dST; •, 253 dST. C; Root size comparison of plants derived from different storage times of acorns harvested in 2007: ♦, 21 dST; ■, 63 dST; ▲, 92 dST; ○, 126 dST; •, 169 dST. Each value represents the mean and standard error with an initial sample of 50 individuals that gradually decreased according to the survival capacity for each period of storage time according to Fig. 2.

Table 1. Root growth at 21 days of plant development: velocity and longitude.

2004			2007		
Storage (day)	Velocity (cm/day)	Longitude at 21DT (cm)	Storage (day)	Velocity (cm/day)	Longitude at 21DT (cm)
48	0.55	9.13 ± 3.58	21	0.58	8.90 ± 3.32
158	0.65	11.03 ± 3.27	63	0.56	9.15 ± 3.86
181	0.52	8.73 ± 4.21	92	0.39	7.44 ± 3.50
216	0.38	6.38 ± 5.19	126	0.32	6.04 ± 4.08
253	0.22*	7.61 ± 2.76	169	0.55	8.16 ± 3.8

These data represent the mean and standard deviation.
∗Considering only the values from 5 to 14DT: 0.47 cm/day; or considering all the days for the development: 0.22 cm/day.

from 1.28 to 2.51 when comparing 48 and 158 dST (Fig. 5C). Only one plant formed a third bud (3°B) after 256 dST with a 1°B/3°B ratio of 1.5.

After 21 dDT, P_{2007} showed a smaller number of 2°B (approximately 20%) compared with P_{2004}; this amount increased from 21 to 63 dST and was maintained up to 126 dST. After this time point, it decreased to its initial value at 169 dST. A greater number of 3°B were observed in the interval from 21 to 123 dST, and these buds were present on no more than 9.3% of the total number of plants (Fig. 5D). The length of the 1°B is 4.27 ± 0.39 cm, which is slightly shorter than the total average for P_{2004} (Fig. 5E). The ratio between the 1°B and the 2°B, or the 1°B and the 3°B increased in the plants from early to intermediate storage times (21 to 92 dST); this result implies that the 2°B and 3°B are smaller in relation to P_{2004} (Fig. 5F).

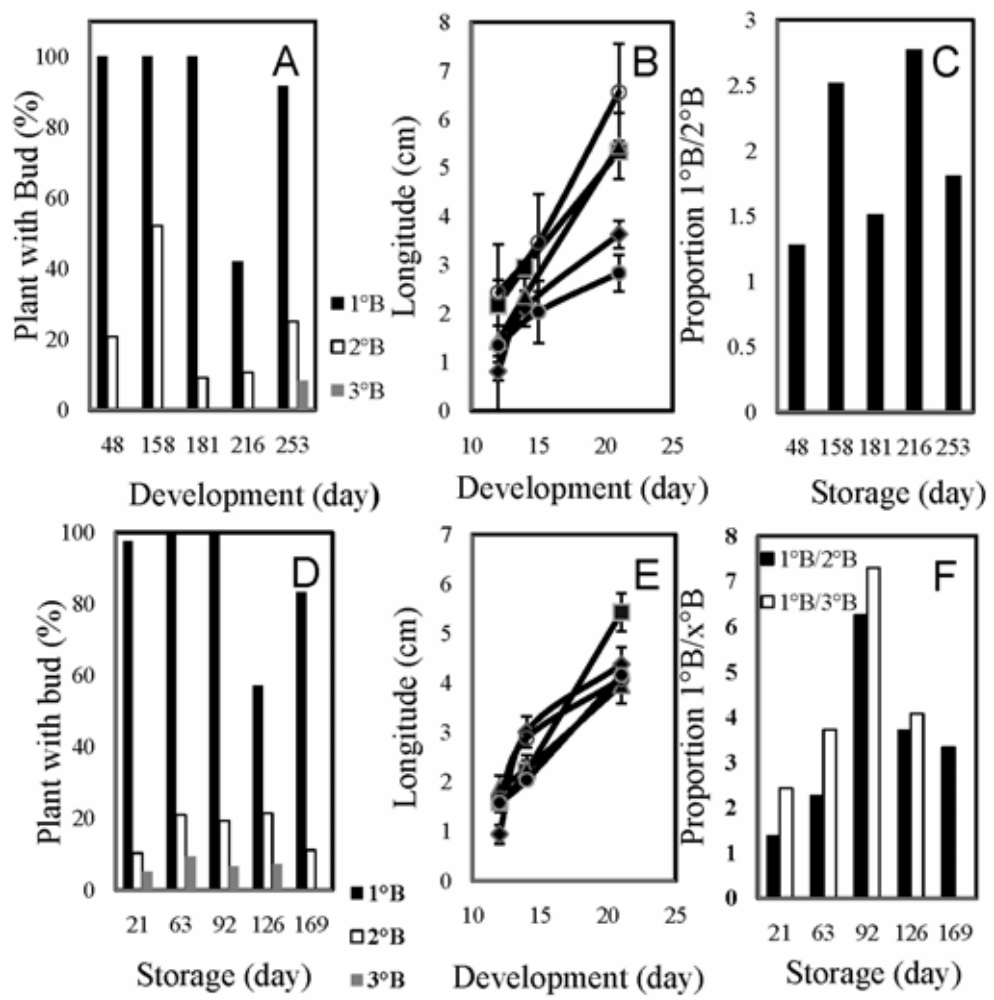

Fig. 5. Buds. The figure shows the percentage of budding plants, the length of the first bud and the ratio between the first, the second or the third bud. A-C: 2004 harvest. D-F: 2007 harvest. A and D: percentage of plants with 1 to 3 buds, after a development of 21 days, generated by acorns with different periods of storage time; B and E; Size of the first bud in plants generated by acorns with different periods of storage time. C and F; Size ratio between the 1° and 2° bud or 1° and 3° bud after 21 days of growth. Symbols: 2004 harvest: ♦, 48 dST; ■, 158 dST; ▲, 181 dST; ○, 216 dST; •, 253 dST; ⊕; for plants at 216 dDT the 2°B dies after 21 dDT and thus the value represents the ratio at 14 dDT. 2007 harvest: ♦, 21 dST; ■, 63 dST; ▲, 92 dST; ○, 126 dST; •, 169 dST. Abbreviations: 1°B: first bud; 2°B: second bud and 3°B: third bud. These data represent the mean and standard error.

An intermediate storage time (92 dST) reached a maximum 1°B/2°B ratio greater than any plant from the P_{2004} harvest (Fig. 5F).

Leaf primordia can first be observed at 14 dDT and increase in number until 21 dDT. Storage increased the number of plants with leaf primordia from 44.8% to 73.9% between the 48 to 158 dST for P_{2004}, after which the longer storage times showed that plants with leaf primordial fluctuated around the initial percentage (Fig. 6A). The number of leaves per plant increases and reaches a maximum value at 181 dST, with an average of 5 leaves per plant, and this value decreases at the end of the storage period (Fig. 6B). In P_{2007}, different storage times show leaf primordia attaining a maximum value of approximately 50% at 63 dST, and a minimum of 23% at 169 dST (Fig. 6C). The average number of primordia was approximately 3 leaves per plant (Fig. 6D).

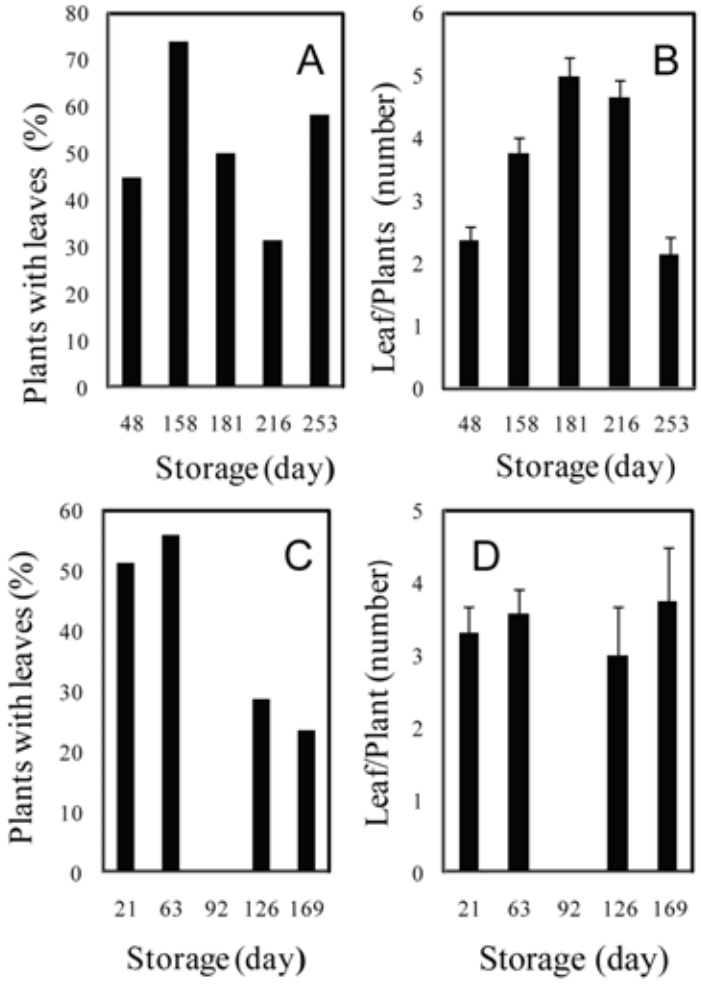

Fig. 6. Number of leaves per plant after 21 days of development produced by acorns with different storage times. A and B; 2004 harvest. C and D; 2007 harvest. A and C: Plant percentage of leaf primordia. B and D: Number of leaves per plant. These data represent the mean and standard error of surviving plants.

DISCUSSION

Storage conditions, germination and viability

A great variety of post-harvest storage conditions have been described in the literature in hopes of extending the period of time that maintains recalcitrant seed viability, and some level of success has been attained with some species. Recalcitrant seeds are metabolically active and will progress from development to germination at the water content typical of shedding. Thus, efforts have focused on the optimisation of short- to intermediate-term storage of recalcitrant seeds by preserving the shedding water content or by allowing desiccation to happen at an optimal rate, which permits the generation of mechanisms capable of minimising the damage produced by water loss that can vary among the different recalcitrant seeds [9, 27, 28].

It has been determined that temperature is a decisive factor to maintain acorn viability during the first year of storage, and the use of low temperatures has been recommended for recalcitrant seeds. Consequently, reports in the literature state that *Q. nigra* acorns have been stored at -2°C, [29, 30]; whereas the European specimens *Q. rubra*, *Q. shumardii and Q. muehlenbergii* have been stored at -1°C or -2°C for a period of up to 5 years [31-33]. Considering that the habitat for *Q. hintonii* is located at a more southern latitude, where

environmental conditions are less harsh than those corresponding to the species growing in more northern latitudes, and according to reports issued by the digital climate atlas of Mexico (version 2.0) proposed by the Centro de Ciencias de la Atmosfera de la Universidad Nacional Autonoma de Mexico [http://atlasclimatico.unam.mx/atlas/edomex/edomex.html], *Q. hintonii* acorns are exposed to temperatures fluctuating between a monthly average of 15°C and 25°C during the months of August and September when the fruit is shed, thus, the mature acorn is not generally exposed to temperatures near or below freezing. Although there are no studies about the possible cold damage suffered by acorns of this species, there is a report of *Q. virginata* acorns that were damaged by cold storage temperatures [30]. There is no general rule or consensus concerning the appropriate storage temperature for acorns. Storing acorns at 7°C makes it possible to reduce the metabolic rate and thus delay the growth of mycoflora that takes place at higher temperatures. Studies of Mexican oaks and acorn storage recommend using temperatures between 5°C and 7°C [2], and the results of the present study indicate that this temperature is adequate to preserve *Q. hintonii* acorn viability in association with controlled relative humidity.

Moisture content remains a crucial factor throughout storage because acorns with moisture levels superior to 30% and temperatures above freezing respire at a faster rate [9]. Germination decreases when water content is below 20-30% [2, 34]. In *Q. nigra* and *Q. pagoda*, the acorn moisture content (shedding water content) are approximately 30% [30, 35], although there is some variation in water content and its effects on germination amongst the different species and subspecies of red oaks (subspecies Erythrobalanus). This finding explains the previous use of controlled moisture chambers containing magnesium chloride and calcium chloride, which in saturated solutions at 7°C, maintains the relative humidity at 33% [26]. Using this type of chamber allowed the preservation of seed viability for a longer period of time, with germination values greater than 85% at 63 dST (2007 harvest), and prolonged storage times of 115 to 183 dST (2007 harvest vs. 2004 harvest) showed a germination capacity of 50%, as opposed to germination values of 4.5% found in acorns stored for 30 days under laboratory conditions [10].

The time needed for an acorn to germinate is 5 days and can be extended up to 14 days for prolonged storage times (253 dST) as observed for the 2004 harvest. However, acorns from the 2007 harvest that were stored for a shorter period of time showed germination times which slowly increased from 5 to 14 days, indicating that although the seed is still viable, vigour is gradually lost despite a healthy appearance (good quality). Acorns of both harvests were produced by the same parent trees, with similar initial FW; hence, the different results between harvests is due to the sensitivity of each acorn to storage time.

Notably, the environmental conditions and humidity differed greatly between 2004 and 2007 when the acorns were harvested. For example, the 2004 harvest was carried out on sunny days, the plant litter-covered soil was practically dry with scant moisture, all the acorns on the ground were apparently healthy, and none of the seeds had germinated in this time. By contrast, acorns from the 2007 harvest had fallen on moist plant litter because there had been rainy days, and the acorns were excess hydrated. It has been documented that the moisture content when acorns are harvested is a decisive factor for the prolongation of seed viability, recalcitrant seeds are transiting between a development one of germination metabolism, thus brief transition phase is one of high sensitivity [9, 27, 28]. One consequence of acorns being exposed to controlled relative humidity and temperature conditions is that it reduces the critical phase in the transition between the development to germination metabolism. It has been suggested that the increase in water-loss rate minimises the damage to the seed and induces a decrease in primary metabolism, which generates a mechanism to counterbalance desiccation damage [9, 36]. However, this hypothesis has been partially rejected because the structure and composition of every recalcitrant seed affects water loss rates ($\Delta\Psi_w/\Delta t$), differentially reduces primary metabolism and the protection of cellular structures [37]. The time required to dehydrate seeds depends on their initial water content. Acorns with higher water content display an extended period of desiccation sensitivity and suffer greater damage. Desiccation damage to

recalcitrant seed was a function of two interrelated parameters: the rate and duration of dehydration [37]. Therefore, the acorns harvested in 2004 were a relatively homogeneous batch, all acorns were in the last stage of the development of the seed, none of them had germinated when they were harvested; thus, the susceptibility period was shorter when the acorns were stored and germinated within a 5 day period and could be stored for a longer time (G½ of 180 dST). However, this was not the case for the acorns harvested in 2007, which exhibited a range of developmental stages, as seen from the large proportion of seeds that had clearly initiated the germination phase at harvest and were excluded from the study. Because the 2007 acorns had a higher water content due to the moisture in the plant litter from the rainfall at harvest, the dehydration time was increased, affecting the desiccation rate needed to reach the equilibrium inside the controlled humidity chamber (33% RH), thus extending the sensitivity window of the seeds in a much wider range than that observed for the acorns harvested in 2004. The acorns show different deterioration levels between the harvest years and an inverse relationship was found between germination capacity and storage time.

Storage effects on vigour

These data show that there are no differences in acorn and plant FW or root size between the 2004 and 2007 acorns. However, the acorns harvested in 2004 were able to be stored for a longer period of time than those harvested in 2007. Once germinated, plant vigour was not affected by acorn storage time, which demonstrates that plant quality and root growth capacity are preserved. This species provides preferential growth capacity to the root over the stem, which demonstrates the fundamental need to establish a root system that allows for water and nutrient absorption while providing structural support for the new plant.

The effect of controlled storage of acorns produced variations in bud production; 1°B and 2°B were generated in plant from both harvests with a greater quantity in the 2004 harvest. The average size of the 1°B is similar in both harvests (4.7 ± 0.90, 2004 harvest *vs*. 4.27 ± 0.39 cm, 2007 harvest). A third bud was only produced in the plants harvested in 2004 that experienced prolonged storage time, whereas plants from the 2007 harvest produced the 3°B from the start. However, stems from the 2004 harvest with intermediate storage times (158 to 216 ST) show an increase in size (5.77 ± 0.8 cm), which decreases with longer storage times to the minimal value of the studies, indicating that storage time not only decreases the amount of viable acorns but also the vigour of the seedling, as shown by the fact that the 1°B is 1.7 times smaller. Plant growth of acorns harvested in 2007 is similar regardless of storage time, and the mean value is maintained between the maximum and minimum values of the 2004 harvest. The growth of the 2°B and 3°B was more affected in 2007, and the buds were smaller, thus affecting the 1°B/2°B ratios, which had a value of 6; the 1°B/3°B ratio were even more affected with a value of 7. After 14 days of development, the seedlings start forming leaves to establish organisms with autonomous metabolism. After 21 days, the plants display several leaf primordia distributed amongst the buds. The 2004 plants show a greater quantity of leaves (5 leaves per plant) compared with those from the 2007 harvest (3 leaves per plant), thus showing a greater photosynthetic capacity. Taken together, these data indicate that acorns from the 2004 harvest have a greater capacity for plant establishment than those harvested in 2007. Thus, acorns harvested in 2004 show increased vigour, indicating that acorns harvested in 2007 were under greater stress, probably due to the time needed to attain equilibrium with the relative humidity inside the chamber; therefore, these acorns were more susceptible to damage. There is no doubt that the acorns from 2007 had certain disadvantages compared with 2004 acorns on the basis of the fact that they differed in number and in germination time, in the seedling survival rate, and therefore in bud quantity, size, proportion in longitude, and leaf quantity.

Given that the desiccation tolerance of recalcitrant seed also decreases with storage time [38-40], this study confirms those results and suggests storage conditions that extend *Q. hintonii* acorn viability, with differences in seedling vigour as a function of the rate and duration of dehydration in conditions that control temperature and

relative moisture. Environmental conditions during development as well as during acorn shedding have critical effects on viability, G½, vigour, survival, stem number and size, and number of leaf primordia, as seen from the comparative study of acorns produced by the same parent trees in different harvests under different environmental conditions at harvest.

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