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ORIGINAL ARTICLE

Viability Loss of Recalcitrant Brant's Oak (*Quercus brantii* Lindl.) Acorns during Overwintering

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A B S T R A C T

The lack of germination success and subsequent seedling recruitment of recalcitrant acorns of Brant's oak (*Quercus brantii*) has been described as one of the main reasons for the cessation of sexual regeneration in Zagros forests. What happens to acorns whose viability is immediately lost is examined in the form of overwintering. In this study, overwintering conditions following seed dispersal were simulated by subjecting freshly harvested acorns with three moisture content levels (85, 90 and 100% fully hydration) at 3°C for up to three months. The results indicated that Brant's oak acorns are totally recalcitrant and show no signs of dormancy. Their viability depended entirely on the moisture content. Fully hydrated acorns maintained a viability rate of more than 80% after three months of overwintering. This time period for acorns with 95% full hydration lasted only one month. At 90% fully hydration, the viability rate of acorns decreased almost immediately to less than 80%, and to less than 50 and 20% after 1 and 3 months, respectively. The passage of time was deteriorating during overwintering and its negative effects accelerated after one month, even for fully hydrated acorns.

Introduction

Brant's oak (*Quercus brantii* Lindl.) occupies a large part of the forests of southwestern Asia. Its high adaptability and wide ecological niche width have made it a notable dominant tree in the region, playing an important role in the food chain of the Zagros forest ecosystems and in the supply of timber and other forest products (Jazireie and Ebrahimi Rostaghi, 2013). In addition to its extraordinary coppicing and sprouting capacity, the tree produces large, highly vigorous acorns, with high food storage and hypogeal germination. These acorns are recalcitrant. Mechanisms of seed dormancy has been discovered in many nut trees, while it's not still that much known in oak (Vahdati *et al.*, 2012).

The fact that a mature Brant's oak acorns are recalcitrant has two basic meanings: intolerance to desiccation, hence difficulty in long-term storage, and lack of a dormant radicle (Alvaninejad *et al.*, 2009). The strategy of the mother tree is to outgrow other seeds by producing fewer large recalcitrant acorns in autumn (Heydari *et al.*, 2012). Like other white oaks (Steele, 2021), and under optimal conditions, these acorns

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overwinter as pre-germinated seeds or tiny seedlings, which are not very sensitive to cold. Acorns that do not germinate easily but retain sufficient moisture content
(MC) will survive the mild winter and germinate in the spring (Johnson *et al*., 2019). In recent decades, however, Brant's oak acorns have increasingly faced the risk of post-dispersal desiccation in the autumn and late summer (Zolfaghari *et al*., 2013; Yadegari and Seyedi, 2019). As a result, there is hardly any visible sexual regeneration of Brant's oaks in the Central Zagros forests (Soltani *et al*., 2020; Fattahi, 1994).

For red oaks, the high MC of dormant acorns has corresponded to successful stratification and high-humidity storability (Suszka, 1980; Finch-Savage and Clay, 1994; Aizen and Woodcock, 1996). Consequently, the idea that recalcitrant acorns may exhibit orthodox behavior has become a major focus of research interest (Noland *et al*., 2013). For Brant's oak, which is not a red oak, it is well known that lowering the MC to less than 40% significantly decreases acorn viability; however unlike some other oaks in the *Cerris* section (Benamirouche *et al*., 2018; Leon-Lobos and Ellis, 2018; Macchia *et al*., 1992), there are no studies on the probable positive effect of cold storage on germination of Brant's oak acorns. Poor storability of these acorns has also been recorded (Akbari *et al*., 2001), nevertheless it is not known how overwintering of these acorns is affected by different MC levels.

My hypothesis is that there is a range of MCs for storing recalcitrant acorns of oak species that best preserve seed viability over winter. The lucky acorns that pass their post-dispersal fate in this MC corridor will avoid desiccation, pre-germination, and freezing of the embryos. Therefore, the aim of this study was to monitor the germination of Brant's oak acorns during overwintering at different MC levels to determine, first, whether the germination of these acorns is more influenced by changes in MC or the overwintering period (OP), and second, which MC is critical for maintaining viability.

Material and Methods

Acorn origin and pretreatments

During the second week of October 2020, greenish-brown acorns were directly collected from 7 single-stem Brant's oak (*Quercus brantii*) trees with an average diameter of 43 cm and height of 9.8 m growing along the Karun River near Helen Forest in the central Zagros (31°50' N, 50°39' E). The pooled lot was enhanced using specific gravity separation, flotation test and fungicide treatment. Acorns were placed in layers of folded wet cloth in a 36-liter cooler at 3°C for two days and regarded as the fully hydrated lot. The MC was measured based on wet basis:

$$(\frac{W_w - W_d}{W_w}) \times 100,$$

where W_w and W_d are the wet and oven-dry weights, respectively (Sagwal, 2020). Drying was carried out at 105° C for 24 hours.

Overwintering

Based on MC percentage, the lot was divided into three portions with zero, ten and fifteen percent less than the fully hydrated acorns. I refer to these three portions as "100%", "90%" and "85%" MCs. Desiccation was carried out in a ventilating cold incubator equipped with an ambient humidity sensor.

The overwintering environment was imitated by placing acorns into single-row polypropylene trays without medium, hermetically sealed with cellophane, incubated in dark at 3°C with adjusted ambient humidity equal to the water content of the acorns (a pilot overwintering test indicated that no per-germination was observed at temperatures below 4°C). The realized viability of the three MC portions was evaluated before and after two- and three-month OP, using a germination test.

The germination test was conducted in four replications of 25 pre-imbibed acorns on transparent egg clamshells, filled with sterilized washed sand in the dark at 20±1°C. The realized viability was considered to be the number of acorns germinated during and at the end of the germination test, expressed as a percentage.

Statistical analysis

The experiments were laid out in a completely randomized design. The germination percentages were arcsine transformed prior to statistical analysis. A paired t-test ($P < 0.05$) was used on each counting day between two MC variables in a row. An analysis of variation (ANOVA) and its related Tukey test ($P < 0.05$) was also applied between final germination values of each MC portion over the three OPs. The MC-OP combinations at each final germination percentage were fitted to contour lines to model viability above and below 50 and 80%.

Results

The MC of the collected lot was high (an average of 49%), and it was regarded as the fully hydrated MC (100%). The other two MC variables were 44 and 41% (after 90 and 85% MC, respectively). Before overwintering, the germination test showed 95.2, 87.1 and 48.3% realized viability values for 100, 90 and 85% MC lots, respectively. The last acorns germinated after 15 days. Although there was no statistical difference between the 100 and 90% MC portions on most counting days, the 85% MC portion showed significantly lower viability values throughout the germination course before being transferred to overwintering conditions (Fig. 1a).

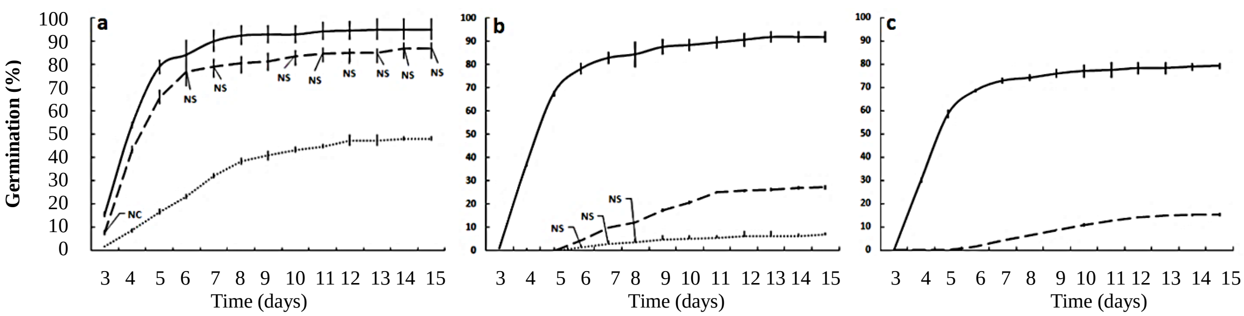

Fig. 1. Daily cumulative germination percentage after three overwintering periods (a: none, b: two months, c: three months). Solid, dashed, and dotted lines represent 100, 90, and 85% hydrated acorns, respectively. Error bars indicate standard deviation. NS: no significant difference at $P < 0.05$ by running a paired t-test for the two moisture content variables at each counting day.

The realized viability of fully hydrated acorns decreased to 91.8% after two-month OP, which was not significantly different from the corresponding lot with no OP (Figure 2). However, two-month OP had a significant negative effect on the viability of acorns with 90 and 85% MC (Fig. 2). These two lots also lagged significantly behind the fully hydrated acorns throughout germination (Fig. 1b). At all moisture levels, the viability of acorns overwintered for three months decreased dramatically. The lower the MC, the lower the viability, so that viability decreased to 15% at 90% MC and was completely impaired by reducing the MC to 85% after three-month OP (Fig. 2). Similar to the acorns that overwintered for two months, germination of three- month OP acorns with 90% MC was delayed by one day. The onset of germination, however, was delayed by six days for all acorns overwintered with 85% MC (Fig. 1c).

The contour line model showed that prior to overwintering, the viability of acorns desiccated to 15% lower than the fully hydrated MC decreased to less than 50%. However, the minimum MC required maintaining 50% viability after one- and two-month OP was 90 and 94% of the fully hydrated MC, respectively. If acorns are to overwinter for three months, a minimum of 96% of fully hydrated MC is required to achieve minimum of 50% viability. With a maximum MC of over 98%, viability of over 80% can be obtained after a two-month OP (Fig. 3).

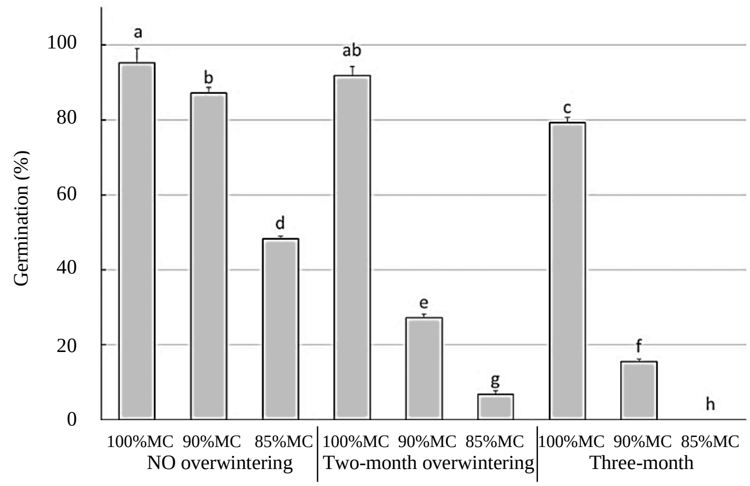

Fig. 2. Average percentage of final seed germination at different moisture contents and overwintering periods. Error bars indicate standard deviation. Columns marked with the same letter are not significantly different at 95% confidence level.

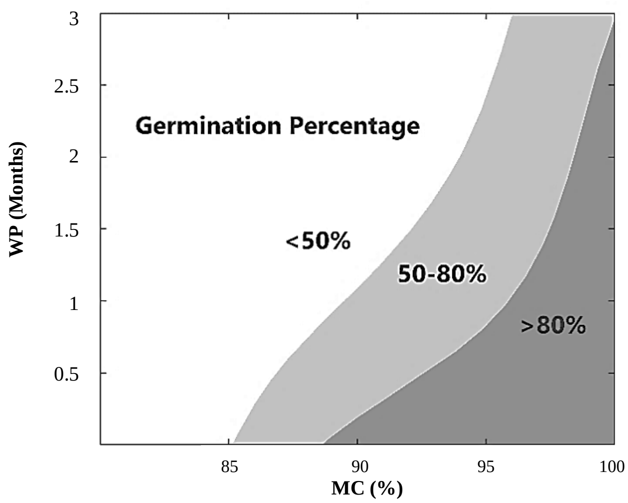

Fig. 3. Contour plots of the final 50 and 80% germination percentage at different MC-OP combinations.

Discussion

Recalcitrant acorns can be divided into two main categories: one that has the characteristics of orthodox seeds and one that does not. Both categories are not desiccation tolerant, but the germination rate of the former improves with cold storage (overwintering), while that of the latter does not (Landis, 1990). The results of this study indicated that acorns of Brant's oak belong to the latter category. Not only were there no signs of orthodoxy in these acorns, but overwintering led to a significant decrease in the viability of those acorns with MS levels below 90%.

The effect of the OP × MC combination on the viability of Brant's oak acorns has not been documented. However, in contrast to previous work on acorn water potential (Alvaninejad *et al*., 2009), the MC reduction in this experiment did not lead to a linear decrease in viability, and after an initial linear decrease, acorns with MC levels below 90% showed a sharp decrease in their germination rate. This nonlinear decrease was also described for acorns from both sections of *Quercus* (Pasquini *et al*., 2011) and *Lobatae* (Sowa and Connor, 2003). Although Brant's oak is known to be a xerophyte species compared to Mediterranean oaks, the results showed that its acorns are more sensitive to desiccation, such that by reducing MC to 41% (85% of total moisture), acorn survival reached below 50% even prior to overwintering. This level of MC is much higher than the comparative values of (Ganatsas and Tsakaldimi, 2013) for drought-tolerant Mediterranean oaks.

There are two main components to overwintering: the passage of time and low temperatures. The results of this experiment show that low temperatures, i.e., those normally applied to cold stratification, do not affect, let alone improve, the preservation of acorns. Like other non-dormant acorns (Matsuda and McBride, 1989), Brant's oak acorns germinated whenever they were exposed to a minimum continuous average daily temperature above 7°C (data not shown in the results). Thus, the only apparent effect of low temperatures on these acorns was to delay germination of healthy ones or to prevent decay of infested acorns (Carvell and Tryon, 1961). For acorns of Brant's oak, the passage of time was deteriorating. The results showed that the viability of fully and non-fully hydrated acorns was significantly reduced after two- and one- month of overwintering, respectively. Therefore, not only is it not necessary for Brant's oak acorns to be overwintered, but OP for more than two months are harmful even fatal to them. The exponential deterioration effect of overwintering accelerates after one month, as the minimum MC with a germination rate of 50% will be as high as 90%. This suggests that these acorns do not adopt an overwintering period of more than one month.

Temperature variability during overwintering was not considered in this study because of the difficulty in drawing conclusions. Consistent with previous studies (Suszka and Tylkowski, 1980), my pilot experiment showed that 3°C was the highest OW temperature to stop acorn pre-germination. Based on data from the nearest synoptic meteorological station in the region since 2019, the diurnal temperature difference in the region during winter varies from 10°C to -2°C (Chaharmahal Va Bakhtiari Meteorological Administration, 2020). However, no research has been done on the actual temperature fluctuation to which acorns buried in topsoil by squirrels or jays are exposed. These temperatures are easily attainable in the region. As a result, few non-germinated acorns remain for winter, and all those that are not hunted or infested will germinate before winter.

The acorns of Brant's oak are adapted to the climatic conditions that have prevailed in the Zagros forests for centuries. These conditions were mainly characterized by wet autumns and mild winters. Over the past decades, climatic conditions have changed. The autumns have become drier and the winters warmer. A warmer climate in the season following seed dispersal may imply more favorable conditions for the germination of recalcitrant acorns, especially since the results of this experiment also suggest that overwintering has little effect on preventing acorn aging. On the other hand, the results of the significant effect of MC reduction on the viability of these acorns imply that dry autumns have some negative impact on the regeneration capacity of these trees.

Conclusions

For acorns of Brant's oak, the interaction between MC and OP depends entirely on the level of MC: when MC values are high, OP has little effect, but as MC decreases slightly, OP becomes significant. The intensity of the decrease in the viability is exponential, especially for the weakest 20% of acorns. In nurseries, when planting of acorns is postponed to late winter or spring, it is important to ensure that MC does not fall below 95%. Acorns with MCs close to fully hydrated values can be overwintered for nearly three months with no more than 20% loss of viability. Nevertheless, acorns dehydrated to 95% MC are likely to retain less than 50% viability after three months of overwintering.

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Conflict of interests

The author has no conflicts of interest to declare.

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