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Research paper

Depredation and acorn germination ability of three seed sources for *Quercus suber* L. in Mediterranean environment

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ABSTRACT The cork oak is a major forest species that occupies a limited natural area in the Mediterranean region. Unfortunately, it is a patrimony that continues to deteriorate under the effect of many factors, leading to the disappearance of large forest areas. Its decline is caused by abiotic factors, including climate change, wildfire, and biotic factors such as invasive species, and pests. In addition, the species faces regeneration difficulties because of lack of forest conservation. We respond to these problems by studying the germination of acorns, as this constitutes the first and crucial phase of vegetative development. We then considered some biotic and abiotic interactions, including the effect of provenance (altitude gradient), health status and biometrics of acorns. The results indicate that there is variability in acorn biometrics by site, that there are no significant correlations between the health status of the acorns and their biometrics, and that carpophageal insects can attack different acorns regardless of their calibre or weight. For germination, healthy acorns with a wider calibre are much better favoured. The impact of acorn depredation on germination is dependent on its intensity: a single perforation, for example, was not found to influence germination; beyond that, this impact becomes significant.

KEYWORDS: cork oak, acorns, health status, germination, Algeria, infestation.

Introduction

The cork oak (*Quercus suber* L.) is a forest species belonging to the *Fagaceae* family, whose origin dates to the Tertiary period (Natividade 1956). It is thus described as a species endemic to the Mediterranean basin, descended from the upper Pliocene flora where it has been present for more than 60 million years (Aafi 2006, Piazzetta 2005, Quezel 2000, Boudy 1950).

The very precise requirements in the climate and soil of cork oak mean that its global natural growth area is established exclusively around the western Mediterranean and the Atlantic façade of Portugal and Morocco (Natividade 1956), between latitudes 31 and 45 degrees north (Quezel and Santa 1962, Boudy 1947). It forms the climax forest on non-calcareous soil in areas with a minimum rainfall band of 600 mm, which essentially corresponds to seven countries: Tunisia, Algeria, Morocco, Spain, France, Italy, and Portugal, offering them great ecological and socio-economic importance (Silva and Catry 2006).

Cork oak holds its nobility for its bark, commonly called cork, which it produces regularly throughout its life. Cork is a material that is particularly light, supple, elastic, waterproof, and non-conductive of heat that has been used since antiquity for various purposes (Boudy 1950). Similarly, its fruits (or acorns) are of major importance, not only for regeneration but also because they are a food much appreciated by livestock and other rodents under natural conditions (Aronson et al 2012).

Unfortunately, cork oak stands have been confronted in recent decades with a loss of vigour, a lack of natural regeneration, and a dieback that threaten the sustainability of this species. The ecological and socio-economic role of cork oak forests will fade over time as natural conditions (climate, soil, vegetation), anthropogenic activities (fires, logging, rangeland), and parasitic attacks increase (Benabdeli et al. 2015, Ghefar 2013).

The regeneration of cork oak by direct seeding, whether artificial or natural, is problematic (Pulido et al. 2013, Aronson et al 2012, González-Rodríguez et al. 2011, Hasnaoui 1998, Messaoudène 1984). The slow germination of acorns would increase the risk of mortality (desiccation, fungal, and entomological attacks) and rodent removal (Merouani et al. 2001a, Merouani et al. 2001b), in addition to its effect on the quality and quantity of acorn production.

Biotic and abiotic constraints therefore oppose regeneration by direct seeding (Arosa et al. 2017, Ibáñez Moreno et al. 2015, Pulido et al. 2013, Herrera 1995, Lorimer et al. 1994). These include problems related to the physiology and ecology of the tree, such as ageing, embryonic dormancy, and irregularity of seedlings (Miguel et al. 2015, Sork and Bramble 1993). In addition, there is the impact of depredation on acorns by fungi and insects, the consequences of drought, and problems induced by stand dieback (Pérez-Ramos et al. 2008, Fuchs et al. 2000, Crawley and Long 1995, Cabral et al. 1993).

Regarding other forest species, the regeneration of cork oak through their seeds raises the problem of germination and the rigor of the plants. This study will aim to master this first key phase of forest development by assessing the impact of depredation and the effect of provenance on the germination and

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acorn emergence to determine the most profitable choice.

Materials and methods

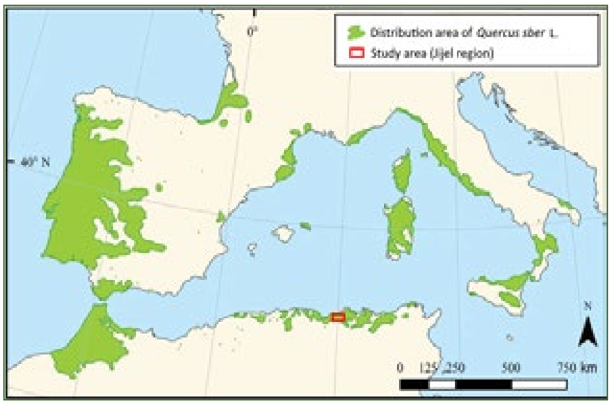
Collection, health status and biometry of acorns

We collected acorns directly from the ground after they matured from their mother trees around mid-November to mid-December 2019. The study area is in the northeast of Algeria, a part of the Mediterranean region and a crucial agroforestry location with a strong history of growing cork oak trees. Specifically, the collecting sites are distributed in the Jijel region, between latitudes 36° 10' and 36° 50' north and longitudes 5° 25' and 6° 30' est. Here we opted for three seed sources according to an altitudinal gradient (Tab. 1 and Fig. 1).

Table 1 - Location of the sites where cork oak acorns were collected.

N° Sites	Forestry canton	Latitude	Longitude	Altitude
P1	Eldjerda	36°39'55.03"N	5°48'55.55"E	887
P2	Elharma	36°40'58.74N	5°47'53.09E	475
P3	Aghzar	36°47'27.13N	5°40'10.04E	51

Figure 1 - Map of cork oak distribution in the Mediterranean region, displaying the study area (region of Jijel in northeastern Algeria) where the acorns are collected.



For each observation site, we had a corresponding batch of acorns, from which we first drew a number of 100 acorns at random, serving for biometric measurements (length, width and weight) and at the same time for an examination of their health status. Each acorn drawn at random was carefully observed to reveal the proportions of the different categories of existing acorns, whether healthy or showing various alterations (rotting, perforation or decapitation) due in particular to biotic interactions (infestations of carpophagous or fungal diseases).

Finaly, to find out which insects are carpophagous, another batch of 300 acorns with perforations were dissected to quantify the rates of species present inside the acorns.

Experimentation and seeding conditions.

Our experiment took place in a multichapel of the above-ground nursery of Kissir, belonging to the forest conservation of Jijel, which is located on the north-eastern coast of Algeria (36.79°N, 5.66°E, altitude 18 m). This multichapel is characterised by the following growing conditions: temperature ranging from 25° C to 28°C, humidity of about 75-80%, a cooling system and a shading net (which captured 5% and reflected 40% of solar energy) and a sprinkler irrigation system.

A total of 630 acorns from three different sources were set to germinate in an above-ground nursery, each in thin plastic containers of the Riedacker WM type, with a volume of 400 cm^3. These containers, used for sowing, were arranged in perforated boxes with dimensions of 50×35×15 cm and were raised 60 cm above ground level. The acorns were soaked for 48 hours at 20°C, disinfected for 10 to 15 minutes in an 80% sodium chloride solution and then germinated in sterile sand (200°C for two hours). They were kept moist, dark conditions for 28 days at 20°C.

The experimental device selected corresponds to a complete randomized block design with three replications. It contains 07 sowing modalities or treatments (03 different provenances + 04 categories of acorn health status) (Tab. 2). Each modality (seeding method) was represented by 90 acorns (30 x 03). Thus, the total number of acorns sown in the experiment was 630 acorns (30 acorns x 07 sowing modalities x 3 replications).

Germination test

The acorns were set to germinate in an order already informed with numbers to allow the follow-up of this germination. An acorn was considered germinated as soon as the radicle pierced the pericarp and showed a positive geotropism. During this experiment, which lasted four weeks (28 days), the rate of germination and elongation of the radicle was noted for all sprouted acorns corresponding to each week.

Statistical analysis

Depending on the objectives of the study; two methods of statistical analysis are used.

- The first was a principal component analysis (PCA), which is one of the methods of analysing multivariate data, to find out which variables influence the health status of the acorns and therefore on germinative ability and emergence. It is an exploratory analysis of data, with the objective of analysing correlations between variables and similarities between observations (glands). For our case, Table 2 describes the various variables considered.

- The second method was to perform an analysis of variance with a one-way analysis of variance (ANOVA) to determine whether there are significant differences for dependent variables considered "radicle elongation" which we want to explain variability by the qualitative explanatory variable "altitudinal gradient" on the one hand, and by the variable "number of perforations" on the other by using its classes as categorical treatments (Tab. 3). This ANOVA test is followed by a test of multiple comparisons which was based on Tukey and Dunnett tests, enabling you to compare modalities with a control.

The data were analysed by the statistical software XLSTAT version 2016.

Table 2 - Description of the variables considered for the principal component analysis (PCA).

Variables	Code	Units of measurement / Rating scales
Acorn perforation	Per	Number
Acorn decapitation	Dec	Proportion (%)
Acorn rotting	Rot	0 (not rotten), 1 (rotten)
Acorn width (mm)	Wid	Millimeter (mm)
Acorn length (mm)	Len	Millimeter (mm)
Acorn weight (g)	Wei	Gram (g)
Rootlet elongation at 4th week of sowing	RoW4	Millimeter (mm)
Acorn emergence at 5th week of sowing	EmW5	0 (not emerged), 1 (emerged)
Altitude	Alt	Metre (m)

Table 3 - Description of the modalities (variable dependent) of the acorns germinated in the two ANOVAs performed.

Qualitative predictor variable	Sowing modalities (Treatments)	Description
Provenance (Altitude)	Per	Number
	Alt50	50 metres above sea level
	Alt475	475 metres above sea level
	Alt887	887 metres above sea level
Health status of the acorns (number of perforations) Altitude	AP0	Healthy Acorns (Control) 0 perforations
	AP1	Acorns with one perforation
	Ap2	Acorns with two perforations.
	AP3	Acorns with three perforations or more
	Alt	Metre (m)

ing the collection period, we opted for a Principal Component Analysis (PCA) to see the possible interactions of the depredation of the acorns in relation to two factors: their biometrics and their provenances (altitude effect; Fig. 2).

This corresponds to a multidimensional analysis, presenting a projection of different variables following a factor plane of two axes, F1 and F2. In our case, the quality of the projection, when switching to a smaller number of dimensions, shows a total inertia rate equal to 62.07. Therefore, we can note a circle showing significant correlations for variables far from the centre of the graph.

The F1 axis groups the variables of the biometrics of acorns (Wid, Len and Wei) with altitude (Alt), pointing to negatively significant correlations. On the other hand, the F2 axis includes only the three types of depredations, which does not represent any significant correlation with either biometrics or altitude.

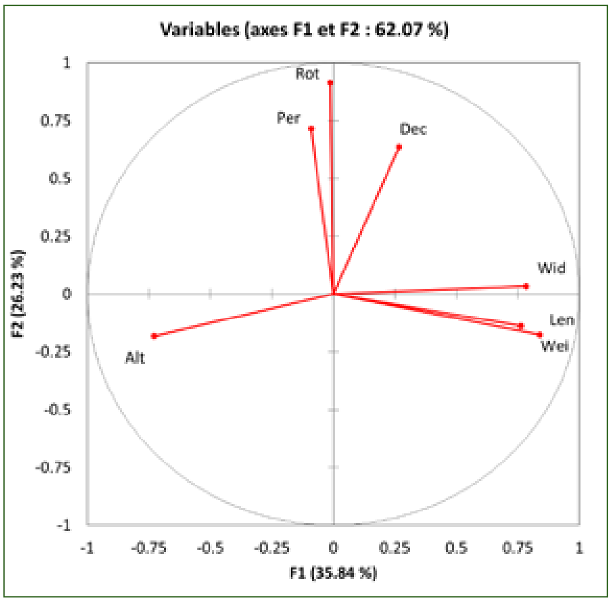
Results

Evaluation of the acorn health status

By looking at the health status of the acorns dur-

The health status of the acorns does not appear to have a significant relationship with their biometrics (Wid, Len et Wei). This differs from the altitude variable, which shows a negatively significant correlation with all biometric parameters (Fig. 2).

Figure 2 - Principal Component Analysis characterising the health status of acorns. Variables are: Perforation [Per], Decapitation [Dec], Rotting [Rot], Width [Wid], Acorn length [Len], Weight [Wei], Altitude [Alt].



Acorn germination ability in relation to altitude

Considering the altitudinal gradient in which the biometrics of the acorns are negatively influenced, it seems that the rate of germination and emergence are also slightly impacted by this gradient, as shown in Figure 3. Low to medium altitudes are therefore better favoured for these two parameters.

In this same altitude criterion, the variance analysis (ANOVA) for the radicle elongation variable on the 28th day of germination revealed highly significant differences (pr<0.0001), as shown in Table 4.

The comparison of the two averages shows that the altitudes between 50 m and 475 m do not represent any significant differences and have the best elongations of the pivot, with 73.08 and 64.13, respectively, belonging to the same homogeneous group (A). The altitude 887 m showed a lower average elongation, evaluated at 47.11, which is classified in a separate homogeneous group (B; Fig. 4 and Tab. 4).

Figure 4 - Average length of pivot based on altitude at 28 days of seeding.

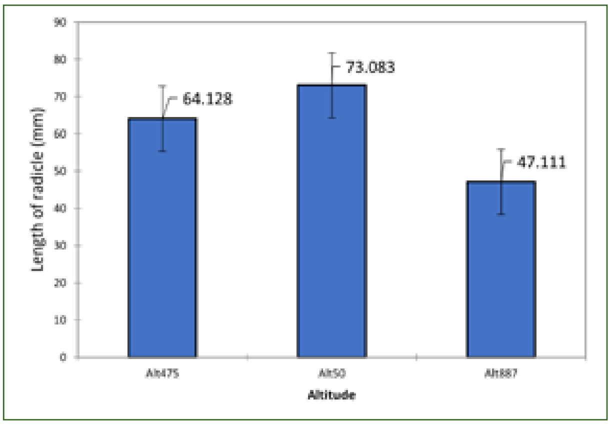


Figure 3 - Germination curves (left) and emergence (right) of acorns depending on time.

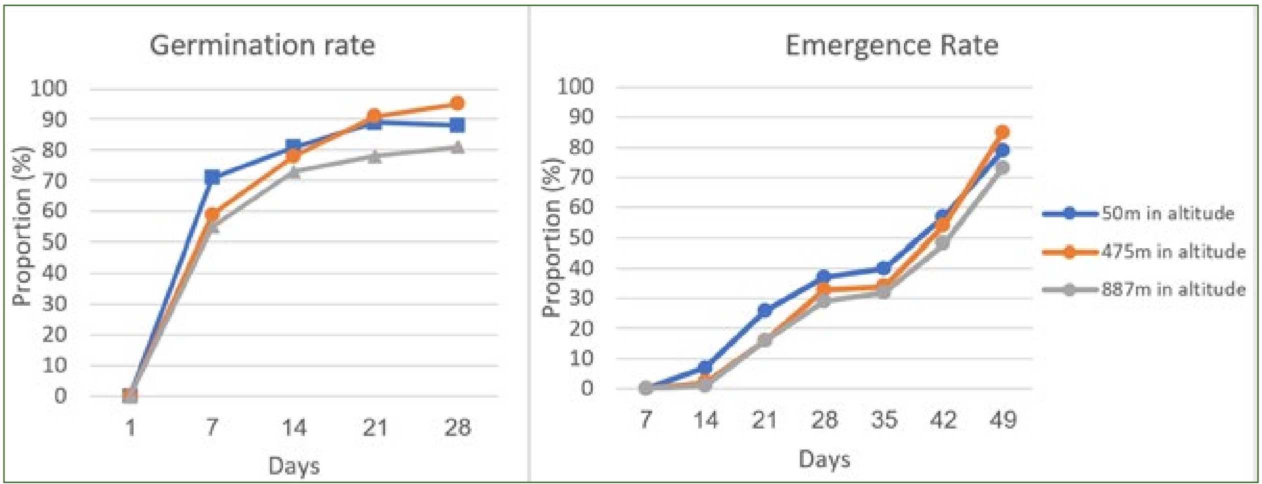


Table 4 - Analysis of variance for pivot elongation on day 28 by altitude (Rad28).

ANOVA criterion	Significance	Modality	Estimated means (mm)	Homogeneous groups
Altitude	Highly significant differences (***) Pr=0.000	Alt50	73.083	A
		Alt475	64.128	A
		Alt887	47.111	B

Influence of acorn biometric parameters on its germination (PCA)

A second PCA (Fig. 5) shows that acorn emergence is positively correlated with the elongation speed of the radicle. The length and weight of these acorns also showed no significant effect, unlike the width which has a more or less positively significant effect. However, the depredation of the acorns, expressed here by the number of perforations, showed a significantly negative effect, which affects the germination and acorn emergence.

Impact of perforation on the germination and emergence of acorns

By focusing on the impact of the number of perforations on the germination and emergence of acorns in relation to a control group (healthy glands), the variance analysis showed that there are highly significant differences (Tab. 5). For radicle elongation, acorns in the control (healthy) group were much better developed than the infested acorns regardless of the number of perforations (Fig. 6). However, for emergence, acorns containing a single perforation are classified in the same group (A) as healthy acorns, and the impact of this depredation on appearance became significant when two or more perforations were present.

Figure 5 - Principal Component Analysis characterising the interactions of biometric parameters of acorns with their germination ability. Variables are: Acorn perforation [Per], Acorn width [Wid], Acorn length [Len], Acorn weight [Wei], Rootlet elongation at 4th week of sowing [RoW4], Acorn emergence at 5th week of sowing [EmW5].

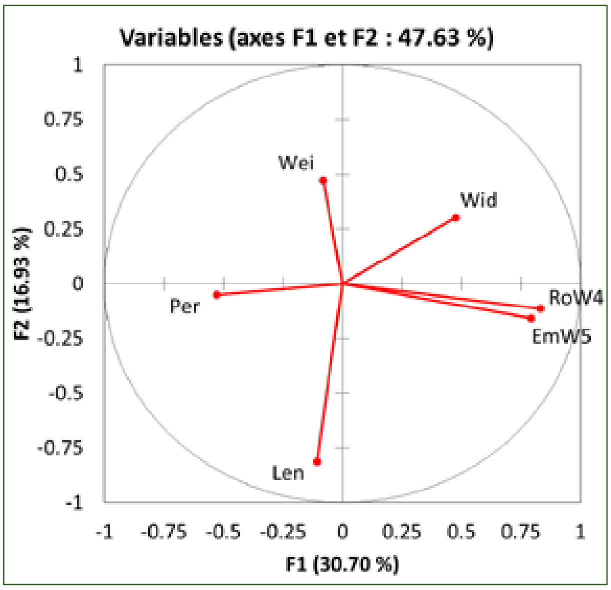
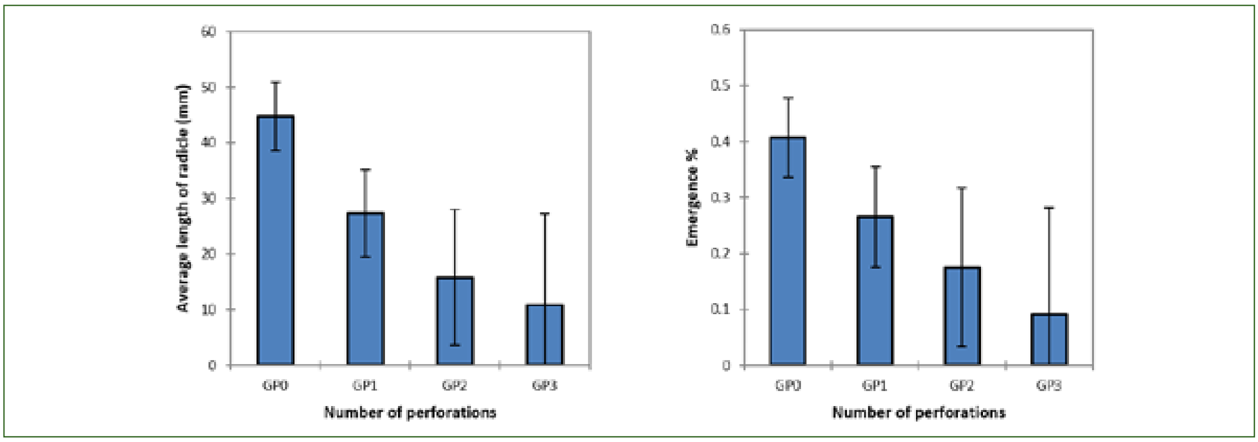


Table 5 - Analysis of the variance for elongation of pivots on the 28th day and the emergence of acorns up to the 49th day, depending on the number of perforations.

ANOVA criterion	significance	Modality	Estimated means	Homogeneous groups
Elongation of the radicle (mm)	Highly significant differences (***) Pr<0.0001	GP0	44.740	A
		GP1	27.308	B
		GP2	15.792	B
		GP3	10.820	B
Acorn emergence (rate)	Highly significant differences (***)Pr = 0.001	GP0	0.406	A
		GP1	0.265	A B
		GP2	0.175	B
		GP3	0.091	B
		Metre (m)		

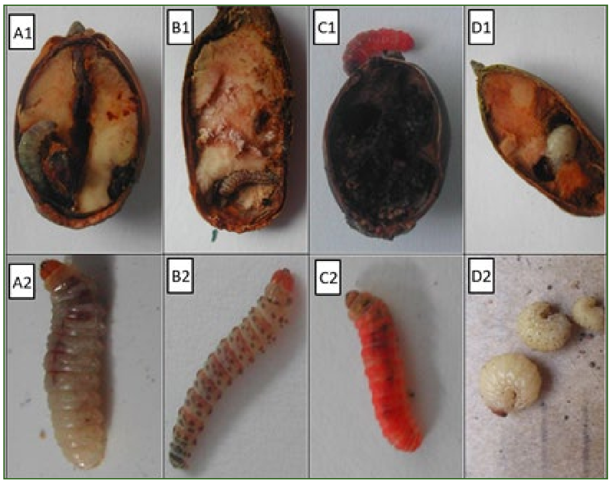
Figure 6 - Elongation of pivots on the 28th day (left) and the emergence of acorns up to the 49th day (right), depending on the number of perforations.



Carpophagous insects observeds

The most prevalent carpophagous insects that infested acorns in our study area are the larvae of *Curculio* sp. (48.7%) and *Cydia* sp. including C. *splendana* (43.5%) and C. *fagiglandana* (only 4.6%). On the other hand, very low attacks are presented by other larvae (3.2%), like *Pammene faciana* (Fig. 7). Out of 300 dissected acorns, 197 contained carpophagous larvae (i.e., 65.7%).

Figure 7 - Photos of carpophagous insects on cork oak acorns.
The species are: *Cydia splendana* [A1, A2], *Pammene faciana* [B1, B2], *Cydia fagiglandana* [C1, C2] and *Curculio sp*, [D1, D2] (photos by Adjami 2016 and Younsi 2019).



Discussion

Evaluating cork oak acorn germination with regards to their biometrics and considering the problem of perforation as the most frequent example of their depredation has led to some recommendations concerning the choice of acorns for the seedling regeneration of this species. Thus, it is possible to locate the collection sites that satisfy the criteria of this choice by prospecting different sources along an altitudinal gradient.

The results obtained confirm what was also reported by Arosa et al. (2017), who stated that specific sizes of healthy acorns act favourably on the development of young seedlings, especially concerning the emergence and radicle elongation reported by our case study. In contrast, for germination ability in general, cork oak acorns do not have a marked effect, although in natural conditions, seed germination depends on several biotic factors (genetics: origin or clone; morphological: weight or size; and physiological: dormancy) and abiotic factors (physico-chemical environmental conditions: temperature, humidity, substrate type, etc.) (Roberts and Hooley 1988, Côme 1970).

The biometric measurements of the acorns collected in the study area show significant correlations that are inversely proportional to altitude. The low altitude acorns are the most developed in length and width, and therefore have a much higher weight. This low altitude provenance provides the advantage that its acorns are the most favoured for the regeneration of cork oak either by direct sowing or for the production of seedlings in nurseries.

However, in considering the depredation problem, based on our results, there are no significant correlations either with the biometrical parameters or with the altitude considered (Fig. 2 and 5). This finding agrees with that of other authors (Bouhraoua 2003, Merouani et al. 2001b), who have noted the different sizes of acorns (large, medium and small) without a significant correlation with the depredation problem. However, Soukkou et al. (2018) found that healthy acorns are generally the largest and heaviest, which counters our finding. Attacks on acorns involve a reduction in weight because larvae inside of them feed on the almond. Branco et al. (2002) also reported that acorns attacked by insects show changes in their physiological state, which register a decrease in fresh weight accompanied by an increase in moisture content.

In terms of evaluating the size of the holes, our results agree with those of Bouchaour-Djabeur (2001). Our results allowed us to distinguish acorns with large holes attacked by beetles and acorns with small holes attacked by Lepidoptera. These same authors also reported that beetles are the most frequent insect pests, a finding validated by other researchers (Stiti 1999, Abidi and Abidi 2009).

Generally, cork oak acorns do not pose a germination problem. Germination can be performed regardless of whether the acorn is in poor health or is small. Also, elevation classes (altitude) influenced neither germination nor emergence. We observed only a small advantage for acorns at medium altitudes (475 m). According to Vinagre et al. (2005) cork oak acorns have high germination rates of more than 80%, while according to Merouani et al. (2001a), acorns on most trees have a final germination rate of more than 92% at the time of dissemination.

At the moment of germination, the size and weight of the acorns could play an important role in the speed of the elongation of the pivot or even in the development of seedlings. In our case, there were positively significant correlations of radicle elongation with the width of the acorns, but their weight and length did not exert a sufficiently interesting effect; that is, the correlations recorded were not significant.

We thus reported a negatively significant correlation between variable acorn width and perforation. The damage that insects cause can disrupt and reduce the regularity and abundance of the acorn crop, but above all, it can directly impact the germination capacity and therefore the natural regeneration of the species. Soil acorns are more often attacked than acorns on the tree (Adjami 2008). Hirka (2003) noted that attacked acorns can also germinate if the embryo is not consumed; they can even produce seedlings, but these do not develop as well as those of healthy acorns.

In Algeria according to several authors, oak acorns are attacked by two species of *Coleoptera*; *Curculio elephas* and *Curculio glandium*, two *Lepidoptera Cydia fagiglandana* and *Cydia splendana* and the gallic, *Callirhytis glandium* (*Hymenopera*, *Cynipidae*) (Adjami et al. 2013, Bouhraoua 2003, Derbal 2000, Benmecheri 1994). Through laboratory observations, we found these larvae in our sample acorns.

It is true that regeneration by the natural seeding of cork oak is subject to numerous constraints: the irregularity of the glands, the loss of fresh acorns due to grazing and embryonic dormancy (Merouani et al. 2001a) and the depredation of acorns by fungi, including the fungus responsible for black rot in acorns (*Ciboria batshiana*) and various insects (Khaldi et al. 1999, Cabral et al. 1993, Crawley and Long 1995, Fuchs et al. 2000).

By using the number of acorn perforations as an index of depredation, the comparison of radicle elongation speed and emergence with the control (healthy) acorns clearly demonstrated the impact of insect infestation on the further development of young seedlings but not on the germination itself. Our results indicated that only acorns with a single perforation are classified in the same healthy rank as healthy acorns, which can also be selected to produce quality plants in the nursery or for regeneration by direct sowing.

The impact of insect attacks on acorns partly explains the deficiency of the natural regeneration of cork oak observed in our forests. Nursery owners must use assisted regeneration to produce plants of a high rates of germination. This performance can only be ensured by the judicious use of non-infested acorns by avoiding, for example, collecting acorns that have fallen to the ground or by quickly germinating them.

Conclusions

The selection of cork oak acorns for regeneration should be based on their state of health, origin, and biometric parameters such as their size and weight.

In our case study, the lower altitudes (between 50 and 100 m) provided acorns with better quality according to their biometrics. However, there appears to be no relationship between the biometric parameters and the altitude variables for the depredation problem. Insects can ultimately attack acorns regardless of their size or origin.

Thus, although healthy acorns provide better germination ability, insect-infested acorns can also germinate and produce viable seedlings, despite the rotting problem. Acorn perforation is our studied case of depredation, for which the impact was on acorns with two or more holes. It has little effect on germination itself or during its first days of vegetative development, but it has a significant impact afterwards, depending on the severity of the damage. They affect the rate of radicle elongation and the rate of seedling emergence, whose disruption restricts regeneration

The study raised some guidelines related to the proper choice of acorns for direct seeding or the production of quality seedlings in the nursery, however, they are still preliminary and limited to one specific area. This study, therefore, needs results for other geographical areas by surveying more collection sites and more environmental descriptors. Thus, we propose that the effects of insect depredation on different stages of plant development be examined. We also suggest that other damages, such as decapitation, have to be studied.

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