

## Effect of the state of cork oak (*Quercus suber* L.) acorns on the emergence and growth of seedlings

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### Abstract

In Algeria, cork oak forests are of great socio-economic and ecological importance due to their great biological and ecosystemic diversity from east to west of the country. Given the decline of these forests and the difficulty of their natural regeneration, this work contributes to knowing some of the causes that affect this regeneration by studying the effect of the state of cork oak acorns (*Quercus suber* L.) on the emergence and growth of seedlings. The 917 cork oak acorns, collected from the township of Ain Lamcene in the Bissa forest, were divided into four batches according to their apparent condition: HeNPG (healthy non pre-germinated), HePG (healthy pre-germinated), HNPG (holed non pre-germinated) and HPG (holed pre-germinated) with respective rates of 36%, 30%, 21% and 13%. Analysis of the biometric parameters of the acorns reveals that the apparent state of health has no effect on the length and diameter of the acorns of the different batches; on the other hand, there is a difference in the weight. However, the difference is minimal and can be related to the consumption of the reserves of the cotyledons. The emergence of acorns was higher in healthy lots sown with a rate greater than 80%, compared to those attacked by insects. The growth rhythm of cork oak plants was similar for the four batches of the sown acorns. In addition, seedlings from healthy acorns showed better development regarding height, number of leaves, and diameter at the collar and root growth than those from acorns attacked by insects.

**Keywords:** acorns; biometric parameters; cork oak; emergence; growth; regeneration

### Introduction

The Mediterranean forests cover an area of approximately 81 million hectares. They are made up of a mosaic of forest species, particularly hardwoods which represent about 60% of the total area (Scarascia-Mugnozza *et al.*, 2000; Koumiche and Benmahioul, 2016). Besides, the cork oak is among the most western

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sclerophyllous oak species of the Mediterranean Basin, covering a total area of about 2,123,000 hectares, 67% of which is in Europe and 33% in Africa (Mechergui *et al.*, 2023). In Algeria they cover an area of about 480,000 hectares, which represents 21% of Mediterranean cork oak forests (Quezel, 2000).

Cork oak may start producing acorns at the age of 15-20 years (Natividade, 1950). Planning of seedling production is an essential step to achieve quality, and field performance is a critical part of planning (Landis, 1993). All cork oak stands have been confronted, for several decades, with a loss of vigor, a lack of natural regeneration and a decline which threaten the survival of this endemic species (Belgherbi and Benadeli, 2015). The fruit production in oak trees (or acorn) varies from year to year. According to Schermer *et al.* (2016), it is characterized by massive fruiting in certain years, followed by years of low fruiting in the stand. In addition, according to Croizeau and Roguet (1976), planting the pre-germinated acorns, in the spring, that are picked up from the ground at the end of winter may appear to be a solution for the artificial reconstitution of oak trees. The stock of cork oak acorns, the main source of repopulation, suffers great losses on the ground and on the tree due to multiple predators: rodents, birds, insects as well as humans and their animals, to this must be added the weak production of acorns, and their irregularity (El Boukhari *et al.*, 2013). All these factors are very active and act both at the level of the seeds and of the young seedlings. The slow germination of acorns would increase the risk of mortality (desiccation, fungal and entomological attack) and/or being picked up by rodents (Merouani *et al.*, 2001). Consequently, the regeneration of the cork oak by seminal way, whether artificial, directs sowing or natural, remains problematic (Messaudene *et al.*, 1998). In this context, our work studies the effect of the state (germination and state of apparent health) of cork oak acorns (*Quercus suber* L.), collected from the forest of Bissa (Figure 1) on the emergence and growth of the seedlings.

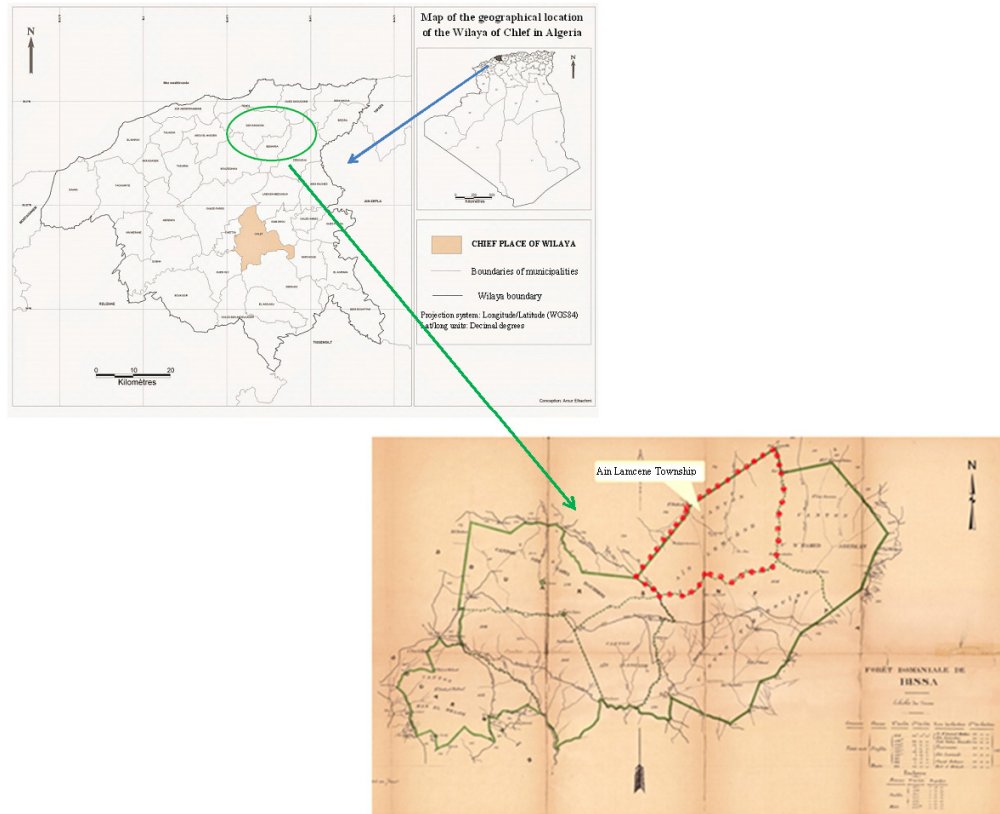


**Figure 1.** Stand of cork oak in the Bissa forest

## Materials and Methods

### *Acorn collection area*

The acorn collection area is a cork oak stand that is located in the township within the domanical forest of Bissa in the region of Chlef in Algeria (Figure 2). The canton occupies the northern slope of the domanical forest of Bissa and is characterized by a unique exposure that is the North (less sunny and more watered) and a sub-humid bioclimatic stage with mild winters.



**Figure 2.** Geographical location of the Ain Lamcene Township

### *Collection and sorting of acorns*

The morphologically mature acorns (brown color) were handpicked randomly from the ground in a cork oak stand (irregular natural stand) towards in the end of December. The acorns were sorted in the laboratory and divided according to their apparent state of germination and the presence of insect pest exit holes into four batches: healthy pre-germinated (HePG), healthy non pre-germinated (HeNPG), holed pre-germinated (HPG) and holed non pre-germinated (HNPG). For all the acorns of each batch we measured the length, diameter and weight.

### *Acorn seeding and growth monitoring*

The sowing of the seeds was carried out by direct sowing of 50 acorns from each batch in containers, which are 10 cm high and 5 cm in diameter. In addition, the containers have a hole in the base and contain forest soil taken from the same station of collecting the acorns. Moreover, the irrigation was carried out twice a week. Growth monitoring is done weekly for each plant of the four batches distributed previously, which gives a total of 200 plants; the measurements and counting of the leaves begin as soon as the stem appears outside the substrate for a period of ten weeks and this as follows:

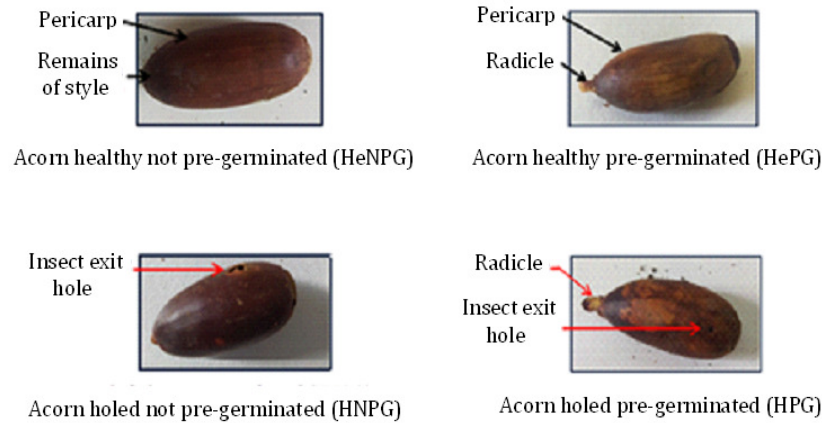
- We made a weekly measurement of the height of the stem from the level of the substrate to the apex using a ruler;
- We weekly counted the leaves of each plant;
- We weekly measured the collar diameter of each plant.
- In the end of the experiment, we measure the length of the root of the seedlings of each batch.

The statistical analysis of the data was carried out by XLSTAT software, to characterize the means and the variances.

## Results and Discussion

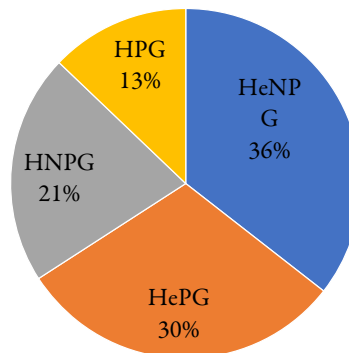
### *Assessment of the apparent condition of the harvested acorns*

The total number of the collected acorns was 917. They were sorted into four categories according to their state of health (insect holes) and their apparent state of germination in four categories (Figure 3).



**Figure 3.** Apparent aspect of *Quercus suber* L. acorns harvested in the township of Ain Lamcène

The rate of the acorns picked up for each batch was calculated based on the total number of the collected acorns. The results are represented in the form of a spectrum (Figure 4) of which the batch HeNPG represents 36%, HePG: 30%, HNPG: 21% and HPG: 13%.



**Figure 4.** Percentage of the number of collected acorns for each batch

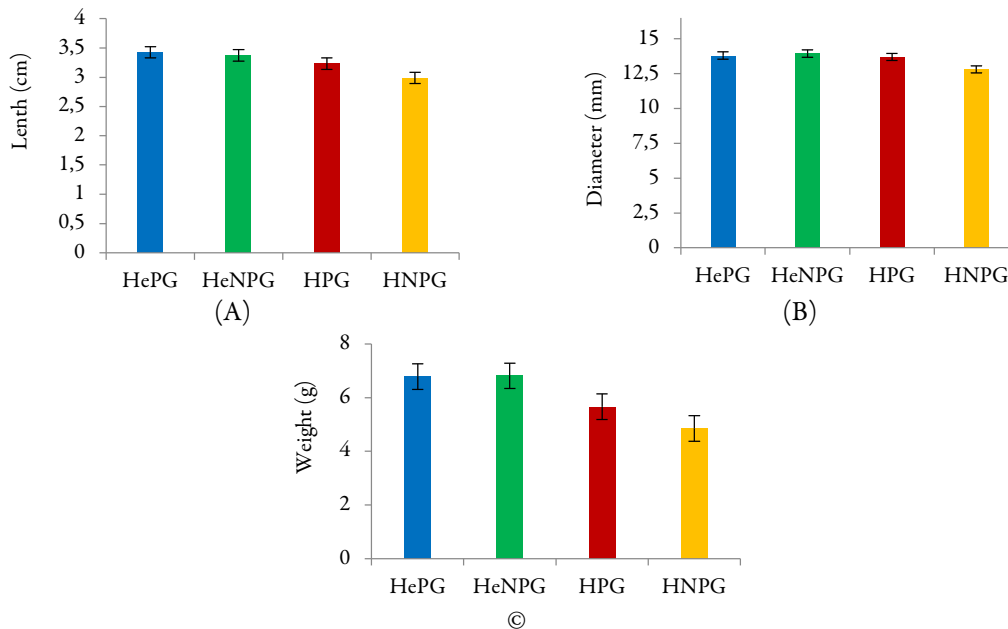
### *Acorn biometrics*

The collected acorns had a maximum length of 4 cm and a minimum length of 1.2 cm. Besides, they had a maximum diameter of 24 mm and a minimum diameter of 10 mm. As for the weight, the maximum was 9.65 g and the minimum was 1.80 g (Table 1).

**Table 1.** Acorns biometric characteristics according to the batches

		HePG	HeNPG	HPG	HNPG
Length (cm)	Mean	3.42	3.37	3.23	2.98
	Max	4	3.8	3.8	3.8
	Min	2.6	3.1	1.2	1.9
	Standard deviation	0.32	0.14	0.41	0.44
Diameter (mm)	Mean	13	13±	13	12
	Max	16	15	24	17
	Min	10	12	11	11
	Standard deviation	0.013	0.007	0.018	0.015
Weight (g)	Mean	6.78	6.81	5.66	4.85
	Max	9.65	8.62	8.07	8.25
	Min	3.86	5.47	3.02	1.80
	Standard deviation	1.371	0.701	1.268	1.756

The healthy acorns were the longest (Figure 5A), with an average length of 3.42 cm for HePG and 3.37 cm for HeNPG, compared to those attacked by insects, they had an average length of 3.23 cm for HPG and 2.98 cm for HNPG. The standard deviations of the four batches are too small and vary between 0.44 and 0.14 (Table 1), indicating that there is a weak distribution around the means and a weak variance among the data. The calculated average diameter is 13 mm for the HePG, HeNPG and HPG batches and 12 mm for the HNPG batch (Figure 5B), the standard deviations of the four batches vary between 0.007 and 0.018 which are close to zero (Table 1), This means that the data are distributed around the means and that the variance among the data is small. The average weight of the acorns of the different batches (Figure 5C), varies between 6.81 g (HeNPG) and 4.85 g (HNPG). As for the standard deviations, they are greater than zero which means that the data are scattered around the mean and that there is a variance among the data of the four batches.

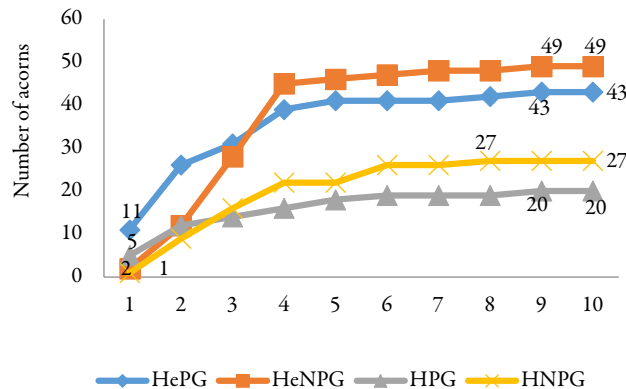
**Figure 5.** Biometric characteristics of the acorns according to the batches (A: average length, B: average diameter and C: average weight)

The study of the biometric characteristics of the collected acorns shows that the healthy acorns were slightly longer, larger, and heavier than the acorns attacked by insects. Our results agree with those obtained by Jdaidi *et al.* (2018) who found that the length of the healthy acorns collected from the ground was more (average between 2.43 to 2.96 cm) than the length of those attacked by insects (average around 2.22 cm), and that these acorns were larger (average between 1.66 and 1.44 cm for healthy acorns and 1.23 and 1.03 cm for acorns attacked by insects) and heavier than (average between 5.09 and 4.91 g for healthy acorns and 3.03 and 2.85 g for acorns attacked by insects).

*Emergence of acorns*

The emergence of the acorns is the beginning of growth of the seedlings where there will be development of the rootlet in the substrate and the stem which rises out of the substrate. Besides, we shall see the first leaves. This represents an early diagnosis of the success of a crop.

According to the results we obtained, the emergence of acorns seems to be affected by their state of health, not the state of pre-germination. The non-pre-germinated and the pre-germinated healthy acorns present an appreciable percentage of acorn emergences with respective rates of 98% and 86% (Figure 6), compared to those of the infested acorns. According to Preney *et al.* (1997), the insects rarely attack the embryo, but the larva is often present in a large gallery and digs in the cotyledons. For our test, the non-pre-germinated and pre-germinated holed acorns present respective emergence rates of 54% and 40%. The results of comparable work show that the non-pre-germinated acorns show less emergence failure compared to pre-germinated ones, with respective losses of 2% and 14% for healthy acorns and 46% and 60% for holed acorns. Besides, the pre-germinated acorns cause high seedling emergence failures, such as 30 to 40 percent losses (Penuelas and Ocana 2000; Almeida *et al.*, 2009). On the other hand, the losses related to the health state of the acorns were much greater, varying between 46% and 60%. According to Ajjami *et al.* (2013), the process of germination of oak acorns and of their growth is a complex physiological phenomenon that depends on the physiological integrity and the caliber of the acorns. The insects disturb this integrity by consuming the endosperm, changing the chemical composition of the acorn, and exerting permanent stress on the acorn. The physiological capacities of the glans are affected, and lead to a deficiency in the germinative capacity.



**Figure 6.** Emergence of the cork oak acorns for the four batches

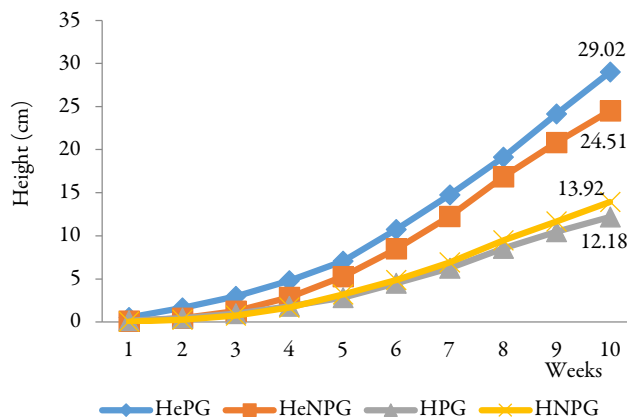
*Seedling growth*

According to Lamhamedi *et al.* (2000), a good forest plant should have a stem of 20 to 25 cm high with a diameter varying from 3 to 4 cm.

The growth in height of the stem

Height is one of the morphological factors that can best predict the performance of seedlings in the nursery (Elboukhari *et al.*, 2013).

There is a significant difference between the stem elongation dynamics of the four sown batches with p-values < 0.0001 and significance level  $\alpha=5\%$ . The kinetics illustrated in the form of curves (Figure 7) take almost the same shape. The two healthy batches showed much more development in height with an average of 29.02 cm for the HePG batch and of 24.51 cm for the HeNPG batch, compared to the batches with holes which showed an average of 13.92 cm for the HPG batch and of 12.18 cm for the HNPG batch. The bilateral test carried out for the growth in height of the plants of the four batches (Table 2) reveals two homogeneous groups, namely A (HPG, h HNPG) and B (HePG and HeNPG).



**Figure 7.** Evolution of height per batch and week

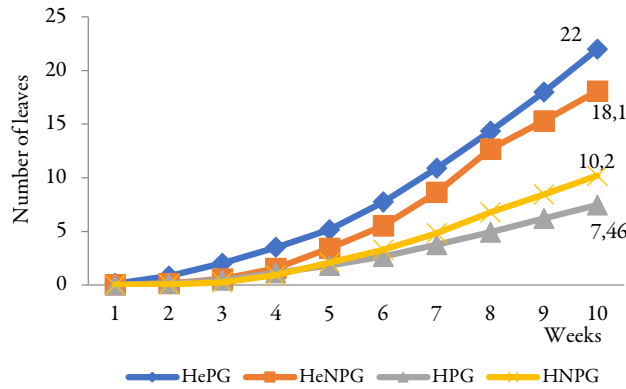
**Table 2.** Homogeneous groups for the height growth of the plants of the four batches

Sample	Effective	Sum of ranks	Rank average	Groups	
HPG	500	388,240.000	776.480	A	
HNPG	500	420,545.000	841.090	A	
HeNPG	500	589,138.500	1,178.277		B
HePG	500	603,076.500	1,206.153		B

Although the seedlings from the four batches of the sown acorns had the same rate of growth in height, the seedlings from the healthy acorns (pre-germinated and non-pre-germinated) were longer than those from the acorns attacked by insects, or with holes (non-pre-germinated and pre-germinated). Bouchaour-Djabeur *et al.* (2011) had results showing that the healthy cork oak acorns, regardless of their origin and the length of their storage, showed better height development than those attacked by insects. According to Sarir and Benmahioul (2017), following a comparative study of vegetative growth and the development of young seedlings from perfectly healthy acorns, the average weekly height does not exceed an average of 4.6 cm/week in the cork oak. By comparison, our seedlings from visibly healthy pre-germinated and non-pre-germinated acorns had an appreciable increase of 2.9 cm/week and 2.45 cm/week, respectively. Those from the acorns attacked by insects, or the holed ones (non-pre-germinated and pre-germinated) had a respective moderate increase of 1.39 cm/week and 1.21 cm/week.

Number of leaves

Like the height growth of the seedlings, the number of leaves is a good index of biomass production. The average number of leaves over the ten weeks (Figure 8) shows a significant difference between the healthy batches and the batches with holes with p-values < 0.0001 and the level of significance  $\alpha= 5\%$ .



**Figure 8.** Evolution of the number of leaves per batch and week

The bilateral test carried out for the development of the number of leaves during the trial period for the four batches (Table 3), reveals two homogeneous groups: A (HPG, HNPG) and B (HePG and HeNPG).

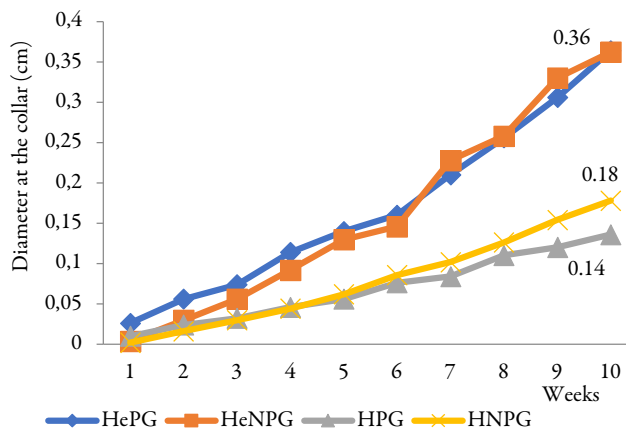
**Table 3.** Homogeneous groups for the evolution of the number of leaves of the plants

Sample	Effective	Sum of ranks	Rank average	Groups
HPG	500	396,906.000	793.812	A
HNPG	500	431,912.500	863.825	A
HeNPG	500	572,265.000	1,144.530	B
HePG	500	599,916.500	1,199.833	B

Thus, the infestation therefore does not have much effect on the embryo itself, which remains viable and able to germinate. However, a major attack on the cotyledons can limit the vigor of the plant and threaten its survival (Bouchaour-Djabeur *et al.*, 2011).

Collar diameter

The diameter of the collar, or the growth in diameter, of the seedlings was relatively low in the four batches (Figure 9). On the other hand, the maximum average diameter calculated for the batches of HePG and HeNPG (Dc= 0.36 cm) is higher than that of the batches of HNPG (Dc= 0.18 cm) and HPG (Dc= 0.14 cm). The difference in the collar diameter development between the healthy batches and those attacked by insects is significant, with p-values < 0.0001 at significance level  $\alpha= 5\%$ .



**Figure 9.** Evolution of the diameter at the collar per batch and week

According to the bilateral test carried out for the development of the diameter at the collar of the four batches (Table 4), two homogeneous groups: A (Dc TPG, Dc TNPG) and B (Dc SPG and Dc SNPG) can be distinguished.

**Table 4.** Homogeneous groups for the increase of the diameter at the collar

Sample	Effective	Sum of ranks	Rank average	Groups	
HPG	500	385,549,500	771.099	A	
HNPG	500	415,229,000	830.458	A	
HePG	500	599,173,000	1198.346		B
HeNPG	500	601,048,500	1,202.097		B

The collar diameter is an important morphological character that can best predict the plant performance after planting (Sarir and Benmahioul, 2017). For our seedlings, those which show an appreciable collar diameter in the start show a good growth in the aerial biomass (height and number of leaves). Besides, the seedlings from healthy pre-germinated acorns (HePG) had a better development in the aerial biomass during the first four weeks than the rest of the seedlings. In this regard, their average diameter in the first week was 0.026 cm, while the seedlings from other batches had an average diameter of 0.002 cm in the first week of growth.

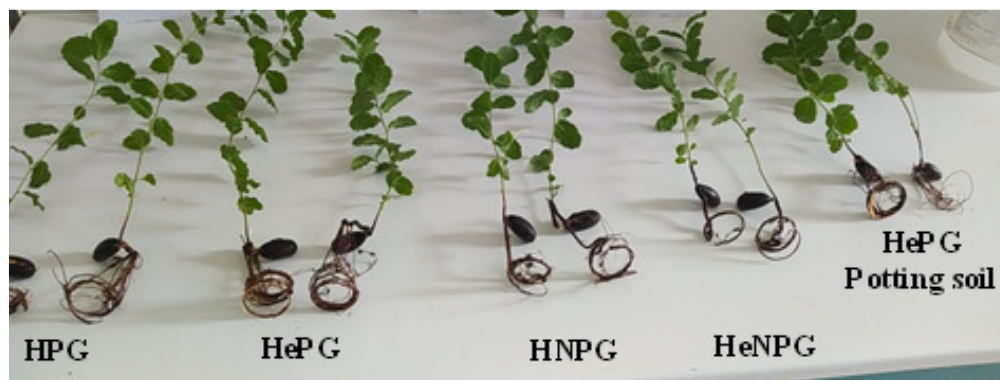
#### Root growth

The root development was better in the seedlings belonging to the batch of healthy non-pre-germinated acorns, which showed an average length of 18.73 cm followed respectively by those HNPG: 17.11 cm, HePG: 15.42 cm and HPG: 15.07 cm, all planted in the ground forest. By comparison, the pre-germinated healthy acorns planted in potting soil (50% OM) had a root development with an average length of 17.66 cm. In order to be able to measure the length of the root, we had to spread it on the graph paper (Figure 10) and read directly.



**Figure 10.** Spreading and root length measurement

The root development was better in the seedlings belonging to the batch of healthy non-pre-germinated acorns (Figure 11), which showed an average length of 18.73 cm, followed respectively by those of HNPG: 17.11 cm, HePG: 15.42 cm, and HPG: 15.07 cm; all planted in the ground forest. By comparison, the pre-germinated healthy acorns planted in potting soil (50% OM), had a root development with an average length of 17.66 cm (Table 5).



**Figure 11.** Comparison of the root growth of seedlings from each batch

**Table 5.** Average length of the root according to the batches

Batch of acorns	Average root length (cm)
HeNPG/ forest floor	18.73
HePG/ forest floor	15.42
HNPG/ forest floor	17.11
HPG/ forest floor	15.07
HePG/ potting soil	17.66

The development of the main root was greater in the seedlings from non-pre-germinated acorns regardless of their apparent state of health. On the other hand, the density of the lateral roots was more remarkable in the seedlings from the healthy acorns. It has been showed that the carbohydrate reserves play an important role in the lateral root emergence, and that the seedling performance depends on the lateral roots speed of emergence (McKay, 1998).

It can be said that the attacked acorns were lighter than the healthy ones; they presented less emergence and growth in height and diameter. Insects consume the reserves contained in the cotyledons and cause troubles in the development and vigor of the seedling. The state of cork oak (*Quercus suber* L) acorns infestation and pre-germination affects the emergence and growth of the seedlings at different degrees. The acorns state of health conditions their emergence more than the state of pre-germination. The seeded healthy acorns (HeNPG and HePG) presented more emergences with respective rates of 98% and 86%, compared to those with holes or attacked by insects (HNPG and HPG), which showed respective rates of 54% and 40%. The state of pre-germination affects the emergence of the seedlings more for the first two weeks of sowing. Then, from the 3<sup>rd</sup> week, the batches of non-pre-germinated acorns showed more emergences than the pre-germinated ones, taking into account the perforated state until the 10<sup>th</sup> week. The consumption of the endosperm by the larvae probably leads to a reduction in the physical pressure on the germ, which results in a slowing down of the average germination time (Montoya and Iranzo, 1997; Merouani *et al.*, 2001; Branco *et al.*, 2002). The importance of attacks on acorns by insect pests partly explains the weakness of the cork oak regeneration. In this context, according to Merouani *et al.* (2001), the seedling growth was strongly affected by the size of freshly harvested seeds.

The growth of the cork oak seedlings is characterized by stem height, cumulative number of leaves and diameter at the collar, with which the performance of the seedlings can be predicted. Our results show that there is a close relationship between the height growth, the number of leaves, and the diameter of the collar. In this regard, the longer and thicker the stem is the more leaves it bears. Cork oak seedlings grow rhythmically. After emergence, the shoot elongation occurs by rapid growing lasting about 2 weeks, which changes with resting periods. This characteristic is already known for almost all Temperate Zone species (Lavarenne *et al.*, 1971). The rhythm of growth of the cork oak seedlings is not affected by the apparent state of health and the

pre-germination of the acorns, but its intensity (which is related to the height and diameter of the stem, and the number of leaves) depends beforehand on the health of the acorns. In the end of the 10th week after sowing, the plants from the acorns of the HeNPG batch reached an average final height of 29.02 cm, an average final diameter of 0.36 cm, and an average cumulative number of leaves of 22. In addition, the plants from the acorns of the HePG batch reached an average final height of 24.51 cm, an average final diameter of 0.36 cm, and an average cumulative number of leaves of 18.1. Moreover, the plants from the acorns of the HNPG batch reached an average final height of 13.92 cm, an average final diameter of 0.18 cm, and an average cumulative number of leaves of 10.2. Finally, the plants from the acorns of the HPG batch reached an average final height of 12.18 cm, an average final diameter of 0.14 cm, and an average cumulative number of leaves of 7.46.

## **Conclusions**

In the end, our findings show that the state of health of cork oak acorns affects their weight due to the consumption of part of the cotyledons by insects, what will consequently have a negative effect on the seedlings emergence, height, and diameter growth. The state of health of the acorns conditions their emergence and the growth of the seedlings at different degrees, more than their state of pre-germination. This does not apply to the root growth, where the development is greater in the seedlings from the non-pre-germinated acorns, regardless their apparent state of health. The rhythm of the cork oak seedlings growth is not affected by the apparent state of health and the acorns pre-germination, but its intensity (which is related to the height and diameter of the stem, and the number of leaves) depends beforehand on the health of the acorns.

## **Authors' Contributions**

Conceptualization and design of the research work: SZ; Execution of field/laboratory experiments and data collection: FH, KR, SZ and YA; Data analysis and interpretation: EL and SZ; Manuscript preparation: SZ; review and validation: SZ, EL, FH, KR and YA.

All authors read and approved the final manuscript

## **Ethical approval** (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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