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Comparative analysis of *Quercus suber* L. acorns in natural and semi-natural stands: Morphology characterization, insect attacks, and chemical composition

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The present study aims to investigate the differences between cork oak acorns from natural and semi-natural stands in terms of morphology, insect attack rate, and acorn chemical composition. Moreover, it examines the metabolic responses induced by insect attacks. The results show that acorns from the semi-natural stand in our study are larger than those from the natural stand. In addition, the insect attack rate was higher in the natural stand (8.25%) than in the semi-natural stand (6.25%). Furthermore, acorns in the semi-natural stand exhibit high total flavonoid content (TFC), whereas those in the natural stand are rich in total phenolic content (TPC). In terms of biochemical changes in acorns, the study revealed a remarkably significant difference in TPC, TFC, and antioxidant activity subsequent to infestation by *Cydia* and *Curculio* insects. *Cydia*-infested acorns from the natural stand had higher TPC levels, with a value of 93.96±0.39 mg GAE/g, showing a 17.7% increase over healthy acorns. Acorns from the semi-natural stand attacked by *Curculio* show the highest TFC with a value of 0.288±0.004 mg EQ/g, showing a 121.5% increase over healthy acorns. Moreover, both DPPH and FRAP methods revealed that antioxidant activity of the acorns from the semi-natural stand attacked by *Curculio* was more effective. This research is crucial for providing a solid foundation for the selection of high-quality cork oak germplasm resources and exploring the potential valorization of insect-affected acorns in the realms of food and agriculture.

Keywords. Acorns; insects; natural stand; *Quercus suber* L.; secondary metabolites; semi-natural stand

1. Introduction

The cork oak is an endemic feature of the Mediterranean basin: it is grown in four European countries (Portugal, Spain, France, and Italy) and three North African countries (Algeria, Morocco, and Tunisia) (Pio *et al.* 2005). In Algeria, cork oak forests originally covered an estimated area between 429,000 and 480,000 hectares. This made it the third-largest area of cork oak forests after Portugal and Spain. The majority of extensive, high-quality cork oak forests are found in eastern Algeria, mainly in humid and sub-humid zones. These regions offer environmental conditions conducive to cork oak growth (Bouchaour-Djabeur *et al.* 2021). *Quercus suber* L. is a valuable natural resource because of its corky bark, which is used in a variety of industries for making bottle caps. Its wood has a high calorific value, making it ideal for use as charcoal or firewood. Additionally, its acorns provide nourishment for both wild and domestic animals (Bouchaour-Djabeur *et al*. 2011). This combination of characteristics makes the cork oak a sought-after species, appreciated for its economic as well as ecological aspects (Maghnia *et al*. 2019).

The *Q. suber* L. fruit plays an important role in the natural regeneration of cork oaks, which depends essentially on the abundance of acorns, the phytosanitary conditions of the acorns, and their size (Merouani *et al*. 2001a). In addition, this fruit also has strong antioxidant properties and high content of phenolic compounds, making it an excellent source of phytochemicals for preventing human diseases as well as a useful functional ingredient in the food industry (Makhlouf *et al*. 2019). In North Africa, the fruit of *Q. suber* L. has traditional medicinal and food applications. However, owing to their high tannic acid content, which imparts a bitter flavor, acorns are typically soaked in water, boiled, or roasted. Once processed, they can be incorporated into bread or couscous preparations after being dried and ground into flour, or roasted as coffee bean substitutes. They can also be combined with honey and used for their stomachic and antidiarrheal properties (Igueld *et al*. 2015; Zarroug *et al*. 2022).

However, these acorns are prone to attacks by fungi and insects, which reduce their germination capacity and consequently the quality and quantity of acorn production (Merouani *et al*. 2001a). Insects have a substantial impact on the reproductive capabilities of oaks and play a significant role in diminishing the viability of acorns. The germination of acorns can be hindered by direct influences on the cotyledons and embryonic nutrition (Csóka and Csókáné Hirka 2006). This is mainly due to the decrease of certain chemical compounds in the glands, such as proteins, carbohydrates, and lipids (Adjami *et al*. 2016). Two main groups of insects can induce these effects: species from the genus *Cydia* within the Tortricidae family of Lepidoptera, and species from the genus *Curculio* within the Curculionidae family of Coleoptera (Branco *et al*. 2002).

Plants have a variety of defence mechanisms against insect pest infestation, including morphophysiological, biochemical, molecular, and hormonal signals (Kumari *et al*. 2022). These include the creation of foliar trichomes, glandular hairs, a wax layer, and endogenous secondary metabolites, which are chemical compounds produced by plants that can be toxic to invading insects, effectively repelling them (Granados-Sánchez *et al*. 2008). These secondary metabolites, including phenolic and flavonoid compounds, function as antioxidants and have various other biological functions. They deter oviposition and feeding, provide protection against predators, and impede insect growth and development (Ikonen *et al*. 2001). Many studies have demonstrated that plants exposed to infestation have increased concentrations of secondary metabolites associated with plant defences (Nagrare *et al*. 2017; Kovalikova *et al*. 2019; Scott *et al*. 2020). Organically grown vegetables and fruits generally contain higher amounts of phenolic compounds than conventionally grown vegetables, which may be related to increased levels of insect stress (Cohen and Kennedy 2010; Scott *et al*. 2020).

Secondary metabolites differ quantitatively and qualitatively between different species and vary between populations of the same species, as well as between different tissues and organs of the same species and within individuals of the same population. When plants are infested, the secondary metabolite content also depends on the infesting insect, its population density, and level of infestation. An efficient method for determining the degree of metabolic defence in various plant tissues is to quantify phenolics (Brenes-Arguedas and Coley 2005; Del Valle *et al*. 2015; Kovalikova *et al*. 2019). Phenolic compounds are widely distributed in various plant parts, such as leaves, stems, and fruits, and they fulfill a diverse range of roles within plant species. Among these phenolic compounds, total phenols and flavonoids are particularly noteworthy, as they possess biologically active properties and powerful antioxidant activities that protect plants against various types of stress, whether of abiotic or biotic origin (Ghasemzadeh and Ghasemzadeh 2011).

Previous research has predominantly focused on examining the characteristics of *Quercus* acorns to identify potential significant differences between various species or to explore the impact of geographical distribution within the same species (Ramírez-Valiente *et al*. 2009; Valero Galván *et al*. 2012; Giertych and Chmielarz 2020; Sun *et al*. 2021). However, no study has specifically investigated acorns from natural and semi-natural stands of *Q. suber* under similar climatic conditions. Additionally, existing studies have primarily determined the chemical composition of healthy acorns and methods for valorization, with limited exploration of the chemical composition of acorns attacked by insects, particularly focusing on secondary metabolites. The current study aimed to address this gap by investigating how different forms of management, or their absence, can affect acorn morphology, insect attack rates, and acorn chemical composition. By providing a comparative analysis between natural and semi-natural stands, we sought to identify potential implications for forest management and conservation of cork oak. Additionally, our study aimed to distinguish the impact of attacks by two different orders of insects, Lepidoptera and Coleoptera, on the chemical composition of acorns, with particular focus on phenolic compounds and antioxidant activity. Therefore, the objectives of the present investigation were as follows: (i) to conduct a comparative biometric study and analyze the chemical composition of acorns in both stands in order to assess the level of insect attacks in each stand, and (ii) to evaluate changes in total phenolic content, total flavonoid content, and antioxidant activity in cork oak acorns following attacks by two insect pests, *Cydia* and *Curculio*. These findings not only complement the existing body of knowledge on natural stands but also address, for the first time, these aspects in semi-natural populations where no similar study has been conducted previously. Furthermore, our results will provide reference for selecting acorns with high antioxidant activity, rich or low in phenolic compounds, for use in areas such as animal nutrition or other applications. Additionally, our study could inform cork oak reforestation programs by helping in the selection of the largest acorns to produce higher-quality seedlings.

2. Materials and methods

2.1 *Presentation of the study area*

Two study sites were chosen, a natural cork oak stand and a semi-natural cork oak stand located in the Errich forest (latitude: 36°24′34.78″N; longitude: 3°51′31.15″E; altitude: 610 m) in the Bouira region. This region is situated in the northern-central region of the country, approximately 120 km southeast of Algiers. The terrain of the Errich canton features fairly good topography, with slopes not exceeding 12%. The geological substrate consists of ancient alluvium, resulting in a moderately deep clay-sandy soil with significant humus content due to the abundance of vegetative matter. The soil pH ranges between 6.5 and 7. The study area falls within the semi-arid bioclimatic stage, experiencing cold winters and hot, dry summers. The lowest humidity levels are recorded during the summer months, ranging from 40% to 49%. Conversely, maximum humidity, exceeding 80%, is recorded during January, February, November, and December. The cork oak forest is rich in undergrowth, predominantly composed of mastic, wild olive, rockrose, lavender, myrtle, and strawberry trees. The natural cork oak forest of Errich is defined as areas where ecological processes occur without significant human intervention and contain a pure natural stand with an average age of approximately 100 years. The composition of the undergrowth varies from place to place and is mainly represented by herbaceous species such as grasses and some shrub species such as cistus and heather. The semi-natural stand of Errich is part of the natural cork oak forest where some form of human management is present, such as sylvicultural practices. It is fenced and protected against the impact of trampling and grazing. The undergrowth is made up of herbaceous species such as grasses.

2.2 *Harvesting of biological material*

The harvest of biological materials took place during the winter in two cork oak forests of Errich. More than 400 acorns were randomly collected from each cork oak forest. On each tree taken as a sampling unit, we manually collected 8 acorns from the ground after their fall at maturity and 8 acorns from the tree, 4 of which were collected from the northern direction and 4 from the southern direction. The sampling method was the same as that adopted by Jdaidi *et al.* (2018). The harvested acorns were transported in paper bags to the laboratory, where they are stored dry at room temperature.

2.3 *Morphological characterization and weighing of acorns*

The length and width of the harvested acorns were measured with an electronic digital caliper, and the weight was determined using a precision electronic balance.

2.4 *Rearing of biological material*

After the biometric study, each acorn was individually stored in a plastic vial, covered with mesh fabric, and kept under natural laboratory conditions. The reared biological material was monitored regularly to detect larval emergence. Figure 1 shows that emergence of all larvae took 56 days, attributed to the absence of any further larval emergences after 14 consecutive days. This monitoring also aids in determining the infestation rate.

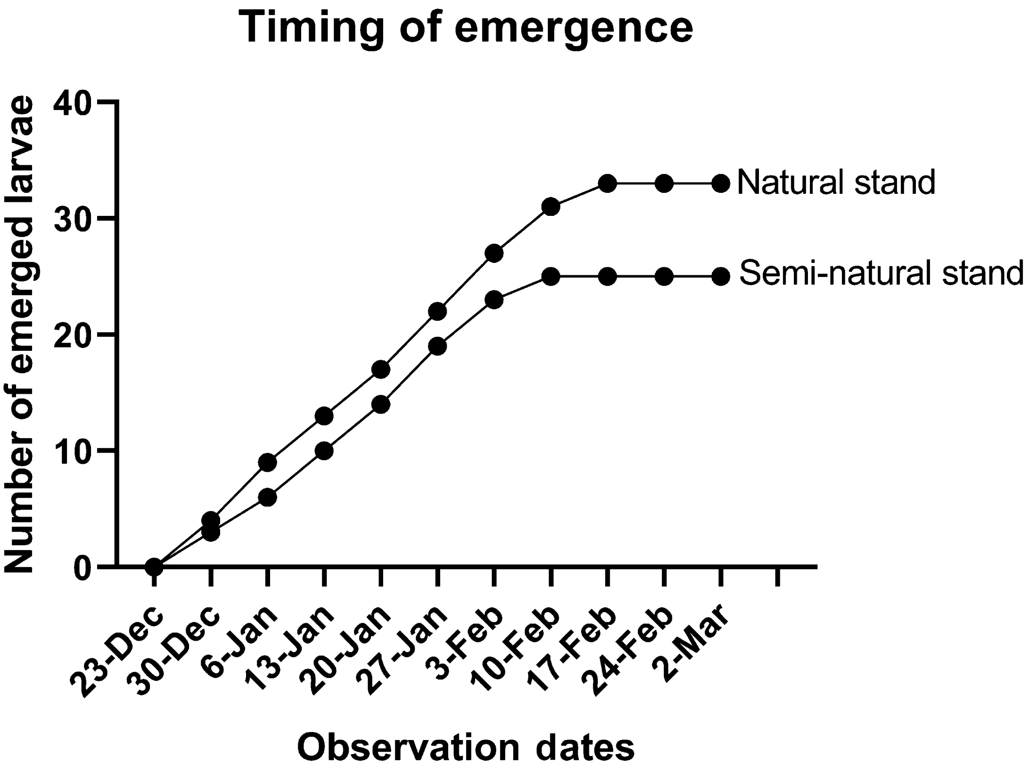

Figure 1. Timing of larval emergence in natural and semi-natural cork oak stands.

2.5 *Biochemical characterization of acorns*

After the emergence of larvae, the acorns were manually sorted as healthy acorns, acorns with a large pest exit hole, attacked by *Curculio*, and acorns with a small exit hole, attacked by *Cydia*. Using a grinder, the acorn kernels were then ground and sieved into a fine powder, which was then extracted using the protocol described by Djeridane *et al.* (2006) with some modifications. To 1 g of acorn flour, 50 mL of 70% ethanol was added and left to react under stirring for 24 h at room temperature. The extract was then centrifuged to recover the supernatant, which was then used for the determination of total phenolic content (TPC) using the adapted Folin–Ciocalteu method described by Singleton and Rossi (1965). Total flavonoid content (TFC) was quantified using the method of Wu *et al.* (2009), which is based on the formation of a flavonoid–aluminum complex, while the antioxidant activity was estimated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method, following the protocol described by Alara *et al.* (2018), and the iron reduction assay (FRAP) was carried out using the method of Oyaizu (1986). Absorbances were measured using a UV/visible spectrophotometer model SP-3000 Nano.

2.6 *Statistical analysis*

The statistical analyses and the graphical representations were processed using GraphPad Prism 8.0.2. The *t*-test was used to test the differences in biometry and weight of acorns harvested from natural and semi-natural cork oak forests. The comparative study of the biometry and weight of healthy and attacked acorns (harvested from soil, north-facing trees, and south-facing trees) was carried out using one-way ANOVA. The analyses of the phenolic compounds and antioxidant activity of cork oak acorns were carried out in triplicate, and the experimental data were reported as means ± standard deviation, and the results were analyzed using analysis of variance, two-way ANOVA, and Tukey's comparison test. Significant differences were established at $p<0.05$.

3. Results

3.1 *Biometrics and weights of acorns harvested from the two stands*

The results of the weighing and biometric study of the acorns harvested in the two stands are summarized in tables 1–3.

Table 1. Variations in the weight of acorns harvested in the two cork oak forests

	Acorns harvested from a natural stand			Acorns harvested from a natural stand		
	Soil	North-facing trees	South-facing trees	Soil	North-facing trees	South-facing trees
Average weight (g)	4.25	5.08	5.56	6.12	6.43	5.97
Standard deviation	1.18	1.27	1.24	1.80	1.82	1.39
Extreme values	1.84–8.63	2.03–10.67	3.35–9.13	2.39–13.01	3.10–13.62	3.11–9.85

Table 2. Variations in the maximum lengths of acorns harvested in the two stands surveyed

	Acorns harvested from a natural stand			Acorns harvested from a natural stand		
	Soil	North-facing trees	South-facing trees	Soil	North-facing trees	South-facing trees
Lengths average (mm)	29.91	32.24	33.38	33.30	33.53	32.37
Standard deviation	3.36	3.37	3.52	4.42	4.53	3.98
Extreme values	20.32–40.89	22.35–43.94	26.16–42.16	21.59–45.21	21.84–44.70	22.09–42.16

Table 3. Variations in maximum widths of acorns harvested in the two cork oak forests surveyed

	Acorns harvested from a natural stand			Acorns harvested from a natural stand		
	Soil	North-facing trees	South-facing trees	Soil	North-facing trees	South-facing trees
Widths average (mm)	13.88	14.69	15.56	15.90	16.58	16.34
Standard deviation	1.66	1.80	1.43	1.86	1.71	1.32
Extreme values	9.40–19.56	8.64–21.34	11.94–19.05	10.66–21.59	12.44–21.08	11.43–19.90

Acorns from the semi-natural stand were significantly larger than those from the natural stand, weighing 6.17 g and 4.97 g, respectively (table 1). Analysis of variance on the samples revealed a highly significant difference in the weight of acorns between the two stands (t=12.58; p<0.0001).

Acorns taken from the semi-natural stand had average lengths of 33.07 mm and average widths of 16.27 mm. In the natural stand, average acorn lengths and widths were 31.84 mm and 14.71 mm, respectively (tables 2 and 3). Analysis of variance revealed a highly significant difference between lengths (t=6.17, p<0.0001) and widths (t=13.55, p<0.0001)

3.2 *Comparative study of biometry and weight of healthy and attacked acorns*

The results of the comparison of acorn weights and measurements are summarized in table 4.

The weights and measurements of the acorns differed according to their state of health, with the average weight of attacked acorns being lower than that of healthy acorns (table 4). For the natural stand, statistical analysis at the 5% significance level indicated that there was a highly significant difference between the weight of healthy acorns harvested from soil, north-facing trees, and south-facing trees (F=35.96;

Table 4. Weights and measurements (means ± SD) of healthy and damaged acorns harvested from the two stands

		Natural stand			Semi-natural stand		
		Weight (g)	Length (mm)	Width (mm)	Weight (g)	Length (mm)	Width (mm)
Soil	Healthy	4.32 ± 1.20	30.08 ± 3.31	13.87 ± 1.69	6.22 ± 1.80	33.63 ± 4.29	15.93 ± 1.87
	Attacked	3.71 ± 0.84	28.51 ± 3.55	14.01 ± 1.36	4.91 ± 1.36	29.40 ± 4.33	15.63 ± 1.89
North-facing trees	Healthy	5.06 ± 1.16	32.22 ± 3.38	14.73 ± 1.69	6.50 ± 1.83	33.66 ± 4.58	16.62 ± 1.73
	Attacked	4.74 ± 1.33	32.32 ± 3.86	13.37 ± 1.88	4.92 ± 0.82	30.67 ± 1.86	15.88 ± 1.50
South-facing trees	Healthy	5.55 ± 1.24	33.31 ± 3.51	15.55 ± 1.44	6.01 ± 1.39	32.53 ± 4.00	16.36 ± 1.25
	Attacked	5.36 ± 0.87	34.54 ± 4.54	15.11 ± 1.13	5.30 ± 1.32	29.90 ± 2.93	16.22 ± 2.40

Table 5. Descriptive analysis of acorns harvested from the two stands

	Acorns harvested from a natural stand			Acorns harvested from a natural stand		
	Soil	North-facing trees	South-facing trees	Soil	North-facing trees	South-facing trees
Healthy acorns	179	91	97	185	96	94
Infested acorns	21	9	3	15	4	6
Infestation rate	10.5%	9%	3%	7.5%	4%	6%

$p<0.0001$). Analysis of the effect of exposure (north and south) revealed a significant difference ($p=0.01$). Similarly, for the weight of attacked acorns, statistical analysis revealed a significant difference between acorns taken from the three harvesting strata ($F=3.56$; $p=0.04$). However, there was no significant difference between the two exposure classes (north and south) ($p=0.78$).

In terms of length, there was also a highly significant difference between the average length of healthy acorns of the three sampling classes ($F=31.87$; $p<0.0001$), whereas there was no significant variation between the two orientations (north and south) ($p=0.07$). For attacked acorns, a significant difference was observed among the three harvesting strata ($F=4.77$; $p=0.01$), while no significant difference was found between the north and south exposures ($p=0.72$).

Statistical analysis showed that in each harvesting class, there was a highly significant difference between the width of healthy acorns ($F=35.42$; $p<0.0001$) and between those of north and south exposures ($p=0.001$). For attacked acorns, however, there was no significant difference between the three sampling classes ($F=1.17$; $p=0.32$), and the same held true for the exposure classes ($p=0.33$).

In the semi-natural stand, analysis of variance showed no significant effect at the 5% level between the weight of healthy acorns sampled from the ground, trees, and between the two exposures, north and south ($F=1.94$; $p=0.14$). The analysis carried out on the weight of unhealthy acorns showed no significant difference between acorns sampled from the ground and acorns sampled from the trees facing north and south ($F=0.20$; $p=0.81$).

Statistical analysis revealed that there was no significant difference ($F=2.34$; $p=0.09$) between the length of healthy acorns collected from the three sampling classes (ground, north-facing trees, and south-facing trees). The same observation was made for acorns with holes ($F=0.18$; $p=0.83$) in the three harvesting states.

The analysis carried out on the width of healthy acorns ($F=5.73$; $p=0.003$) showed a highly significant difference between the three sampling strata, while between the two sampling classes (north and south), the difference was not significant ($p=0.52$), and for attacked acorns, there was no significant variation between the three harvesting strata ($F=0.18$; $p=0.82$). For the two classes, north and south, no significant effect at the 5% level was observed ($p=0.96$).

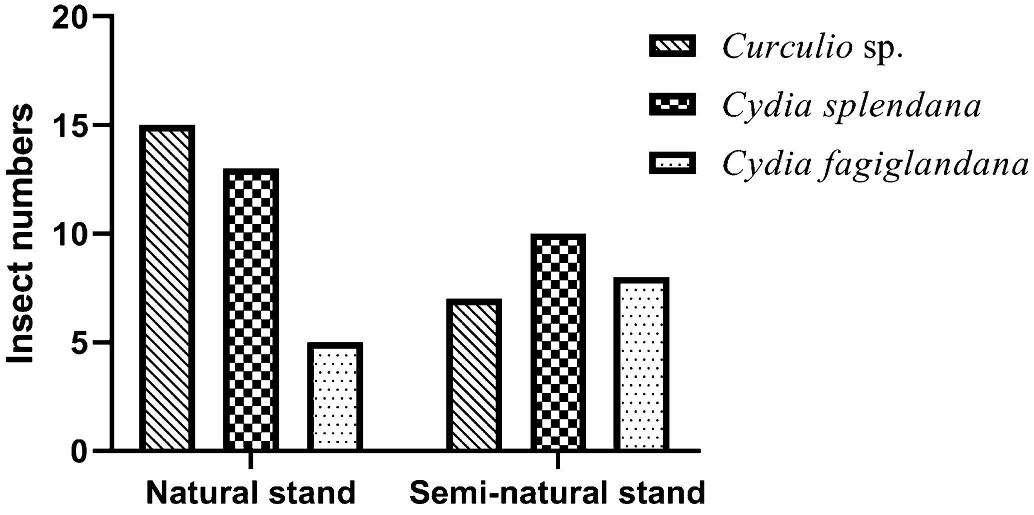

Figure 2. Variation in numbers of the three acorn pest species in the two stands.

3.3 *Analysis of condition of acorns harvested*

The results of analysis of the condition of the acorns harvested from the two stands are summarized in table 5.

The results of the analysis of the health status of the acorns show that the rate of healthy acorns is higher than that of attacked acorns (table 5), with an attack percentage of no more than 8.25% in the natural stand and 6.25% in the semi-natural stand. It is on the ground that the number of attacked acorns is greater than on trees. In the natural stand, the attack rate for acorns harvested from trees with northern exposure was higher (9%) than for acorns with a southern exposure (3%). In the semi-natural stand, we recorded 4% and 6%, respectively, for the two exposures, north and south (table 5).

3.4 *Emergence of acorn insects during rearing period*

The biological material studied enabled the emergence of three insect species belonging to two orders: Coleoptera and Lepidoptera. Lepidoptera was represented by two species, *Cydia fagiglandana*, and *Cydia splendana*, while Coleoptera was represented

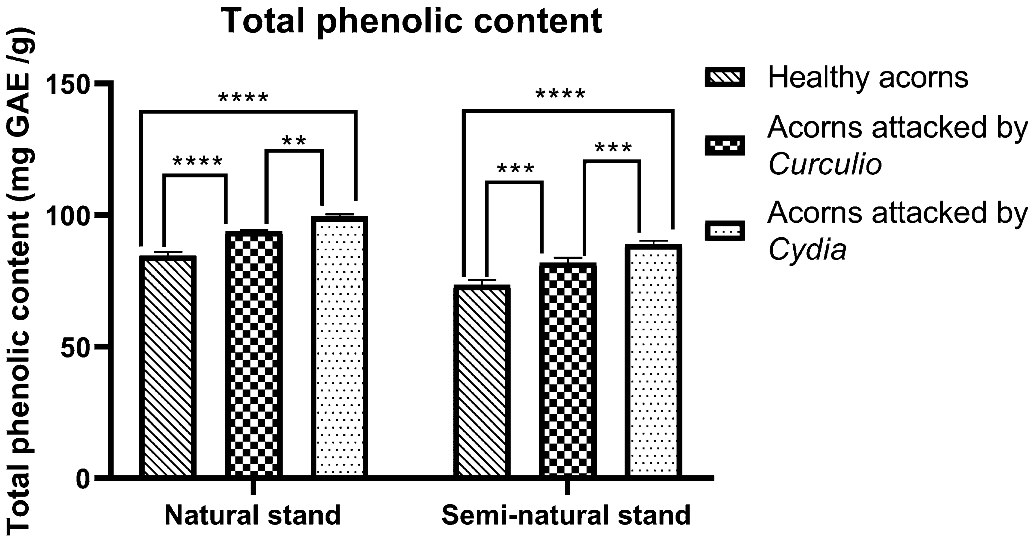

Figure 3. Variations (means ± SD) in the total phenolic content of healthy and attacked acorns in the two stands. Different asterisks (*) indicate significant differences at $p<0.05$ according to two-way ANOVA and Tukey's multiple comparison tests.

by *Curculio* sp. Figure 2 shows the numbers of the three species found in the two stands.

According to figure 2, *Curculio* sp. has the highest number with 45.45%, followed by *Cydia splendana* with 39.39%, and in the third position, *Cydia fagiglandana* with 15.15% in the natural stand. In the semi-natural stand, infestation by *Cydia splendana* and *Cydia fagiglandana* were higher at 40% and 32%, respectively, followed by *Curculio* sp. at 28%.

3.5 *Biochemical composition of different acorn types*

The phenolic compounds and antioxidant activity of cork oak acorns vary according to whether the acorn is healthy, attacked by *Cydia*, or attacked by *Curculio*, as well as whether it comes from a natural or semi-natural stand.

3.6 *Determination of total phenolic content (TPC)*

As depicted in figure 3, a highly significant difference in TPC exists between acorns harvested from the two stands (p<0.0001); this content was greater in acorns from the natural stand. Another highly significant difference (p<0.0001) was observed among the three types of acorns (healthy, *Curculio*-attacked, and *Cydia*-attacked). According to figure 3, the TPC of acorns with small holes was higher than that of acorns with large holes and healthy acorns, with respective values of 99.58±0.74 mg EAG/g of dry weight (DW), 93.96±0.39 mg GAE/g DW, and 84.55±1.53 mg GAE/g DW in the natural stand, and TPC of 88.86±1.37 mg GAE/g DW, 81.87±1.93 mg GAE/g DW, and 73.51±1.98 mg GAE/g DW in the semi-natural stand.

3.7 *Determination of total flavonoid content (TFC)*

The TFC of acorns is illustrated in figure 4. When the acorn is healthy, the TFC in the kernel is lower than in attacked acorns; acorns with large holes have the highest TFC, which was of the order of 0.214±0.005 mg QE/g of DW in the natural stand and 0.288±0.004 mg QE/g DW in the semi-natural stand, followed by acorns with small holes, with levels equal to 0.129±0.002 mg QE/g DW in the natural stand and 0.174±0.002 mg QE/g DW in the semi-natural stand. For healthy acorns, the TFC recorded in the natural and semi-natural stands was 0.097±0.002 mg QE/g DW and 0.130±0.003 mg QE/g DW, respectively, with this difference in content between the three acorn types being highly significant (p<0.0001). Statistical analysis also revealed that there was a highly significant difference between acorns from the two stands (p<0.0001) and that acorns from the semi-natural stand were richer in TFC than acorns harvested from the natural stand.

3.8 *Assessment of antioxidant activity*

Two methods were used in this study: DPPH and FRAP.

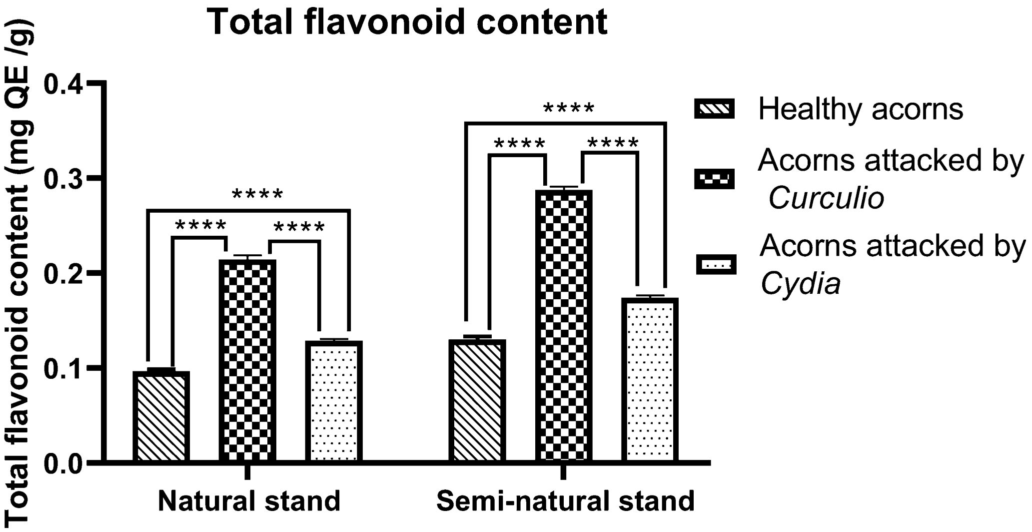

Figure 4. Variations (means ± SD) in the total flavonoid content of healthy and attacked acorns in the two stands. Different asterisks (*) indicate significant differences at p<0.05 according to two-way ANOVA and Tukey's multiple comparison tests.

Table 6. Antioxidant power expressed by the IC50 of acorn extracts (Means ± SDs)

IC50 value of DPPH (μg/mL)	Healthy acorns	Acorns attacked by *Cydia*	Acorns attacked by *Curculio*
Natural stand	150.92 ± 0.02	138.87± 0.94	111.33 ± 0.95
Semi-natural stand	111.77 ± 2.26	110.46 ± 0.27	99.78 ± 2.17

DPPH method: The DPPH radical scavenging assays for the various extracts studied are reported in terms of the quantity of extract required to reduce the initial DPPH concentration by 50%. For each extract, IC50 was determined, and the results are shown in table 6.

Analysis of antioxidant activity showed a highly significant variation ($p<0.0001$) between the IC50s of extracts from acorns harvested from the two cork oak forests and that extracts from acorns from semi-natural stands had higher antioxidant activity. Analysis of the condition of the healthy and attacked acorns revealed a highly significant difference between the IC50s of the extracts ($p<0.0001$), with the extract from acorns attacked by *Curculio* showing the highest antioxidant activity at 99.78±2.17 μg/mL DW in the semi-natural stand and 111.33±0.95 μg/mL DW in the natural stand (table 6), followed by acorns attacked by *Cydia* and lastly by the healthy acorns.

FRAP method: The reducing power of cork oak acorn extracts was measured by monitoring the direct reduction of ferric iron (Fe^{3+}) from the ferricyanide complex ($Fe^{3+}(CN)_6$) to ferrous iron (Fe^{2+}) at different concentrations (25–1000 μg/mL). The reducing powers obtained for the extracts are shown in figures 5 and 6, as the increase in absorbance measured at 700 nm. Figures 5 and 6 show that the reducing power of Fe^{3+} is proportional to concentration for both healthy and attacked acorns in the two stands.

In the semi-natural stand, extracts from healthy acorns, acorns attacked by *Cydia*, and acorns attacked by *Curculio*, at a concentration of 600 μg/mL, showed absorbances of 2.331±0.049, 2.585±0.010, and 3±0.000, respectively (figure 5).

For extracts from natural stands, healthy acorns, acorns attacked by *Cydia*, and acorns attacked by *Curculio* showed absorbances equal to 1.880±0.014, 2.568±0.019, and 3±0.000, respectively, at a concentration of 800 μg/mL. The results indicate that the reducing power of extracts from attacked acorns is higher than that of healthy acorns (figure 6).

Statistical analysis reveals that there is a highly significant difference between healthy acorns and acorns attacked by *Curculio* and *Cydia* in both stands ($p<0.0001$) and also a highly significant variation between the different concentrations ($p<0.0001$). Figure 7 shows that, comparing the reducing power of extracts from the two stands, the ability of extracts from semi-natural stands to reduce iron was more effective than that of extracts from natural stands.

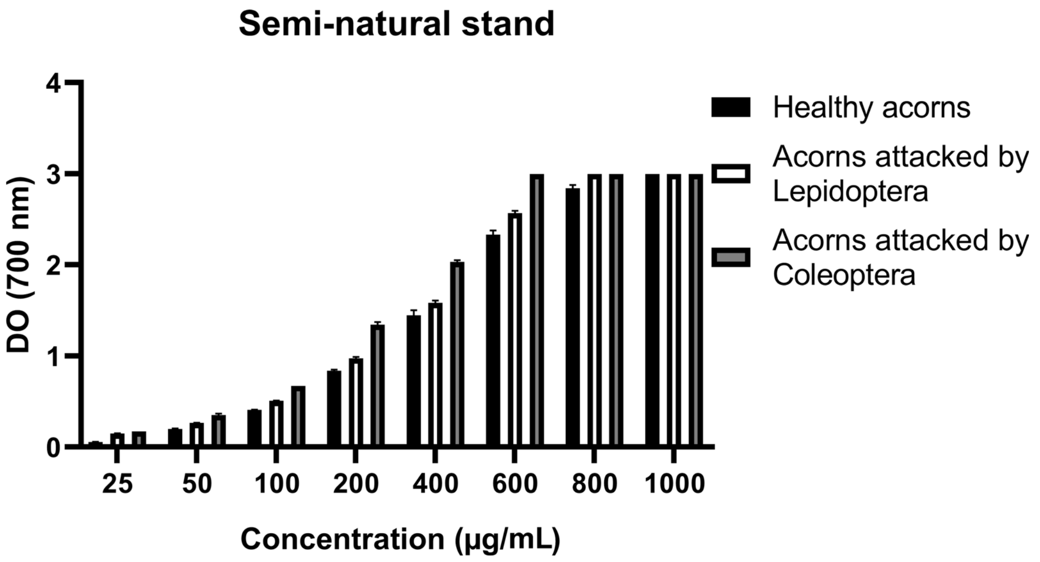

Figure 5. Variation (means ± SD) in reducing power absorbance at different concentrations of healthy and attacked acorns in the semi-natural stand.

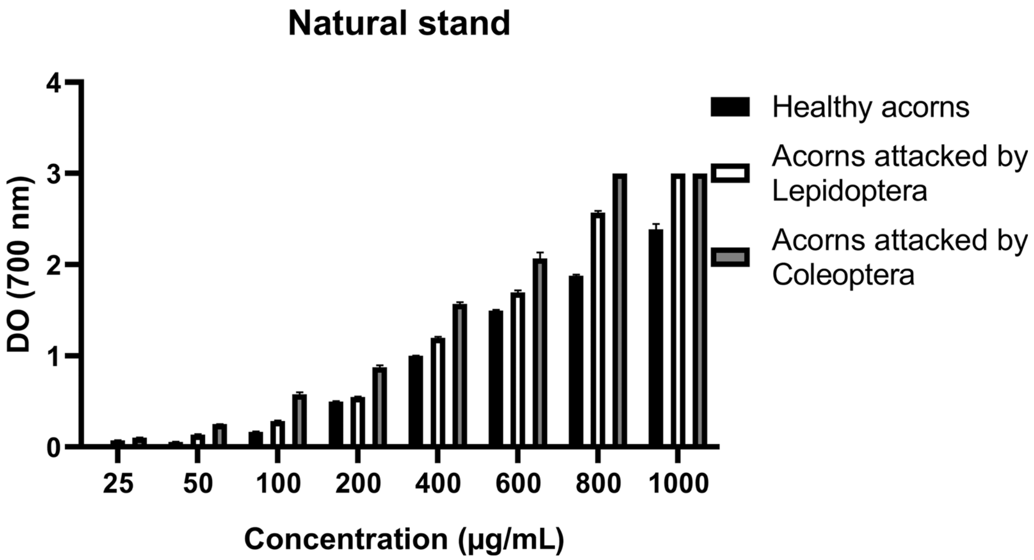

Figure 6. Variation (means ± SD) in reducing power absorbance at different concentrations of healthy and attacked acorns in the natural stand.

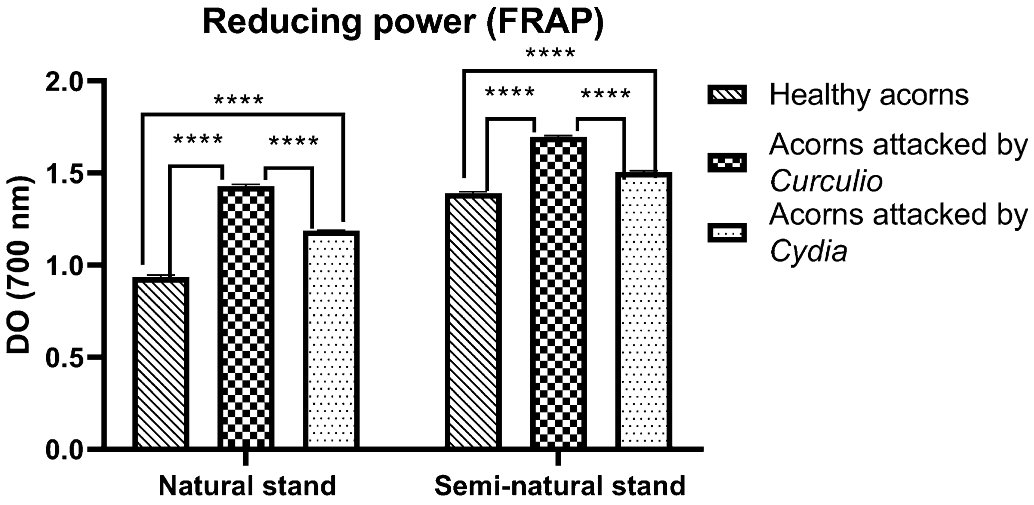

Figure 7. Variation (means ± SD) in reducing power of healthy and attacked acorns in the two stands. Different asterisks (*) indicate significant differences at $p<0.05$ according to two-way ANOVA and Tukey's multiple comparison tests.

4. Discussion

The *Quercus* genus is known for its high inter-specific and intra-specific variability in acorn size. Giertych and Chmielarz (2020) found very high variation in acorn size across 15 *Quercus* species. Others found a difference within the same species, Ramírez-Valiente *et al*. (2009) in *Q. suber* L. acorns, and Sage *et al.* (2011) in *Q. lobata* acorns. In our study, analysis of the biometric results revealed a greater weight of acorns harvested from the semi-natural stand, as did their length and width, with average values of 6.17 g for weight, 33.07 mm for length, and 16.27 mm for width. Biometric analysis of the acorns from the natural stand revealed lower weight and dimensions than those taken from the semi-natural stand (weight=4.97 g, length=31.84 mm, width=14.71 mm). Statistical tests revealed a highly significant difference in the measurements of cork oak acorns between the two stands, even though both cork oak stands were situated at the same site. This disparity can be attributed to the maintenance practices employed at the semi-natural stands. The semi-natural cork oak stand of Errich is located in a closed, protected area that benefits from regular irrigation by forestry authorities and various measures, including pruning and cork harvesting, aimed at enhancing the health and productivity of trees.

Larger cork oak acorns are better adapted to a hot, dry climate (Ramírez-Valiente *et al*. 2009). Acorn size and weight play an important role in increasing germination rates and accelerating seedling growth and survival (Merouani *et al*. 2001b; Gómez 2004; Pesendorfer 2014; Shi *et al*. 2019); the largest acorns produce larger seedlings with better growth (Tecklin and McCreary 1991; Sage *et al*. 2011). This can be used in cork oaks reforestation programs to produce plants with very high above-ground and below-ground biomass (Mechergui *et al*. 2021). It appears that the acorns collected in our study in central Algeria are relatively larger than those collected from eastern Algeria; the average biometry varied between 2.90 and 3.88 g for weight, 24. 7 to 29 mm for length, and 14 to 15.2 mm for width (Adjami *et al*. 2013); similarly, for western Algeria, the average biometry was 2.94 g for weight, 27.33 mm for length, and 14.9 mm for width (Bouchaour-Djabeur *et al*. 2011). Jdaidi *et al*. (2018), in a biometric study of acorns sampled in Tunisia, found mean measurements of 4.36 g for weight, 27.68 mm for length, and 14.83 mm for width. These results are less significant than those found in this study; unlike in Spain, where the average recorded weight of acorns is 7.74 g, the average length is 38.52 mm, and the average width is 17.30 mm (Ramírez-Valiente *et al*. 2009), we note that the weight and size of acorns differ from year to year and also from region to region.

On the other hand, large seeds are not necessarily the best because post-dispersal acorn predators, such as wild boar and wood mice, prefer larger acorns (Gómez 2004). Also, insects are more likely to attack trees that produce large acorns, with the possibility of several insect larvae developing in the same acorn (inter- and intra-specific insect competition) during low harvest years (Mezquida *et al*. 2021). However, a larger seed size can be beneficial, as more reserves increase the chances of embryo survival following larval damage (Bonal *et al*. 2007; Yi and Yang 2010). The selective role of predators in acorn size is still debatable.

Healthy acorns are heavier than attacked ones. Cotyledons, which hold reserves for seedling growth, are consumed by insect larvae, resulting in reduction in acorn weight and also seedling quality with slower growth rate (Branco *et al*. 2002; Bonal *et al*. 2007; Xiao *et al*. 2007; Yi and Yang 2010; Bartlow *et al*. 2018). It should also be noted that insect attacks prevent acorns from developing optimally, as they are prematurely abscised and fall earlier (Bonal and Munoz 2008; Canelo *et al*. 2021).

Cork oak acorns can be attacked mainly by *Cydia* spp. moths (Lepidoptera: Tortricidae) and *Curculio* spp. weevils (Coleoptera: Curculionidae) (Tiberi *et al*. 2016). In all the acorns studied, we were able to identify three species of insects living in the fruit kernels: *Curculio* spp., *Cydia splendana*, and *Cydia fagiglandana*. In our study, *Curculio* attacked around 45.45% of infested acorns in the natural stand and 28% in the semi-natural stand, while *Cydia* attacked 54.55% in the natural stand and 72% in the semi-natural stand. Our results differ from those found by Bouchaour-Djabeur *et al*. (2011) and Adjami *et al*. (2013), where *Curculio* were most represented in acorn infestations, since the estimated insect attack rate varies from year to year and from region to region (infestation rate is influenced by weather conditions). According to Canelo *et al*. (2021), weevil infestations can be minimized by rain delays in late summer, as soil hardness hinders adult emergence from underground cells. On the other hand, delayed rains may increase *Cydia* infestation on acorns; precipitation could prevent adult flight. According to the same authors, in the years when late summer storms started later, total insect infestation rates were lower; this may explain the low infestation rate found during our sampling. According to Canelo *et al*. (2021), infestation rates will be lower if the Mediterranean region experiences prolonged droughts.

Acorn fruits are packed with valuable nutrients such as carbohydrates, protein, lipids, minerals, and fiber. In addition to these essential nutrients, they also contain a variety of bioactive compounds, including tannins, gallic acids, and several flavonoids known for their powerful antioxidant activity, which can also play a role in reducing the risk of cardiovascular disease (CVD), cancer, human immunodeficiency virus (HIV), diabetes, and inflammatory diseases (Vinha *et al*. 2016). These characteristics make them a food of choice for human consumption as well as a valuable resource for livestock feed (Stiti *et al*. 2021). In addition to the benefits outlined above, metabolites are essential in the life cycle of plants. Primary metabolites, such as carbohydrates, proteins, and lipids, are essential for root and leaf growth and tissue construction and are involved in plant development (Schaaf *et al*. 1995). At the same time, secondary metabolites offer protection to plants against abiotic and biotic stresses by accumulating bioactive chemicals. Secondary metabolites serve as a deterrent to herbivores, a barrier against disease invasion, and a source of antioxidants (Jan *et al*. 2021).

The chemical composition of acorns can highlight variations between species, such as the difference in total phenolic and flavonoids content between *Q.*

mongolica and *Q. variabilis* from 44 populations across China (Sun *et al*. 2021), and even within the same species, as in 13 populations of *Q. ilex* from Spain (Valero Galván *et al*. 2012), where this variation may be due to pedoclimatic factors, such as climate and soils (Tejerina *et al*. 2011). According to Sun *et al*. (2021), with increasing latitude, acorn sizes and soluble sugars increase while total phenolic content decreases. In our study, we observed that acorns from semi-natural stands were larger and richer in TFC, while acorns from natural stands were smaller and richer in TPC. These variances were remarkable, even though both stands were located at the same latitude and under identical climatic conditions. These differences are probably due to acorn sizes and sampling sources: acorns harvested from natural and semi-natural stands. Human management practices in semi-natural stands, such as weed suppression and water resource management, could also impact the production of TPC and TFC. These management practices may influence tree health and metabolic response, potentially explaining the observed differences in acorn chemical composition between the two types of stands.

The results on biochemical compounds in *Q. suber* L. acorns show that there is a highly significant difference in TPC, TFC, and antioxidant activity due to infestation by *Cydia* and *Curculio*. The presence of these insects, especially *Cydia*, significantly increased the TPC of infested acorns compared with healthy ones. Many studies have reported higher TPC after insect infestations. A significant increase in the amounts of phenolic compounds was observed in *Helicoverpa armigera* and *Aphis craccivora* infestations of groundnut as compared with healthy plants. Groundnuts infected with *H. armigera* had much higher increases in TPC than those infected with *A. craccivora* (War *et al*. 2013). Similar increases in TPC were seen in rice genotypes as a response to infestation by the rice leaf folder *Cnaphalocrocis medinalis* (Punithavalli *et al*. 2013). TPC increased by 185.7% with cotton mealybug *Phenacoccus solenopsis* Tinsley infestation on cotton plants (Nagrare *et al*. 2017). Another study conducted by Golan *et al*. (2017) aimed to investigate the changes in biochemical plant responses to insect feeding. The study revealed that the two-spotted spider mite *(Tetranychus urticae* Koch) and the grape mealybug (*Pseudococcus maritimus* Ehrh.) caused significant increase in TPC in comparison with the controls of leaves of strawberry and orchid, respectively, at different periods of herbivory (from 1 h to 2 weeks).

The determination of flavonoid content is another extensively used analytical method in stressed plants (Kovalikova *et al*. 2019). In our study, TFC was elevated in acorns exposed to insect infestation, mainly by *Curculio.* According to Nouira (2012), TFC level increases in the leaves of *Quercus coccifera, Erica multiflora, Erica arbórea,* and *Pistacia lentiscus,* damaged by *Orgyia trigotephras* (a polyphagous caterpillar that attacks cork oak). According to the study by Kovalikova *et al*. (2019), it has been observed that infestations by *Phyllotreta nemorum* and *Pieris brassicae* induce a defence response in cabbage plants, characterized by increase in superoxide, phenylalanine, phenols, and flavonoids. The level of TFC was noticeably higher in cabbage plants that had been subjected to 50 individuals of *Phyllotreta nemorum*. Among the monitored molecules, quercetin levels were the highest, followed by luteolin and kaempferol. According to Nocchi *et al*. (2020), after 13 days, *Nymphoides humboldtiana* and its herbivore, *Biomphalaria glabrata,* responded by increasing antioxidant activity with a correlated increase in flavonoid levels.

Pests produce oxidative stress, which is characterized by increased ROS production. Antioxidant systems prevent chain oxidation by eliminating partially reduced oxygen species such as superoxide and hydrogen peroxide (Mitra *et al*. 2021). In this study, in the acorns infested by *Cydia* and *Curculio*, two methods were used to assess antioxidant activity: DPPH and FRAP. Acorns attacked by *Curculio* recorded higher antioxidant activity compared with healthy acorns, according to the two available assessment methods. Horsáková *et al*. (2013) state that the fruits of peach trees infected with the plum pox virus (PPV) recorded higher antioxidant activity when assessed with the DPPH and FRAP methods: the antioxidant activity had increased by 13.2% (DPPH) and 26.7% (FRAP) in comparison with non-infected trees. It was also found that antioxidant activity correlated positively with total phenolic content (which increased by 30.4% compared with the control). Another study carried out by Seljåsen *et al*. (2013) investigated the effect of carrot psyllid attack on the quality and antioxidant capacity of carrots. The results showed that increasing psyllid attack increased antioxidant capacity, but this increase may have little practical significance due to deterioration in size and shape of affected carrots, which will be discarded. Consequently, the potential benefits of the extra antioxidants in these carrots cannot be fully exploited by consumers.

In this study, higher antioxidant activity was detected in insect-infested acorns of *Q. suber* L., but its main functional components still require additional research in subsequent studies.

5. Conclusion

In conclusion, the results indicate that natural cork oak acorns are different from semi-natural cork oak acorns in their morphology and chemical composition. Acorns from semi-natural stands are characterized by their larger size and richness in TFC, while acorns from natural stands are smaller but rich in TPC, which is a requirement for screening high-quality *Q. suber* germplasm resources. Insect attack, mainly by *Cydia* and *Curculio*, have a negative impact on acorn weight but also lead to an increase in phenolic compounds and antioxidant activity in response to pest attacks. This increase differs according to insect genus, with *Cydia* favoring an increase in TPC and *Curculio* favoring an increase in TFC and antioxidant activity. This natural increase in antioxidants in acorns could be exploited by the food industry and for animal nutrition. In addition, the high levels of phenolic compounds observed in attacked acorns could be valorized for their potential biological activity, such as insecticidal, antifungal, and antibacterial properties, which could contribute to the biological protection of plants. A more comprehensive investigation of specific phenolic compound alterations is required to elucidate their significance in the defence response. Furthermore, a future perspective of this research involves the characterization, both qualitatively and quantitatively, of bioactive compounds using high-performance liquid chromatography (HPLC), which will provide deeper insights into the unique properties of these acorns and their potential applications.

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Conceptualization, investigation, methodology, and writing – original draft: SK. Conceptualization, resources, and supervision: DM. Methodology and visualization: SD. Methodology and investigation: NB. All the authors participated in the review and editing of the manuscript.

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References

Adjami Y, Daas H, Ghanem R, *et al.* 2013 Effets des attaques d'insectes sur les glands de chêne-liège: Impact sur le pouvoir germinatif. *Geo-Eco-Trop* **37** 201–210

Adjami Y, Ghanem R, Daas H, *et al.* 2016 Influence of carpophagous attack on metabolites of cork oak (*Quercus suber*) acorns. *Turk. J. For.* **17** 51–57

Alara OR, Abdurahman NH, Ukaegbu CI, *et al.* 2018 *Vernonia cinerea* leaves as the source of phenolic compounds, antioxidants, and anti-diabetic activity using microwave-assisted extraction technique. *Ind. Crop. Prod.* **122** 533–544

Bartlow AW, Agosta SJ, Curtis R, *et al.* 2018 Acorn size and tolerance to seed predators: the multiple roles of acorns as food for seed predators, fruit for dispersal and fuel for growth. *Integr. Zool.* **13** 251–266

Bonal R and Munoz A 2008 Seed growth suppression constrains the growth of seed parasites: premature acorn abscission reduces *Curculio elephas* larval size. *Ecol. Entomol.* **33** 31–36

Bonal R, Munoz A and Díaz M 2007 Satiation of predispersal seed predators: the importance of considering both plant and seed levels. *Evol. Ecol.* **21** 367–380

Bouchaour-Djabeur S, Benabdeli K, Bejamaa ML, *et al.* 2011 Déprédation des glands de chêne liège par les insectes et possibilités de germination et de croissance des semis. *Geo-Eco-Trop* **35** 69–80

Bouchaour-Djabeur S, Benabdeli K and Taib N 2021 Glands de chêne-liège de la subéraie Hafir-Zarieffet (Tlemcen, Algérie): caractéristiques, état sanitaire et infestation par les insectes. *Geo-Eco-Trop* **45** 599–615

Branco M, Branco C, Merouani H, *et al.* 2002 Germination success, survival and seedling vigour of *Quercus suber* acorns in relation to insect damage. *For. Ecol. Manage.* **166** 159–164

Brenes-Arguedas T and Coley PD 2005 Phenotypic variation and spatial structure of secondary chemistry in a natural population of a tropical tree species. *Oikos* **108** 410–420

Canelo T, Gaytán Á, Pérez-Izquierdo C, *et al.* 2021 Effects of longer droughts on holm oak *Quercus ilex* L. acorn pests: consequences for infestation rates, seed biomass and embryo survival. *Diversity* **13** 110

Cohen SD and Kennedy JA 2010 Plant metabolism and the environment: implications for managing phenolics. *Crit. Rev. Food Sci. Nutr.* **50** 620–643

Csóka G and Csókáné Hirka A 2006 Direct effects of carpophagous insects on the germination ability and early abscission of oak acorns. *Acta Silv. Lign. Hung.* **2** 57–67

Del Valle JC, Buide ML, Casimiro-Soriguer I, *et al*. 2015 On flavonoid accumulation in different plant parts: variation patterns among individuals and populations in the shore campion (*Silene littorea*). *Front. Plant Sci.* **6** 939

Djeridane A, Yousfi M, Nadjemi B, *et al*. 2006 Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* **97** 654–660

Ghasemzadeh A and Ghasemzadeh N 2011 Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *J. Med. Plants Res.* **5** 6697–6703

Giertych MJ and Chmielarz P 2020 Size variability in embryonic axes, cotyledons, acorns and seedlings in fifteen species of the genus *Quercus*. *Trees* **34** 593–601

Golan K, Sempruch C, Górska-Drabik E, *et al*. 2017 Accumulation of amino acids and phenolic compounds in biochemical plant responses to feeding of two different herbivorous arthropod pests. *Arthropod-Plant Interact.* **11** 675–682

Gómez JM 2004 Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. *Evolution* **58** 71–80

Granados-Sánchez D, Ruíz-Puga P and Barrera-Escorcia H 2008 Ecología de la herbivoría. *Rev. Chapingo Ser. cienc. For. y del Ambient.* **14** 51–63

Horsáková J, Sochor J and Krška B 2013 Assessment of antioxidant activity and total polyphenolic compounds of peach varieties infected with the Plum pox virus. *Acta Univ. Agric. Silvic. Mendel. Brun.* **187** 1693–1701

Igueld SB, Abidi H, Trabelsi-Ayadi M, *et al*. 2015 Study of physicochemicals characteristics and antioxidant capacity of cork oak acorns (*Quercus suber* L.) grown in three regions in Tunisia. *J. Appl. Pharm. Sci.* **5** 026–032

Ikonen A, Tahvanainen J and Roininen H 2001 Chlorogenic acid as an antiherbivore defence of willows against leaf beetles. *Entomol. Exp. Appl.* **99** 47–54

Jan R, Asaf S, Numan M, *et al*. 2021 Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. *Agronomy* **11** 968

Jdaidi N, Chaabane A, Toumi L, *et al*. 2018 Influence de l'état sanitaire des glands sur la régénération de *Quercus suber* en Tunisie. *Rev. Ecol.-Terre Vie*. **73** 71–79

Kovalikova Z, Kubes J, Skalicky M, *et al*. 2019 Changes in content of polyphenols and ascorbic acid in leaves of white cabbage after pest infestation. *Molecules* **24** 2622

Kumari A, Goyal M, Mittal A, *et al*. 2022 Defensive capabilities of contrasting sorghum genotypes against *Atherigona soccata* (Rondani) infestation. *Protoplasma* **259** 809–822

Maghnia FZ, Abbas Y, Mahé F, *et al*. 2019 The rhizosphere microbiome: A key component of sustainable cork oak forests in trouble. *For. Ecol. Manage.* **434** 29–39

Makhlouf FZ, Squeo G, Barkat M, *et al*. 2019 Comparative study of total phenolic content and antioxidant proprieties of Quercus fruit: flour and oil. *North Afr. J. Food Nutr. Res.* **3** 148–155

Mechergui T, Pardos M and Jacobs DF 2021 Effect of acorn size on survival and growth of Quercus suber L. seedlings under water stress. *Eur. J. for. Res.* **140** 175–186

Merouani H, Branco C, Almeida MH, *et al*. 2001a Comportement physiologique des glands de chêne liège (*Quercus suber* L.) durant leur conservation et variabilite inter-individus producteurs. *Ann. For. Sci.* **58** 143–153

Merouani H, Branco C, Almeida MH, *et al*. 2001b Effects of acorn storage duration and parental tree on emergence and physiological status of cork oak (*Quercus suber* L.) seedlings. *Ann. For. Sci.* **58** 543–554

Mezquida ET, Caputo P and Acebes P 2021 Acorn crop, seed size and chemical defenses determine the performance of specialized insect predators and reproductive output in a Mediterranean oak. *Insects* **12** 721

Mitra A, Kataki S, Singh AN, *et al*. 2021 Plant stress, acclimation, and adaptation: a review; in: *Plant growth and stress physiology* (Eds.) Gupta DK and Palma JM (Cham: Springer International Publishing) pp 1–22

Nagrare V, Sheeba JA, Bhoyar P, *et al*. 2017 Biochemical changes in cotton plants due to infestation by cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). *Appl. Nat. Sci.* **9** 382–388

Nocchi N, Duarte HM, Pereira RC, *et al*. 2020 Effects of UV-B radiation on secondary metabolite production, antioxidant activity, photosynthesis and herbivory interactions in *Nymphoides humboldtiana* (Menyanthaceae). *J. Photochem. Photobiol. B Biol.* **212** 112021

Nouira S 2012 Relation entre les chenilles d'Orgyia trigotephras (Lepidoptera, Lymantriidae), insecte polyphage ravageur du chêne-liège, et ses plantes hôtes en Tunisie. *IOBC/WPRS Bull.* **76** 271–278

Oyaizu M 1986 Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr. Diet.* **44** 307–315

Pesendorfer MB 2014 The effect of seed size variation in *Quercus pacifica* on seedling establishment and growth. *Gen. Tech. Rep. PSW-GTR-251* 407–412

Pio C, Silva P, Cerqueira M, *et al*. 2005 Diurnal and seasonal emissions of volatile organic compounds from cork oak (*Quercus suber*) trees. *Atmos. Environ.* **39** 1817–1827

Punithavalli M, Muthukrishnan N and Rajkuma MB 2013 Defensive responses of rice genotypes for resistance against rice leaffolder *Cnaphalocrocis medinalis*. *Rice Sci.* **20** 363–370

Ramírez-Valiente J, Valladares F, Gil L, *et al*. 2009 Population differences in juvenile survival under increasing drought are mediated by seed size in cork oak (*Quercus suber* L.). *For. Ecol. Manage.* **257** 1676–1683

Sage RD, Koenig WD and McLaughlin BC 2011 Fitness consequences of seed size in the valley oak *Quercus lobata* Née (Fagaceae). *Ann. For. Sci.* **68** 477–484

Schaaf J, Walter MH and Hess D 1995 Primary metabolism in plant defense (regulation of a bean malic enzyme gene promoter in transgenic tobacco by developmental and environmental cues). *Plant Physiol.* **108** 949–960

Scott ER, Li X, Wei J-P, *et al*. 2020 Changes in tea plant secondary metabolite profiles as a function of leafhopper density and damage. *Front. Plant Sci.* **11** 636

Seljåsen R, Vogt G, Olsen E, *et al*. 2013 Influence of field attack by carrot psyllid (*Trioza apicalis* Forster) on sensory quality, antioxidant capacity and content of terpenes, falcarindiol and 6-methoxymellein of carrots (*Daucus carota* L.). *J. Agric. Food Chem.* **61** 2831–2838

Shi W, Villar-Salvador P, Li G, *et al*. 2019 Acorn size is more important than nursery fertilization for outplanting performance of *Quercus variabilis* container seedlings. *Ann. For. Sci.* **76** 1–12

Singleton VL and Rossi JA 1965 Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **16** 144–158

Stiti B, Khalfaoui M, Bahri S, *et al*. 2021 Towards optimizing acorn use as animal feed in Tunisia: evaluation and impact on natural regeneration. *Bois Forets des Tropiques* **348** 17–26

Sun J, Shi W, Wu Y, *et al*. 2021 Variations in acorn traits in two oak species: *Quercus mongolica* Fisch. ex Ledeb. and *Quercus variabilis* Blume. *Forests* **12** 1755

Tecklin J and McCreary D 1991 Acorn size as a factor in early seedling growth of blue oaks. *USDA For. Serv. Gen. Tech. Rep. PSW.* **126** 48–53

Tejerina D, García-Torres S, de Vaca MC, *et al*. 2011 Acorns (Quercus rotundifolia Lam.) and grass as natural sources of antioxidants and fatty acids in the "montanera" feeding of Iberian pig: Intra-and inter-annual variations. *Food Chem.* **124** 997–1004

Corresponding editor: RADHIKA VENKATESAN

Tiberi R, Branco M, Bracalini M, *et al*. 2016 Cork oak pests: a review of insect damage and management. *Ann. For. Sci.* **73** 219–232

Valero Galván J, Jorrin Novo JJ, Cabrera AG, *et al*. 2012 Population variability based on the morphometry and chemical composition of the acorn in Holm oak (*Quercus ilex* subsp. *ballota* [Desf.] Samp.). *Eur. J. For. Res.* **131** 893–904

Vinha AF, Barreira JC, Costa AS, *et al*. 2016 A new age for Quercus spp. fruits: review on nutritional and phytochemical composition and related biological activities of acorns. *Compr. Rev. Food Sci. Food Saf.* **15** 947–981

War AR, Paulraj MG, Ignacimuthu S, *et al*. 2013 Defensive responses in groundnut against chewing and sap-sucking insects. *J. Plant Growth Regul.* **32** 259–272

Wu N, Fu K, Fu Y-J, *et al*. 2009 Antioxidant activities of extracts and main components of pigeonpea [*Cajanus cajan* (L.) Millsp.] leaves. *Molecules* **14** 1032–1043

Xiao Z, Harris MK and Zhang Z 2007 Acorn defenses to herbivory from insects: implications for the joint evolution of resistance, tolerance and escape. *For. Ecol. Manage.* **238** 302–308

Yi X and Yang Y 2010 Large acorns benefit seedling recruitment by satiating weevil larvae in *Quercus aliena*. *Plant Ecol.* **209** 291–300

Zarroug Y, Boulares M, Sfayhi D, *et al*. 2022 Structural and physicochemical properties of Tunisian *Quercus suber* L. starches for custard formulation: a comparative study. *Polymers* **14** 556