



# Article Potential Factors behind the Decline of *Pinus pinea* Nut Production in Mediterranean Pine Forests

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**Abstract**: Mediterranean stone pine nut is appreciated for its high economic and nutritional value. Starting in 2012, *Pinus pinea* nut production declined throughout the Mediterranean area. The dry cone syndrome associated with this decline and the introduction of *Leptoglossus occidentalis* occurred simultaneously. This study aims to evaluate potential reasons behind the decline in pine nut production in Lebanon, considering climatic factors and the invasion of *L. occidentalis*. Correlation analysis was used to examine a potential relationship between cone yield and the percentage of damaged seeds per cone. Climatic variables were also tested. Two time periods were considered for analysis: before and after 2012. Cone production and the percentage of damaged seeds were negatively correlated (r = -0.42). From 2012 to 2017, cone production declined by 50% and the percentage of damaged seeds increased on average from 3% in 2012 up to 60% in 2017. Correlations were detected between cone production and the temperature of the hottest three months of the year of harvesting, and between cone production and average temperatures during the year of cone initiation. A conjunction of factors that include *L. occidentalis* and climatic factors might have affected the pine nut production in Lebanon.

Keywords: Pinus pinea; pine nuts; Leptoglossus occidentalis; climate

# 1. Introduction

Distributed around the Mediterranean basin and coastal Portugal, stone pine (*Pinus pinea* L.) occupies a wide range of climatic and soil conditions. The tree is valued for its timber, resin and bark, and it protects soil against erosion in addition to its other environmental and aesthetic purposes [1]. However, since the Paleolithic era, its edible seeds have been the most economically important non-wood forest product (NWFP) obtained from Mediterranean forests [2,3]. Pine nuts are a high-value product in the region, with current retail prices exceeding 100 EUR/kg in Europe [4] and ranging between 35 and 55 EUR/kg (retail price) in Lebanon [5]. In some rain-fed areas (i.e., Mediterranean areas), harvesting pine nuts provides a much higher income in the short term than harvesting wood after long rotations [6,7]. However, Mediterranean pine forests are threatened by a drought-prone environment, difficult socioeconomic conditions and overexploitation, all of which affect *P. pinea* forest growth, health and pine nut production [8].

The cone development cycle of *P. pinea* requires at least three years, with the tree bearing its first-, second- and third-year cones at the same time [9]. Pollination of female



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). cones occurs on the first-year cones, whereas fecundation occurs on the third-year cones before maturation and harvesting [10]. In addition, the stone pine is known to be a masting species with different cone and flower formation mainly affected by weather conditions [2,11].

Located on the eastern edge of the Mediterranean Sea, Lebanon is a country where stone pine forests are economically important, contributing directly to the livelihood of at least 1000 rural families [12]. Stone pine forests extend over approximatively 12,740 ha and represent 9.3% of the total forest cover of the country [13–15]. Umbrella pine is a typical masting habit species, showing huge interannual cone production variability mainly ruled by climatic factors, especially rainfall events occurring at key moments in the cone development cycle [11]. Pine nut production is variable and ranges from <200 to >5000 kg/ha [16]. During the last decade, the main producing countries in the Mediterranean basin have reported a dramatic decline in cone production per hectare and a high percentage of empty kernels per cone [2,17]. From 2012, similar symptoms have been detected in Lebanon; dry cone syndrome (DCS), associated with empty pine seeds and dried conelets, has been found in many stone pine stands across the country [12]. These symptoms are reported to be caused either by the invasive conifer seed bug *Leptoglossus* occidentalis [18–23], or by other biotic and abiotic factors as a result of climate change and droughts, as reported by others [2,4,24–26]. Additionally, *Pinus pinea* is threatened by the fungus Diplodia pinea, and an association between L. occidentalis and this fungus was detected in Mediterranean forests [27].

Leptoglossus occidentalis Heidemann (Heteroptera Coreidae) is a polyphagous cone and seed predator, commonly known as the western conifer seed bug. It is native to the western part of North America, from British Columbia in the north to Veracruz in Mexico in the south; Montana, Wyoming and Colorado correspond to the eastern boundaries [28,29]. In North America, its feeding has been described as a likely cause of conelet abortion. It has also caused a significant reduction in seed production that is critical for pine reforestation [30–35]. Moreover, it has affected pine nut production in Mediterranean Europe by damaging pine seeds directly or indirectly when vectoring the fungus Diplodia sapinea [7]. The pest was first recorded in Europe in 1999 in Italy [36], where years later, serious losses in *P. pinea* nut production of up to 90% were reported [18,37]. In the Iberian Peninsula, the pest was reported in 2004 in Catalonia by Ribes and Oscala [38], afterwards throughout the Spanish Regions [19,39–41] and reached Portugal in 2010 [42,43]. The species has invaded almost the entire continent of Europe only in 10 to 15 years [44-46]; this fast invasion in Europe was enhanced by multiple introductions and human-eased transportation [47]. The insect then continued its expansion towards other Mediterranean and Asian countries, and was recorded in eastern Turkey [48], east Asia [49–51], and in North Africa in Tunisia [52] and Morocco [53]. In 2015, it was detected in Lebanon [12] and, more recently, in Crete and in the northern part of the Golan Heights [54,55]. The invasion continues and new records from different countries in Africa, South America and Europe have been recorded from 2019 until 2021 [56–63].

The main objective of this study is to quantify the recent decline in cone production and kernel nuts by investigating the relationship between cone production and prevailing climatic factors in Lebanon between 2009 and 2017. The study also aimed to compare pine cone production and the percentage of empty seeds in two periods, before and after 2012, considering a probable establishment of *L. occidentalis* in the country around that year.

#### 2. Materials and Methods

## 2.1. Investigated Areas and Data Collection

*Pinus pinea* forests in Lebanon cover mainly two regions, which are similar in terms of their environmental conditions, management traditions and practices. Data were collected yearly from 2008 to 2017 in 24 forest sites located in two different districts in Mount Lebanon: 16 sites in Baabda district and 8 sites in Jezzine district (Figure 1). Cone production was calculated per hectare. Data concerning cone production and empty seeds in the last 9 years



were provided by the "Lebanese Pine Farmers Association". The values are presented in the form of average  $\pm$ SD.

Figure 1. Map of the studied forest sites in Lebanon.

## 2.2. Post Harvesting Phase and Data Analysis

Cone harvest is performed yearly by specialized tree climbers between mid-November and the end of February. After harvest, the cones are gathered and stored either indoors until mid-May in aerated bags or on the ground over thick nylon layers in the forests; this provides both moisture and good aeration. Around mid-May, the cones are laid out and exposed to sunlight for 10 to 15 days until ripening. The cones are then ground in mills, and pine nuts are subsequently separated from scales and fragments using sieves and cyclones. Shelled pine nuts are then immersed in large water basins for two purposes: water-soaked shells are more elastic, and thus, less prone to kernel breakage, and empty nuts floating on the surface can be easily discarded. Empty nuts are considered a waste product and may serve as fuel for residential heating.

The cone production unit was standardized to Mg/ha/year and the percentage of empty seeds in the final kernel yield was calculated by volume. Climatic data were obtained from the Department of Meteorology—General Direction of Civil Aviation in Lebanon. Annual rainfall ranged from 850 to 1350 mm per year in Baabda and Jezzine, and the annual mean temperatures ranged between 17 and 20 °C. Each site received an average of 200 mm of rainfall during spring and summer seasons, with the exception of the year 2010, where rainfall during both seasons was 50 mm (Figure 2).

Statistical analyses and plots were performed using R 3.5.1 [64] software in RStudio 1.1.463 [65]. Line plots were generated using the package g.gplot2 [66].

Prior to performing the correlation analysis, the assumptions for parametric statistics were checked. Normality of data distribution was tested using the package fitdistrplus [67] in combination with a Shapiro–Wilk test and Kolmogorov–Smirnov test, and homoscedasticity was tested using Bartlett's test and Levene's test. Repeated measures two-way ANOVA on transformed data (log(Production) and arcsin(sqrt(Empty Seeds)) was performed, to study the differences in cone production and empty seeds between the districts and according to the time where a drop in cone yield and an increase in empty seeds was observed, i.e., 2012–2013. Observations on the same pine stands each year were considered as repeated measures. District (two levels: Baabda and Jezzine) and time period (two levels: 2008–2012 and 2013–2017) were used as fixed factors in ANOVA.



**Figure 2.** Averages of mean annual rainfall (mm), growing season and cone initiation (March–July), rainfall (mm) and temperature (°C) from 2006 to 2016. Reference: Department of Meteorology—General Direction of Civil Aviation in Lebanon.

The Pearson correlation between annual cone production and percentage of damaged/empty seeds was tested. Correlations were studied between climatic conditions and cone yield production and empty seed percentages. The climatic variables considered were as follows: the total annual rainfall, the average temperature of the three hottest months (June, July, and August), and the average temperature of the three coldest months (January, February, and March). The latter variables were measured in the year of maturation (t), one year before (t-1) and two years before (t-2). In addition, total seasonal rainfall and average temperatures measured during cone initiation (t-2) were reported from March to July.

#### 3. Results

#### 3.1. Pine Production 2008-2017

Between 2008 and 2012, cone production in the selected forests gave an average yield of  $3.1 \pm 3.1$  (Mg/ha) and a relatively low percentage of damaged/empty seeds ( $6.5 \pm 3.3$ ) (Figure 3). However, cone production dropped to  $1.5 \pm 1.7$  Mg/ha between 2012 and 2017, and the percent of damaged empty seeds averaged  $41.8\% \pm 20.8\%$  of the total annual yield (Figure 3). Our results show that time periods significantly affected both cone production (df = 1; F =8 6.1; *p* < 0.0001) and empty seeds (df = 1; F = 296.995; *p* < 0.0001). There was a significant negative correlation between production of pine cones and damaged/empty seed percentages (r = -0.42; *p* < 0.001). The percentage of damaged/empty seeds increased and cone production decreased in 2012/2013 in Baabda and in 2013/2014 in Jezzine (Figure 3). There was no significant difference between districts, neither for production (df = 1; F = 0.001; *p* = 0.654) nor for empty seeds (df = 1; F = 2.597; *p* = 0.1227).



**Figure 3.** Pine cone production (in blue) and percentage of damaged/empty seeds (in red) over the years in two districts of Lebanon: Baabda (N = 16) and Jezzine (N = 8). Lines represents the mean of replicates (Forest sites), and shades are the 95% confidence interval of the mean.

# 3.2. Climatic Variables Effect

A significant positive correlation was found between cone production and average temperatures during the year of cone initiation (March–July) (r = 0.54, p = 0.032). Empty seed percentages were correlated with temperature in the hottest three months of the year of harvesting (t) (p = 0.019). Empty seeds and temperature in the hottest months (t-2) were negatively but not significantly correlated (r = -0.45, p = 0.079). Rainfall in the year of harvesting (t) was positively but not significantly correlated with cone production (r = 0.47; p = 0.068) (Figure 4). Similarly, rainfall was negatively but not significantly correlated with the percentage of empty seeds (r = -0.47; p = 0.068) (Figure 5).



**Figure 4.** Summary of Pearson correlation between pine cone production and climatic variables in Lebanon. t—year of harvesting; t-1—one year before the year of harvesting; t-2—two years before the year of harvesting; CM—coldest months in the year of harvesting; CM-1—coldest months one year before the year of harvesting; CM-2—coldest months two years before the year of harvesting; HM—hottest months in the year of harvesting; HM-1—hottest months one year before the year of harvesting; HM-2—hottest months two years before the year of harvesting; HM-2—hottest months two years before the year of harvesting; HM-2—hottest months two years before the year of harvesting.



**Figure 5.** Summary of Pearson correlation between the percentage of empty seeds and climatic variables in Lebanon. t—year of harvesting; t-1—one year before the year of harvesting; t-2—two years before the year of harvesting; CM—coldest months in the year of harvesting; CM-1—coldest months one year before the year of harvesting; CM-2—coldest months two years before the year of harvesting; HM—hottest months in the year of harvesting; HM-1—hottest months one year before the year of harvesting; HM-2—hottest months two years before the year of harvesting; HM-2—hottest months two years before the year of harvesting; HM-2—hottest months two years before the year of harvesting.

Cone production and empty/damaged seeds were not significantly correlated with most climatic variables investigated during the three-year cycle of cone development (Figures 4 and 5).

# 4. Discussion

Following the decline in pine nut production in Lebanon, abiotic factors were investigated to determine the principal reasons for this decline. This study, therefore, analyzed the relationship between the decline in pine nut production, the increase in damaged/empty seeds, and climatic factors during the last decade.

The reported data show that this decade can be divided into two distinct phases: (1) normal cone production with negligible empty seed percentages before 2012, and (2) a sharp decline in cone production (Mg/ha/year) associated with a sudden increase in

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damaged/empty seeds after 2012. This striking collapse in pine nut yield can be attributed to several factors, including unsuccessful pollination or fertilization, and ovule abortion due to climatic factors.

Our data showed that cone yield production per hectare and percentage of damaged/empty seeds were significantly negatively correlated. There is a simple direct relationship between cone production (Mg/ha) and the percentage of empty seeds, as the latter affects the weight of the cone. Thus, an increase in the number of empty seeds would mean a decrease in cone weight. However, nut yield represents 3.5–5% of the total cone weight [68–70], and the observed decrease in Lebanon's pine cone production between the different periods of years (before and after 2012–2013) amounts to 50% (Figure 3), suggesting that this decrease is mainly due to the abortion of conelets rather than to empty seeds. Although the presented data confirm that the decline in nut production started in 2012, there is growing evidence that the causal agent of this decline could be attributed to a biotic causal agent and particularly L. occidentalis [71], an insect already detected in Lebanon. As this insect was reported in Turkey in 2009 [44], Nemer [12] (2015) suggested that it could have been introduced and established in Lebanon several years before its first detection. According to the scientific literature, damage caused by L. occidentalis on conifers include conelet abortion, total seed per cone reduction, and empty or partially filled seeds [22,25,29,30,35,45,72-74].

Further studies on pine species demonstrated that climatic conditions during seed cone initiation may strongly influence seed and cone production [2,75,76]. Although most correlations did not show a tangible significant effect of temperatures and rainfall on production, the influence of climate should not be neglected, since the introduction of infectious diseases and alien pests into new countries has often been associated with climate change, human ecology, and increased global trade [77]. Although not significant in our study, rainfall in the year of harvesting (t) may have had a positive effect on cone production (Figure 4) and the decrease in the number of empty seeds (Figure 5), suggesting that rainfall is probably among the factors that regulate cone production, in agreement with the work of Calama et al. [69] and Loewe-Munoz et al. (2016). In addition, a reduction in rainfall in Jezzine after 2013 may have affected the increase in empty seeds (Figure 3). In agreement with Loewe-Munoz et al. [78], average temperature during the period of cone initiation t-2 positively affects cone production (Figure 4). However, Redmond et al. [76] stated that increases in temperature lead to a decrease in cone and seed production. Moreover, the influence of climate on the phenology of stone pine in Lebanon should not be automatically excluded. For example, there is evidence that a temperature increase in the last month of winter affected the phenology of trees in Germany [79]. Furthermore, Loewe-Muñoz et al. [78] showed a significant effect of climate on pine growth in Chile, although these effects were not reflected in cone productivity. Therefore, although the climate variables' effect may not be tangible, their effect should be taken into consideration, since they may influence other physiological processes.

Several studies have attempted to determine the cause of such a decline in pine nut production. Most of them attributed the cause to an infestation of *L. occidentalis*, which may affect the cones and conelets as well [25,31,33].

A one-year delay in the decline in pine nut production was observed between the two forest sites, suggesting that the infestation may have extended to the south of Lebanon. The sudden increase in empty seeds in Jezzine in south Lebanon was observed in 2013, whereas the same rapid increase was observed in Baabda forests in 2012. This could indicate that a progressive infestation may have played a decisive role in the decline in cone production. Moreover, the decrease in rainfall observed in 2013 in Jezzine could have had an impact on production (Figure 2).

Since Lebanese pine stands are characterized by tall tree canopies, harvesting may be complex and often requires skilled tree climbers. This means that labor is often expensive, and this is a further reason for the high price of the final product. The recent scarcity of pine nut kernels has, therefore, generated additional labor costs to maintain the same levels of profitability. This problem places the pine nut value chain in a challenging situation.

The decline in pine seed production in Lebanon is a major concern involving a wide variety of socioeconomic and environmental aspects. Not only could the fragile rural economic sector be affected, but the decline may negatively affect the vegetative regeneration ability of *Pinus pinea*.

In light of our results, losses in pine nut yield may not be correlated to climatic factors alone; the presence of *L. occidentalis* may also play a role in threatening pine nut production in Lebanon. For this reason, there is a need for further investigations, and it is particularly important to examine the phenology of *P. pinea* in Lebanon.

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