

Article

Water Supply on Grafted Stone Pine: Effects on Growth and Mating

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Abstract

The pine nut, the seed of the stone pine, is highly valued in local cuisine and dietetics for its nutritional qualities. These nuts still come from forest stands, which results in their limited and irregular presence on the market. Domesticating the species through orchard management practices could increase its production. In this context, two irrigation schedules were tested in a grafted stone pine orchard that was planted in 2009 and has been under drip irrigation since 2011. Water needs were calculated weekly using the water balance method (ETc-P). The treatments considered were as follows: T₁, irrigation from April to the end of summer; T₂, irrigation from April to the end of June; and T₀, rainfed as the control. Monitoring of the pines, considering vegetative and reproductive responses, was carried out from 2015 (7th leaf) to 2023. T₁ and T₂ increased primary and secondary growth and resulted in higher strobili production compared to T₀. The tree's ability to sustain the reproductive load was enhanced under irrigation versus rainfed treatment. The longest irrigation schedule increased productivity. However, when the environmental cost of supplying twice as much water in the Mediterranean region—an annual average of 900 m³ ha^{−1} (T₂) versus 1900 m³ ha^{−1} (T₁)—is considered, the overall assessment changes. The irregularity of stone pine mating was not prevented by a regular water supply, but it was mitigated, promoting sustainable production.

Keywords: *Pinus pinea*; *Pinus halepensis*; sustainable irrigation; stand management; rootstocks; pine nut production



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1. Introduction

The stone pine (*Pinus pinea* L.) is a genuinely Mediterranean species that has been widespread across the Iberian Peninsula since 10 kyr BD [1] and currently covers more than 600,000 hectares, which represents 80% of the world's *Pinus pinea* growing area [2]. Pine nuts are one of the most appreciated nuts due to their nutritive features, and the most expensive nut crop [3–6]. Currently, its production derives from naturalized stands in monospecific or mixed forests in the Mediterranean countries of France, Italy, Lebanon, Portugal, Spain, Tunisia and Turkey but also in other parts of the world such as Chile, New Zealand and Argentina where the species has been more recently introduced [2,7–14]. However,

traditional adult stands tend to exhibit low productivity with strong interannual variations and many difficulties in harvest management [13,15–18]. In addition, the production of *P. pinea* pine nuts is limited compared to the world average production of edible pine nuts and represents only 1.5% of the 41,000 t of kernels sold in 2022 [5]. For this reason, there is an urgent need to help this species to avoid its replacement in the market by other pine nuts with organoleptic and nutritional characteristics that are inferior to *P. pinea* pine nuts [2,3,19].

Plant biodiversity in the Mediterranean is at risk. This temperate region of the world has been affected by historical and recurrent human activities. In recent times it has suffered severe droughts, resulting in an extremely fragile environment, with a great number of woody species affected by environmental stress in their natural forest stands [20]. Although stone pine is a characteristic species of the Mediterranean drylands [9,10,12,21], its growth is being adversely affected by the current climatic scenario, with water scarcity posing a significant threat, particularly in the marginal soils where *P. pinea* is usually confined [11,13,22]. For this reason, it does not seem feasible to expect a natural recovery of pinecone production in the forest stands. Although several studies have attempted to understand the factors that determine its erratic productive response [7,16,23], changing the current reproductive dynamics of *P. pinea* through forest management is unlikely to provide short-term results. Therefore, to keep this nut on the market, the species should be managed similarly to other fruit and nut trees and planted in well-managed orchards that aim to increase production in terms of precocity, regularity and quantity [8,24,25].

Numerous initiatives have aimed to domesticate the species through agricultural management strategies designed to establish new plantings. In this context, orchards should guarantee productive regularity and facilitate harvesting [13,24–26]. The first major steps already taken have been (i) to select highly productive clones [27–29] and (ii) to obtain grafted plants that reduce the non-productive period of the species by more than 10 years [24,30]. The species tends to grow well on sandy soils with low water retention capacity, and indeed these pines have been widely used for dune stabilization throughout the Mediterranean basin. Many of the current naturalized stands originate from this practice [10,13,22]. Extending the cultivation area in the Mediterranean region involves planting in soils that are typically calcareous and poorly drained, leading to the use of rootstocks other than *P. pinea*. In this context, the next challenge is to select the right rootstocks with consideration of the soil conditions in which the orchard will grow. At the present time, research is focused on evaluating the adaptive improvements of the use of *P. halepensis* as a rootstock for *P. pinea* [6].

In various countries during the last 10 years, clear evidence from the reproductive point of view has been observed on *P. pinea* response to basic management stimuli such as pest and disease control, mineral inputs, water supply or clean-up pruning [31–38].

However, until now, it has not been possible to pinpoint explanations for the irregular production of this species [7,16]. The reproductive biology of *P. pinea* is closely linked to climatic conditions, particularly to spring water supply [8,39,40]. In this species, successful mating depends on a complex and prolonged reproductive cycle, which lasts over 42 months from floral differentiation to harvest [41,42]. The coexistence of three developmental stages of female organs in the same tree (Figure S1), while simultaneously maintaining growth and floral differentiation, represents a significant challenge when managing these new plantations [6,24,40]. Knowledge of the reproductive physiology of the species and the interrelationship between the different organs is essential when making management decisions [6,43].

The effect of water supply on production has been the subject of numerous and extensive studies on many species of nuts and in numerous countries where quantities of

irrigation and optimization of applications have been investigated in consideration of the scarcity of this natural resource [44–46]. However, stone pine is not a typical horticultural species; it is a pine tree and a species normally found in unirrigated forests. Further, because stone pine is also usually planted on limiting/marginal agricultural soils [32,34,35], the combination of these constraints makes it particularly challenging to study of the effect of water supply on production. Improving water status is usually beneficial for any species, and with this in mind, determining the benefits this could have for *P. pinea* has already been the subject of some recent studies, particularly in Chile [25,32,33] and Portugal [34]. These studies support the idea that irrigation and fertirrigation in *P. pinea* promote growth, influence the characteristics of annual production and help achieve a certain degree of sustainability in production. However, in a species with a reproductive cycle of almost four years, long data series are needed to properly assess the response of the trees. It is also necessary to know the minimum water supply required and the best time to provide it in order to sustain orchard production when water scarcity is a reality.

This work was focused on analyzing the growth and reproductive response of 14-year-old pine trees grafted onto Aleppo pines, under a management regime aimed at pine nut production. The main objective was to assess the productive and vegetative response of these pine trees to different water supply schedules and to determine whether water supply could play a significant role in the regulation of the mating in this species.

2. Materials and Methods

2.1. Planting Site and Plant Material

The plantation was established in Caldes de Montbui (30 km from Barcelona) in Torre Marimon, an IRTA experimental farm. The general characteristics of the planting site are summarized in Table 1. The soil characterization values correspond to the results of the analysis carried out by a specialized company. Fertility was defined as low, but EC was not a limiting factor. Content of nitric N and P (Olsen) was analyzed using spectrophotometry UV-VIS, (N: 3.5 mg·kg^{−1} and P: 16.2 mg·kg^{−1}), presence of K, Ca and Mg (102 mg·kg^{−1}, 2777 mg·kg^{−1}; and 120 mg·kg^{−1}, respectively) and Na (not detected) was analyzed on ammoniac acetate substrate using spectrometry ICP-OES. Water retention capacity was very low, with soil texture being sandy loam; following USDA classification, the soil contained up to 84% sand down to a depth of 60 cm depth.

Table 1. Planting site characteristics.

Localization		Annual Climate Data (Average 2009–2023)			Soil Data		
UTM	msl	Tm °C	P mm	Description	pH	% OM	Texture
31N: 431,204; 4,607,394	163	14	664	Mediterranean climate. Field at 20 km from the sea	8.3	1.3	Sandy loam

In 2008, the scattered existing trees in the field were uprooted, and then the plot was stumped and subsoiled. In 2009, grafted pines were planted in a soil previously prepared, tilled and milled, covering a trial area of about 3500 m².

The rootstocks used in the experiment were *Pinus halepensis* seedlings, which were grown over three years in 3 L pots and then grafted at the national nursery of Forestal Catalana SA. Scions for grafting were collected from several stone pine trees identified for their consistent cone production by local farmers in the ‘Catalunya Litoral’ Spanish region of provenance [47]. The grafted plants, scions aged less than one year and grafted onto four-year-old rootstock, were planted in the trial plot in spring. After planting, trees

were manually watered twice, with additional hydric support applied four times during spring–summer for the first and the second vegetative periods, at a rate of 50 L per tree.

2.2. Plot Management and Trial Design

The pines were planted in eight rows of 12 trees at a spacing of 6×6 m. The drip irrigation system was installed at the beginning of the third growing season, 2011, to ensure a uniform water supply within plants. Each tree was equipped with two drippers located at the planting line, approximately 75 cm from the trunk on both sides, providing at a rate of two liters per hour. The date and duration of the irrigation were scheduled by ensuring that the water penetrated over 60 cm into the soil profile.

The amount of water (AW) to be applied weekly by irrigation was calculated using the standard water balance method, following the equation $AW = (E_{to} \times K_c) - P$, being P effective above 10 mm and K_c the crop coefficient. E_{to} and rainfall data were obtained directly from the Torre Marimon weather station. The K_c values were derived from FAO data on Mediterranean pine and holm oak forests, then adjusted based on the IRTA team's experience on managing these species (0.85, 0.90, 0.92, 0.83, 0.62 and 0.6, from April to September, respectively). The irrigation efficiency coefficient of the drip irrigation considered was $E_f = 0.9$. The canopy width in square meters was measured monthly to apply irrigation on the basis of the shaded area under the tree.

The soil was kept clean by mechanical weeding twice a year, and clean-up pruning was performed on the pines in 2019 and 2022 to remove all dried branches and improve light penetration into the canopy. From 2018 onwards, *Leptoglossus occidentalis* was chemically controlled with two treatments a year, using a product registered for *P. pinea* in Spain (deltamethrin 2.5% [EC] P/V). The first treatment was applied at the emergence of the winter generation, and the second occurred at the peak of the first generation of the year [38].

The trial design consisted of six rows for the water-supplied treatments, and two rows for the reference control. Three replicates were marked transversally to the irrigation treatments (Figure 1). Two water regimes were implemented: a long period of watering, from April until the end of summer (T_1), and a shorter period, stopping the irrigation at the end of June (T_2). Watering began in April, with the same amount applied weekly and at the same frequency in T_1 as in T_2 , although the corresponding water balance varied the amount supplied and, with it, the duration of irrigation. On the 1st of July, only T_1 continued to be watered until the end of September. The second treatment was chosen for two reasons: (a) water is usually available in the Mediterranean basin until late spring, and (b) by that time, the third-year cones have already reached their maximum growth. The control treatment did not receive water (T_0) as *P. pinea* and its rootstock are usually rainfed species. The T_0 lines were complete for this treatment (currently, 23 trees) while the T_1 and T_2 treatments were alternated every four trees within the same row. Two parallel hoses were installed to provide the appropriate differential schedule of irrigation in the central six rows of the plot (Figure 1). The annual extra water received in the trial plot by irrigation and the rainfall amount in Caldes de Montbui from 2015 to 2023 are summarized in Table 2.

Table 2. Amount of water received by each treatment.

Year	Rainfall (mm)			Irrigation Support (mm)	
	Annual	January to April	April to September (T_0)	T_1	T_2
2015 *	387	82	190	72	24
2016	488	91	233	72	45

Table 2. Cont.

Year	Rainfall (mm)			Irrigation Support (mm)	
	Annual	January to April	April to September (T_0)	T_1	T_2
2017	478	193	189	100	45
2018	754	239	259	131	52
2019	506	13	284	140	52
2020	810	391	391	180	67
2021	336	187	188	236	125
2022	392	246	246	253	131
2023	280	230	230	218	126
Mean	492	186	245	156	74

* Year monitoring started.

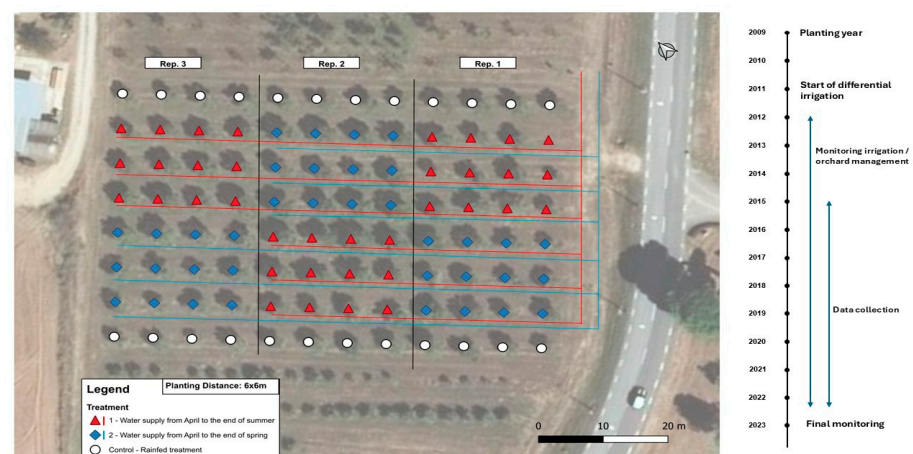


Figure 1. Trial design for the different irrigation schedules applied to the stone pine trees planted in Torre Marimon, Caldes de Montbui. Planted 2009. Drip irrigation system installed in 2011.

2.3. Recorded Data and Sample Handling

The monitoring of the trial was conducted from 2015 to 2023. The vegetative growth, trunk diameter (below grafting on the rootstock) and total tree height were measured annually using a caliper and a telescopic sight. At harvest, all the ripe cones were counted, and the fresh weight production per tree (FWP) measured. The cones were placed in mesh bags and hung in a ventilated environment of a warehouse for further processing. The production of each tree was oven-dried ($<45\text{ }^{\circ}\text{C}$) until the cones opened and pine nuts could be extracted and weighed (WPN). From 2018 onwards, only a sub-sample of six cones, representative of the tree cone size production, was evaluated. The empty pine nuts were discarded using a flotation test, and the full nuts were dried at room temperature and weighed again (WFPN). Monitoring of production started in 2015, but analysis at the pine nut-level was not performed until 2016. In 2017, the harvest data could not be analyzed due to theft of ripe cones from the field.

The number of female strobili (P_1), 2nd-year cones (P_2) and 3rd-year cones (P_3) was recorded annually from 2011 (Figure S1). The number of P_1 was counted visually at the end of spring. However, from 2018 onwards, P_1 was counted using RGB images taken by unmanned aerial vehicles (UAVs) (Phantom 4.0) flying at an altitude of approximately 15 m. The camera captured the aerial images at a resolution of 20 megapixels. The images

were then processed using ImageJ v152k. Prior to using this system, tests were carried out on different plots of *Pinus pinea*, confirming that the correlation between visual and image counting was high and significant ($R^2 = 0.72$). P_2 and P_3 were recorded visually from the ground until the trees reached 10 years, and later an elevated platform was used for proper observation. P_3 , ripe cones, were evaluated again at harvest. Data were analyzed from 2015, when trees initiated their productive phase (defined as when at least five strobili per tree could be counted), through to 2023.

2.4. Statistical Analysis

Growth and productive parameters were analyzed using the mixed model approach, accounting for repeated measures because the same trees were monitored annually. The fixed factors considered were the water supply treatment and the year. All sources of variation were considered in the REML model. In the case of any significant interaction, the analysis was carried out separately by year. The statistical differences were made using the post hoc Tukey's HSD test. Multivariate analysis and correlations were used to establish potential links among variables. The different analyses were performed using SAS v.9.4 software.

3. Results

3.1. Amount of Water

During the last six years of the study (from 2018 until 2023), the trees reached their “adult” phase as they produced 1500 kg ha^{-1} on average per year. The highest water consumption was in 2022, with $1300 \text{ m}^3 \text{ ha}^{-1}$ and $2500 \text{ m}^3 \text{ ha}^{-1}$ received from irrigation in T_2 and T_1 , respectively (Table 2). In other words, the water consumption in T_2 was almost half the water consumption in T_1 . Considering the annual water input during these six years, the results showed a similar trend, with the average amount of consumption being $922 \text{ m}^3 \text{ ha}^{-1}$ in T_2 and $1930 \text{ m}^3 \text{ ha}^{-1}$ in T_1 .

3.2. Growth Versus Amount of Water

The basal growth section (B_s) evolution showed a strong interaction of Year $\times$ Treatment and was clearly quantitative for both factors. Pines grew regularly every year and, as expected, increased water supply led to greater B_s ($T_0 < T_2 < T_1$). However, considering the total height of the trees (H_T), the interaction of Year $\times$ Treatment was not significant, while the effect of water supply was significant because T_0 always had smaller trees in all years. T_1 trees had higher growth in all years, but it was not until 2021, six years after the start of irrigation treatments, that this difference relative to T_2 became significant (Tables 3 and 4).

Table 3. ANOVA results. (a) Mixed model analysis. (b) Analysis of Treatment (T_1 , T_2 and T_3) per year.

(a)							
	df	¹ B_s	¹ H_T	¹ FWP	² WRC	² WPN	³ WFPN
Treatment	2	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p = 0.0023$	$p < 0.0001$	$p < 0.0001$
Year	8	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Treatment $\times$ Year	16	$p < 0.0001$	$p = 0.594$	$p < 0.0001$	$p < 0.0001$	$p = 0.0003$	$p = 0.0004$
(b)							
Year		¹ B_s	¹ H_T	¹ FWP	² WRC	² WPN	^{2,3} WFPN
2015		$p < 0.001$	$p < 0.001$	ns	ns	ND	ND
2016		$p < 0.001$	$p < 0.001$	ns	ns	ns	ns
2017		$p < 0.001$	$p < 0.001$	ND	ND	ND	ND
2018		$p < 0.001$	$p < 0.001$	ns	ns	ns	ns

Table 3. *Cont.*

2019	$p < 0.001$	$p < 0.001$	ns	ns	ns	ns
2020	$p < 0.001$	$p < 0.001$	$p < 0.001$	ns	ns	ns
2021	$p < 0.001$	$p < 0.001$	ns	ns	ns	ns
2022	$p < 0.001$	$p < 0.001$	ns	ns	$p < 0.001$	ns
2023	$p < 0.001$	$p < 0.001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.001$

ns: not significant $p > 0.05$; ND: no data; ¹ data per tree; ² data from all cones; ³ data of six representative cones from 2019 ahead. Bs: trunk section; H_T: tree height; FWP: weight of fresh production; WRC: weight per ripe cone; WPN: weight of inshell pinenuts per cone; WFPN: weight of filled pinenuts per cone.

Table 4. Mean ($\pm$ SE) of trunk section (Bs), tree height (H_T), weight of fresh production (FWP), weight per ripe cone (WRC = FWP/number of cones), weight of inshell pine nuts per cone (WPN) and weight of filled pine nuts per cone (WFPN), per tree by watering treatment. Trial plot sited in Torre Marimon (Caldes de Montbui-Spain).

Year	Water Supply	N	Bs (cm ²)	H _T (cm)	FWP ¹ (g)	WRC ¹ (g)	WPN ² (g)	WFPN ³ (g)
2015	T ₀	24	181 $\pm$ 9 b	350 $\pm$ 9 b	747.5 $\pm$ 230.6	331 $\pm$ 19	-	-
	T ₁	35	241 $\pm$ 8 a	388 $\pm$ 8 a	1069.1 $\pm$ 260.0	351 $\pm$ 17	-	-
	T ₂	36	233 $\pm$ 8 ab	374 $\pm$ 7 a	1640 $\pm$ 373.7	362 $\pm$ 13	-	-
2016	T ₀	24	235 $\pm$ 13 c	382 $\pm$ 11 b	1432.7 $\pm$ 380.9	462 $\pm$ 30	77.98 $\pm$ 6.14	62.09 $\pm$ 7.27
	T ₁	35	340 $\pm$ 12 a	433 $\pm$ 8 a	2287.7 $\pm$ 438.2	472 $\pm$ 22	82.22 $\pm$ 4.25	66.51 $\pm$ 5.04
	T ₂	36	322 $\pm$ 11 b	421 $\pm$ 9 a	1641.7 $\pm$ 401.4	466 $\pm$ 18	78.23 $\pm$ 4.64	65.01 $\pm$ 5.49
2017	T ₀	24	293 $\pm$ 15 c	409 $\pm$ 9 b	-	-	-	-
	T ₁	35	428 $\pm$ 15 a	454 $\pm$ 8 a	-	-	-	-
	T ₂	36	406 $\pm$ 14 b	446 $\pm$ 9 a	-	-	-	-
2018	T ₀	24	358 $\pm$ 20 c	437 $\pm$ 10 b	4638.0 $\pm$ 1058.8	532 $\pm$ 9	98.76 $\pm$ 3.50	87.03 $\pm$ 4.43
	T ₁	35	526 $\pm$ 17 a	480 $\pm$ 10 a	5261.3 $\pm$ 724.4	502 $\pm$ 11	90.40 $\pm$ 2.83	75.35 $\pm$ 3.59
	T ₂	36	481 $\pm$ 15 b	473 $\pm$ 10 a	5354.5 $\pm$ 682.1	522 $\pm$ 11	96.70 $\pm$ 2.79	78.08 $\pm$ 3.53
2019	T ₀	24	403 $\pm$ 21 c	477 $\pm$ 10 b	13,314.2 $\pm$ 1677.2	458 $\pm$ 12	92.97 $\pm$ 3.42	77.86 $\pm$ 3.92
	T ₁	35	602 $\pm$ 22 a	536 $\pm$ 10 a	15,493.7 $\pm$ 1586.7	475 $\pm$ 17	94.67 $\pm$ 2.85	72.94 $\pm$ 3.27
	T ₂	36	556 $\pm$ 18 b	517 $\pm$ 10 a	16,933.9 $\pm$ 1298.2	461 $\pm$ 9	95.26 $\pm$ 2.77	75.47 $\pm$ 3.17
2020	T ₀	24	497 $\pm$ 25 c	503 $\pm$ 11 b	16,952.5 $\pm$ 1855.4 b	526 $\pm$ 24	108.63 $\pm$ 3.05	93.78 $\pm$ 3.22
	T ₁	35	732 $\pm$ 23 a	570 $\pm$ 10 a	28,085.9 $\pm$ 2721.0 a	447 $\pm$ 14	95.54 $\pm$ 3.11	80.40 $\pm$ 3.36
	T ₂	36	662 $\pm$ 20 b	544 $\pm$ 11 a	25,373.6 $\pm$ 1892.0 a	490 $\pm$ 14	105.75 $\pm$ 2.82	87.70 $\pm$ 2.98
2021	T ₀	24	543 $\pm$ 26 c	535 $\pm$ 11 c	7108.8 $\pm$ 1074.8	349 $\pm$ 20	58.20 $\pm$ 3.00	41.08 $\pm$ 2.94
	T ₁	35	807 $\pm$ 24 a	617 $\pm$ 10 a	8367.9 $\pm$ 1119.1	336 $\pm$ 10	50.55 $\pm$ 2.52	34.05 $\pm$ 2.74
	T ₂	36	724 $\pm$ 22 b	577 $\pm$ 12 b	7386.9 $\pm$ 889.8	374 $\pm$ 10	59.44 $\pm$ 2.52	44.20 $\pm$ 2.47
2022	T ₀	23	575 $\pm$ 35 c	565 $\pm$ 10 c	4090 $\pm$ 659	372 $\pm$ 10	58.47 $\pm$ 2.96 a	48.06 $\pm$ 2.95
	T ₁	34	855 $\pm$ 25 a	649 $\pm$ 9 a	5220 $\pm$ 842	337 $\pm$ 10	46.42 $\pm$ 2.38 b	37.50 $\pm$ 2.37
	T ₂	36	759 $\pm$ 22 b	617 $\pm$ 11 b	4877.2 $\pm$ 649.6	387 $\pm$ 10	57.92 $\pm$ 2.37 a	46.66 $\pm$ 2.34
2023	T ₀	23	675 $\pm$ 42 c	584 $\pm$ 19 c	6077 $\pm$ 862 b	289 $\pm$ 10 b	56.90 $\pm$ 2.67 b	41.90 $\pm$ 3.25 b
	T ₁	34	910 $\pm$ 33 a	679 $\pm$ 14 a	14,187 $\pm$ 1542 a	339 $\pm$ 8 a	70.86 $\pm$ 2.23 a	55.02 $\pm$ 2.71 a
	T ₂	36	761 $\pm$ 22 b	657 $\pm$ 13 b	11088.3 $\pm$ 1261.9 a	377 $\pm$ 12 a	75.91 $\pm$ 2.13 a	57.90 $\pm$ 2.59 a

N: number of trees. ¹ data per tree; ² data from all cones; ³ data of six representative cones from 2019 ahead. T₀, T₁ and T₂: rainfed, irrigation from April to September and irrigation from April to the end of June, respectively. Different letters mean significant differences between treatments in the corresponding year. The statistical differences were calculated using the post hoc Tukey's HSD test.

3.3. Production Versus Amount of Water

All the production parameters studied showed a strong Year $\times$ Treatment interaction (Table 3a). The narrow link between year and production in FWP, WPN or WFPN was clear because this species, even when grafted, has a marked annual irregularity in producing cones [6,23,35]. Furthermore, the results showed that the rainfed treatment (T₀) was always the least productive and that none of the productive variables studied improved significantly with the application of the longer irrigation period, T₁. It was only in 2023 that the WRC, and also WPN and WFPN, showed a dependency on the amount of water received, although the results from the previous years suggest that this behavior should be evaluated in more detail (Table 4).

3.4. Water Supply Effect on Mating

The ability to produce and maintain the different female organs (strobili and cones) on the trees in the same year indicated a Year $\times$ Treatment interaction ($p < 0.001$). The influence of water supply was analyzed by considering the years as replicates, and water supply increased not only the presence of strobili (P_1) but also the reproductive load ($P_1 + P_2 + P_3$) measured at the beginning of summer in each tree (Table 5).

Table 5. Annual average of number of female strobili per tree (years 2013–2023 (P_1), 2nd year conelets (P_2), ripe cones (P_3) and total productive load in July under the three watering treatments, April to September irrigation (T_1), April to June irrigation (T_2) and rainfed (T_0). Trial plot sited in Torre Marimon (Caldes de Montbui-Spain).

Water Supply	N	Num. P_1	Num. P_2	Num. P_3	$P_1 + P_2 + P_3$ July
T_0	262	24.5 ± 1.2 c	18.7 ± 1.0 b	13.4 ± 0.9 b	56.6 ± 2.2 c
T_1	383	36.1 ± 1.4 a	25.4 ± 1.2 a	19.3 ± 1.2 a	80.4 ± 2.8 a
T_2	396	29.5 ± 1.0 b	22.0 ± 1.0 ab	16.9 ± 1.0 ab	68.1 ± 2.1 b
Treatment		$p < 0.0001$	$p = 0.0003$	$p = 0.0012$	$p < 0.0001$

Different letters mean significant differences between values of reproductive parameters. N: number of data points per treatment.

The evolution of female organs across cohorts is illustrated in Figure 2. It shows the progression from the strobili produced in year- n to second-year cones in year- $n + 1$ and to ripe cones in year- $n + 2$. The mating irregularity of this species appears to cycle across four and five years, excluding 2015, because the trees were still only in their 7th leaf (juvenile phase). In 2016 and 2017, the number of female strobili increased, with a maximum production in 2018 and declining in 2019 and 2020. After 2020, a new upward cycle began, but it appeared to truncate again in 2023. Although there are still insufficient data to fully explain the reproductive cycle at this site, the presence of all female reproductive organs was consistently associated with water supply in all years. However, when calculating the reproductive efficiency of each cohort, no effect of the amount of water supply was observed in this ratio (Table 6).

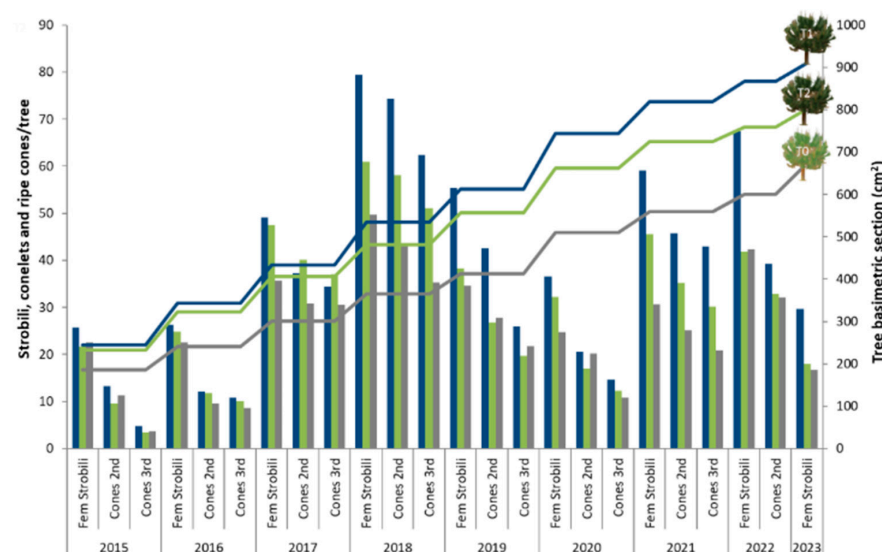


Figure 2. Cohort evolution from 2015 to 2023. Number of reproductive female organs held per tree. Growth in basimetric section (Bs) (lines), cone production (bars) and watering treatments, April to September irrigation (T_1 in blue), April to June irrigation (T_2 in green) and rainfed treatment (T_0 in brown).

Table 6. Mating efficiency. Percentage of strobili converted to harvestable ripe cones three years later in each watering treatment, April to September irrigation (T₁), April to June irrigation (T₂) and rainfed (T₀). Trial plot sited in Torre Marimon (Caldes de Montbui-Spain).

Cohort	2015	2016	2017	2018	2019	2020	2021
T ₀	19.8	38.4	81.8	73.7	60.6	46.9	67.4
T ₁	22.2	43.1	67.0	78.3	47.2	41.8	72.3
T ₂	16.3	42.7	77.1	83.2	51.4	47.0	66.4
Treatment	ns						

ns: no significant difference, $p > 0.05$.

3.5. Expected Production of *Pinus pinea*

Measuring the reproductive efficiency in stone pine means understanding the linkage between the strobili of year $n = 1$ and the harvest of year $n = 3$ in the same cohort. Pearson correlation showed a significant linkage between the different female organs of the pine in the same cohort (Table 7). The different transitions in the same cohort were highly significant resulting in 0.83, 0.88 and 0.94 for the transitions from P₁ to P₃, from P₁ to P₂ and from P₂ to P₃, respectively.

Table 7. Person correlation coefficients of mating parameters recorded from 2011 to 2023 in the trial plot of Torre Marimon (Caldes de Montbui). Numbers of female strobili (P₁) year n1, 2nd year conelets (P₂) in year n2 and ripe cones (P₃) in year n3 belonging to the same cohort.

	P ₂ n ₂	P ₃ n ₃
P ₁ n ₁	R = 0.88; $p < 0.0001$	R = 0.83; $p < 0.0001$
P ₂ n ₂	-	R = 0.94; $p < 0.0001$

Figure 2 shows the cumulative production of the 93 studied pines during the period 2015–2023. Despite being a grafted plantation, not all pines met the same productivity expectations. The scions used for grafting in the nursery came from a mixture of phenotypically outstanding individuals of the *P. pinea* Spanish Provenance Region of ‘Cataluña Litoral’, but their productivity was uneven. Some pines produced over 70 cones across 8 harvests, averaging less than 10 ripe cones per year, whereas other genotypes achieved cumulative production of over 300 cones, averaging more than 35 ripe cones per year. With an average of 30 strobili per tree and an estimated loss rate of less than 50% to ripe cones (Table 6), annual average production in the trial plot was approximately 1500 kg ha^{−1}. With these results, *P. pinea* cone production can be considered a profitable crop for farmers, despite the reproductive irregularity. Given these productive constraints, >30 strobili and >1500 Kg ha^{−1} (Figure 3), only 43 of the 93 trees studied could be deemed suitable for grafted plantations. Thus, selecting the appropriate clone(s) for plantation is a crucial decision for farmers.

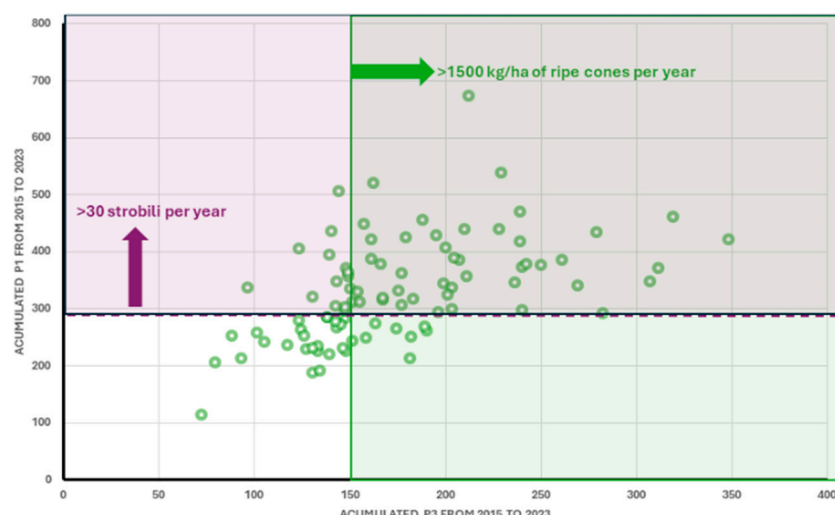


Figure 3. Cumulative number of ripe cones (ΣP_3) versus cumulative number of strobili (ΣP_1) per studied tree in the trial plot of Torre Marimon (Caldes de Montbui) from 2015 to 2023. For the X, Y coordinates of each point, X corresponds to P_1 and Y corresponds to P_3 for each of the 93 trees included in the trial plot of Torre Marimon (Caldes de Montbui-Spain). On average >30 strobili per year are needed to produce more than 1500 kg ha⁻¹ of ripe cones.

4. Discussion

4.1. Constraints in the Mediterranean Area

In today's world, combining adaptation to the environment with high productivity in agricultural crops has become the major challenge in most types of fruit and nut production since the last century. Water scarcity is one of the main traits of the Mediterranean climate, but in recent years, it has worsened and production that previously thrived with irrigation is now facing serious threats [20]. The future climate scenario for the world's Mediterranean climates is not particularly optimistic (IPCC Sixth Assessment Report), and the use of water for irrigation is increasingly being questioned by society. The new targets in agriculture are the development of cropping systems that promote greater water savings, and the use of species naturally adapted to rainfed conditions.

At present, *P. pinea* holds significant productive interest due to its highly valued nut production and its ability to thrive as a rainfed species. The stone pine is a well-adapted species to the Mediterranean climate. Its characteristics, including a deep root system, resilience to drought and an ability to thrive in poor and rocky soils, have enabled the species to grow in marginal areas since ancient times [1,9,10]. The aim of the study is to find out whether water management based on nut production criteria can achieve improvements in pinecone production that justify the water use.

In the two irrigation schedules versus the rainfed treatment (T_0), water consumption under T_1 was twice that of T_2 in all the years considered, and irrigation from July onwards proved to be a luxury usage of a very precious and scarce resource, with little contribution to improving production (Figure 2 and Table 4).

Deficit irrigation systems are used for many nut trees, such as almonds, pistachios and walnuts, which are usually grown in semi-arid areas. The aim of implementing these irrigation systems is to avoid excessive consumption by providing only enough water to ensure a good harvest that year without affecting the following year's harvest. There are numerous references found in other nuts about managing water savings, such as pistachios [44], almonds [45,48] and walnuts [46,49], which are species also cultivated in semi-arid areas of the world. These strategies must be adapted to each area and to the reproductive biology of the crop. Significant water savings can be achieved for certain

crops [48,49]. The results of this study are in line with this approach of trying to use only the water that is necessary. In the case of a strictly dryland species such as the stone pine, this consideration is even more relevant.

4.2. The Use of Aleppo Pine Rootstock

The root system of *P. halepensis* exhibits vigorous development from an early age [50]. It is one of the Mediterranean pines best adapted to the recurrent droughts typical of the region and to soils of low agronomic quality [51]. Although the effects of water stress on Mediterranean pines are generally described in broad terms, significant phenotypic differences between provenances of *P. halepensis* have been reported. When selecting rootstocks for grafted plantations, it is crucial to understand their behavior, in particular the differences in water use efficiency WUE [50,51]. WUE is a critical trait in *P. halepensis*, because it directly affects the tree's ability to balance water uptake during growth, thus optimizing growth under limited water availability [52,53]. The grafting of *P. halepensis* in the nursery is considered more challenging, primarily due to its slower radial growth compared to *P. pinea* at the same age. However, this is likely a matter of grafting technique rather than inherent difficulties. In fact, in plantations where grafting has been used, no incompatibility problems have been observed [54]. In limestone soils with low permeability, *P. halepensis* has shown better growth and productivity than *P. pinea* [6]. Based on the current information, its use as rootstock appears reasonable for extending the presence of *P. pinea* in many parts of the Mediterranean region [55,56]. The use of this rootstock may help to mitigate the projected northward and altitudinal shift expected for *P. pinea* over the next 50 years under current climate scenarios [56]. Another key factor in favor of selecting Aleppo pine as a rootstock is its rapid entry into production. This may be due to the heteroblastic nature of the graft. It should be noted that some authors have stated that this combination is not recommended, although such observations correspond to plantations outside the usual range of *P. halepensis* [6,57].

4.3. Growth Versus the Water Supply

B_s and H_T increased indicating the characteristic pattern of regular growth over the years for the Aleppo pine [6,23]. The SE of B_s and H_T were slightly higher than 5% and 2%, respectively, in the early years (Table 4), but a trend towards heterogeneity in B_s from T_0 to T_1 and T_2 was observed over the years, with its value also gradually increasing. Irrigation seems to have maintained a high regularity in the growth on the plot. *P. halepensis* has demonstrated the ability to adapt to different water supply conditions as reported by several authors [50,51].

Clearly, water supply significantly benefited growth in B_s , resulting in an increase of 669 cm² over 9 years (1.9 cm year^{−1} in DBH) at T_1 and 528 cm² (1.6 cm year^{−1} in DBH) at T_2 , compared to 494 cm² (1.5 cm year^{−1} in DBH) under rainfed conditions. Nonetheless, this growth on Aleppo pine rootstock was greater and more homogenous than the references reporting on stands of *P. pinea* [7,8,13]. However, it is important to note that this study was conducted in a good area for *P. halepensis* growth and in a managed plantation, where herbaceous competition was controlled biannually.

In terms of height, there was less growth in the rainfed treatment than the other two treatments from 2015 onwards. However, no significant differences between T_1 and T_2 were observed until 2021, 10 years after the initiation of differential irrigation. This is in line with the fact that *P. halepensis* typically grows well under the usual water supply conditions within its range but is ultimately affected by recurrent water shortages [50]. It is worth noting that the growth shift observed in 2021 was associated with particularly low rainfall from April to September during that year (Table 2).

4.4. Production Versus Water Supply

In all the years studied, production (FWP) was always higher in the water-supplied trees *versus* rainfed treatment (Table 4). However, the analysis performed by year only showed significant differences between treatments in 2020 and 2023. Both years can be classified as “on” years, characterized by high production, (Figure 2) although the average production per tree in 2020 was more than double that in 2023. Extrapolating the data to Kg ha^{-1} , in 2020 (an exceptional productive year) yielded 4700 Kg ha^{-1} , 7700 Kg ha^{-1} and 6900 Kg ha^{-1} for T_0 , T_1 and T_2 , respectively. In 2023 the yields were 1700 Kg ha^{-1} , 3900 Kg ha^{-1} and 3100 Kg ha^{-1} , for T_0 , T_1 and T_2 . The differences in production between T_1 and T_2 were up to 10% and 17%, in 2020 and 2023, respectively. If we also consider that water savings in 2020, a rainy year in the area, were one third in T_2 and that in 2023, a very dry year, they were slightly less than half (Table 2), the benefit of extending irrigation is seriously questionable.

Considering the average production of the last 6 years counted from the tree age of 7, when a grafted plantation reaches its productive phase [58] the production means were 9 kg, 13 kg and 12 kg per tree for T_0 , T_1 and T_2 , respectively. Thus, the small difference in production of only 1 kg per tree between irrigation treatments, combined with the overall water consumption, clearly favors T_2 . Furthermore, the fact that providing water in the Mediterranean beyond June is both environmentally and economically costly reinforces the benefits of T_2 even more. On the other hand, by the end of June, third-year cones have already reached their maximum growth and, like other nuts, the transformation and accumulation of fatty acids in the kernel begins. This process is typically less water demanding, as documented in other nut crops [44–46,56,57].

4.5. Tree Growth Versus Production

The results of this study indicated that tree growth explained the increase in production of just over 9% (R^2 values, Figure S2). Despite the species’ irregular production, it increases over the years according to growth, as expected. So, the results are consistent with Shestokova et al. (2021) [23] where no clear trade-off between growth and reproduction in stone pine was observed.

Considering this, Sánchez-Bragado et al. (2022) [43] suggested, as a preliminary hypothesis that because pinecones remain green into their third year, they display mixotrophic behavior. They act as a green organ with photosynthetic capacity that may support the function of the needles during the growth season. Furthermore, in this trial there was no evidence that the size of the cone, WRC, decreased with the amount of harvest. This reinforces the idea that the green cone can play an important role in the final stage of seed development.

4.6. Water Versus Mating

Increasing water supply favored the production of female strobili (P_1), as indicated by other authors [25,59], but its effect on the maintenance of second- and third-year cones (P_2 and P_3) was less clear. However, the total reproductive load assessed in July was significant and positively influenced (Table 5), i.e., a greater number of cones of all ages remain on the tree due to the water supply. When considering the cohorts, we can clearly see in Figure 2 that water supply promoted the number of strobili in any given year. However, water intake did not appear to affect reproductive efficiency, i.e., the changes that allow the transition of reproductive organs over more than three years, which depended only on the cohort (Table 6). The key factor seems to be that the reproductive cycle needs to start with a good production of strobili, and a supply of water in spring helps to achieve this. However,

extending irrigation until September does not result in reproductive improvement for the corresponding cohort.

Trials in Portugal based on a five-year follow-up have also indicated that water supply, and even fertirrigation, does not seem to improve mating [34]. Other studies carried out in Chile [32] and based on data collected by dendrometers, that detected each tree's level of wellbeing in real time, concluded that water supply does mitigate interannual growth variability, which is consistent with the results obtained in the present study over nine years. Perhaps the masts can be controlled indirectly by ensuring that the trees do not suffer water stress during key points in the reproductive and vegetative period. These points are well known in other nut species [48,49] but have yet to be determined in stone pine.

Other authors have pointed out that mating of *P. pinea* is significantly influenced by several factors, both endogenous (hormones or even genotype) and exogenous (climatic, seasonal or even management practices) [7,8,23,35,40], during stone pine's long reproductive process. It is highly likely that water support is just another one of these factors.

4.7. Stone Pine as a Nut Tree

One of the key objectives for the future of this species is to ensure stable production, so it can be considered as an alternative crop for agronomically poor soils with few possibilities of significant water supply.

Nevertheless, a lot can happen between flower differentiation and harvest in this species, representing a 42-month period, and thus it is demanding to establish a good understanding of stone pine's reproductive physiology to best manage it as a productive crop [2,23,24,43,60].

As illustrated in Figure 3, a genotype must not only be capable of producing a high number of strobili but also maintain cones of different ages on the tree until harvest, which occurs three years later. When selecting a plant, both aspects are crucial to minimize the irregularity of mating as much as possible [24]. Until now, this behavior has not been considered in selection because of the difficulties involved in observing and evaluating it. The loss of cones occurs at all ages and cannot be attributed to the physiology of the tree alone because there are many biotic and abiotic factors involved, often beyond our control. A thorough investigation of these aspects is essential for the effective management of this species for pine nut production.

As expected, the individual performance of genotypes plays a significant role in determining the potential profitability of grafted plantations. In Spain, materials selected for their high productivity are already available [27,28], and several 'plus' trees have been identified in other Mediterranean countries [29]. Consequently, some genotypes with favorable productive traits are now available for use in new plantations.

Furthermore, in the present study the water supply did not appear to clearly mitigate the alternating pattern of *P. pinea* in bearing, but it did provide improved reproductive conditions. The water contributions made in spring helped to increase the P_1 load in particular, which means that the cohort starts in a good position.

5. Conclusions

The water supplied as in T_1 (watering from April to September) and T_2 (watering from April to the end of June) applied to stone pine grafted onto *P. halepensis* caused positive differences in both vegetative and productive behavior compared to the rainfed treatment. Although the final size of the cones was unaffected by the increased water supply.

Irrigation up to $900 \text{ m}^3 \text{ ha}^{-1}$ per year increased the production of female strobili, but the extra cost of extending irrigation until the end of summer does not seem to result in a sustainable productive benefit.

Despite this, the scheduled water supply did not mitigate the annual production irregularities typical of this species in the Mediterranean area. However, it did enhance the total production over a full productive cycle, which has been estimated in this orchard to be between 3 and 5 years, and represented an average annual production of around 1500 kg of green cones per hectare. That productivity of properly managed stone pine plantations makes *P. pinea* pine nuts a sustainable agricultural alternative in semi-arid Mediterranean regions.

The method used to calculate irrigation requirements, based on weekly water balance using specific Kc values, appears to align with the agronomic requirements of the species. However, further research is needed in semi-arid areas—where these pines usually grow—as has been conducted for other nut crops. Water conservation and achievement of sustainable production are key objectives, as is ensuring the long-term viability of the species.

P. halepensis rootstocks performed well under the tested conditions, but the water use efficiency of *P. pinea* should also be assessed on different rootstocks.

Although the agronomic management of *P. pinea* has a long way to go, its optimization will depend on a deeper understanding of its reproductive physiology.

In view of the current climate scenario and the foreseeable increase in the cost of water, the scarcity of irrigation water means that we must carefully assess the use of water in the domestication of a species considered to be strictly rainfed.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/su17219854/s1>, Figure S1: Reproductive load in July on the tree; Figure S2: Tree basimetric growth versus ripe cone cumulative production per studied tree in the trial plot of Torre Marimon (Caldes de Montbui) from 2015 to 2023.

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