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Effect of fertilization on *Pinus pinea* cone to seed and kernel yields

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ABSTRACT

Stone pine (*Pinus pinea* L.) is a species of economic interest mainly due to its pine nuts, the most expensive nut in the world. Despite the high value of pine nuts, cones are mainly harvested from unmanaged forests. Therefore, research and its applications are needed to develop nutritional management techniques among other cultivation practices intened to boost production. This study evaluated the effect of fertilization on cone production and quality (cone weight and size, number of seeds and kernels, and health status), which in turn may affect cone to seed and cone to kernel yields. Fertilization consisted of two applications of macro and micronutrients in two adult plantations twice in the growing season (in spring and fall). Morphometric variables, cone production per tree and cone health were evaluated during two years after fertilization. Fertilization effect on cone production was positive and significant in both years, with variations between years. Most of the morphometric variables were positively impacted by fertilization, including cone diameter (3.5% increase), kernel weight and length (7.7% and 2.7% increase, respectively), and total seed and kernel weight per cone (20.9% and 18.6% increase, respectively). Fertilized stone pine trees increased production and quality of cones, seeds and kernels, but did not increase cone to seed or kernel yields. However, fertilization increased type II seed damage by 45.4% and 18.9% in 2020 and 2021, respectively. The results obtained in this research may help producers adopt cultural practices to boost pine nut production.

1. Introduction

Stone pine (*Pinus pinea* L.) is appreciated for its highly demanded edible pine nuts, the most expensive nut worldwide (up to € 80 kg^{-1} at the wholesale) (INC, 2020). Since 2012, prices have increased by 1% a year (Jaouadi et al., 2021). Pine nuts are not only delicious, but also a source of healthy components, such as high-quality fats, proteins, vitamins, potassium, phosphorus, magnesium, zinc, iron, and copper (Nergiz and Dönmez, 2004; Evaristo et al., 2010; Bolling et al., 2011; Lutz et al., 2017). Currently, pine nuts from several species are traded internationally besides *P. pinea*, including P. *koraiensis, P. gerardiana* and *P. sibirica.*

Stone pine was found to be present outside its native range as far back as 2,800 years ago. It was cultivated by Phoenicians, who contributed to the spread of the species (Moricca et al., 2021). In Chile, where stone pine is an emergent crop with over 4,000 ha of new plantations, management guidelines to optimize pine nut production are increasingly needed. However, unlike in other fruit tree species, the effect of fertilization on cone to kernel yield is mostly unknown.

Fertilization is a management practice usually applied in fruit orchards to adjust soil nutrients, thereby fostering fruit production and quality (Marschner, 2012; Sansavini and Ranalli, 2012). Stone pine growth was found to be sensitive to soil nutritional conditions (Court-Picon et al., 2004). This limitation can be tackled through fertilizer supply (Loewe-Muñoz et al., 2020a); however, the optimal soil fertility level for this species is unknown (Freire et al., 2019). Positive effects of fertilization on growth have been reported for *P. pinaster* (Zas and Fernández-López, 2005), *P. taeda* (Maggard et al., 2016) and *P. pinea*, both in the short term (Loewe et al., 2017) and in the long term (Loewe-Muñoz et al., 2020a; Loewe-Muñoz et al., 2020c).

The impact of this cropping technique on cone production has been scarcely studied in conifers; the few records available indicate an increase in cone production in *P. edulis* and *P. monophylla* (McLain and Frazier, 2008), as well as in the production of *P. pinea* one-year-old conelets (Loewe et al., 2017) and mature three-year-old cones (Loewe-Muñoz et al., 2020a). In stone pine producing areas, however, fertilization is only marginally used (Özden et al., 2022).

In a previous study, we highlighted that cone to kernel yield

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Table 1
Characterization of the studied stone pine plantations.

Characteristic	Plantation	
	Toconey	Cáhuil
Age (years)*	16	30
Latitude	35°24′ S	34°29′ S
Longitude	72° 3′ W	72° 0′ W
Altitude (m a.s.l.)	56	116
Trial		
Area (ha)	1.47	0.74
Plot size (m^2)	2,450	1,225
Basal area (m^2/ha)	10.2	4.4
Mean DBH (cm)	27.0	16.4
Climate		
Annual average temperature (°C)	14.2	13.2
Autumn maximum temperature† (°C)	20.1	18.7
Thermal oscillation (°C)	16.6	13.0
Annual rainfall (mm)	570	382
Soil		
Sand (%)	29	58
Lime (%)	45	38
Clay (%)	26	4
pH (H2O)	6.2	6.2
Salinity, EC (mmhos cm^{-1})	0.04	0.06
Organic matter (%)	2.1	1.7
N (mg kg^{-1})	26.5	10.0
P (mg kg^{-1})	4	2
K (mg kg^{-1})	100	89
Ca exch (cmol + kg^{-1})	2.51	5.41
Mg exch (cmol + kg^{-1})	0.78	5.04
Na exch (cmol + kg^{-1})	0.34	0.36
K exch (cmol + kg^{-1})	0.38	0.23
CIC (cmol + kg^{-1})	12.7	12.9
Cu (mg kg^{-1})	0.35	2.31
Zn (mg kg^{-1})	0.21	0.75
Mn (mg kg^{-1})	9.7	55.9
Fe (mg kg^{-1})	25.0	24.5
B (mg kg^{-1})	0.40	0.33

† Autumn (March 21st to June 20th) maximum temperature was found to be a significant variable for seed number $cone^{-1}$ by Loewe et al. (2016). Climatic data were obtained from the Chilean National Environmental Information System (https://www.dga.cl; https://www.inia.cl).
*At trial establishment.

Table 2
Average tree size of Toconey and Cáhuil trials at age 29 and 40 respectively.

Average size	Toconey		Cáhuil	
	Control	Fertilized	Control	Fertilized
DBH (cm)	40.3±1.5	41.1±1.0	23.7±0.2	25.0±0.2
Height (m)	13.8±0.4	15.3±0.3	6.8±0.1	6.8±0.1
Crown diameter (m)	6.2±0.3	7.1±0.6	4.5±0.0	4.8±0.1
Basal area (m^2 ha^{-1})	26.7±2.2	28.1±1.2	12.8±0.2	14.2±0.3

Mean ± SE.

depended primarily on the number of kernels per cone and secondarily on kernel weight (Loewe-Muñoz et al., 2019), while Balekoglu et al. (2020) reported the effect of both cone size and weight on cone to kernel yield. Cone to kernel yield, a relevant commercial trait of stone pine cropping, was reported to be higher in Chile than in the main producer countries (Loewe-Muñoz et al., 2019). However, the effect of fertilization on cone quality and how it further affects cone to kernel yield, has been even less explored. Differences in dietary fiber and minerals of pine nuts grown in different regions were reported (Evaristo et al., 2010; Vanhanen and Savage, 2013; Loewe-Muñoz and Noel, 2021), suggesting that the environment, especially soils, might have an important influence. The long-term effect of fertilization on stone pine fruiting was

Table 3
Stone pine fruit traits.

Traits	Abbreviation	Unit	Measurement procedures
Morphometric			
Cone weight	CW	g	3-year-old cones were weighed in a Mettler (Toledo, Spain) AJ150†
Cone length	CL	mm	Measured with a digital caliper
Cone diameter	CD	mm	Measured with a digital caliper in the largest section
Seed weight	SW	g	Each seed was weighed in a Mettler (Toledo, Spain) AJ150
Seed length	SL	mm	Measured with a digital caliper
Seed diameter	SD	mm	Measured with a digital caliper in the largest section
Kernel weight	KW	g	Each kernel was weighed in a Mettler (Toledo, Spain) AJ150††
Kernel length	KL	mm	Measured with a digital caliper
Kernel diameter	KD	mm	Measured with a digital caliper in the largest section
Total seed weight per cone	SWC	g $cone^{-1}$	All seeds per cone were weighed in a Mettler (Toledo, Spain) AJ150
Total kernel weight per cone	KWC	g $cone^{-1}$	All kernels per cone were weighed in a Mettler (Toledo, Spain) AJ150
Cone to kernel yield	KY	%	KY = ((KN × KW)/CW) × 100
Cone to seed yield	SY	%	SY = ((SN × SW)/CW) × 100
Seeds per cone	SN	# $cone^{-1}$	All seeds were extracted from each cone and counted
Health			
Aborted seeds per cone	AS	# $cone^{-1}$	All aborted seeds were extracted from each cone and counted
Healthy kernels per cone	KN	# $cone^{-1}$	All healthy kernels were extracted from each cone and counted
Type I damaged kernels per cone	D1	# $cone^{-1}$	All type I damaged kernels were extracted from each cone and counted
Type II damaged kernels per cone	D2	# $cone^{-1}$	All type II damaged kernels were extracted from each cone and counted
Type III damaged kernels per cone	D3	# $cone^{-1}$	All type III damaged kernels were extracted from each cone and counted
Empty/Damaged seeds per cone	DS	%	DS = (SN – PN)/SN
Productive			
Cones per tree	CN	# $tree^{-1}$	All cones were counted in each tree

† Fresh weight at harvest.
†† Kernels were dried to 6% of humidity at 40 °C in a Red Line Binder oven (Tuttlingen, Germany).

studied in an area of poor soils with macro- and micronutrient deficiency in Chile (Loewe-Muñoz et al., 2020a), showing that fertilization has a positive impact on cone production (up to 3.3 times), and on cone size-weight distribution.

In the last decade, a severe decline in cone production and cone to kernel yield has been observed in all producing countries due to abiotic stresses (Calama et al., 2020; Guàrdia et al., 2021; Özden et al., 2022) and/or to biotic agents such *as Leptoglossus occidentalis* (Farinha et al., 2021; Loewe-Muñoz et al., 2020a; Calama et al., 2020). Farinha et al. (2018) reported that under irrigation, cones from fertilized stone pine trees are more susceptible to seed damage by *L. occidentalis* than from cones of non-fertilized trees.

Therefore, increasing attention is being given to cone quality (Santos, 2020a) and cone to kernel yield (Santos, 2020b). Cone to kernel yield is an important pine nut production trait impacting commercialization; furthermore, in the market cones have been traditionally sold by their weight instead of their kernel content

Table 4
Mean of morphometric, health and productive variables in fertilized and non-fertilized stone pine trees by plantation and year.

Variable	Unit	Toconey 2020		Toconey 2021		Cáhuil 2020		Cáhuil 2021	
		Control	Fertilized	Control	Fertilized	Control	Fertilized	Control	Fertilized
Morphometric									
CW	g	242.4±8.4	326.5±8.1	227.1±12.4	310.6±13.0	206.8±9.3	212.5±13.1	292.1±10.6	329.5±13.3
CL	mm	93.0±2.2	108.1±1.4	89.0±2.2	94.0±2.1	98.1±2.3	97.5±3.0	97.2±1.9	104.0±2.5
CD	mm	77.1±1.2	82.6±0.7	74.5±1.2	74.9±1.0	70.6±1.0	73.0±1.2	77.0±1.0	79.1±1.0
SW	g	0.52±0.02	0.61±0.01	0.64±0.02	0.70±0.02	0.41±0.01	0.43±0.02	0.69±0.01	0.74±0.01
SL	mm	15.5±0.2	16.4±0.2	15.9±0.2	16.4±0.2	13.8±0.1	13.9±0.2	17.2±0.1	17.3±0.1
SD	mm	7.7±0.1	7.9±0.1	8.4±0.1	8.4±0.1	7.1±0.1	7.2±0.1	8.1±0.1	8.4±0.0
KW	g	0.12±0.01	0.14±0.00	0.17±0.01	0.18±0.00	0.08±0.00	0.08±0.00	0.13±0.00	0.14±0.00
KL	mm	11.4±0.1	12.1±0.2	11.9±0.2	12.1±0.1	9.9±0.1	10.0±0.1	12.0±0.1	12.1±0.1
KD	mm	4.4±0.1	4.5±0.0	4.9±0.1	4.8±0.0	3.9±0.0	3.9±0.0	4.2±0.0	4.4±0.0
SWC	g	33.4±1.7	44.1±2.4	22.3±2.2	33.5±2.4	36.1±2.1	39.6±2.7	48.4±2.7	52.2±3.2
KWC	g	5.4±0.4	7.0±0.5	4.1±0.5	6.4±0.5	5.4±0.5	5.6±0.7	8.5±0.5	9.0±0.6
KY	%	2.2±0.2	2.2±0.1	1.8±0.2	1.9±0.1	2.6±0.2	2.4±0.2	2.9±0.1	2.7±0.1
SY	%	13.8±0.5	13.5±0.6	9.5±0.7	9.8±0.5	17.7±0.8	18.7±0.6	16.4±0.5	15.6±0.5
SN	#	71.4±3.2	79.5±3.8	38.2±3.9	51.5±3.3	95.0±5.1	99.8±4.4	75.7±4.3	72.0±3.5
Health									
AS	#	13.6±2.0	9.2±1.8	38.5±2.9	24.0±1.5	8.5±2.7	4.6±0.9	9.4±1.2	11.9±1.4
KN	#	46.1±3.7	49.2±3.3	24.5±2.5	33.4±2.4	66.9±5.3	61.2±5.8	64.5±4.3	62.4±3.5
D1	#	5.7±0.8	2.6±0.8	0.9±0.2	2.0±0.4	6.0±0.7	5.2±0.6	1.3±0.7	1.4±0.7
D2	#	16.1±2.6	23.3±2.9	9.1±1.6	12.9±1.5	20.6±2.9	30.2±5.0	8.1±1.1	8.0±1.0
D3	#	3.4±0.6	4.4±1.1	3.8±1.4	3.1±0.4	1.5±0.3	3.3±0.8	1.8±0.5	0.1±0.1
DS	%	36.3±3.8	37.7±3.4	33.4±3.4	33.2±2.5	30.5±3.8	38.0±5.4	16.1±2.2	13.9±1.8
Productive									
CN	# $tree^{-1}$	2.4±0.0	8.7±0.0	7.8±0.0	17.1±0.0	1.2±0.0	4.8±0.0	2.9±0.0	5.1±0.0

CW: cone weight, CL: cone length, CD: cone diameter, SW: seed weight, SL: seed length, SD: seed diameter, KW: kernel weight, KL: kernel length, KD: kernel diameter, SWC: total seed weight per cone, KWC: total kernel weight per cone, KY: cone to kernel yield, SY: cone to seed yield, SN: seeds per cone, AS: aborted seeds per cone, KN: healthy kernels per cone, D1: type I damaged kernels per cone, D2: type II damaged kernels per cone, D3: type III damaged kernels per cone, DS: empty/damaged seeds per cone, CN: cones per tree.
Mean values ± standard error.

(Nunes et al., 2016). In the last years though, cone to kernel yield has been used as a cone quality indicator, with a higher price being paid for higher yield cones (Santos, 2020b). Furthermore, the need to propose management practices to maximize pine nut production has been highlighted (Calama et al., 2007; Freire et al., 2019; Schneider et al., 2020; Loewe-Muñoz et al., 2020a).

Despite the market demand for pine nuts, application of cropping techniques to the species as a fruit tree has started only recently (Guàrdia et al., 2021), and cones are mostly harvested from unmanaged stands (INC, 2019).

Fertilization studies in this species are particularly challenging due to the long cone development cycle that takes 42 months from differentiation to harvest, with one-, two- and three-year-old mature cones developing in a tree at the same time, posing additional challenges in comparison with other tree species (Loewe et al., 2016). Improving the understanding of cone to kernel yield variability would enhance management effectiveness. Thus, the objective of this work was to assess the effects of fertilization on stone pine cone to kernel yield in central Chile.

2. Material and methods

2.1. Material

A fertilization study was established in two non-irrigated adult stone pine plantations located in Toconey and Cáhuil, in central Chile. Location, climatic and soil variables of these plantations are presented in Table 1. Measurements from last inventories (2022) are presented in Table 2.

The Toconey trial was started in a 16-year-old plantation in 2009, thinned to 204 trees ha^{-1} and pruned before fertilization. In spring 2009, a first fertilization of 260 g per tree was applied in fertilized plots, including: phosphoric acid (60 g), calcium phosphate (60 g), zinc sulfate (10 g), sodium borate (20 g), potassium sulfate (60 g) and carbamide (50 g). Fertilizer was applied at 5 cm soil depth on two lateral strips located at the edge of the crown projection. The Cáhuil trial was established in 2012 in a 30-year-old plantation thinned and pruned first at age 20 (2002, −50%) and again at age 30 (2012, −65%), leaving 285 trees ha^{-1} fertilization.

The climate in the region is typically Mediterranean, characterized by long dry summers and short and intense rainfalls in winters, with maritime influence of the Pacific Ocean. A soil survey was conducted in each trial area following a systematic zigzag pattern in three randomly located zones. At each sampling point, soil from three depths (up to 60 cm) was collected to build a compotrial sample. Soil analyses (Table 1) showed that the Toconey trial is on a loam, non-salty soil with neutral pH, and medium organic matter content. Mineral composition showed a very low phosphorus, zinc, sulphur and boron content; low calcium content; and medium nitrogen, potassium, magnesium, copper, iron and manganese content. The Cáhuil trial is on a sandy-loam, non-salty soil with neutral pH, and medium organic matter content. Mineral composition was very low in phosphorus, boron and sulphur content; low in nitrogen and potassium content; medium in copper, zinc and iron; and high in manganese content.

In Toconey, two treatments were established, involving fertilized and non-fertilized plots (control), with six replicates for the former treatment and two replicates for the latter. The experimental units were plots of 25 and 10 trees (7×7 m spacing) for fertilization and non-fertilization treatments, respectively. In Cáhuil, a randomized complete block design with two treatments and three replicates was assessed: fertilization and control (no fertilization). The experimental units were conformed by 25 trees. In both trials, a ~7×7 m spacing was used and data from border trees of each plot were excluded from the statistical analysis.

Fertilized plots received macronutrients (nitrogen, phosphorus,

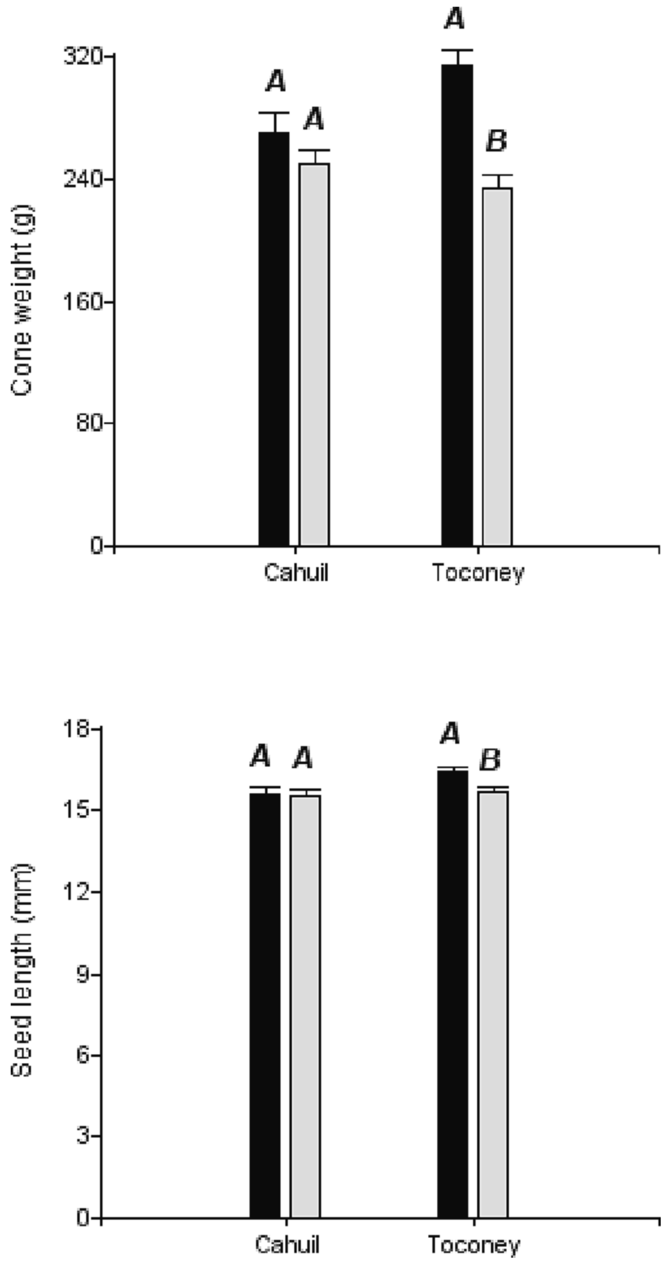

Fig. 1. Significant treatment by trial interactions for morphometric cone and seed variables (fertilized: *black*, control: *grey*). Different letters indicate statistical significance at $\alpha = 0.05$.

potassium, calcium, magnesium and sulphur) and micronutrients (boron, iron and zinc). Fertilization consisted of the application of the following products and doses (g tree^{-1}): Novatec N-max (1,500 g plant^{-1}), zinc sulfate (20 g plant^{-1}) and borax (60 g plant^{-1}) in spring, and a second fertilization application with triple super phosphate (350 g plant^{-1}) and carbamide (200 g plant^{-1}) in the following autumn to induce nutrient load (Li et al., 2016). In Cáhuil, fertilization was applied in spring of 2013, 2014, 2015 and 2018, and in autumn of 2014, 2015, 2016 and 2019, and in Toconey, in spring of 2018 and in autumn of 2019. In both plantations, fertilizers were applied locally at 5 cm depth on two lateral strips located at the edge of the crown projection.

Both trials were monitored for two years (2020 and 2021) to assess the effect of fertilization on cone production and quality (weight, size, number of seeds and health status). A random sample of 10 mature 3-year-old cones was collected from each plot of both trials in July each year; cones were immediately weighed to determine cone to kernel yield, as reported by Mutke et al. (2015). All cones were dried at room temperature and opened, and in-shell seeds and kernels were extracted. Cone size (length and diameter), seed number per cone, and seed and kernel size (length and diameter) and weight were recorded following the procedures reported in Table 3. Seed yield and kernel yield were determined using the formulas specified in Table 3.

To monitor seed health status, we counted damaged kernels and assigned kernels to one of the following categories proposed by Farinha et al. (2018): (i) healthy (intact kernel, tegument and embryo), (ii) partially damaged kernel, with different color/serous appearance; embryo with variable damage (type I), (iii) completely shrunken and dry embryo with no endosperm (type II), and (iv) empty shell without embryo or endosperm (type III). The number of aborted seeds was also counted (Ponce-Herrero et al., 2017). Cone number was recorded in each tree by harvesting all cones (Toconey) or by counting them from a standing position (Cáhuil).

2.2. *Statistical analyses*

Cone number and other count data were evaluated across trials using generalized linear mixed models (Stroup, 2012). The generalized mixed linear models included the fixed effects of treatment, year, trial and their interactions, and a random replicate within trial effect. Count data were modeled as Poisson using a log link. Morphometric variables of cones, seeds and kernels and the derived ratios were analyzed using the same linear mixed model and assuming Gaussian distribution and identity link. Mean comparisons among treatments at each trial and year combination were performed using an LSD test ($\alpha = 0.05$). Statistical analyses were performed using the software Infostat (Di Rienzo et al., 2023) and its interface with R to run mixed linear models (https://www.r-project.org).

3. Results

Trait means for both treatments for each trial and year are presented in Table 4. Treatment × trial × year interactions were mostly non-significant ($p > 0.05$) for morphometric and productive variables, but statistically significant for health status (Table 4). Second-order interactions of treatment with trial were statistically significant for two morphometric variables: CW and SL (Table S1, Fig. 1). Seed number per cone significantly increased with fertilization in Toconey (by 22.6%), but not in Cáhuil. All the differences in morphometric variables between treatments were consistent in both years (Table S1). The treatment × year interactions influenced cone production per tree (Fig. 2). However, CN increased in both plantations under fertilization (276.6% in 2020 and 96.8% in 2021).

No interactions with treatments were observed for CD, seed weight and size (SW, SL and SD), kernel weight and length (KW and KL) or total weight of seeds and kernel per cone (SWC and KWC) ($p > 0.05$). For all these traits, the main effect of fertilization was statistically significant (Table S1, Fig. 3). No interactions among trials, years and treatments were observed for KY or SY; in addition, the effect of fertilization on KY and SY was non-significant.

Treatment effect on aborted seeds and damaged kernels varied across plantations and years, since all interactions were statistically significant ($p < 0.05$) (Table S1). However, on average, the number of aborted seeds decreased under fertilization (39.3% in 2020 and 11.1% in 2021), and the number of type II damaged pine nuts increased under fertilization in both years (45.4% in 2020 and 18.9% in 2021) (Fig. 2).

4. Discussion

This study provides information on plantations of this exotic nut tree as orchard crop in central Chile, that might offer a profitable alternative land use, contributing to the species cropping by assessing the impact of fertilization on cone quality through morphometric and health

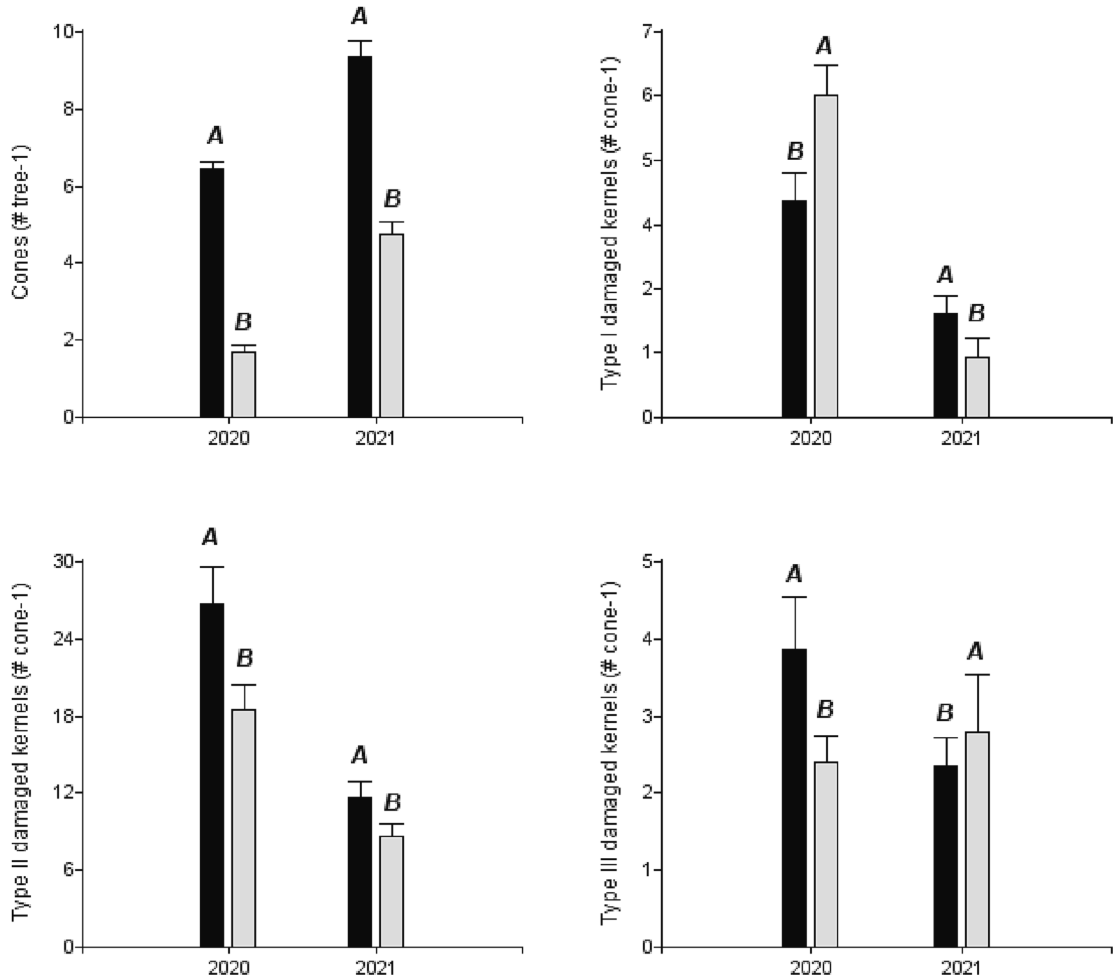

Fig. 2. Significant treatment × year interactions for cone production and seed health variables (fertilized: *black*, control: *grey*). Different letters indicate statistical significance at α = 0.05.

variables, cone production, and cone to kernel yield in stone pine plantations. Regarding morphometric variables, we found a positive main effect of fertilization on cone and seed weight and diameter, and on kernel weight and length, some of them determining cone to kernel yield (Loewe-Muñoz et al., 2019; Balekoglu et al., 2020). A positive fertilization effect was also observed on the number of seeds per cone, but it varied between trials and years. This positive impact of fertilization might have boost cone to kernel yield, but we did not find such effect. In spite of this, total kernel production per cone was increased by 18.6% in fertilized trees due to the higher number of seeds per cone and kernel weight than in control trees.

In this study, average cone weight (260 g in Cáhuil and 289 g in Toconey) was lower than previously in the same trials in 2018 and 2019 (408 g in Cáhuil and 498 g in Toconey) (Loewe-Muñoz et al., 2021). This reduction may decrease kernel quantity and quality (Freire et al., 2019). Since cone weight is related to the climatic conditions in the spring of the last year of cone maturation (Loewe et al., 2016; Calama et al., 2020; Santos, 2020a), this drop is probably due to the severe continual mega-drought affecting the country, with rainfall drops of up to 40% (Garreaud et al., 2020). Despite the low values of cone weight recorded, fertilization increased cone weight by 21.8%. This result shows that the effect of fertilization is independent of the hydric status, as reported by Kilci et al. (2016), and that it improves fruit quality, in agreement with Freire et al. (2019).

Cone to seed and cone to kernel yields (13.5% and 2.2%, respectively) were lower than values reported for previous years in both trials (17.7% and 4.1%, respectively; Loewe-Muñoz et al., 2019), as also recorded in the main producing countries (Özden et al., 2022). This result could be attributed to the independent or combined action of biotic (megadrought, climate change) and abiotic factors, such as the *L. occidentalis* attack (Loewe-Muñoz et al., 2020a; Calama et al., 2020; Farinha et al., 2021). Further studies that help to elucidate the impact of both stressors on tree resilience could contribute to decision-making for the species management, providing valuable information for a climate-smart cropping.

Seeds per cone (69 units) was lower than in previous years (107 units) (Loewe-Muñoz et al., 2021); this trait was positively affected by fertilization only in Toconey, where it increased by 22.5%, probably due to the higher precipitation and soil quality in that trial.

Regarding health, fertilization induced a 26.6% decrease in the quantity of aborted seeds, which would suggest that stone pine trees make some sort of decision to optimize its seed crop on an annual basis. Despite this positive impact, fertilization increased the quantity of type II damaged kernels by 31.2%. This result is in agreement with findings of Farinha et al. (2018), who reported that cones from stone pine trees fertilized through an irrigation system are more susceptible to seed damage by *L. occidentalis* than from cones of non-fertilized trees.

Damaged seeds reached on average 30.5%, with no differences induced by fertilization. This value is higher than records reported for the same trials in 2018/19 (12.3%, Loewe-Muñoz et al., 2021). However, damaged seeds percentage was lower than reported for Spain (39%, Sousa et al., 2012 and 50%, Mutke and Calama, 2016), Turkey (46%, Balekoglu et al., 2020), and Lebanon (60%, El Khoury et al., 2021). We found on average 4.1% of empty seeds (type III damaged

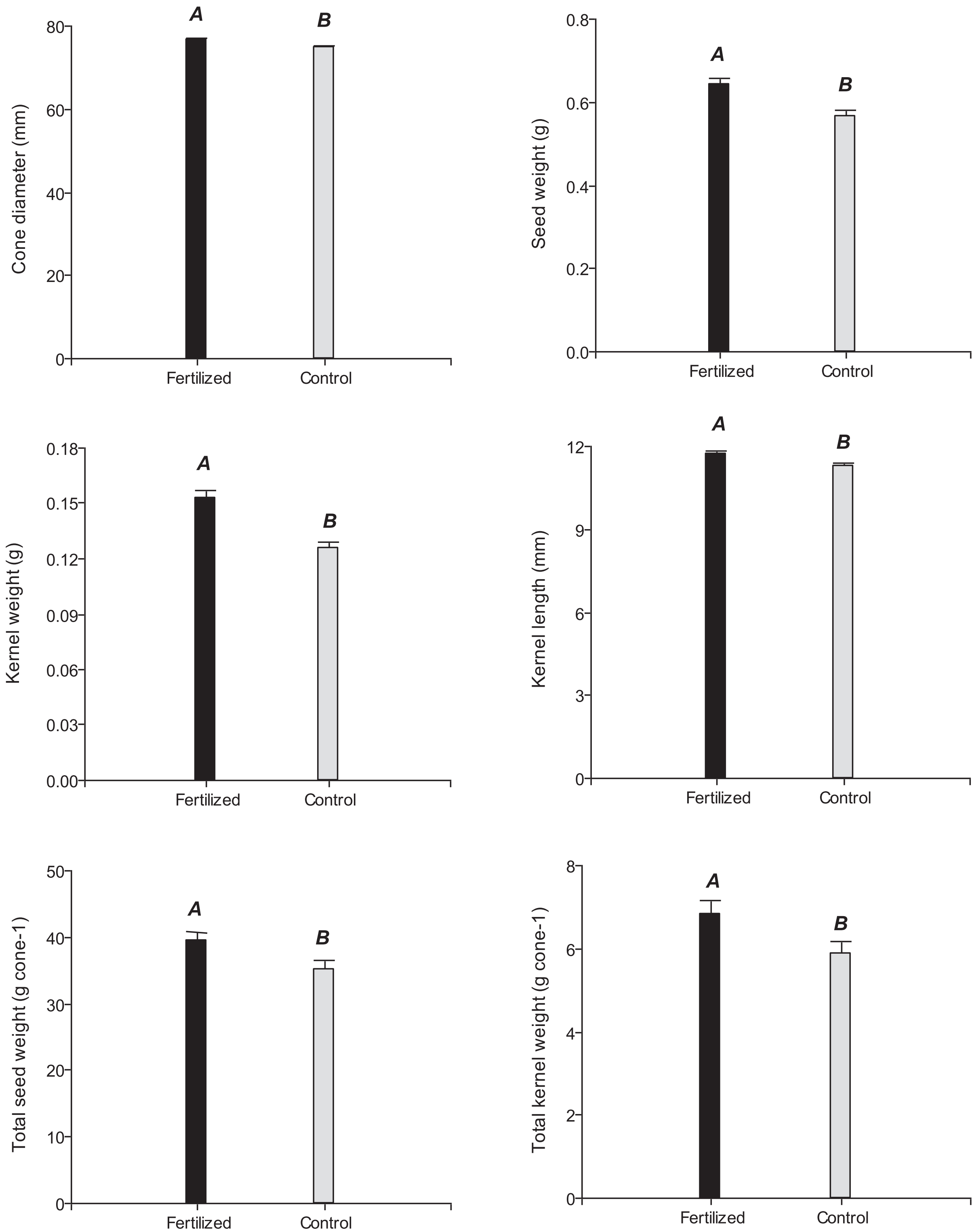

Fig. 3. Significant effects of fertilization on cone diameter, seed weight, kernel weight and length, seed weight per cone and kernel weight per cone (fertilized: *black*, control: *grey*). Different letters indicate statistical significance at α = 0.05.

kernels), whereas Balekoglu et al. (2020) reported 46% of empty seeds in Turkey.

A decrease in KY, from a stable 3.8% to 0.25%, was reported in Spain (Mutke et al., 2017), and from 3.5% to 0.5% in Italy (Agri-Ciência - Consultores de Engenharia, 2014), a trend also observed in Portugal (Piqué et al., 2017) and Lebanon (Parlak, 2017). The authors attributed these decreases to an increase of empty or damaged seeds, and to a higher number of aborted seeds. Despite this significant drop, in the last years a recovery of KY has been reported in Portugal, where the average KY was 2.98% in the 2019/20 season (Santos, 2020b). In the Northern Hemisphere, a decline in cone production driven by climate warming and/or drought has been reported for *P. edulis* (Redmond et al., 2012) and *P. pinea* (Guàrdia et al., 2021). In the case of *P. pinea*, the impact of *L. occidentalis* attack causes an additional decrease in cone production, with a combined effect that is challenging the species fruiting in the main producing countries (Calama et al., 2020; Guàrdia et al., 2021). Since heavier cones have a lower number of damaged seeds (Calama et al., 2020), the impact of fertilization on cone weight could favor KY. However, in this study fertilization did not induce any changes in KY or SY.

The estimated average cone production during dry years in the studied plantations in Chile, located in the Southern Hemisphere, would reach 306 kg ha^{-1}, a higher value than the estimated production in Northern Hemisphere countries, but lower than in previous years, which could be explained by the species' typical masting habit (Prades et al., 2005; Loewe-Muñoz et al., 2020b; Shestakova et al., 2021), reported also in other pines (Wion et al., 2020). The most relevant drivers of masting include processes related to resource dynamics, pollen limitation and genetic and hormonal regulation (Bogdziewicz et al., 2020). In the case of stone pine, mast years have been associated with climate (Loewe et al., 2016; Calama et al., 2019). In particular, an annual thermal oscillation below 12 °C favors cone production (Loewe et al., 2016), a climatic condition that does not occur in the monitored trials.

Nevertheless, besides a significant treatment × year interaction, cone production per tree increased on average 2.7 times due to fertilization. Such effect has also been reported in several species bearing dry fruits such as almond (*Prunus dulcis* (Mill.) (Muhammad et al., 2018), walnut (*Juglans regia* L.) (Wani et al., 2016) and hazelnut (*Corylus avellana* L.) (Tous et al., 2005), and in stone pine in Portugal (Freire et al., 2019) and Spain (Calama and Montero, 2007), as well as in other pines that bear edible seeds in the US (McLain and Frazier, 2008). Our results show a higher impact of nutrient supplementation than the 1.4 times higher cone production obtained by Kilci et al. (2013) after a single application of 2,360 g tree^{-1} of macronutrients. Indeed, Loewe-Muñoz et al. (2020a) concluded that it is necessary to repeat fertilization to enhance cone production and weight; further studies on this topic are necessary for an efficient development of this practical tool.

In Toconey, the 3.6 and 2.2-fold increase in cone number per tree in the fertilized plots in 2020, corresponding to conelets pollinated in spring 2017, and in 2021, corresponding to conelets pollinated in 2018, are only partially explained by the fertilizations applied in 2018 and 2019. The first fertilization applied ten years before triggered tree growth (Loewe-Muñoz et al., 2020a), thus indirectly boosting cone production. In Cahuil, the fertilization applied twice a year during four years (6 to 2 years before cone sampling) translated into a higher cone production than in control trees, 4.0-fold superior in 2020 and 1.8-fold higher in 2021, impacting mainly cone number per tree; tree growth was positively affected by fertilization, but with a lower magnitude (2.0 and 5.5% superior DBH, 5.2 and 10.9% superior basal area, in Toconey and Cáhuil respectively). This would indicate that the observed effects on cone production depend not only upon fertilization but also on sites characteristics and tree size, being Toconey trees bigger than Cáhuil trees.

A positive effect of fertilization on stone pine fruiting was confirmed in both studied areas with poor soils in central Chile. This result could be at least partially explained by the impact of the micronutrients supplied, especially boron and iron, whose deficiency has been shown to decrease *P. pinea* cone production (Malchi and Shenker, 2011). The positive impact of spring fertilization on cone induction was reported in another pine, *P. elliotti* (Shoulders, 1968).

Management of stone pine plantations is a worthy investment, since the crop has potential for the development of rural areas (Jaouadi et al., 2021), enhancing the value of marginalized lands, with fertilization being one of the most feasible techniques. However, an economic evaluation of management including fertilization, taking into account the reality of the countries where the species is being cropped (Freire et al., 2019), and input market value, should be performed to confirm its feasibility. The results of the study are not only relevant for strict agronomic plantations, but also for a broader understanding of the relationship between nutrient status and reproductive effort in this tree, and the implication for susceptibility to seed damaged by the exotic *L. occidentalis*.

5. Conclusions

Fertilization is a useful silvicultural practice to increase the production and quality of stone pine cones, seeds and kernels, but not cone to seed or kernel yields. Nevertheless, the results point out the advantages of studying fertilization to increase cone, seed and kernel size and weight, and consequently pine nut production, contributing to the species cropping for pine nut production.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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