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RESEARCH PAPER

Variability of stone pine (*Pinus pinea* L.) fruit traits impacting pine nut yield

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Abstract

• ***Key message*** **Cone to pine nut yield (PY), an important commercial feature of stone pine cropping, was higher in Chile than in main producer countries. PY is highly variable along years and depends on pine nut number inside cones, followed by pine nut weight. Cone morphometry is not a good indicator of PY, thus selecting cones for size/weight will not improve it.**

• ***Context*** Stone pine nuts are highly appreciated; however, pine nut yield (total pine nut weight as percentage of cone weight), important feature for the species cultivation, is variable and decreasing worldwide.

• ***Aims*** Evaluating inter-annual and spatial variability of fruit traits impacting pine nut yield.

• ***Methods*** Across years and plantation variability of fruit features were estimated from a multi-environment study monitored during 6 years in Chile. Variance component restricted maximum likelihood estimates were calculated for 14 fruit traits. Classification and Regression Tree (CART) was used to identify the variable best explaining pine nut yield.

• ***Results*** Pine nut yield (3.6–5%) was higher than in main producer countries. Cone weight (521 g), length and diameter were correlated to most of seed and pine nut traits, but not to pine nut yield. The most important fruit trait in determining pine nut yield was pine nut number per cone, followed by pine nut weight. Pine nut yield showed high inter-annual and within plantation variability, whereas pine nut weight more spatial than temporal variability. Pine nut yield was superior in cones containing over 78 pine nuts.

• ***Conclusion*** Pine nut yield has high inter-annual variability, with cone morphometry not being a good indicator, thus selecting cones for size/weight will not improve cone to pine nut yield.

Keywords Kernel yield · Cone weight · Inter-annual variability · Between plantations variability · Stone pine · *Leptoglossus occidentalis* Heidemann · Pine nuts

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Contribution of co-authors VL designed the study, directed the project that funded the study, and wrote most of the paper. MB designed, supervised, and performed the statistical analysis. CD was in charge of field activities and contributed to the paper elaboration. AA was in charge of morphometric measurements, elaborated figures, and collaborated in information gathering and practical issues of the publication. All authors discussed the results and implications and commented on the manuscript at all stages.

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1 Introduction

The nut-bearing stone pine (*Pinus pinea* L.) is a well-known species for the high nutritional value of its edible nuts (commonly known as pine nuts or pinoli), being a good source of unsaturated high quality fats, protein, vitamins, minerals, and bioactive compounds (Bolling et al. 2011; Evaristo et al. 2013). Thus, pine nuts are highly appreciated and increasingly in demand by the food industry, reaching high prices worldwide (Fady et al. 2004; International Nut and Dried Fruits 2012). Despite their high demand and being one of the most important nut species in the world, it has not been domesticated nor have varieties or cultivars been defined or used for production purposes, with limited efforts having been made for its cultivation (Mutke et al. 2007). This lack of research could be attributed to its biological characteristics; in

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particular, the long reproductive cycle of this species (42 months) may lead to cone, ovule, or embryo abortion (Krannitz and Duralia 2004). Additionally, there is a cultural barrier to considering it as a fruit crop, since pine nuts are regarded as a non-wood forest product (Loewe 2016).

Currently, an important reduction in the stone pine production in European producer countries has been reported due to the attack of the insect *Leptoglossus occidentalis* Heidemann and, as a consequence, pine nut demand and prices have rapidly increased in the last few years (Lonja de Reus 2018). The species has shown a good agroecological adaptation in Chile (Loewe et al. 2015, 2016), where potential cultivation areas were assessed, with over 4.8 million hectares found suitable for pine nut cropping (Ávila et al. 2012). In this country, the species show good health but *L. occidentalis* has recently been detected (Faúndez et al. 2017). Furthermore, agroclimatic conditions are suitable for the species growth (Loewe et al. 2015) and fruiting (Loewe et al. 2016), and important efforts are being made to improve stone pine cropping to maximize pine nut production (Loewe and Delard 2012). This is particularly important because only a very small fraction of cone weight corresponds to pine nuts (Montero et al. 2008).

According to Gordo et al. (2012), cone quality is related to cone size and weight, since bigger cones are associated with a higher number of seeds (unshelled pine nuts), higher yield, and bigger pine nuts (shelled white pine nuts, the edible component). Thus, pine nut production depends upon three main variables: number of cones, cone weight, and cone to pine nut yield (percentage of total pine nut weight in relation to cone weight), all of them being affected by climatic conditions (Calama et al. 2007), especially by spring and summer rainfall. No reference had been found regarding stand age on fruit characteristics. However, Calama et al. (2011) reported a high inter-annual variability in cone production.

The importance of fruit variability studies, especially of those focusing on cone to pine nut yield, has been growing because of the increasing presence of empty and damaged seeds (Mutke et al. 2015a). This phenomenon has been attributed to damage associated with increasing droughts or phenological shifts due to climatic change (Mutke et al. 2014), as well as with the infestation with *Leptoglossus occidentalis* Heidemann (Mutke et al. 2012; Sousa et al. 2012). In the European Mediterranean, low precipitation and continental climate are expected to cause a decrease in tree growth and cone production (Gordo et al. 2005; Mutke et al. 2006; Calama et al. 2016).

Apparently, healthy cones may contain up to 50% of empty seeds (Mutke et al. 2014). This phenomenon has driven to a severe loss of seed yield since 2001, with drops in cone to seed yield from 17 to 5%, and in cone to pine nut yield from 4 to 2%, or even less. In Chile, where *L. occidentalis* have only been recently detected, the observed variability should not be confused with the effect of pest infestation. The decrease in cone to pine nut yield is particularly relevant because companies usually buy cones by their weight rather than by pine nut content; determining cone to pine nut yield has still not been possible to date, even though serious attempts have been made (Nunes et al. 2016).

In a cross-sectional study (at a specific point in time), we characterized cone production and fruit traits along a latitudinal gradient in Chile (Loewe et al. 2016). However, longitudinal studies (repeated observations performed over several years) in healthy plantations, important to estimate the relative contribution of temporal and spatial components of fruit trait variability, are still missing. Consequently, we hypothesized that cone to pine nut yield is stable along years and variable among sites. Accordingly, the main goals of this study were to evaluate morphometric fruit traits to determine: (1) inter-annual variability, (2) spatial variability, and (3) identifying fruit traits that impact on pine nut yield.

2 Material and methods

2.1 Material

Five non-irrigated plantations (Rosario, Cáhuil, Toconey, Mulchén and Antiquina) and one irrigated plantation (Quilvo) were chosen at random from the 36 stone pine plantations existent among the O'Higgins and Araucania regions, Chile, sampling a 16.6% of the species plantations in the area. The location of the plantations and 10-year (2003–2013) average climatic variables are presented in Table 1 and Fig. 1. Climatic data were obtained from the Chilean National Environmental Information System (www.dga.cl; www.inia.cl).

These six plantations were monitored during 6 years (winters 2010–2015). Since a previous study (Álvarez 2010) reported coefficient of variation smaller than 15% for most of the fruit traits within plantations in Chile, ten healthy 3-year-old cones were randomly harvested per plantation during each year. Cones were collected from different trees and immediately weighted (fresh weight); the standard methodology is reported by several authors (Calama and Montero 2007; Mutke et al. 2015b).

A total of 360 cones were processed at INFOR's laboratory to extract seeds (in-shell pine nuts) and pine nuts (Fig. 2). Cone size (length and diameter), seed number per cone as well as seed and pine nut size (length and diameter) and weight were measured in the lab following the procedures reported in Table 2. Seed yield and pine nut yield were determined using the expressions specified in the same table. Empty and damaged seeds were quantified to monitor fruit health.

Statistical analyses Mixed linear models (West et al. 2014) were fitted with each fruit trait as response variable (Table 2). Each mixed model included plantation (P) and year (Y) effects as random, as well as an error term associated to

Table 1 Characterization of the studied stone pine plantations

Plantation	Age (years in 2015)	Geographical location			Annual average temperature (°C)	Autumn maximum temperature† (°C)	Annual rainfall (mm)
		Latitude	Longitude	Altitude (m.a.s.l.)			
Rosario	20	34° 20′ S	70° 51′ W	352	13.6	18.6	300
Cáhuil	32	34° 29′ S	72° 0′ W	116	13.2	18.7	382
Quilvo	26	34° 55′ S	71° 7′ W	330	14.2	18.0	459*
Toconey	22	35° 24′ S	72° 3′ W	56	14.2	20.1	570
Mulchén	46	37° 39′ S	72° 15′ W	408	13.2	17.5	1150
Antiquina	18	38° 04′ S	73° 23′ W	100	11.5	15.2	815

*With irrigation in spring and summer

† Autumn (March 21 to June 20) maximum temperature was found to be a significant variable for seed number per cone by Loewe et al. (2016)

Fig. 1 Latitudinal distribution of the stone pine plantations studied in Chile

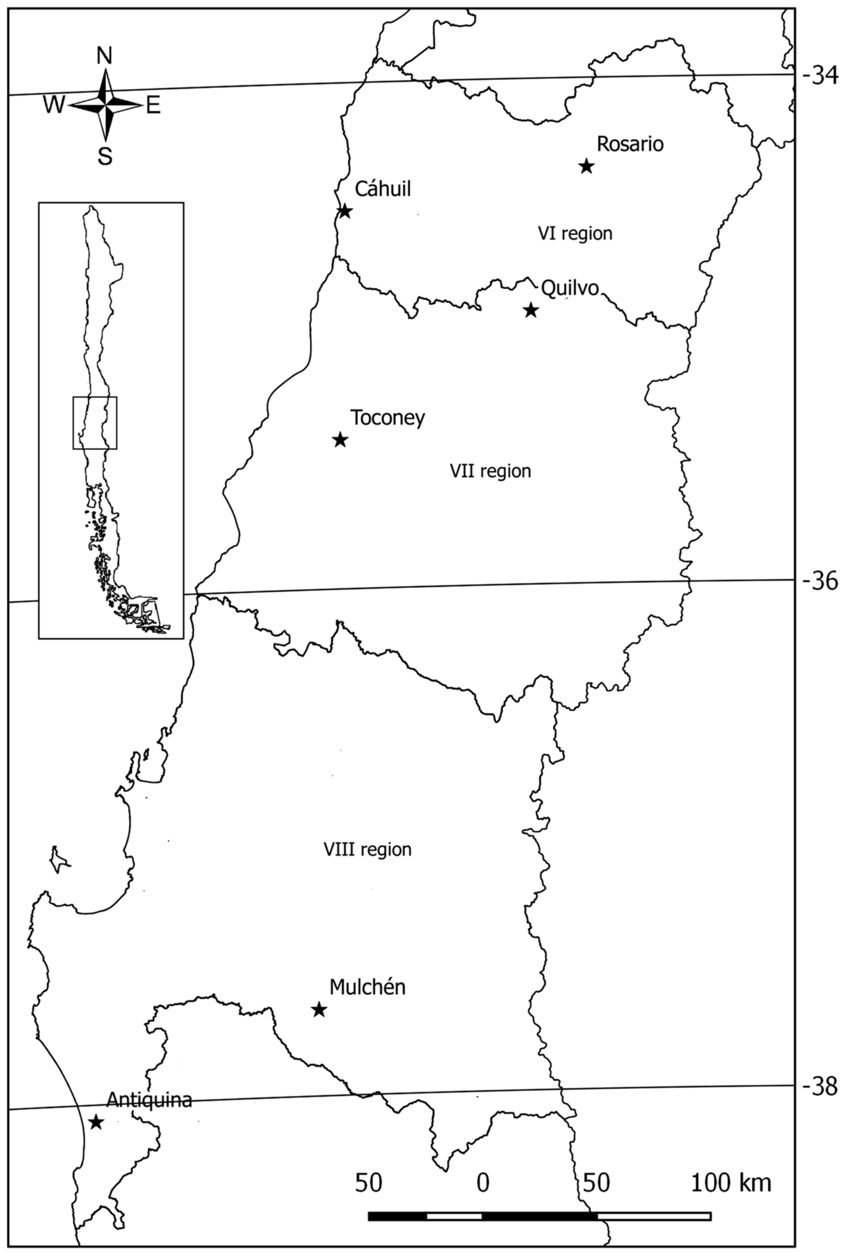


Fig. 2 *Pinus pinea* cones (left), seeds (center), and pine nuts (right)

residual variance. Restricted maximum likelihood (REML) estimates (Patterson and Thompson 1971) of P and Y effects were interpreted as indicator of spatial and temporal variability, respectively. The residual variability was interpreted as variability within plantation in a given year (residual). The estimated variance components were expressed as standard deviation between plantations, between years and within plantation, and as percentage of total variability.

In order to explore correlations among traits, the Pearson correlation coefficient (Pearson 1920) was calculated for each pair of fruit traits. Variability of pine nut yield (PY) as a function of morphometric traits was explored using a Classification and Regression Tree (CART) algorithm (Breiman et al. 1984) in order to identify the fruit trait that had most impact on PY. CART analysis creates a predictive model for a continuous response variable based on the recurrent classification of the studied cases into groups according to the values (threshold) of the predictor variables. The result of this recursive binary partitioning is a model whose structure can be displayed as a tree-like graph, with each split in the tree labeled according to the variable and threshold used for split definition. Additionally, we performed a non-parametric ANOVA (Conover 1999) to evaluate the statistical significance ($\alpha = 0.05$) of differences between groups suggested by the thresholds of the RT main nodes (nodes with more than 20 observations). Statistical analyses were performed using the software Infostat (Di Rienzo et al. 2015) and its interface with R to run mixed linear models (www.r-project.org).

Table 2 Stone pine fruit traits

Traits	Abbreviation	Unit	Measurement procedures
Cone weight	CW	g	3-year-old cones were weighted in a Mettler (Toledo, Spain) AJ150[†]
Cone length	CL	cm	Measured with a digital caliper
Cone diameter	CD	cm	Measured with a digital caliper in the largest section
Seeds per cone	SN	No.	All seeds were extracted from each cone and counted
Seed weight	SW	g	Each seed was weighted 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
Seed yield	SY	%	$SY = ((SN \times SW) / CW) \times 100$
Pine nuts per cone	PN	No.	All pine nuts from each cone were counted
Pine nut weight	PW	g	Each pine nut was weighted in a Mettler (Toledo, Spain) AJ150[††]
Pine nut length	PL	mm	Measured with a digital caliper
Pine nut diameter	PD	mm	Measured with a digital caliper in the largest section
Pine nut yield	PY	%	$PY = ((PN \times PW) / CW) \times 100$
Empty/damaged seeds	DS	%	$DS = (SN - PN) / SN$

[†] Fresh weight at harvest

[††] Previously pine nuts were dried to 6% of humidity at 40 °C in a Red Line Binder oven (Tuttlingen, Germany)

3 Results

Pine nut yield, which includes pine nut weight (PW) and pine nuts per cone (PN), as well as cone weight (CW) as components, was high in all plantations, ranging from 3.6 to 5% (Table 3). Quilvo had the biggest and heaviest cones (675.9 g) and pine nuts (0.27 g) and a PY of 4.7%. Antiquina had the highest seeds per cone (SN) (125.7 units) and PN (116.8 units), but the smallest seed weight (SW) (0.67 g) and PW (0.15 g), and an average PY value of 4.1%. In Rosario, with the lowest PN (94.6 units), the highest PY (5.0%) was observed, with a PW high in relation to CW. Damaged seeds (DS) reached 9.2% on average, ranging from 5.4% in Quilvo to 13.8% in Toconey.

The correlation analysis (Table 4) showed that PY was positively correlated to number and size of pine nuts and negatively correlated to DS. Meanwhile, CW and cone size (CD and CL) were positively correlated to most of the morphometric characteristics, but not to PY. SN was positively correlated to seed yield (SY), PN, and PY. PW was correlated to all fruit traits, with the exception of SN, PN, and DS.

The variance component estimated from the fitted mixed model analysis indicated that PY had a higher inter-annual variability (41.3% of total variability) than spatial or across plantations variability (4.7%). Number and size of seeds and pine nuts also showed high inter-annual variability (44.8 and 77.3% respectively). Moreover, SN, PN, and DS, and consequently PY, showed high variability within plantations in a single year (over 50%). However, SW and PW had a higher variability across plantations than across years, with these traits being the least affected by inter-annual variability (Table 5). As found for PY, SY also showed a higher temporal variability than spatial variability (34.6% vs 3.8%).

DS had a very high inter-annual variability (41.5%) and high variability within plantations in a single year (58.5%). Morphometric cone traits showed high inter-annual variation (> 40%).

PY variability as a function of fruit traits considering all cones and explored by CART analysis showed that PN and PW were the most influential variables to determine PY (Fig. 3). A threshold of 78.2 PN was found showing that the PY of cones with more than this value had a relatively high value. Differences of PY between cones with PN below or above that threshold (2.7 vs 4.4%) were statistically significant ($p < 0.0001$). Pine nut yield, expressed as kernel weight (g) per cone weight (kg), was 17 g kg^{-1} higher on average in cones with more than 78 pine nuts than in the opposite case (Fig. 4). For PW, a threshold of 0.135 g was detected with statistical differences ($p < 0.0001$) in PY between cones containing pine nuts lighter or heavier than this threshold. PY was 58.6% higher when PW was above this threshold (4.6 vs 2.9%).

4 Discussion

The results of this study showed that stone pine PY values in Chile (3.6 to 5.0%) are at least equal to the 3.6% reported for Italy in 1910 (Peruzzi et al. 1998), and higher than those recorded in the Valladolid area, Spain, which ranged between 2.7 and 4.4% in 1996/1997 and have drastically decreased recently (1.8% in 2012/2013 and 2.1% in 2013/2014) (Calama et al. 2014). Chilean PY values were also higher than those recorded in Cataluña (1.1%) and Meseta Norte (2.1%) in 2013/2014 (Calama et al. 2015) and in Portugal (1.7%) in 2003/2004 (Evaristo et al. 2008). This could be due to the absence of negative effects of *Leptoglossus occidentalis* Heidemann in Chile so far, and to the adequate climatic characteristics of the area in which stone pine is cultivated, especially the low hydric deficit which is the variable that best explains the quantity of seeds per cone (PN) according to Loewe et al. (2016), which coincidently in this study is also the best indicator for predicting PY.

Table 3 Stone pine fruit traits by plantation (average of 6 years, 2010–2015)

Plantation	Cone			Seed						Pine nut				
	CW g	CL cm	CD cm	SN no.	SW g	SL mm	SD mm	SY %	DS %	PN no.	PW g	PL mm	PD mm	PY %
Rosario	436.0	10.0	10.3	107.0	0.88	18.6	9.3	21.7	10.9	94.6	0.23	14.0	5.6	5.0
Cáhuil	474.3	11.2	9.0	107.9	0.77	17.1	8.6	17.7	10.2	96.9	0.18	12.2	4.7	3.6
Quilvo	675.9	14.7	11.7	118.8	1.23	21.0	10.2	22.6	5.4	112.6	0.27	15.5	5.7	4.7
Toconey	466.4	11.3	8.6	109.8	0.76	16.5	8.4	17.8	13.8	97.0	0.22	12.6	4.7	4.6
Mulchén	635.8	13.0	9.8	109.4	1.08	19.0	10.0	18.7	7.1	100.2	0.25	13.3	5.8	3.9
Antiquina	436.7	12.2	8.9	125.7	0.67	15.8	8.3	19.3	7.6	116.8	0.15	14.3	5.4	4.1
Overall mean	520.8	12.1	9.7	113.1	0.90	18.0	9.1	19.6	9.2	103.0	0.22	13.6	5.3	4.3

CW cone weight, *CL* cone length, *CD* cone diameter, *SN* seeds per cone, *SW* seed weight, *SL* seed length, *SD* seed diameter, *SY* seed yield, *DS* empty/damaged seeds, *PN* pine nuts per cone, *PW* pine nut weight, *PL* pine nut length, *PD* pine nut diameter, *PY* pine nut yield

Table 4 Stone pine fruit traits correlations

	CW	CL	CD	SN	SW	SL	SD	SY	DS	PN	PW	PL	PD
CL	0.73*												
CD	0.57*	0.68*											
SN	0.41*	0.46*	0.27*										
SW	0.69*	0.50*	0.50*	0.03									
SL	0.47*	0.38*	0.54*	− 0.07	0.61*								
SD	0.38*	0.29*	0.44*	− 0.17*	0.44*	0.82*							
SY	− 0.01	0.15*	0.17*	0.50*	0.37*	0.08	− 0.11						
DS	− 0.17*	− 0.08	0.08	− 0.16*	− 0.10	− 0.10	0.002	− 0.04					
PN	0.41*	0.42*	0.19*	0.89*	0.05	− 0.05	− 0.15*	0.42*	− 0.57*				
PW	0.53*	0.36*	0.36*	− 0.06	0.75*	0.48*	0.35*	0.21*	0.03	− 0.08			
PL	0.20*	0.20*	0.35*	0.004	0.31*	0.42*	0.41*	0.14*	− 0.12	0.04	0.31*		
PD	− 0.002	0.04	0.18*	− 0.23*	0.14*	0.36*	0.51*	− 0.06	− 0.06	− 0.18*	0.18*	0.61*	
PY	− 0.13	− 0.02	− 0.09	0.37*	0.05	− 0.07	− 0.22*	0.62*	− 0.37*	0.46*	0.36*	0.15*	0.02

CW cone weight, *CL* cone length, *CD* cone diameter, *SN* seeds per cone, *SW* seed weight, *SL* seed length, *SD* seed diameter, *SY* seed yield, *DS* empty/damaged seeds, *PN* pine nuts per cone, *PW* pine nut weight, *PL* pine nut length, *PD* pine nut diameter, *PY* pine nut yield

*Statistically significant correlation among traits ($p < 0.05$)

Cone weight, an important quality attribute in most fruit crops (Wetzstein et al. 2011), reached a high average value (521 g), confirming what previously reported by Loewe et al. (2016). In fact, cones in Chile are heavier than in Tunisia (Boutheina et al. 2013), Portugal (Evaristo et al. 2008; Gonçalves and Pommerening 2012) and Spain (Gordo et al. 2012; Mutke et al. 2012). However, the studied plantation with lowest cone weight (436 g) had the highest PY; therefore, CW by itself should not be used as the unique fruit indicator of PY. Our results showed that the average number of pine nuts per cone is a key trait in determining PY. In average, we measured 103 pine nuts per cone, a higher value than those reported in Turkey (Bilir 2009), Italy (Ciancio et al. 1990), and Portugal (Evaristo et al. 2008; Calama et al. 2015).

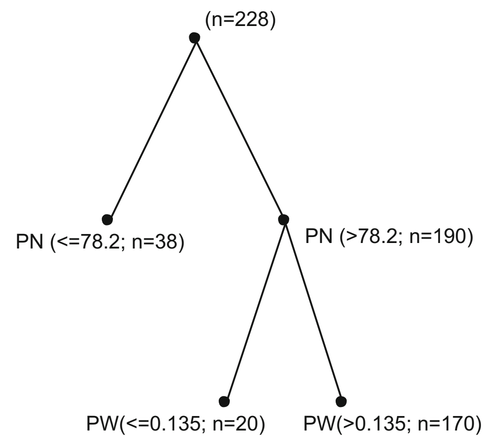
Furthermore, the variance component analysis showed that the PY inter-annual variability was high (41.3%) probably caused by the impact of meteorological variations on CW and SN. Loewe et al. (2016) showed that the annual average temperature, annual rainfall and hydric deficit significantly affect CW, and autumn maximum temperature and hydric deficit impact SN. PY within-plantation variability was also high (54%), and related to high SN and PN variability between 3-year-old cones from the same plantation. By contrast, Montero et al. (2008) indicated that despite large spatial and temporal variability in cone production, PY remains almost constant.

In agreement with Mutke et al. (2005) and Calama et al. (2007), we found a high inter-annual variability also in CW (46%). The relationship between climate and CW was reported in a previous study conducted in numerous plantations across Chile (Loewe et al. 2016), where annual average temperature and annual rainfall were found to be positively

Table 5 Stone pine fruit trait variance components (expressed as standard deviation and percentage of total variability between parentheses)

Effect	Cone			Seed						Pine nut				
	CW g	CL cm	CD cm	SN no.	SW g	SL mm	SD mm	SY %	DS %	PN no.	PW g	PL mm	PD mm	PY %
P	87.3	1.4	0.9	0.0	0.2	1.6	0.5	0.0	<0.01	1.9	0.0	0.5	<0.01	0.3
	(26.8)	(36.8)	(26.3)	(0.0)	(54.2)	(32.8)	(10.1)	(3.8)	(0.0)	(0.4)	(47.1)	(4.1)	(0.0)	(4.7)
Y	114.2	1.4	1.3	17.6	0.1	2.0	1.3	0.0	7.7	18.5	0.0	2.2	1.1	0.8
	(46.0)	(40.1)	(59.3)	(44.8)	(22.9)	(52.2)	(75.7)	(34.6)	(41.5)	(44.9)	(26.5)	(77.3)	(62.3)	(41.3)
Residual	87.9	1.1	0.7	19.5	0.1	1.1	0.6	0.0	9.1	20.4	0.0	1.1	0.8	1.0
	(27.2)	(23.2)	(14.4)	(55.2)	(22.9)	(15.0)	(14.2)	(61.5)	(58.5)	(54.6)	(26.5)	(18.6)	(37.7)	(54.0)

CW cone weight, *CL* cone length, *CD* cone diameter, *SN* seeds per cone, *SW* seed weight, *SL* seed length, *SD* seed diameter, *SY* seed yield, *DS* empty/damaged seeds, *PN* pine nuts per cone, *PW* pine nut weight, *PL* pine nut length, *PD* pine nut diameter, *PY* pine nut yield, *P* plantation effect (spatial variability), *Y* year within plantations effect (inter annual variability), *Residual* variability within combination of plantation and year



Predictor variable	PY (%)
PN (≤78.2)	2.7
PN (>78.2)	4.4
PW (≤0.135)	2.9
PW (>0.135)	4.6

Fig. 3 Stone pine fruit traits that best explain cone to kernel yield. Average PY for each node is reported in the embedded table. PN pine nuts per cone, PW pine nut weight, PY pine nut yield

correlated with this fruit trait and with high maximum temperature during embryo development (a favorable factor). Interestingly, a higher CW did not translate into a higher PY, and a lower CW does not necessarily translate into a lower PY, in agreement with Calama et al. (2015), who stated that there is no effect of CW on PY. For example, the plantation in Rosario, with the lowest CW, had the highest yield (5.0%). This indicates that selecting cones by weight will not improve PY, as also indicated for pomegranate (Wetzstein et al. 2011), a species whose fruit is formed from one flower and contains several seeds, as it also occurs in stone pine. In fact, our results showed that PN is the most determining variable for PY. High values of PN are highly required to enhance cone filling, as indicated for other crops such as wheat (Wiegand and Cuellar 1981; Fischer et al. 1977), maize (Borrás and Otegui 2001; Otegui et al. 1995), rice (García et al. 2010), sunflower (Mercau et al. 2001), and peanut (Giayetto et al. 2012; Phakamas et al. 2008), even though maximum weight is not reached.

In fact, a partial correlation between the number of pine nuts and its weight has been reported in many crop species (Grashoff and D'Antuono 1997; Board et al. 1999; Borrás and Otegui 2001; Zapata et al. 2004; Phakamas et al. 2008). The average number of pine nuts per cone reported in this work (103) was similar to the value reported by Álvarez (2010) (107), with almost no variation across plantations (0.4%) but high variation among years (44.9%) and within plantations in the same year (54.6%). An increase of more than 60% (as shown in Fig. 3) in PY was detected in cones that contained more than 78 pine nuts. Therefore, cones with many pine nuts will be those with the highest PY. However, PY was also associated to the weight of pine nuts, which is positively correlated with a high PY. PW was on average 0.22 g and showed high spatial variability (47.1%). Plantations with biggest and heaviest cones were also those with highest PW, in agreement with Calama et al. (2015).

Regarding cone to seed yield, we observed an average of 19.6% (ranging from 17.7 to 22.6%), similar to the value reported in Portugal (19.2%) (Silveira 2012) and in Italy (20 to 26%) (Ciancio et al. 1990) before the arrival of *L. occidentalis* from the USA. In Chile, we found a greater SY temporal variation than spatial variation (34.6 vs 61.5%, respectively). Calama et al. (2014) reported values between 8.8 and 18.2% for the inter-annual variability of this trait. Moreover, our results indicate that SY is also highly variable within plantations in a given year (61.5%). SY was found to be positively correlated to seed number and weight, as indicated by Gordo et al. (2012). According to our results, SY was not correlated to empty or damaged seeds, probably due to the healthy condition of the species in Chile (González 2012),

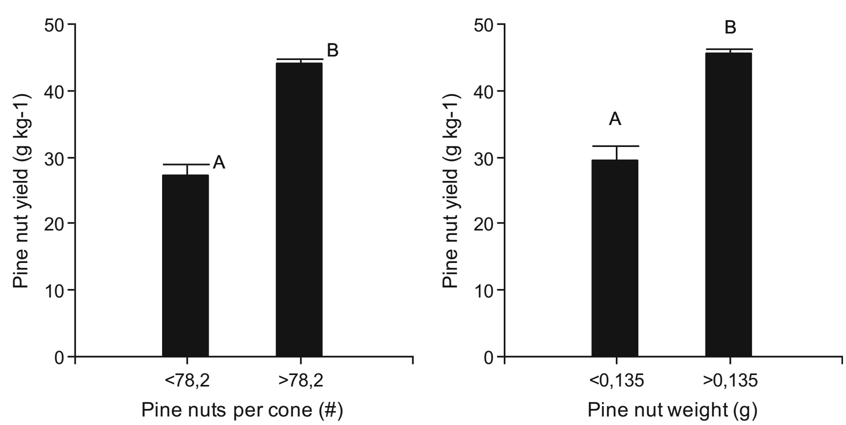


Fig. 4 Fruit morphometric traits affecting cone to pine nut yield in stone pine plantations. Each threshold was detected by Regression Tree analysis. Different letters indicate statistical differences between groups of pine nuts ($p < 0.0001$)

where *L. occidentalis* only recently has been detected (Faúndez et al. 2017). In fact, no damages to the endosperm with developed embryo, classified as type II by Calama et al. (2015), were observed. Total non-viable seeds reached on average 9.2%, lower than values reported in Croatia (10.8% and 4.6% of empty and rotten seeds, respectively) (Jakovljevic et al. 2009), in Tunisia (19.3%) (Boutheina et al. 2013) and in Spain, where empty seeds have increased from below 10% (Sousa et al. 2012) to 50% (Mutke et al. 2016) or even 60% (Calama et al. 2017). The increasing presence of non-viable seeds has been related to the attack of *L. occidentalis* (Calama et al. 2015) and to climatic variables such as annual rainfall (Calama et al. 2014). Our results showed that this trait was highly variable among cones even within the same plantation.

SW (0.9 g on average) was higher than the 0.4 to 0.6 g reported by other authors (Mutke et al. 2012; Calama et al. 2014) which could be due to the cooler habitat that allows the species to live even with a lower hydric availability (Loewe et al. 2015) and to show a high cone yield in Chile (Loewe et al. 2016). Our study showed more SW spatial than temporal variability (54.2 vs 22.9%). High inter-annual variability of SW has been reported in Spain (Calama et al. 2014). SW variation was correlated to SY in our study as Mutke et al. (2005) reported, but SW variation was not correlated to PY.

Given the PN high inter-annual and within plantation variability, and since it is the main determinant in PY, practices oriented to increase average values and reduce variability in PN might increase PY. Therefore, PN should be a characteristic of tree selection in the species breeding programs. Additionally, cultivation practices such as irrigation (Lobell et al. 2005) and fertilization (Loewe and Delard 2015), which increases PN, should be further explored to improve PY.

To our knowledge, no studied have reported the variables impacting pine nut number per cone. The closest information comes from Loewe et al. (2016) who identified climatic variables that significantly influence seed number per cone (annual water deficit and the autumn maximum temperature) and also the biometeorological variables that account for physio-phenological phases involved in this trait determination (accumulated rainfall during induction of male primordia; maximum average temperature during 2-year-old cone growth, and accumulated rainfall during 3-year-old cone growth and cone ripening). Since pine nut number per cone is so determinant in PY, we suggest addressing in future studies both climatic variables and cultivation practices that could help in maximizing pine nut number in stone pine cones.

5 Conclusions

Cone to pine nut yield, an important commercial feature of stone pine cropping, ranged from 3.6 to 5% in Chile, higher values than the current values reported in the main pine nut producing countries. The most important fruit trait in determining PY was pine nut number per cone, followed by pine nut weight. PY showed both a high inter-annual variability and within plantation variability, similarly to PN, whereas PW showed more spatial than temporal variability. PY was not correlated to cone weight and size. Cone morphometry is not a good indicator of PY, and thus, selecting cones for size or weight will not improve cone to pine nut yield.

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Data availability The datasets generated and analyzed during the current study are not publicly available due to institutional guidelines but are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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