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Impact of *Leptoglossus occidentalis* on *Pinus pinea* cone to pine nut yield in Chile

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Abstract

Stone pine nuts are highly appreciated and increasingly in demand. However, pine nut yield expressed as percentage of nut weight over cone weight, has decreased (from 4% to 2% or even less) in the European producer countries, due to the attack of the insect *Leptoglossus occidentalis*, which produces an increase in the presence of empty (up to 50%) and damaged seeds. The species shows a good adaptation in Chile, with over 2,000 hectares of new plantations. *L. occidentalis* here was first detected in 2017, with increasing captures in the last years. The objective of this study was to assess fruit traits and pine nut yield before and after the arrival of the insect, and to quantify its damages (types I (endosperm/embryo damage), II (endosperm elimination) and III (empty seeds)). Seven plantations were monitored in 2018 and 2019 and compared to data from previous years (2010/15). Cone size, seed number per cone, seed and pine nut size (length and diameter) and weight were measured at each plantation and year and pine nut yield was determined. Results showed average pine nut yield of 4% without decreases along time. In a hotspot site for the insect, a significant decrease in cone weight, seed weight and pine nuts per cone was quantified. A significant increase in damages type I and II was found in all sites after 2017. Consequently, the need of monitoring the advance of the insect in the country and its effects on stone pine cropping is relevant. Biological or chemical control will be required for adequate orchard management.

Keywords: kernel yield, cone health, stone pine, insect attack.

Introduction, scope and main objectives

Pinus pinea L. is appreciated for the high value of its edible nuts, the pine nuts, which are a good source of unsaturated fats, protein, vitamins, minerals and bioactive compounds (Evaristo et al. 2013). Pine nuts are highly demanded, reaching high prices (INC 2020).

Despite its market value, stone pine has not been domesticated, and limited efforts have been made for its cultivation (Mutke et al. 2007). In Chile, the species has shown to grow well and bear a high cone production (Loewe et al. 2015, 2016), with over 4.8 million hectares of potential areas for its crop (Ávila et al. 2020). Important efforts have been made to develop management techniques to enhance pine nut production (Loewe et al. 2020), with over 2,500 hectares of plantations established in the last years.

In the producer countries, a severe drop in cone production and cone to kernel yield has been reported, attributed to increasing percentages of empty seeds (Mutke et al. 2016b). Consequently, pine nut prices have further increased (Lonja de Reus 2020).

The Western Conifer Seed Bug (*Leptoglossus occidentalis*) is native to the US, where it is considered a pest in conifer seed orchards (Blatt and Borden 1996). *L. occidentalis* was introduced to Italy two decades ago, rapidly spreading to Europe during early 21st century (Mutke et al. 2016a); currently this pest occurs from Portugal to Russia and from North Africa to Scandinavia, and in China, Japan and Korea (Lesieur et al. 2014).

The Western Conifer Seed Bug feeds on conifer seeds, especially on *P. pinea* seeds, whose large size and nutrient content attract it. Ponce-Herrero et al. (2017) reported severe damage to *P. pinea* cones, seeds and pine nuts in controlled experiments.

Damages caused by Western Conifer Seed Bug include massive abortion of conelets and a high percentage of empty seeds in ripened cones (Recuenco et al. 2016). Adults of *L. occidentalis* feed on mature seeds by piercing the hard and thick coat (consumed kernels appeared skunked and wrinkled), with a low consumption per individual, showing a collaborative feeding process (Farinha et al. 2018a).

In Chile, *L. occidentalis* was first detected in 2017 (Faúndez et al. 2017a, b), being the first record of this insect in the southern hemisphere, with increasing captures in the last years (Sandoval and Ide 2018; Ide and Rojas 2020). The scope of this work was to assess changes in fruit traits and pine nut yield caused by *L. occidentalis* to monitor the effects of this pest on stone pine fruiting.

Methodology/approach

Material

Seven plantations were randomly chosen from adult stone pine plantations inventoried in Chile (Table 1), and monitored in winter during 2010 to 2015 (before *L. occidentalis* arrival) and from 2018 to 2019. In total, 140 three-year-old cones were collected and immediately weighed to determine cone to pine nut yield (Mutke et al. 2015a), and cone size (length and diameter) was measured. After drying and opening, seeds (in-shell pine nuts) and pine nuts were extracted. Cone size, seed number per cone, and seed and pine nut size (length and diameter) and weight were measured (Table 2). Seed and pine nut yield were determined by using the expressions specified in Table 2.

To monitor fruit health, we quantified three types of pine nut damages (Farinha et al. 2018b), separating kernels into four categories: (i) healthy (healthy kernel, tegument and embryo), (ii) partially damaged kernel; variable damage of embryo (type I), (iii) embryo shrunken/dry with no endosperm (type II), and (iv) empty shells, no embryo or endosperm (type III).

Table 1. Characterization of the studied stone pine plantations

Plantation	Age (years in 2019)	Geographical Location			Annual average temperature (°C)	Annual rainfall (mm)
		Latitude	Longitude	Altitude (m a.s.l.)		
Santo Domingo	20	33°38' S	71°36' W	31	13.4	459
Rosario	24	34°20' S	70°51' W	352	14.9	491
Cáhuil	36	34°29' S	72° 0' W	116	13.8	641
Quilvo	30	34°55' S	71° 7' W	330	14.5	630*
Toconey	26	35°24' S	72° 3' W	56	14.7	762
Mulchén	50	37°39' S	72°15' W	408	13.4	1.153
Antiquina	22	38°04' S	73°23' W	100	12.7	1.304

* with spring and summer irrigation. Climate variables from Santibáñez (2017).

Table 2. Measurement procedures for stone pine fruit traits

Traits	Unit	Measurement procedures
Cone weight (CW)	G	3-year-old cones were weighed in a Mettler balance †
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)	#	All seeds were extracted from each cone and counted
Seed weight (SW)	G	Each seed was weighed in a Mettler balance
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 × SW) / CW) × 100
Pine nuts per cone (PN)	#	All pine nuts from each cone were counted
Pine nut weight (PW)	G	Each pine nut was weighed in a Mettler balance ††
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 × PW) / CW) × 100
Healthy pine nut (HP)	#	Observed visually as intact kernel (endosperm), tegument and embryo.
Damaged seeds (DS)	%	Percentage of total seeds presenting any type of damage, excluding abortions
Damage type I	#	Observed as having different color/serous appearance, embryo with variable damage
Damage type II	#	Observed as completely shrunken and dry embryo with no endosperm
Damage type III	#	Observed as empty shell without embryo or endosperm
Aborted seeds (AS)	#	Counted as seeds of less than 9 mm in length.

† Fresh weight at harvest. †† Pine nuts were previously dried to 6% of humidity at 40 °C in a Red Line Binder oven.

Statistical analyses

A generalized linear mixed model (Stroup 2013) with binomial distribution and a logit function was modelled to compare the proportion of damaged seeds before and after *L. occidentalis* detection. The same modelling but assuming normal distribution, was used to compare metric fruit traits between 2010/15 and 2018/19. Statistical analyses were performed using the InfoStat software (Di Rienzo et al. 2020) and its interface with R (www.r-project.org).

Results

Before the arrival of *L. occidentalis* in Chile, pine nut yield (PY) was high in all plantations (3.6 to 5.0%), as well as after that (2.8 to 4.3%) (Table 3), with no statistical differences for mean values (4.2 vs 3.7%). No significant decrease in PY was registered in any site. In Santo Domingo, cone length (11.3 to 9.2 cm, $p<0.0001$) and CD (10.3 to 6.9 cm, p=<0.0001) decreased significantly after the insect arrival. Quilvo had the biggest and heaviest cones both before and after the pest arrival; Mulchén had significantly lower values of CW (635.8 g before and 393.0 g after, $p=0.0009$), SW (1.1 to 0.9 g, $p=0.0003$) and PN (100.2 to 63.5 units, $p<0.0001$) after the insect detection. Antiquina had the highest SN and PN but the lowest SW, independently of *L. occidentalis*.

The distribution of damage types was statistically different before and after *L. occidentalis* first detection ($p<0.0001$) (Table 4). Regarding damaged pine nuts, a significant almost four-fold increase was observed in Mulchén (8.4 to 27.8%, $p<0.0001$) and 67% in Toconey (11.5% to 19.2%, $p=0.0048$). In all plantations, pine nut damage types I and II were first observed after *L. occidentalis* introduction, increasing over time. On average, across sites, DS significantly increased after insect detection, from 10.3% to 15.1% ($p=0.0041$).

Table 3. Stone pine fruit traits and yield before (2010/15) and after (2018/19) the first detection of *L. occidentalis* in Chile

Plantation	Period	Cone			Seed					Healthy Pine nuts				
		CW	CL	CD	SN	SW	SL	SD	SY	PN	PW	PL	PD	PY
		g	cm	cm	#	g	mm	mm	%	#	g	mm	mm	%
Santo Domingo	Before	452.4 a	11.3 a	10.3 a	119.0 a	0.8 a	17.4 a	10.0 a	20.7 a	98.0 a	0.2 a	12.3 a	5.6 a	3.4 a
	After	416.0 a	9.2 b	6.9 b	122.5 a	0.8 a	17.5 a	8.6 a	23.2 a	88.0 a	0.1 b	12.1 a	4.2 a	2.8 a
Rosario	Before	436.0 a	10.0 a	10.3 a	107.0 a	0.9 a	18.6 a	9.3 a	21.2 a	94.7 a	0.2 a	14.0 a	5.6 a	5.0 a
	After	492.9 a	10.4 a	8.7 a	110.5 a	1.0 a	19.6 a	8.9 a	21.5 a	99.0 a	0.2 a	14.4 a	5.0 a	4.3 a
Cáhuil	Before	474.3 a	11.2 a	9.0 a	107.8 a	0.8 a	17.1 a	8.6 a	17.6 a	97.0 a	0.2 a	12.2 a	4.7 a	3.6 a
	After	407.6 a	10.6 a	8.0 a	99.0 a	0.8 a	17.3 a	8.3 a	18.5 a	93.5 a	0.2 a	12.3 a	4.5 a	3.6 a
Quilvo	Before	675.9 a	14.7 a	11.7 a	119.0 a	1.2 a	21.0 a	10.2 a	21.8 a	112.7 a	0.3 a	15.5 a	5.7 a	4.4 a
	After	564.0 a	11.6 a	9.6 a	111.0 a	1.1 a	20.8 a	9.3 a	21.6 a	103.5 a	0.2 a	15.2 a	5.0 a	4.3 a
Toconey	Before	466.4 a	11.3 a	8.6 a	109.7 a	0.8 a	16.5 a	8.4 a	18.2 a	97.0 a	0.2 a	12.6 a	4.7 a	4.6 a
	After	498.1 a	10.7 a	8.4 a	115.0 a	0.8 a	14.3 a	8.6 a	19.0 a	92.0 a	0.2 a	13.5 a	4.8 a	3.6 a
Mulchén	Before	635.8 a	13.0 a	9.8 a	109.4 a	1.1 a	19.0 a	10.0 a	18.5 a	100.2 a	0.3 a	13.3 a	5.8 a	3.8 a
	After	393.0 b	10.7 a	8.7 a	88.0 a	0.9 b	19.0 a	9.2 a	20.2 a	63.5 b	0.2 a	14.1 a	5.2 a	3.7 a
Antiquina	Before	436.7 a	12.2 a	8.9 a	125.4 a	0.7 a	15.8 a	8.3 a	19.3 a	116.8 a	0.2 a	14.3 a	5.4 a	4.1 a
	After	413.5 a	11.0 a	8.4 a	137.0 a	0.7 a	16.8 a	8.1 a	22.4 a	123.5 a	0.1 a	11.9 a	4.2 a	3.8 a
Mean	Before	511.1 a	11.9 a	9.8 a	114.0 a	0.9 a	17.9 a	9.3 a	19.7 a	102.4 a	0.2 a	13.4 a	5.3 a	4.2 a
	After	455.0 a	10.6 a	8.4 b	111.9 a	0.9 a	17.9 a	8.7 a	20.9 a	94.7 a	0.2 a	13.3 a	4.7 b	3.8 a

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, PN: pine nuts per cone, PW: pine nut weight, PL: pine nut length, PD: pine nut diameter, PY: pine nut yield. Different letters indicate statistically significant differences among periods for each plantation ($p<0.05$).

Table 4. Damages on stone pine nuts before (2010/15) and after (2018/19) the first detection of *Leptoglossus occidentalis* in Chile

Site	Period	Damaged pine nuts cone^{-1} (%)	Damage type (%)		
			I	II	III
Santo Domingo	Before	17.7 a	0.0	0.0	100.0
	After	28.1 a	43.0	56.1	0.9
Rosario	Before	11.5 a	0.0	0.0	100.0
	After	10.4 a	45.4	53.0	1.6
Cáhuil	Before	10.2 a	0.0	0.0	100.0
	After	5.6 a	13.7	68.7	17.6
Quilvo	Before	5.3 a	0.0	0.0	100.0
	After	6.3 a	17.4	63.5	19.1
Toconey	Before	11.5 b	0.0	0.0	100.0
	After	19.2 a	26.0	59.0	15.0
Mulchén	Before	8.4 b	0.0	0.0	100.0
	After	27.8 a	17.0	78.9	4.1
Antiquina	Before	7.5 a	0.0	0.0	100.0
	After	9.8 a	19.6	76.6	3.8

Discussion

Studies on cone to pine nut yield have been growing in native areas because of the increasing presence of empty/damaged seeds (Mutke et al. 2015b), a phenomenon associated with *L. occidentalis* (Sousa et al. 2012) and with droughts (Mutke et al. 2014). The insect spread in Europe has caused a severe decrease of cone to seed yield since 2001 (17 to 5%, Mutke et al. 2016b). Before *L. occidentalis* introduction into Chile, average SY was 19.7% and has not significantly decreased.

In *P. pinea* native habitat, the insect caused a reduction in cone to pine nut yield from 4 to 2%, or less (Ponce-Herrero et al. 2017). In Spain, losses of up to 65% in attacked stands have been reported (De la Mata et al. 2019) with a significant drop in the final cone to kernel yield, with reductions of over 50% in the most affected areas (Calama et al. 2020), with increasing rates of damaged pine nuts. Pine nut yield decrease is important, since companies usually buy cones by their weight rather than by pine nut content. We compared data from a six-year period before *L. occidentalis* first detection in Chile in 2017 with data from 2018/19, and found an average pine nut yield of 4%, with no significant reductions over time.

In Mulchén, close to a *L. occidentalis* hotspot, a significant reduction of 62% in cone weight was recorded; this is in agreement with the 48% drop in cone weight in attacked stands with respect to protected ones (De la Mata et al. 2019).

As per damages, we quantified an average of 15.1% of damaged seeds in the two-year period after the first insect record, with significant increases in Toconey and Mulchén. In our study, in the two-year period after the first record of the insect, we quantified an average of 1% of empty seeds (damage type III) across sites

As per damages, we quantified an average of 15.1% of damaged seeds in the two-year period after the first insect record, with significant increases in Toconey and Mulchén. In the two-year period after the first record of the insect, we quantified an average of 1% of empty seeds (damage type III) across sites, which is far from the 50% of empty seeds observed in externally healthy cones by Mutke et al. (2014). Before the insect detection, this type of damage was also described, being attributed to the lack of pollen availability in many sites along Chile (Loewe-

Muñoz et al. 2018). Recuenco et al. (2016) indicated that *L. occidentalis* must be considered the most likely cause of all of described damages. Considering that accidentally introduced species, as *L. occidentalis* in Southern Latin America, spread faster than intentionally introduced ones (Roques et al. 2016), further damages by *L. occidentalis* in stone pine can be expected.

The results obtained in an exotic habitat evidence the need for monitoring the insect spread in the Southern Hemisphere and its effects on stone pine cultivation. Biological or chemical control, ensuring the compliance of environmental safeguards provided by the national agency SAG, would be required for adequate orchard management. Studies on the insect population dynamics are also needed to determine when the insect becomes active, its biological cycle and host preferences.

Conclusions

Pine nut damage by *L. occidentalis* in central Chile has still not translated into a reduction of pine nut yield. Monitoring the spread of the insect in Southern Hemisphere countries and stone pine productivity losses are recommended. Plantations should be managed including authorized biological or chemical control to limit the impact of the insect on cone and seed weights, and on cone filling.

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