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Damage caused by *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae) on *Pinus pinea* L. cones varies with feeding season

Laura Ponce-Herrero[1,2] | **Ana Ponce Díaz**[1,2] | **Valentín Pando Fernández**[1,3] | **Fernando Manuel Alves-Santos**[1,2] | **Juan Alberto Pajares Alonso**[1,2]

[1]Department of Vegetal Production and Forest Resources, University of Valladolid – La Yutera Campus, Palencia, Spain

[2]Sustainable Forest Management Research Institute, University of Valladolid – La Yutera Campus, Palencia, Spain

[3]Statistics and Operations Research Department, University of Valladolid – La Yutera Campus, Palencia, Spain

Correspondence
Laura Ponce-Herrero, Department of Vegetal Production and Forest Resources, University of Valladolid – La Yutera Campus, Avda. de Madrid 57, 34004 Palencia, Spain.
Email: laura.ponce@uva.es

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Abstract

1. The commercialization of pine nuts generates major economic benefits for the Mediterranean basin. This has been reduced due to an increase in cone and seed damage associated with the spread of the western conifer seed bug (WCSB) throughout the Mediterranean region and the Dry Cone Syndrome appearing on pine cones. Studies on several conifer species have associated cone and seed damage with WCSB feeding.
2. To relate cone and seed damage of stone pine to WCSB feeding, one exposure and exclusion trial of cones to the feeding of the natural WCSB population was carried out. To determine the seasonality of cone and seed damage caused by this pest, another trial consisting of bagging cones with insects was performed with first-, second- and third-year cones at different stages of cone development.
3. The natural WCSB population was associated with cone abortion in second-year cones (unprotected: 43.6% vs. protected: 2.7%), a decreased number of seeds per cone (82.0 vs. 105.3), kernel yield (1.8% vs. 3.8%) and proportion of sound kernels (35.6% vs. 73.6%), and numerous types of kernel damage.
4. Cone mortality in first- and third-year cones bagged with insects decreased as the bagging date advanced. A temporal sequence of kernel damage in third-year cones (aborted, dry embryo without endosperm, and totally or partially damaged endosperm) was reported for seed development and the timing of WCSB feeding.
5. Our findings may be useful in the management of WCSB populations to prevent significant damage to the stone pine nut crop.

KEYWORDS

cone mortality, forest management, kernel damage, kernel yield, stone pine, Western conifer seed bug (WCSB)

INTRODUCTION

Leptoglossus occidentalis Heidemann (Hemiptera: Coreidae), known as the Western conifer seed bug (WCSB), is a conifer cone pest that is native to western North America (Heidemann, 1910). Since the second half of the 20th century, this pest has been associated with economic losses from seed orchards to produce seeds for genetically superior trees (Bates, Strong, & Borden, 2002; Blatt & Borden, 1996b; Connelly & Schowalter, 1991; Koerber, 1963; Schowalter, 1994; Strong et al., 2001). WCSB nymphs and adults feed on developing and mature cones by inserting their proboscis between cones scales and secreting digestive substances, such as lipases and proteases, from the salivary glands, to dissolve the cone tissues and suck them up.

The damage caused by WCSB cannot, however, be visually diagnosed in the field since the external feeding marks made by the WCSB are not detectable on the cone surface (Bracalini et al., 2013; Campbell & Shea, 1990; Farinha, Silva, et al., 2018; Pasek & Dix, 1988; Pimpão et al., 2017). Symptoms of WCSB feeding were found in insect bagging experiments at different stages of cone development of the *Pinus contorta* var. *latifolia*, *Pinus monticola*, *Pinus ponderosa* and *Pseudotsuga menziesii* conifer species. These symptoms ranged from cone abortion, reduced seed set, seed fusion with cone scales and seed abortion when WCSB feeding occurred early in the growing season, with partially filled seeds being associated with late season feeding (Bates et al., 2001; Bates, Borden, Kermode, & Bennett, 2000; Bates, Lait, et al., 2002; Bates, Strong, & Borden, 2002; Connelly & Schowalter, 1991; Pasek & Dix, 1988; Schowalter & Sexton, 1990; Strong, 2006).

The WSCB has been found to feed on the cones of over 40 conifer species and the seeds of hardwood species such as *Pistacia vera*, *Pistacia lentiscus* and *Amygdalus communis* (Fent & Kment, 2011; Lesieur et al., 2014; Uyemoto et al., 1986). Its polyphagous nature, combined with the high flight capacity of the adults, and anthropogenic activities (e.g., intercontinental movement of goods and people) makes the WCSB a successful global invader. This pest spread rapidly first outside its native range in North America and then across Europe, Asia, Africa and South America (Fent & Kment, 2011; Gall, 1992; Kim et al., 2020; Koerber, 1963; Olivera et al., 2020). In Europe, the WCSB has spread rapidly across most of the continent in only a decade (Lesieur et al., 2019) since it was first recorded in northern Italy in 1999 (Taylor et al., 2001). Recent records have indicated that the pest continues to spread across Asia (Kalashian et al., 2021), South Africa (Giliomee & Rayner, 2021), Europe (Golub et al., 2020; Van der Heyden, 2020) and Central and South America (Faúndez & Silvera, 2019; Kun & Masciocchi, 2019). This spread has raised questions as to the type of damage that it can impose on newly invaded ecosystems, such as damage to the natural regeneration of European coniferous forests (Lesieur et al., 2014; Tamburini et al., 2012) or economic damage to the stone pine nut sector of the Mediterranean basin (Bracalini et al., 2013). Here, stone pine nuts have made up part of the human diet since the Mesolithic period thanks to their nutritional properties (Evaristo et al., 2010; Salas-Salvadó et al., 2011). Today, they are considered a gourmet product due to their flavour and nutritional values (Mutke et al., 2013). The stone pine nut supply is limited given the strong masting habit of the species, resulting in excessively long intervals between good seed harvests (Calama & Montero, 2007), the method of collecting cones that are still harvested in the wild (Mutke et al., 2012) and the decline in kernel-per-cone-yield recorded since 2012. This yield loss, defined as 'Dry Cone Syndrome' (DCS), has been reported by cone pickers and processors in several Mediterranean countries and it refers to an increase in aborted unripe cones, empty seeds and seeds containing withered kernel remnants (Calama et al., 2020; Mutke et al., 2017).

In Spain, the production of stone pine nuts in shell decreased from more than 7000 tonnes in 2012 to less than 500 tonnes in 2020. This led to an increase in prices from 2.5 €/kg in 2012 to 6 €/kg in 2020 (MITECO, 2012, 2020), reaching a final kernel price over 60–70 €/kg (Calama et al., 2020). The high demand for stone pine nuts has been met by edible kernels of other pine species having higher exports and lower prices (Evaristo et al., 2010; INC, 2020), but with different flavour, nutritional values and processing quality (Mutke et al., 2013). Therefore, DCS is an important threat to the viability of the stone pine sector in the Mediterranean basin and has been associated with the WCSB (Bracalini et al., 2013; Calama et al., 2020; Farinha, Silva, et al., 2018; Roversi et al., 2011). Based on the distribution pattern of DCS, it could be associated with WCSB distribution in the main stone pine nut-producing countries of the Mediterranean basin (Bracalini et al., 2013; Calama et al., 2020; Mutke et al., 2017): Italy (1999), Spain (2003), Turkey (2009), Portugal (2010), Lebanon (2015) and Morocco (2015) (Fent & Kment, 2011; Gapon, 2015; Grosso-Silva, 2010; Nemer et al., 2019; Ribes & Escolà, 2005; Taylor et al., 2001). Damages described as DCS had not been recorded in studies on pine nut production before the arrival of WCSB in any country or region (Afonso et al., 2020; Loewe-Muñoz et al., 2019). However, in the Central Plateau, the area with the highest stone pine nut production in Spain (80%; MITECO, 2020), the historical kernel-per-cone yield decreased from 3.5% (1992–1999) to 1.8% following the WCSB invasion (2012–2016). In addition, cone and seed damage associated with DCS has been documented in stone pine stands in Spain and Italy following WCSB invasion (Bracalini et al., 2013; Calama et al., 2020; Roversi et al., 2011), and in controlled experiments with *Pinus pinea* L. cones bagged with insects (Farinha, Silva, et al., 2018). However, these authors were not able to prove that WCSB is the main causative agent of DCS.

Stone pine cones have a long ripening period (Abellanas, 1990). This favours the accumulation of cones and seeds damaged by extreme weather events (rainfall, winter frost or unusually low or high temperatures in key phases of cone development; Mutke et al., 2005a), resource depletion and cone and bud pests namely *Pissodes validirostris* (Coleoptera: Curculionidae), *Dioryctria mendacella* (Lepidoptera: Pyralidae), *Rhyacionia buoliana* (Lepidoptera: Tortricidae) and *Rhyacionia duplana* (Lepidoptera: Tortricidae) (Baixeras et al., 1996; Huerta et al., 2002; Naves et al., 2022; Sousa et al., 2017), and/or diseases such as *Sphaeropsis sapinea* (Ascomycota: Botryosphaeriaceae) (Luchi et al., 2012). In the Central

Plateau (Spain), the long-shoot buds of the stone pine begin to develop from April to June of year 0 of the growing season. By mid-May of year 1, the buds begin to develop female cones. Conelets are wind pollinated in early June when the cone scales begin to lignify. Second-year conelets begin to grow by late June of year 2. Finally, third-year cones grow from mid-April to mid-June of year 3, when they reach their final size and begin to lignify. Seeds are fertilized in late-April of the third year and fully mature by late fall (November) of that year when they can be marketed. In the field, cone opening and seed dispersal occur the following spring (Abellanas, 1990; Abellanas & Pardos, 1989; Mutke, 2000; Mutke et al., 2005b). Thus, during this maturation period, cones are also susceptible to feeding by the WCSB (Koerber, 1963). Knowing the type of damage caused by WCSB feeding at different stages of cone development is essential for making pest management decisions and preventing major crop damage (Strong, 2006). We hypothesize that WCSB feeding causes different types of damage to cones and seeds, depending on their phenological stage at the time of feeding. This hypothesis was tested by means of two experiments. The first trial consisted of bagging insects with cones of all ages at different phenological stages. The main objective was to identify the seasonal trends in cone mortality, cone yield, seed number and type of kernel damage. The second trial was based on the protection and unprotection of cones from feeding by the natural WCSB population throughout their phenological development. The main objective was to compare the above parameters between protected and unprotected cones to relate them to the WCSB feeding.

MATERIALS AND METHODS

Study site

Trials were conducted on a plot of grafted stone pine planted in 2008 in the Central Plateau (clone bank 'El Molinillo', Tordesillas, Valladolid, Spain; 41°29′57.8″ N 4°56′50.6″ W WGS84; 675 m). The plot consisted of 144 stone pines measuring 1–3 m in height, planted at a density of 6 × 6 m (278 trees/hectare). Lateral pollination of the pines was ensured by the old pine forests in the surrounding area. The plot was not fertirrigated, but weeds were removed annually. The climate of the study area is typical Mediterranean-Continental, with average annual temperatures ranging from 10.1 to 13.5°C and absolute values from −22 to 44°C. The average annual precipitation is 460 mm (range from 275 to 700 mm), with frost potentially occurring from September to May (Calama et al., 2011).

Bagged insect trials: Seasonal damage

Three trials were conducted, each with different age cones: third, second and first year, respectively (Table 1). Stone pine selection was based on the availability of cones of the same age (Bates et al., 2001), regardless of clones (Bates, Strong, & Borden, 2002; Strong, 2015). In March 2015, 20 pines with at least five third-year cones each were selected (Trial 1). These pines were divided into two groups to assign insect bagging dates. At the same time, two groups of 10 pines with a minimum of 3 or 5 second-year cones were selected (Trial 2). In March 2016, 10 additional pines with at least three third-year cones were selected to complete the sequence of insect bagging dates (Trial 1). As for the first-year cones (Trial 3), 10 pines with at least three cones of this age were selected in late June 2015 and 2016, after pollination in the Central Plateau (Mutke, 2000), to avoid a high proportion of empty seeds due to insufficient pollen due to the barrier of the bag (Schowalter, 1994), or even a high abortion of first-year cones caused by the large proportion of unpollinated seeds (Abellanas, 1990). Cones were randomly selected to be bagged with insects or to be bagged as protected control (one control per tree). The latter served as a reference for healthy cones and seeds (Bates, Strong, & Borden, 2002). Cones of all ages were bagged at the time of selection in grey polyethylene bags (1 × 1 mm mesh), since the bag's microclimate does not affect cone yield (Strong et al., 2001).

Two female imagoes, which cause more damage than males (Bates et al., 2001; Bates, Borden, Kermode, & Bennett, 2000; Bates, Lait, et al., 2002; Bates, Strong, & Borden, 2002), were bagged for 2 weeks with one third- or second-year cone (Trial 1 and 2). To avoid a decrease in predation, females were checked weekly and those that

TABLE 1 Parameters of bagged insect trials (WCSB *N* and stage, cone age, number of stone pines, number of cones bagged with insects and cones protected from the natural WCSB population per pine and insect bagging dates).

Trial	WCSB *N* and stage	Cone age	*N* pines	*N* cones per pine (bagged: protected)	Insect bagging dates
1	Two female imagoes	Third	10	4:1	Apr. 10–24; May 7–21; Jun. 4–18; Jul. 2–16
			10	4:1	Apr. 24–May 7; May 21–Jun. 4; Jun. 18–Jul. 2; Jul. 16–30
			10	2:1	Aug. 4–18; Sep. 1–15
2	Two female imagoes	Second	10	4:1	May 7–21; Jun. 4–18; Jul. 2–16; Aug. 20–Sep. 3
			10	2:1	Aug. 4–18; Sep. 1–15
3	Two nymphs	First	10	2:1	Jul. 2–9; Aug. 20–27
			10	2:1	Jul. 14–21; Jul. 28–Aug. 4

died or were missing were replaced. Lost insects were assumed to be dead (Bates, Strong, & Borden, 2002). Trials with females began in mid-April, when WCSB imagoes emerge from hibernation and begin to reproduce (Barta, 2016; Ponce-Herrero et al., 2022; Tamburini et al., 2012). Two nymphs (N3 and N4 stages) were bagged with one first-year cone for 1 week (Trial 3). Early stage nymphs (N1 and N2 stages) were not used as these are the least damaging stages (Strong, 2006). Trials with nymphs began in early July after the first-year cones are pollinated and lignified (Mutke, 2000).

Female WCSB imagoes and nymphs were obtained from a permanent colony of WCSB reared in the laboratory of the Department of Vegetal Production and Forest Resources (University of Valladolid, Palencia, Spain). Since 2014, these insects have been collected annually by foresters in the Castile and Leon region (Spain). The colony has been kept under laboratory conditions and with natural daylight in rectangular breeding boxes (47.5 × 47.5 × 93 cm, 160 μm mesh) (Entomopraxis, Barcelona, Spain). Ad libitum shelled *P. pinea* seeds were provided as a food source, while several young potted *Pinus halepensis* Mill. (2–3 years old) was used as water source and oviposition substrate.

Cone mortality was recorded weekly during the growing season in which the cones were bagged with insects. Simultaneously, external macroscopic marks on the surface of the cones were searched for. These could be associated with WCSB feeding by comparison with controls. Mortality of second- and first-year cones was also recorded at the beginning of the next stages. First- and second-year cones were classified as aborted when the scales appeared shrunken and greyish in colour or when the cone could be detached with a light touch. Third-year cones were classified as aborted if they were small compared to the rest, if their scales appeared shrunken or if they were not greenish (Figure 1).

Exclusion trial: Damage caused by the natural WCSB population

Thirty pines with at least 10 first-year cones were randomly selected after pollination in late July 2016. Five cones were bagged (protected cones: $n = 150$), and five were marked with a label (unprotected cones: $n = 150$). Cone mortality was recorded in February 2017, at the beginning of the second developmental year, in March 2018, at the start of the third developmental year, and in November 2018, after maturation of the cones, when surviving cones were harvested. The WCSB was present in the study area, but its population density was not defined.

Cone and seed processing

Once mature, all cones from the three trials were collected in mid-November. Three of the 10 second-year cones bagged with insects from 24 April to 7 May ($n = 7$) and from 21 May to 4 June ($n = 7$) were collected unripen for another experiment (unpublished).

Cones were examined for symptoms of infestation with *P. validirostris*, *D. mendacella* (Calama et al., 2017) or *S. sapinea* (Tiberi, 2007). Cones without these symptoms were placed individually in a bag with an identification label and were processed based on Calama et al. (2020). Cone fresh weight (FW) was recorded the day after harvest, while cone dry weight (CDW) was recorded after the drying process (45°C until the cones were fully opened). A scale with an accuracy of 1 g was used. All seeds were manually extracted from the cones and counted. Those less than 6 mm in length, mainly from the non-fertile basal bracts (Abellanas, 1990), were considered as empty for normal reasons and removed. All seeds longer than 6 mm in length were extracted from the testa (hereafter referred to as kernels) using a manual nutcracker. Kernels were classified according to the categories defined by Farinha, Silva, et al. (2018) as aborted or undeveloped and empty seeds, without embryo and endosperm; type I or sound embryo and damaged endosperm. This category was divided into type Ip or partially damaged endosperm, and type It or totally deformed, wrinkled or degraded endosperm; type II or dry embryo with no endosperm. This category was divided into type IIf or tegmen fully fused to testa, and type IIu or not fused and deformed tegmen (Figure 2). Sound kernels were weighed with 0.1 g precision.

FIGURE 1 Aborted cones. (a) Third-year cone bagged with two female imagoes on 10–14 April (left) next to a third-year cone not bagged with insects (right), photographed on 21 May. (b) Second-year cone bagged with two female imagoes on 7–21 May (right) next to a second-year cone not bagged with insects (left), photographed on 4 June. (c) First-year cone bagged with two nymphs on 2–9 July. Healthy cone photographed on 9 July (left). Aborted cone photographed 1 week later (right).

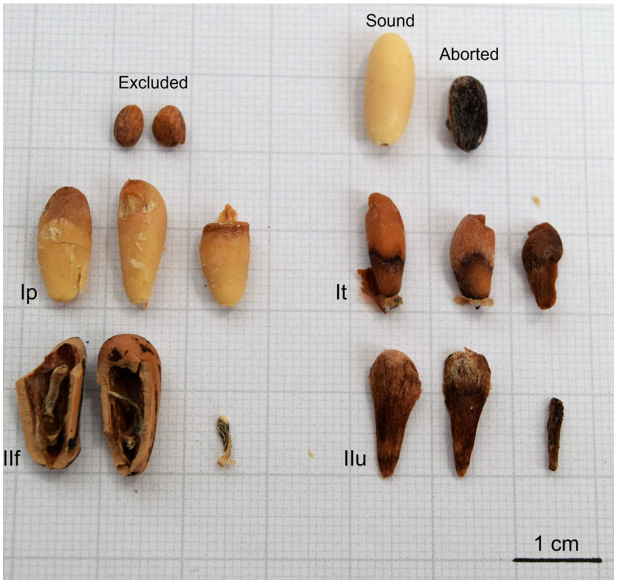

FIGURE 2 Classification of seeds and kernels. (Excluded) Seed less than 6 mm in length; (Sound) marketable kernels; (Aborted) undeveloped and empty seeds, without embryo and endosperm; (I) sound embryo and damaged endosperm. This category was divided into (Ip) partially damaged endosperm or (It) totally deformed, wrinkled or degraded endosperm; (II) dry embryo with no endosperm. This category was divided into (IIf) tegmen fully fused to testa or (IIu) not fused and deformed tegmen.

Definition of the parameters and statistical analysis

WCSB survival was calculated as the percentage of 2-week mean survival for Trials 1 and 2 and as the percentage of mean survival for Trial 3 (1 week).

Cone mortality was calculated as the percentage of aborted cones divided by the total number of cones by bagging date. This parameter was analysed using a generalized linear mixed model with binary distribution and logit link function for the probability of death (GLIMMIX procedure in the SAS software) in bagged insect trials. Cones were the experimental units; pines were considered as a random factor and bagging dates were the between-subjects factor. For the exclusion trial, cone mortality was analysed using a GLIMMIX for probability of death with binomial distribution. The dependent variables were the number of aborted cones by age. Cones were the experimental unit, and the treatment (protected and unprotected) and the age of death (first-year, second-year and third-year, alive) were the within-subject factors.

Mean of seeds was expressed as the mean number of seeds by bagging date in the bagged insect trials or by treatment in the exclusion trial.

Kernel-per-cone yield (ρ), hereafter referred to as *kernel yield*, was calculated per cone as the *final kernel-per-cone-yield in the fresh cone* using Equation (1) and presented as mean by treatment. Aborted cones were excluded.

$$\rho = (\mathrm{SCW}/\mathrm{CFW}^{*})\cdot 100 \tag{1}$$

where SCW = weight of sound kernels (g); CFW* = standardized cone fresh weight (g), defined in Equation (2) as the cone weight at 37% humidity (November harvest) (Calama et al., 2020).

$$\mathrm{CFW}^{*} = \mathrm{CFW}\cdot[(1-H)/0.63], \tag{2}$$

where CFW = cone fresh weight (g); H = humidity, which is defined in Equation (3).

$$H = (\mathrm{CFW}-\mathrm{CDW})/\mathrm{CFW}, \tag{3}$$

where CDW = cone dry weight (g).

Mean of seeds and *kernel yield* were analysed using a GLIMMIX with a Gaussian distribution and the identity link function. Live cones were the experimental units. Pines were considered a random factor, and bagging dates or treatments were the between-subjects factors.

Percentage of kernels was calculated as the number of kernels by type divided by the total number of kernels for cones and presented as the mean (95% confidence limits) by bagging date or treatment. All of these parameters were analysed with a GLIMMIX procedure with binomial distribution. Dependent variables were the number of kernels per type and cone. Live cones were the experimental units, bagging dates or treatments were the between-subject factors and pines were considered a random factor.

Statistical analysis was performed using SAS 9.4. software (http://www.sas.com, Statistical Analysis System, RRID:SCR_008567).

RESULTS

No external macroscopic marks were recorded on the surface of the cones that were bagged with insects as compared to the protected control cones. No cones showed symptoms of *S. sapinea* or *P. validirostris*. Four protected and one unprotected cones were discarded due to *D. mendacella* infestation in the exclusion trial.

Bagged insect trials: Seasonal damage

Trial 1: Third-year cones bagged with female imagoes

Figure 3a,b shows the mean values of cone mortality (%) and kernel yield (%), while Table 2 shows the mean values of seeds, and type of kernels (%) per insect bagging date for Trial 1. Values of p-value and t-value for each parameter and insect bagging date are shown in supplementary S1 to S9.

Damage caused by WCSB feeding decreased as cone development progressed during the third year (Figure 4). Cone mortality was

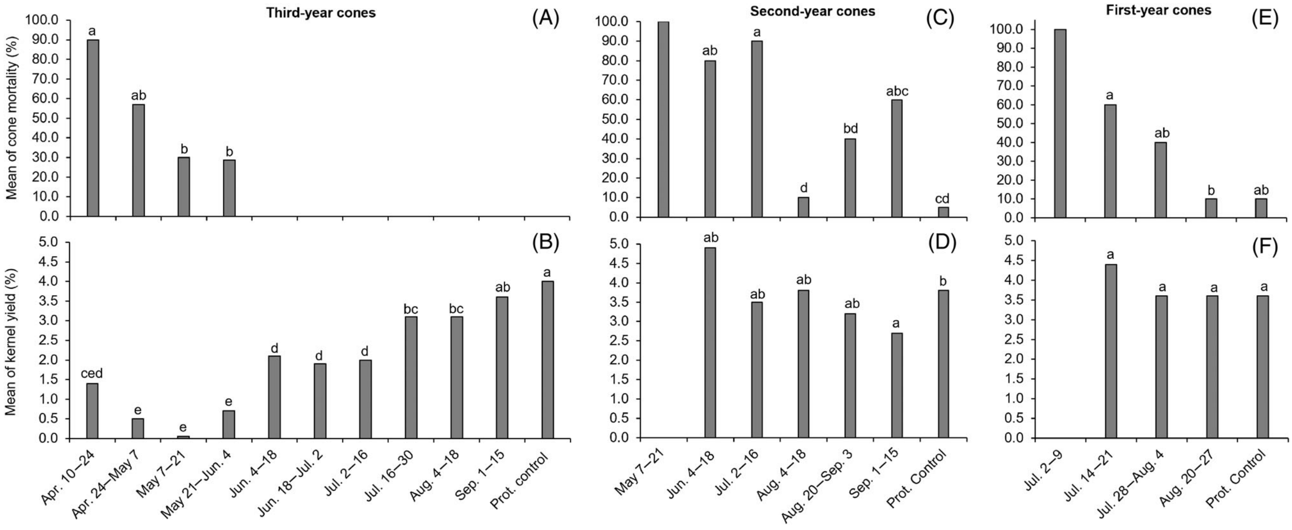

FIGURE 3 Cone mortality and kernel yield. Mean of cone mortality (%) and kernel yield (%) of the Trial 1: third-year cones bagged with two female imagoes (a and b); Trial 2: second-year cones bagged with two female imagoes (c and d) and Trial 3: first-year cones bagged with two nymphs (e and f). Columns in the same graphic with the same letter are not significantly different (*t* test; *p*-value < 0.05).

TABLE 2 Trial 1: Third-year cones bagged with two female imagoes (mean of number of seeds and mean percentage [95% confidence limits] of type of kernels [%] by insect bagging date according to the severity of the damage).

Insect bagging date (*n* = cones)	Seeds	Type of kernels					
		Aborted	IIf	IIu	It	Ip	Sound
Apr. 10–24 (*n* = 10)	51.0 (9.9, 93.5) bdef	70.5 (16.7, 96.6) abcd	0.0	2.0 (0.0, 76.5) abce	0.0	0.0	27.5 (3.6, 79.5) acfg
Apr. 24–May 7 (*n* = 7)	98.6 (73.9, 123.3)abe	85.5 (61.7, 95.6) ab	0.0	2.0 (0.2, 13.6) cf	0.0	0.0	12.5 (3.9, 33.8) fg
May 7–21 (*n* = 10)	76.8 (60.1, 93.5) bcdef	93.2 (78.0, 98.2) a	0.7 (0.0, 0.1) cd	1.1 (0.1, 7.9) defg	0.0	0.2 (0.0, 14.0)a	4.8 (1.2, 17.9)g
May 21–Jun. 4 (*n* = 7)	89.9 (70.4, 109.4)af	68.5 (49.3, 82.9) b	10.0 (4.6, 20.3)c	0.9 (0.1, 10.0) cgh	0.2 (0.0, 5.4) aef	3.8 (1.3, 10.6)a	16.6 (7.4, 33.4) fg
Jun. 4–18 (*n* = 10)	94.6 (80.3, 108.8)ad	16.0 (8.6, 27.8) ce	37.5 (29.5, 45.0)a	1.0 (0.2, 4.9) efh	0.5 (0.1, 2.2) bcdf	1.7 (0.6, 5.1)a	43.3 (32.1, 55.3)ce
Jun. 18–Jul. 2 (*n* = 10)	102.9 (88.6, 117.2)a	32.8 (22.1, 45.6) d	24.7 (18.1, 32.7)b	6.1 (3.3, 11.2) cd	2.1 (1.0, 4.2) ac	3.5 (1.6, 7.2)a	30.8 (21.2, 42.5)def
Jul. 2–16 (*n* = 10)	92.3 (78.0, 106.5)ae	17.4 (9.6, 29.6) de	2.3 (0.7, 7.0)d	32.0 (24.9, 40.0)a	2.4 (1.2, 4.7) ab	2.1 (0.8, 5.6)a	43.8 (32.4, 55.8)cd
Jul. 16–30 (*n* = 10)	102.1 (87.8, 116.4)ag	20.8 (12.3, 33.0) de	0.5 (0.0, 5.3)d	17.8 (12.5, 24.7)b	2.0 (1.0, 4.1) ad	2.6 (1.1, 6.1)a	56.3 (44.5, 67.4)bc
Aug. 4–18 (*n* = 10)	74.7 (60.5, 89.0) befg	13.9 (6.4, 27.6)e	1.5 (0.3, 7.3)d	9.0 (4.9, 15.9) c	4.0 (2.2, 7.1) a	4.1 (1.8, 9.0)a	67.5 (53.7, 79.9)ab
Sep. 1–15 (*n* = 10)	97.3 (83.1, 111.6)ac	10.7 (4.9, 21.8)e	0.8 (0.1, 5.4)d	9.1 (5.4, 14.9) c	1.0 (0.4, 2.9) bcde	4.5 (2.3, 8.7)a	73.9 (62.5, 83.0)a
Prot. control (*n* = 30)	96.7 (88.1, 105.3)a	14.7 (10.2, 20.9) e	0.4 (0.1, 2.0)d	4.9 (3.2, 7.4) ce	0.3 (0.1, 1.0) ef	3.2 (2.0, 5.0)a	76.5 (70.1, 81.8)a

Note: (II) Kernels with dry embryo with no endosperm. This category was divided into (IIf) kernels with tegmen fully fused to testa and (IIu) not fused and deformed tegmen. (I) Kernels with sound embryo and damaged endosperm. This category was divided into (Ip) kernels with partially damaged endosperm and (It) totally deformed, wrinkled or degraded endosperm. Means in the same column for stage followed by the same letter are not significantly different (*t* test; *p*-value < 0.05).

only recorded on cones bagged with insects from early April to early June. The lowest mean kernel yield was recorded at this time, and from April till August, the mean kernel yield was significantly lower in the bagged cones compared to control. This trend was not observed in the end of the season in September. The mean number of seeds per cone was only significantly lower than the protected controls in

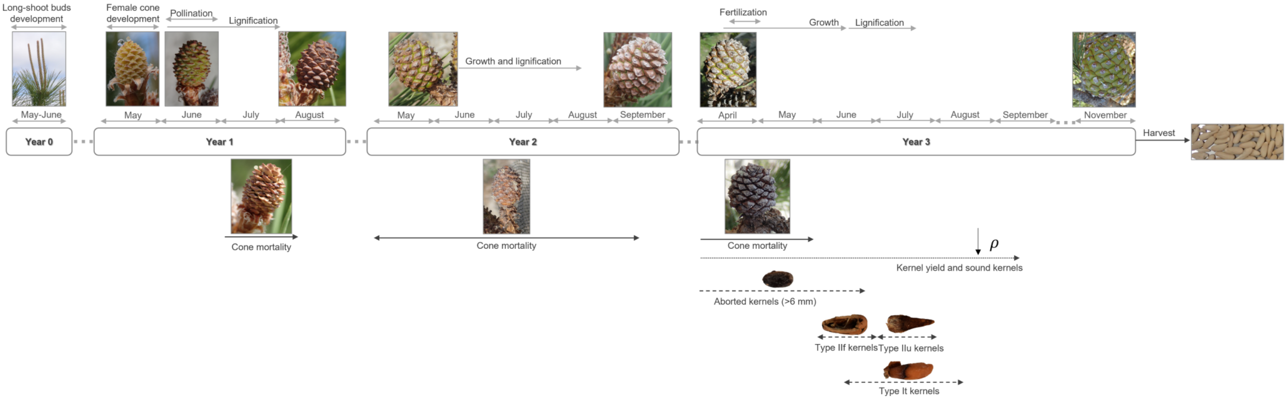

FIGURE 4 Sequence of damage along phenological development of pine cones caused by western conifer seed bug feeding.

the cones bagged in early August, early May, and in April, noting that for the latter date only one cone survived. The mean proportion of sound kernels of cones bagged with insects was significantly lower than that of protected cones from April to late July. This proportion gradually increased from the beginning of July. Except for the bagging dates of 4–18 June and 2–16 July, when IIf and IIu damage were the highest, respectively, the predominant type of damage was aborted kernels, even in the protected controls. Focusing on each type of damage, the mean proportion of aborted kernels was significantly higher than for the protected control in cones bagged with insects from April to early July, except for the 18 June–4 July date. In June, the mean percentage of type IIf kernels was significantly higher in the cones bagged with insects than in the protected controls, with the highest proportion of type IIu kernels being recorded in July, when it was also significantly different from the protected controls. Finally, the mean proportion of type It kernels in the bagged controls was significantly higher than protected controls from mid-June to mid-August.

Trial 2: Second-year cones bagged with female imagoes

Mean values of cone mortality (%) and kernel yield (%) are given in Figure 3c,d, while mean values of seeds and type of kernels (%) per bagging date of Trial 2 are given in Table 3. Values of *p*-value and *t*-value for each parameter and insect bagging date are shown in supplementary S10 to S18.

Cone mortality was the greatest damage caused by WCSB feeding on second-year cones. The mean proportion of kernel yield was only significantly lower for cones bagged with insects in early September as compared to protected controls, while the mean number of seeds and the mean proportion of sound kernels did not significantly decrease at any of the bagging dates compared to the controls. Considering the bagging dates with at least three surviving cones, such as those from August, even in the protected controls, the predominant damage was aborted kernels. Focusing on each type of damage, the mean percentage of aborted kernels was significantly higher in the cones bagged with insects in early August and early September as compared to the protected controls. Differences between Ip and control damage in July were not considered because only two cones survived at that time.

Trial 3: First-year cones bagged with nymphs

Mean values of cone mortality (%) and kernel yield (%) are given in Figure 3e,f, while mean values of seeds and type of kernels (%) per bagging date for Trial 3 are shown in Table 4. Values of *p*-value and *t*-value for each parameter and insect bagging date are shown in supplementary S19 to S27.

Cone mortality was the greatest damage caused by WCSB feeding on first-year cones and decreased as cone development progressed.

Considering the bagging dates with at least three surviving cones, such as those from August, even in the protected controls, the predominant damage was aborted kernels. Focusing on each type of damage, the mean proportion of aborted kernels was significantly higher than that of the protected control in cones bagged with insects in early September. For cones bagged with insects at the end of July, the mean percentage of type IIf and It kernels was significantly higher than that of the protected cones.

Exclusion trial: Damage caused by the natural WCSB population

Mean values of cone mortality (%), seeds, kernel yield (%) and type of kernels (%) per bagging date for Exclusion trial are shown in Table 5. Values of *p*-value and *t*-value for each parameter and treatment are shown in supplementary S28.

Cone mortality was significantly higher for unprotected cones than for protected ones in the first and second year of development. The mean proportion of kernel yield, number of seeds per cone and sound kernels were significantly lower in unprotected cones, while

TABLE 3 Trial 2: Second-year cones bagged with two female imagoes (mean of number of seeds and mean percentage [95% confidence limits] of type of kernels [%] by insect bagging date according to the severity of the damage).

		Type of kernel					
Insect bagging date (n = cones)	**Seeds**	**Aborted**	**IIf**	**IIu**	**It**	**Ip**	**Sound**
May 7–21 (n = 10)	-	-	-	-	-	-	-
Jun. 4–18 (n = 10)	102.7 (67.8, 137.6)a	6.5 (2.1, 18.5)bc	1.2 (0.0, 35.0)a	9.9 (3.4, 25.6)a	3.3 (0.4, 21.1)a	0.008 (0.01, 6.4)b	78.2 (55.9, 91.0)ab
Jul. 2–16 (n = 10)	90.5 (43.9, 137.1)a	9.4 (2.2, 31.7)ab	0.0	2.8 (0.1, 39.2)a	0.9 (0.0, 75.0)a	9.3 (3.7, 21.7)a	77.6 (42.3, 94.2)ab
Aug. 4–18 (n = 10)	82.4 (62.8, 102.0)a	23.7 (17.0, 32.1)a	3.9 (1.1, 13.2)a	4.8 (1.9, 11.9)a	1.1 (0.1, 8.9)a	0.0	66.5 (53.1, 77.6)b
Aug. 20-Sep. 3 (n = 10)	100.9 (78.3, 123.4)a	6.2 (3.1, 11.8)b	1.0 (0.0, 10.6)a	5.6 (2.4, 12.4)a	0.1 (0.0, 30.7)a	1.1 (0.4, 3.2)b	86.0 (74.8, 92.7)a
Sep. 1–15 (n = 10)	89.5 (63.9, 115.0)a	17.2 (9.6, 28.9)c	0.3 (0.0, 67.4)a	12.2 (5.3, 25.7)a	4.5 (0.9, 18.0)a	0.9 (0.2, 5.0)b	64.9 (45.8, 80.2)b
Prot. control (n = 20)	96.8 (81.3, 112.4)a	8.0 (5.6, 11.4)b	1.3 (0.4, 4.9)a	7.7 (5.6, 11.4)a	2.8 (1.3, 6.2)a	0.9 (0.4, 1.9)b	79.3 (72.1, 84.9)ab

Note: (II) Kernels with dry embryo with no endosperm. This category was divided into (IIf) kernels with tegmen fully fused to testa and (IIu) not fused and deformed tegmen. (I) Kernels with sound embryo and damaged endosperm. This category was divided into (Ip) kernels with partially damaged endosperm and (It) totally deformed, wrinkled, or degraded endosperm. Means in the same column for stage followed by the same letter are not significantly different (t test; p-value <0.05).

TABLE 4 Trial 3: First-year cones bagged with two nymphs (mean number of seeds and mean percentage [95% confidence limits] of type of kernels [%] by insect bagging date according to the severity of the damage).

		Type of kernel					
Insect bagging date (n = cones)	**Seeds**	**Aborted**	**IIf**	**IIu**	**It**	**Ip**	**Sound**
Jul. 2–9 (n = 10)	-	-	-	-	-	-	-
Jul. 14–21 (n = 10)	104.8 (82.4, 127.1)ab	9.2 (5.2, 15.7)b	0.5 (0.0, 9.1)ab	1.3 (0.2, 10.2)a	0.5 (0.0, 17.8)ab	0.3 (0.0, 98.0)a	88.3 (67.4, 97.5)a
Jul. 28–Aug. 4 (n = 10)	105.07 (86.6, 123.5)a	11.5 (7.9, 16.7)b	5.2 (2.5, 10.2)a	9.2 (5.0, 16.3)a	6.6 (3.0, 14.0)a	0.3 (0.0, 75.7)a	67.2 (48.3, 81.7)a
Aug. 20–27 (n = 10)	80.6 (65.3, 95.8)b	19.5 (14.6, 25.5)a	0.9 (0.2, 4.6)ab	9.4 (5.2, 16.4)a	0.9 (0.1, 7.0)ab	0.6 (0.0, 44.4)a	68.7 (50.5, 82.7)a
Prot. control (n = 20)	94.5 (83.4, 105.6)ab	13.5 (10.7, 16.7)b	1.2 (0.5, 2.9)b	8.2 (5.6, 12.2)a	0.7 (0.2, 3.1)b	6.8 (1.0, 11.6)a	72.7 (61.5, 81.6)a

Note: (II) Kernels with dry embryo with no endosperm. This category was divided into (IIf) kernels with tegmen fully fused to testa and (IIu) not fused and deformed tegmen. (I) Kernels with sound embryo and damaged endosperm. This category was divided into (Ip) kernels with partially damaged endosperm and (It) totally deformed, wrinkled or degraded endosperm. Means in the same column for stage followed by the same letter are not significantly different (t test; p-value <0.05).

TABLE 5 Exclusion trial (mean of cone mortality [%], mean of seeds, mean of kernel yield [%] and mean percentage [95% confidence limits] of type of kernels [%] by treatment according to the severity of the damage).

Treatment (*n* = cones)	Mortality			Kernel yield	Seeds	Type of kernel					
	First	Second	Third			Aborted	IIf	IIu	It	Ip	Sound
Prot. cones (*n* = 146)	0.7a	2.7a	8.2a	3.8a	105.3a	14.5 (11.5, 18.2)a	4.9 (3.5, 6.8)a	1.8 (1.1, 2.9)a	2.5 (1.5, 4.1)a	0.6 (0.4, 0.9)a	73.6 (68.2, 78.3)a
Unp. cones (*n* = 149)	18.1b	43.6b	8.7a	1.8b	82.0b	19.0 (14.7, 24.3)b	15.1 (10.2, 21.6)b	17.5 (11.6, 25.6)b	3.7 (1.8, 7.3)a	4.2 (2.8, 6.2)b	35.6 (27.9, 44.0)b

Note: (II) Kernels with dry embryo with no endosperm. This category was divided in (IIf) kernels with tegmen fully fused to testa and (IIu) not fused and deformed tegmen. (I) Kernels with sound embryo and damaged endosperm. This category was divided in (Ip) kernels with partially damaged endosperm and (It) totally deformed, wrinkled or degraded endosperm. Means in the same column for stage followed by the same letter are not significantly different (*t* test; *p*-value <0.05).

the percentage of kernel damage was significantly higher in unprotected cones, except for type It kernels.

DISCUSSION

WCSB feeding on stone pine cones caused damage ranging from cone abortion at all ages to a reduction in the number of marketable kernels, depending on the phenological development of the cones (Lesieur et al., 2014). This damage is consistent with that associated with the DCS (Calama et al., 2020; Farinha et al., 2021; Farinha, Silva, et al., 2018; Mutke et al., 2017). According to Bates, Lait, et al. (2002) and Bates, Strong, and Borden (2002), WCSB can cause cone and sound seed loss throughout cone development. Thus, as Bracalini et al. (2013) reported, it is 'one of the most harmful pests for stone pine nut production' in the Mediterranean basin.

Cone mortality

Seasonal trends in cone mortality were observed in first- and third-year cones bagged with insects. A high cone mortality being reported early in the growing season and decreasing as the bagging date advanced (Figure 4), according to Farinha, Silva, et al. (2018), who reported that third-year cones bagged with insects from early June to mid-September survived. These trends have also been reported on cones of distinct conifer species (Bates, Borden, Kermode, & Bennett, 2000; Bates, Strong, & Borden, 2002; Connelly & Schowalter, 1991; Schowalter & Sexton, 1990) and are probably due to the progressive lignification of the scales (Mutke, 2000) during the cone development. No seasonal trends were reported for cone mortality of second-year cones bagged with insects (ranging from 40.0% to 100.0%), including the value reported by Roversi et al. (2011) for stone pine cones (80.0%). The low cone mortality recorded in early August (10.0%) was closer to that recorded in Portugal for the same month (18.4%), which was associated with the physiological or reproductive stage of the WCSB (Farinha, Silva, et al., 2018). It is not possible, however, to confirm this condition given the higher proportion of aborted kernels and It kernels associated with the WCSB at that time (Tables 2, 3, and 4).

In the exclusion trial, cone mortality of the unprotected cones in the first (18.1%) and third (8.7%) year was not related to feeding by the natural WCSB population. That is because these values were lower than those recorded in our bagged insect trials (first-year cones: range from 40.0% to 100.0% in July: third-year cones: range from 28.6% to 90.0% in April and May). Even though we do not know the natural WCSB population density, the influence of the cone mortality of the first-year cones could not be completely excluded since the cone mortality of unprotected cones (18.1%) was significantly higher than that of protected cones (0.7%). Finally, second-year cone mortality was clearly related to this pest, with the high mortality rate of the unprotected cones (43.6%) being into the range recorded by us in the bagged insect trials (range from 40.0% to 100.0%, except in early August).

Number of seeds

In the exclusion trial, the unprotected cones had significantly fewer seeds (82.0 ± 2.8) than the protected ones (105.3 ± 1.6), as was the case in the studies on *P. ponderosa* and *P. contorta* stands with natural WCSB populations (Pasek & Dix, 1988; Strong et al., 2001). However, no seasonal trends were observed in our insect bagging trials. Only third-year cones bagged from 10–24 April, 7–21 May and 4–18 August had significantly lower mean seed contents than the protected controls (note that only one cone survived the first date). In contrast, other pine species such as *P. monticola* (23.0%) or *P. contorta* (75.0%) showed a significant reduction in total seeds per cone when immature cones were exposed to WCSB feeding, even on a seasonal basis for the latter species (Bates, Strong, & Borden, 2002; Connelly & Schowalter, 1991; Strong, 2006).

Kernel yield and type of kernels

In the exclusion trial, the proportion of kernel yield (3.8%) and sound kernels (73.6%) of the protected cones was significantly higher than unprotected cones (kernel yield: 1.8%; sound kernels: 35.6%). This is in accordance with the data from the Central Plateau following the WCSB invasion (kernel yield: 1.8%) (Calama et al., 2020), but it is lower than that recorded by Farinha, Silva, et al. (2018) in protected (sound kernels: 94.0%) and unprotected (70.0%) stone pine cones in Portugal. This last comparison could not be related to WCSB population density among the study sites because we did not define this parameter.

Seasonal trends in the proportion of sound kernels, and therefore in kernel yield, were not observed for the first- and second-year cones bagged with insects. In contrast to other studies on *P. contorta*, *P. monticola* and *P. menziesii* cones (Bates, Lait, et al., 2002; Bates, Strong, & Borden, 2002).

On the other hand, seasonal trends were observed in the third-year cones bagged with insects (Figure 4), in accordance with the bagged insect trials conducted by Connelly and Schowalter (1991) on *P. monticola* cones. The proportion of sound kernels gradually increased from early July, equalling the protected controls from August. This increase may be related to seed development, as sclerotesta lignification and seed reserve accumulation begin in mid-July and reach full maturity in the late fall (Mutke, 2000). The kernel yield also increased gradually over the growing season (Figure 3) as of mid-June (from 1.9% to 3.6%), but never reached the proportion of the protected controls (4.0%). Considering the kernel-per-cone-yield recorded in the Central Plateau before (3.5%) and after (1.8%) the WCSB invasion (Calama et al., 2020), we can conclude that the kernel yield of third-year cones is affected by the feeding of the WCSB.

In the exclusion trial, the total proportion of damaged kernels of the unprotected cones (64.4%) was higher than that recorded in the Central Plateau after the WCSB invasion (49.2%). So, it could be associated with the feeding of the natural WCSB population. By type of kernels, the proportion of aborted kernels was significantly lower in the protected cones (14.0%) as compared to the unprotected ones (19.0%). This result was in line with similar studies on *P. ponderosa* and *P. menziesii* cones in North America (Pasek & Dix, 1988; Schowalter, 1994), but unlike the findings of Farinha, Silva, et al. (2018) who reported a proportion of aborted kernels of under 2.0% for protected and unprotected cones. We agree, however, with these authors, in terms of the proportion of type II kernels in exposed cones (20.6%) as compared to protected ones (4.6%). For type I kernels, significant differences were only found for type Ip kernels. Once again, Farinha, Silva, et al. (2018) also considered this type of damage as an indicator of seed bug activity (exposed cones: 7.1%; protected cones: 0.8%).

Seasonal trends by type of kernels were not observed in first- and second-year cones bagged with insects. Occasionally, the proportions of one type of kernel were higher than the protected controls. These may be the result of mechanical damage to cone tissue in the immature cones (Abellanas, 1990), probably associated with WCSB feeding.

Seasonal trends for type of kernels were evident in third-year cones bagged with insects: aborted, IIf, IIu, It and Ip (Figure 4). Damaged kernels may be associated with WCSB feeding from April to early July (ranging from 95.2% to 56.2%), as the addition of the proportion of all types of damaged kernels was higher than that recorded in the Central Plateau stands after the WCSB invasion (49.2%; Calama et al., 2020). The total proportion of damaged kernels (ranging from 26.1% to 43.7%) from mid-July onwards was not as clearly associated with WCSB feeding. This total proportion of damaged kernels was similar to that recorded for the protected controls of the bagged insect trials (23.6%) and in the Central Plateau stands prior (13.4%) and following (49.2%) the WCSB invasion.

Early season treatments (from April to early June) revealed high proportions of aborted kernels (ranging from 68.5% to 93.2%), according to similar studies with *P. contorta* and *P. menziesii* cones (Bates, Borden, Kermode, & Bennett, 2000; Connelly & Schowalter, 1991; Schowalter & Sexton, 1990; Strong, 2006; Strong et al., 2001). However, the proportion of aborted kernels reported by Farinha, Silva, et al. (2018) from August (1.5%) was lower than that recorded by us (13.9%).

The highest proportions of kernels type IIf were recorded from late May to early July (ranging from 10.0% to 37.4%) when sclerotesta begins to lignify. Given that the highest proportions of kernels type IIu were recorded in early (32.0%) and late July (17.8%), when the physiological process of protective tissue differentiation is more advanced (Abellanas, 1990), the degree of protective tissue collapse (Pasek & Dix, 1988) appears to depend on the time of feeding (Bates, Borden, Kermode, & Bennett, 2000). It is possible that this last type of damage (no endosperm) may have been associated with WCSB feeding before the appearance of storage product precursors in the seeds (Bates et al., 2001), making it impossible to initiate the accumulation of reserve substances (endosperm). Despite the lignification of the sclerotesta, damaged kernels were recorded starting in July, which is possible since WCSB adults can introduce the proboscis into the hard tissues of pine nuts (Farinha et al., 2017).

The appearance of kernels with damaged endosperm (I) was like that recorded by Farinha, Durpoix, et al. (2018) in laboratory feeding trials. The proportion of this type of kernels was increased from early August, in line with Connelly and Schowalter (1991) in *P. contorta* trials. The largest percentage of this type of damage was found in early August (It: 4.0%; Ip: 4.1%), closer to the proportion recorded by Farinha, Silva, et al. (2018) in August (6.9%). Endosperm damage could be related to WCSB feeding after activation of storage product precursors (Bates et al., 2001), causing these substances to occupy the entire seed volume (Abellanas, 1990). *Pseudotsuga menziesii* seeds can resume lipid and protein synthesis before WCSB feeding until the middle of cone development (Bates et al., 2001). Therefore, it is possible that the pine seeds that were fed upon by the WCSB were able to continue to develop the endosperm even with partial damage. Because in the cones bagged with insects from 1 to 15 September, the proportion of kernels type Ip was maintained (4.5%) and that for type It decreased to 1.0%.

Finally, considering the decrease in kernel damage intensity observed as the growing season progressed (Figure 4), the intensity of kernel damage may depend on the development of the seed that was fed on by the WCSB (Bates et al., 2001; Connelly & Schowalter, 1991; Schowalter & Sexton, 1990) and less on the intensity of the WCSB feeding (Bates, Borden, Savoie, et al., 2000). Although this intensity cannot be excluded (Strong, 2015) since seasonal damage may depend on the voracity of the nymphs and adults throughout their biological cycle. For example, it is considered higher for females during the reproductive period given their greater need for nitrogen for egg production (Bates et al., 2001; Bates, Lait, et al., 2002; Bates, Strong, & Borden, 2002), as compared to their needs at the onset of wintering (Bates et al., 2001; Bates, Borden, Kermode, & Bennett, 2000; Strong, 2006).

Note that all types of damaged kernels were recorded in the protected controls, so it cannot be excluded that some of the damage was due to climatic conditions, tree physiology or other biotic causes (Calama et al., 2020; Farinha, Silva, et al., 2018).

Seasonal damages and WCSB management

Our results may be useful for management decisions aimed at reducing WCSB damage in cultivars and stands of stone pine located in the Central Plateau. According to our results and those of Bates, Lait, et al. (2002) and Bates, Strong, and Borden (2002), controlling WCSB populations early in the growing season may help prevent severe cone and seed damage. Thus, control methods should be applied early in the season (from April to early June), coinciding with the end of the overwintering period of WCSB adults (Ponce-Herrero et al., 2022). WCSB population management, however, should ensure cone protection throughout the growing season since cultivars are susceptible to reinfestation by adults from nearby stands, and even other pine species such as *Pinus pinaster* in the Central Plateau, due to the high dispersal capacity of the adult given its polyphagous nature and its ability to fly (Connelly & Schowalter, 1991; Fent & Kment, 2011; Koerber, 1963).

Domestication of the stone pine, based on the grafted experimental plantations established over the past 50 years in several Mediterranean countries (Mutke et al., 2005b; Mutke et al., 2012), may increase the final yield of stone pine crops by planting highly productive genotypes (Mutke et al., 2005a). The establishment of cultivars may enable the management of the stands for seed production (Afonso et al., 2020; Farinha, Silva, et al., 2018; Loewe-Muñoz et al., 2023), the application of specific horticultural techniques (Mutke et al., 2013) and even the control of the local WCSB population through mass trapping (Blatt & Borden, 1996a; Millar et al., 2022), biological control (Ponce-Herrero et al., 2022, 2023; Roversi et al., 2011) or spot application of insecticides or pollinator bags as protective barriers (Bracalini et al., 2013; Farinha et al., 2021; Strong, 2006; Strong, 2015; Strong et al., 2001). However, to make effective management decisions, it is necessary to develop an effective monitoring tool that can detect, quantify and define the population dynamics and density of the natural populations of the WCSB, since these populations are highly variable from year to year (Farinha et al., 2021; Ponce-Herrero et al., 2022; Schowalter, 1994; Strong, 2015). This would permit the prediction of yield losses (Bates & Borden, 2005). This tool may be based on WCSB population attraction to the newly discovered sesquiterpene leptotriene (Millar et al., 2022).

CONCLUSION REMARKS

- The severity of damage caused by *L. occidentalis* feeding varies with the cone and kernel development at which it occurs, decreasing as development progresses.
- Cone mortality of first- and third-year cones follows a seasonal trend, decreasing with lignification, while mortality of second-year cones is high throughout the season.
- The yield and percentage of sound kernels increase progressively with cone development, while the severity of kernel damage decreases in the following order: aborted cones, IIf, IIu, It and Ip.
- The population of *L. occidentalis* is responsible for the mortality of immature cones, the decrease in the number of seeds per cone, the kernel yield and the percentage of sound kernels and an increase in the percentage of damaged kernels.

AUTHOR CONTRIBUTIONS

Laura Ponce-Herrero: Data curation; funding acquisition; investigation; methodology; visualization; writing – original draft; writing – review and editing. **Ana Ponce Díaz:** Data curation; methodology. **Valentín Pando Fernández:** Data curation; formal analysis; resources; software; writing – original draft; writing – review and editing. **Fernando Manuel Alves-Santos:** Writing – original draft; writing – review and editing. **Juan Alberto Pajares Alonso:** Conceptualization; funding acquisition; methodology; project administration; supervision.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Mendely data at Ponce-Herrero (2023) Cone and seeds damage, Mendeley Data, V1, https://doi.org/10.17632/x32njpsnyw.1.

ORCID

Laura Ponce-Herrero https://orcid.org/0000-0001-5253-781X

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Supplementary S1. Trial 1: Third-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of cone mortality (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S2. Trial 1: Third-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of kernel yield (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S3. Trial 1: Third-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of number of seeds compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S4. Trial 1: Third-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of aborted kernels (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S5. Trial 1: Third-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of kernels with tegmen fully fused to testa (IIf) (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S6. Trial 1: Third-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of kernels with not fused and deformed tegmen (IIu) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S7. Trial 1: Third-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of kernels with totally deformed, wrinkled, or degraded endosperm (It) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S8. Trial 1: Third-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of kernels with partially damaged endosperm (Ip) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S9. Trial 1: Third-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of sound kernels compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S10. Trial 2: Second-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of cone mortality (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S11. Trial 2: Second-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of kernel yield (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S12. Trial 2: Second-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of number of seeds compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S13. Trial 2: Second-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of aborted kernels (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S14. Trial 2: Second-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of kernels with tegmen fully fused to testa (IIf) (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S15. Trial 2: Second-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of kernels with not fused and deformed tegmen (IIu) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S16. Trial 2: Second-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of kernels with totally deformed, wrinkled, or degraded endosperm (It) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S17. Trial 2: Second-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of kernels with partially damaged endosperm (Ip) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S18. Trial 2: Second-year cones bagged with two female imagoes. Values (v) of *p*-value and *t*-value of mean of sound kernels compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S19. Trial 3: First-year cones bagged with two nymphs. Values (v) of *p*-value and *t*-value of mean of cone mortality (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S20. Trial 3: First-year cones bagged with two nymphs. Values (v) of *p*-value and *t*-value of mean of kernel yield (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S21. Trial 3: First-year cones bagged with two nymphs. Values (v) of *p*-value and *t*-value of number of seeds compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S22. Trial 3: First-year cones bagged with two nymphs. Values (v) of *p*-value and *t*-value of mean of aborted kernels (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S23. Trial 3: First-year cones bagged with two nymphs. Values (v) of *p*-value and *t*-value of mean of kernels with tegmen fully fused to testa (IIf) (%) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S24. Trial 3: First-year cones bagged with two nymphs. Values (v) of *p*-value and *t*-value of mean of kernels with not fused and deformed tegmen (IIu) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S25. Trial 3: First-year cones bagged with two nymphs. Values (v) of *p*-value and *t*-value of mean of kernels with totally deformed, wrinkled, or degraded endosperm (It) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S26. Trial 3: First-year cones bagged with two nymphs. Values (v) of *p*-value and *t*-value of mean of kernels with partially damaged endosperm (Ip) compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S27. Trial 3: First-year cones bagged with two nymphs. Values (v) of *p*-value and *t*-value of mean of sound kernels compared by insect bagging date. *Significantly different (*t*-test; *p*-value < 0.05).

Supplementary S28. Exclusion trial. Values (v) of *p*-value and *t*-value of mean of cone mortality (%), mean of kernel yield (%), mean of seeds, and type of kernels (%) by treatment (unprotected cones vs. protected cones) according to the severity of the damage. (II) kernels with dry embryo with no endosperm. This category was divided in (IIf) kernels with tegmen fully fused to testa and (IIu) not fused and deformed tegmen. (I) kernels with sound embryo and damaged endosperm. This category was divided in (Ip) kernels with partially damaged endosperm and (It) totally deformed, wrinkled, or degraded endosperm (*t*-test; **p*-value < 0.05).

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