Laboratory evaluation of *Ooencyrtus obscurus* (Mercet) (Hymenoptera: Encyrtidae) on *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae) eggs

Laura Ponce-Herrero [a,b,*], Valentín Pando Fernández [a,c], Fernando Manuel Alves Santos [a,b], Juan Alberto Pajares Alonso [a,b]

[a] Department of Vegetal Production and Forest Resources, University of Valladolid – La Yutera Campus, Avda. de Madrid 57, 34004 Palencia, Spain
[b] Sustainable Forest Management Research Institute, University of Valladolid - La Yutera Campus, Avda. de Madrid 57, 34004 Palencia, Spain
[c] Statistics and Operations Research Department, University of Valladolid – La Yutera Campus, Palencia, Avda. de Madrid 57, 34004 Palencia, Spain

A B S T R A C T

Leptoglossus occidentalis Heidemann (Hemiptera: Coreidae), known as the western conifer seed bug (WCSB), is a significant pest of the *Pinus pinea* L. kernel sector in the Mediterranean basin. The egg parasitoid species *Ooencyrtus obscurus* (Mercet) (Hymenoptera: Encyrtidae) has been recently recorded on WCSB eggs in Central Spain. This study defines the influence of the egg cluster size on the *parasitism rate* of *O. obscurus*, its reproductive and demographic parameters with the goal of defining the potential of this species to be a biological control agent. *Ooencyrtus obscurus* is weakly synovigenic, with the parasitism period concentrating in the first two weeks, reaching its maximum level of intensity on the 1st day (30.2 %). The mean *oviposition period* was 2.3 days, with the mean female longevity being 16.9 days. The mean rate of *pre-imaginal survival* was high (96.7 %), with the mean *pre-imaginal development time* being significantly higher for females than for males. The *sex ratio (% females)* was 41.2 %, while the reproductive strategy was defined as being pseudo-arrhenotokous parthenogenesis after comparing mated and unmated female offspring. Regarding demographic parameters, a single *O. obscurus* female was able to produce more than 3 female offspring (R_0) in a generation time (T) of 23.96 days, multiply its population 1.05 times a day (λ), and double it in 14.34 days (T_d). The comparison between these results and those established for the main egg parasitoid of the WCSB in its native area could allow for decisions to be made on the type of biological control program to be applied in Europe.

1. Introduction

Leptoglossus occidentalis Heidemann (Hemiptera: Coreidae), known as the western conifer seed bug (WCSB), is a species native to western North America (Heidemann, 1910) and is considered to be a significant economic pest for conifer seed orchards in the area (Schowalter, 1994). The polyphagous character of the WCSB (Fent and Kment, 2011), together with the anthropogenic activities, has led to the rapid dispersion of this pest around the world (Kim et al., 2020; Olivera et al., 2020), with it reaching the Mediterranean basin several decades ago (Taylor et al., 2001), and where it has become the most significant pest of the *Pinus pinea* L. kernel sector (Roversi et al., 2011b; Mutke et al., 2017; Parlak, 2017; Calama et al., 2020). The WCSB oviposits in rows on the needles and the nymphs pass

through five instars (Koerber, 1963) and feed on the cones (Pasek and Dix, 1998), which causes the abortion of the immature ones as well as the abortion or deformation of the seed (Farinha et al., 2018). The economic losses for the kernel sector could be reduced through management decisions based on the dynamics and the density of the WCSB population (Bates and Borden, 2005) through integrated pest management programs, which are based on controlling the damage caused by the pest populations through a combination of cultural, biological, and chemical strategies (Landis and Dumrose, 2014). Although no control methods for WCSB populations have yet been described so far (Blatt and Borden, 1996; Blatt, et al., 1998), it is advisable to establish biological control approaches instead of approaches based on the use of chemical products, which are considered unsustainable (Baker et al., 2020).

* Corresponding author at: Department of Vegetal Production and Forest Resources, University of Valladolid – La Yutera Campus, Avda. de Madrid 57, 34004 Palencia, Spain.
  E-mail addresses: laura.ponce@uva.es (L. Ponce-Herrero), vpando@uva.es (V. Pando Fernández), fmalsa@uva.es (F.M. Alves Santos).

Biological control programs use natural enemies, called biological control agents, such as parasitoids, predators, or pathogens, to manage pest populations (Beddington et al., 1978; Orr, 2009). Biological control is subdivided into four strategies that are defined as: Classical biological control, which involves the introduction of an exotic biological control agent; Inoculation biological control, Inundation biological control, and Conservation biological control (Eilenberg et al., 2001). WCSB egg parasitoid observations have been conducted in both North America and Europe. The species *Hadronotus pennsylvanicus*, which was previously reported as *Gryon pennsylvanicum* (Ashmead) (Hymenoptera: Scelionidae) (Straser et al., 2022), has been defined as the main WCSB egg parasitoid in its native area together with secondary species of the genera *Anastatus* and *Ooencyrtus* (Bates and Borden, 2004; Maltese et al., 2012). In Europe, species of these two genera and of the genus *Baryscapus* were recorded on WCSB eggs in several pine forests (Lesieur and Farinha, 2021; Niccoli et al., 2009; Roversi et al., 2011a).

In recent WCSB egg parasitoid observations conducted in Central Spain, the species *Ooencyrtus obscurus* (Mercet) (Hymenoptera: Encyrtidae) was recorded for the first time on WCSB eggs together with the species *Ooencyrtus pityocampae* (Mercet) (Hymenoptera: Encyrtidae) (Ponce-Herrero et al., 2022), which was previously recorded in the WCSB European observations.

H. pennsylvanicus was proposed as a biological control agent in biological control programs in Europe due to its suitable qualities, which were defined in laboratory assessments (Sabbatini Peverieri et al., 2012; Sabbatini Peverieri et al., 2013; Roversi et al., 2011b), and its low negative effects on non-target European host species (Roversi et al., 2014). However, this species has not yet been introduced in Europe. The availability of WCSB egg parasitoids native to Europe allows for the development of biocontrol programs that avoid the risks associated with the introduction of exotic enemies (Van Lenteren et al., 2003) to reduce the WCSB population levels and, therefore, the damage caused to the kernel sector. In light of the results of the WSCB egg parasitoid observations in Central Spain (Ponce-Herrero et al., 2022), laboratory assessments have been conducted to develop the most accurate biological control strategy in Europe by comparing the *parasitism rate*, *sexual reproductive strategy*, lifetime fecundity, life history, and demographic parameters of the main egg parasitoid in the region (*O. obscurus*) with those defined for the main WCSB egg parasitoid in its native area, *H. pennsylvanicus*. Previous assessments were conducted to: (I) measure the effect of the WCSB egg cluster size on the *parasitism rate*; confirm the (II) *sexual reproductive strategy* of *O. obscurus*; and define its (III) reproductive and demographic parameters. In addition, the (IV) external appearance of the WCSB parasitized eggs throughout the development of parasitism was described.

2. Material and methods

2.1. Origin and rearing of the egg parasitoids and WCSB egg masses

The *O. obscurus* colony was established from parasitized WCSB egg masses obtained in two previous trials conducted from mid-May 2019 to mid-September 2019 in a grafted *P. pinea* clone bank (Tordesillas, Valladolid, Spain; latitude: 337. 458; longitude: 4.596.035 ETRS89, UTM 30 N) and in a *P. pinea* pine forest (M.U.P. 17, Nava del Rey, Valladolid, Spain; latitude: 328.547; longitude: 4.590.594, ETRS89, UTM 30 N) (Ponce-Herrero et al., 2022).

Parasitized egg masses were collected and placed individually in test tubes (10 cm long, 1.5 cm dia.) that were closed with a paper cap and kept under laboratory conditions (21 ± 2 °C; RH 40 ± 10 %) and with natural daylight. The emergence of the parasitoids was checked daily. Parasitoids were identified and sexed according to Mercet (1921) under a stereomicroscope and individualized in test tubes (10 cm long, 1.5 cm dia.) that were closed with a cotton cheesecloth (1 × 1 mm)

and kept under laboratory conditions and with natural daylight. A thin coat of pure honey was provided *ad libitum* as food source and was placed on surface of the tube (Sabbatini Peverieri et al., 2012). All parasitoids that emerged during the parasitism assessments were also added to the parasitoid colony. Voucher specimens were stored at the Department of Vegetal Production and Forest Resources of the University of Valladolid (Palencia, Spain).

WCSB eggs used in the parasitism assessments were obtained from a permanent WCSB colony reared since 2014 in the laboratory at the Department of Vegetal Production and Forest Resources. Insects collected in the region, by forestry agents, and in the clone bank by us, were added to the colony on an annual basis. They were kept under laboratory conditions and with natural daylight, and rectangular breeding boxes were used (47.5 × 47.5 × 93 cm, 160 μm mesh) (Entomapraxis, Barcelona, Spain). *Ad libitum* shelled *P. pinea* kernels were provided as food source and several young potted *Pinus halepensis* Mill. (2–3 years old) were provided as water source and ovipositional substrate. WCBS egg masses were collected daily by removing the entire needle from the pines of the colony. Egg masses that were not used on the collection day were stored in test tubes (10 cm long, 1.5 cm dia.) that were closed with a plastic cap and kept at 4 ± 1 °C for up to 7 days (Binazzi et al., 2013).

2.2. Assessment design

Two previous assessments were conducted to measure the effect of the WCSB egg cluster size on the *O. obscurus parasitism rate* (First) and its *sexual reproductive strategy* (Second), with the aim of designing the main assessment (Third) that would define the lifetime fecundity, life history, and demographic parameters of *O. obscurus* on WCSB eggs , and additionally, the external appearance of the WCSB parasitized eggs along the development of parasitism .

General assessment conditions: assessments were conducted under a cold light source (16:8 L:D) (Sabbatini Peverieri et al., 2012) under laboratory conditions (Martel et al., 2019). The parasitoids tested were those that had newly emerged (<24-hour old) (Sabbatini Peverieri et al., 2012). Pine needles with the WCSB eggs (up to 7 days old; Binazzi et al., 2013) were stapled onto a paper strip (Bates and Borden, 2004) and introduced into test tubes (15 cm long, 2.5 cm dia.) closed with a cotton cheesecloth (1 × 1 mm). Pure honey was provided as food source, as described above.

First assessment. Effect of the WCSB egg cluster size on the *parasitism rate*: one female and one male were paired and housed with an egg cluster consisting of between 5 and 9 eggs (n pairs = 30; n eggs = 212) and between 10 and 13 eggs (n pairs = 30; n eggs = 306) in a single 96-hour exposure event to measure the effect of egg cluster size on the *parasitism rate*.

Second assessment. *Sexual reproductive strategy*: one unmated female (n = 30), with no previous contact with males (no simultaneous emergence), was housed with an egg cluster consisting of between 5 and 14 eggs (n = 281) in a single 96-hour exposure event. The *sex ratio (% females)* of the offspring of unmated females was compared with that obtained for the offspring from the First assessment, in which females were paired with males, and, consequently, it is assumed that they were fertilized.

Third assessment. Lifetime fecundity, life history, and demographic parameters: one female and one male were paired (n = 40) and housed with an egg cluster consisting of between 5 and 15 eggs (n = 6,430) which were replenished every 48 – 72 h (Tunca et al., 2019) until the female died. Males that died were replaced to maintain the copulation potential (Sabbatini Peverieri et al., 2012).

Parasitism detection: in the three assessments, the parasitized WCSB eggs per female were recorded daily under a stereomicroscope through stalks detection on the host egg chorion during the host egg exposure time. Stalks are tubes that connect the parasitoid ovipositor

with the lumen of the host egg (Maple, 1947). The number of stalks per host egg and female was also recorded daily during this period. The external appearance of parasitized host eggs was recorded daily both during and after the exposure time and until nymph hatching or parasitoid emergence in order to describe the appearance of stalks, black dots and/or black lines, and detritus under the chorion (Ponce-Herrero et al., 2022). The number of offspring per host egg and female was recorded daily after the exposure time to detect the gregarious character of the parasitoid species. The parasitoids that emerged were sexed and added to the parasitoid colony. The hatched WCSB nymphs were added to the mixed-age colony of WCSB. Unparasitized eggs without nymph hatching and parasitized eggs without parasitoid emergence holes were dissected after 45 days (Anderson and Kaya, 1974) as no diapause period was detected in WCSB eggs for *O. obscurus* under laboratory conditions (Ponce-Herrero et al., 2022). The dissected eggs were classified as either unfertilized by WCSB, aborted nymph, aborted parasitoid, aborted larvae, or without parasitoid development but with a granulated translucent mass (Maple, 1947; Ponce-Herrero et al., 2022).

2.3. Parameter definition and statistical analysis

For all assessments, females that did not produce offspring were discarded (Martel et al., 2019).

The *parasitism rate* was calculated as the number of parasitized eggs divided by the total number of eggs, and this was presented as a percentage and as mean ± SE per female.

Fecundity was defined as the number of offspring per female and was presented as mean ± SE.

Pre-imaginal survival was defined as the number of parasitized eggs with parasitoid emergence holes divided by the total number of parasitized eggs, and this was presented as a percentage.

The *emergence ratio* was calculated by dividing the total number of offspring by the number of parasitized eggs with parasitoid emergence holes and was presented as mean ± SE (Ponce-Herrero et al., 2022). The relationship between the number of offspring and the number of parasitized eggs with emergence holes was defined by analysis regression using PROC GLIMMIX with Poisson distribution and log link function.

The offspring *sex ratio (% females)* was calculated as the number of female offspring divided by the total offspring and was presented as a percentage. The relationship between the age of females and offspring *sex ratio (% females)* was defined by linear mixed regression.

Lifetime fecundity was defined through daily observations of female *longevity* (days) and *fecundity* (mean ± SE). The relationship between *fecundity* and *longevity* was defined by mixed linear regression. *Life expectancy* was defined as the day on which 50 % of females were still alive. The *ovigeny index* was calculated as the fraction of the parasitized eggs oviposited at the time of parasitoid emergence (oviposition < 24 h) per female, and this was presented as mean ± SE (Jervis et al., 2001).

The life history parameters were presented as mean ± SE (Table 1). *Fertility* was defined as the eggs laid by parasitoid females, which were identified through the stalks located on the egg chorion (Tracy and Nechols, 1987). *Aborted offspring* was calculated by subtracting the number of stalks by the number of offspring per female. *Pre-imaginal development time* was defined as the number of elapsed days between the stalk detection and the parasitoid emergence; this parameter was defined by sex and compared using a Students *t*-test.

Demographic parameters were defined regarding Sabbatini Peverieri et al. (2012)and Jervis et al. (2007):

Intrinsic rate of increase: $r_m = \ln(\sum l_x \cdot m_x)/T$.

Finite rate of increase: $\lambda = e^{rm}$

Mean generation time: $T = \sum x \cdot l_x \cdot m_x/R_0$

Doubling time: $T_d = (\ln 2)/r_m$

Table 1
Life history. Mean parameters (±SE) of *Ooencyrtus obscurus* reared on WCSB eggs under laboratory conditions (21 ± 2 °C; RH 40 ± 10 %) and 16:8 L:D photoperiod and provided with honey *ad libitum* as food source. Third assessment data (n = 27).

Parameter	Mean value (±SE)
Adult female longevity (days)	16.9 ± 1.7
Pre-reproductive period (days)	3.9 ± 0.7
Oviposition period (days)	2.3 ± 0.5
Post-reproductive period (days)	10.6 ± 1.7
Fecundity (offspring/female)	7.7 ± 1.0
Fertility (stalks/female)	8.2 ± 1.0
Aborted offspring (unemerged offspring/female)	0.5 ± 0.2
Emergence ratio (offspring/egg)	1.7 ± 0.1
Mean pre-imaginal development time females (days)	21.6 ± 0.3
Mean pre-imaginal development time males (days)	20.6 ± 0.3
Sex ratio (% females)	41.2

Net reproductive rate: $R_0 = \sum l_x \cdot m_x$

Gross reproductive rate: $GRR = \sum m_x$

Where x = age in days, l_x = age-specific survival rate and m_x = age-specific fecundity, were measured experimentally.

The effect of the egg cluster size on the *parasitism rate* was defined by regression analysis using a generalized linear mixed model (GLIMMIX procedure in the SAS software) with binomial distribution and logit link function. Parasitized eggs were the experimental unit.

The effect of the egg cluster size on the offspring *sex ratio (% females)* was defined with a regression analysis, using a generalized linear mixed model (GLIMMIX procedure in the SAS software) with a Gaussian distribution and the identity link function. Parasitized eggs were the experimental unit.

The external appearance of the parasitized eggs was presented as mean ± SE, based on the data from the Third assessment.

Statistical analysis was conducted with the software SAS 9.4. (https://www.sas.com, Statistical Analysis System, RRID:SCR_008567).

3. Results

Original data of this study are available at Mendeley Data (https://doi.org/10.17632/dywxhmmczc.1).

<u>First assessment</u>. **Effect of the WCSB egg cluster size on the *parasitism rate*:**

Of the 60 tested females, 6 were discarded for getting stuck in the honey (n pairs = 54), and 19 of them parasitized at least one WCSB egg (35.2 %). Of the 188 eggs examined, 34.0 % were successfully parasitized (*parasitism rate*), producing a total of 116 offspring. The probability of a WCSB egg being parasitized increases non-significantly with the egg cluster size (*t-value* = 0.07 with *p-value* = 0.9452). The mean number of eggs parasitized per female was 3.4 ± 0.5 (ranged from 1 to 7). The mean offspring (*fecundity*) per female was 6.1 ± 1.0 individuals (ranged from 1 to 15). *Pre-imaginal survival* was high (92.2 %), with only 5 eggs experiencing the development of parasitism and without parasitoid emergence holes. The *emergence ratio* was 2.0 ± 0.14 parasitoids per egg (ranged from 1 to 6), and it decreased as the number of parasitized eggs increased in a not significant way (*t-value* = -0.26 with *p-value* = 0.7986). The *sex ratio (% females)* was 56.0 % and this was not significantly influenced by the size of the egg cluster size (*t-value* = -1.54 with *p-value* = 0.1307).

<u>Second assessment</u>. *Sexual reproductive strategy***:** *Ooencyrtus obscurus* may reproduce sexually, producing both male and female offspring, and may also reproduce asexually through pseudo-arrhenotokous parthenogenesis, producing only male offspring (Frank and Gillett-Kaufman, 2006).

All the unmated females tested (n = 30) died naturally, and 9 of them parasitized at least one WCSB egg (30.0 %). Of the 77 eggs examined, 37.7 % were successfully parasitized (*parasitism rate*), producing a

total of 58 offspring. The mean number of offspring (*fecundity*) per single female was 3.2 ± 0.6 individuals (ranged from 2 to 16). *Pre-imaginal survival* was high (86.2 %), with only 4 eggs experiencing the development of parasitism and without parasitoid emergence holes. The *emergence ratio* was 2.3 ± 0.2 parasitoids per egg (ranged from 1 to 4). The *sex ratio (% females)* was 0.0 %.

Third assessment. Lifetime fecundity, life history and demographic parameters: all females tested (n = 40) died naturally, and 27 of them parasitized at least one WCSB egg (67.5 %). Of the 4,476 eggs examined, 2.8 % were successfully parasitized (*parasitism rate*), producing a total of 209 offspring. The mean of WCSB eggs parasitized per female was 4.5 ± 0.5. The life history parameters are presented in Table 1. *Pre-imaginal survival* was high (96.7 %), with only 4 eggs experiencing the development of parasitism but without parasitoid emergence holes. They contained one aborted larva (n = 3) and a granulated translucent mass (n = 1) associated with parasitism (Maple, 1947). *Fertility* was higher than *fecundity*, and 0.5 ± 0.2 of the parasitized eggs failed to successfully develop. *Fecundity* per female ranged from 2 to 23 eggs. The *pre-imaginal development time* at 21 ± 2 °C was significantly longer (Students test; *t-value* = 2.53 with *p-value* = 0.0122) for females (21.6 days) than for males (20.6 days). The mean *sex ratio (% females)* was 41.2 % (ranged from 0 to 100 %) and this increased significantly with female aging (*t-value* = 2.48 with *p-value* = 0.024 in the linear mixed regression, Fig. 1).

Regarding Fig. 2, parasitization was concentrated in the first two weeks of the lifetime fecundity, especially on the 1st day when 30.2 % of the eggs were parasitized and the maximum mean (±SE) *fecundity* produced 2.3 ± 0.6 offspring per female. On the 1st day, 28.8 % of parasitoid oviposition was laid, while 50.0 % and 90.0 % were laid on the 2nd and 11th day, respectively. The *ovigeny index* was 0.27 ± 0.08. *Fecundity* decreased progressively until the 6th day when it was intermit-

tent. *Fecundity* and *longevity* were not significantly correlated (*t-value* = -1.93 with *p-value* = 0.0709). Females started to die from the 2nd day and mortality decreased steadily until the 18th day and stabilized until the 26th day when it decreased again. Female *life expectancy* was 14 days.

The demographic parameters are presented in Table 2. The *intrinsic rate of increase* (r_m) was 0.05 females/female/day, so the parasitoid population was able to multiply 1.05 times a day (λ) with a *doubling time* of 14.34 days (T_d). A single *O. obscurus* female produced more than 3 offspring females in a *generation time* (T) of approximately 23.96 days.

Parasitism detection: parasitized eggs presented four external appearances (Fig. 3): (I) stalks on the chorion; (II) black dots and/or black lines under the chorion; (III) detritus under the chorion; and (IV) parasitoid emergence holes.

The mean development time from the detection of the first stalk was 3.9 ± 0.1 days for phase II (dots); 8.5 ± 0.2 days for phase III (detritus) and 21.0 ± 0.2 days for phase IV (emergence holes).

4. Discussion

The biological and reproductive parameters of the egg parasitoid *O. obscurus* on WCSB eggs under standard laboratory conditions are reported for the first time in this study. These findings could be a guide to defining the most suitable strategy for a possible biological control program in Europe in terms of the biological control agent to use: exotic or native.

The WCSB parasitoid community in Europe consists of the species *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae), *Baryscapus servadeii* Domenichini (Hymenoptera: Chalcidoidea), *Ooencyrtus telenomicida* Vassiliev (Hymenoptera: Encyrtidae), *O. pityocampae*, and *O. obscurus* (Lesieur and Farinha, 2021; Niccoli et al., 2009; Ponce-
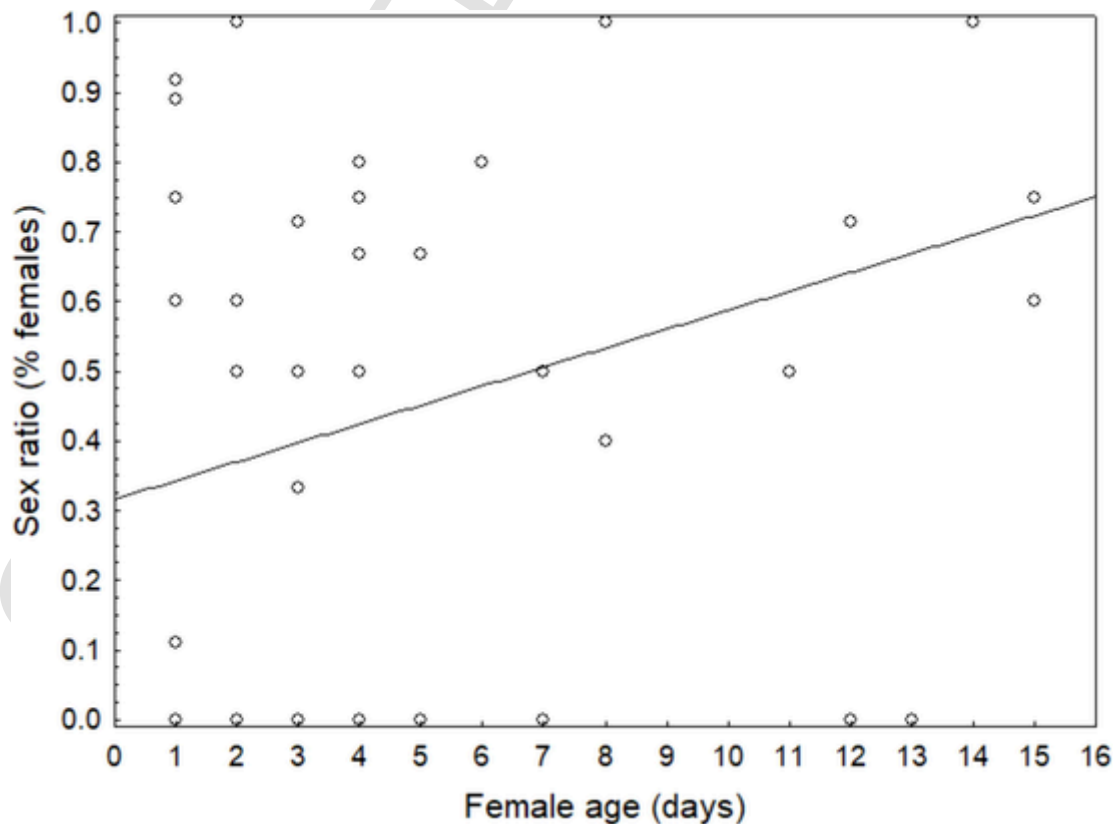

Fig. 1. Relationship between female age and offspring *sex ratio (% females)*. Linear mixed regression analysis (*t-value* = 2.48 with *p-value* = 0.024) of female age and offspring *sex ratio (% females)* for *Ooencyrtus obscurus* reared on WCSB eggs under laboratory conditions (21 ± 2 °C; RH 40 ± 10 %) and 16:8 L:D photoperiod and provided with honey *ad libitum* as food source. Third assessment data (n = 27).
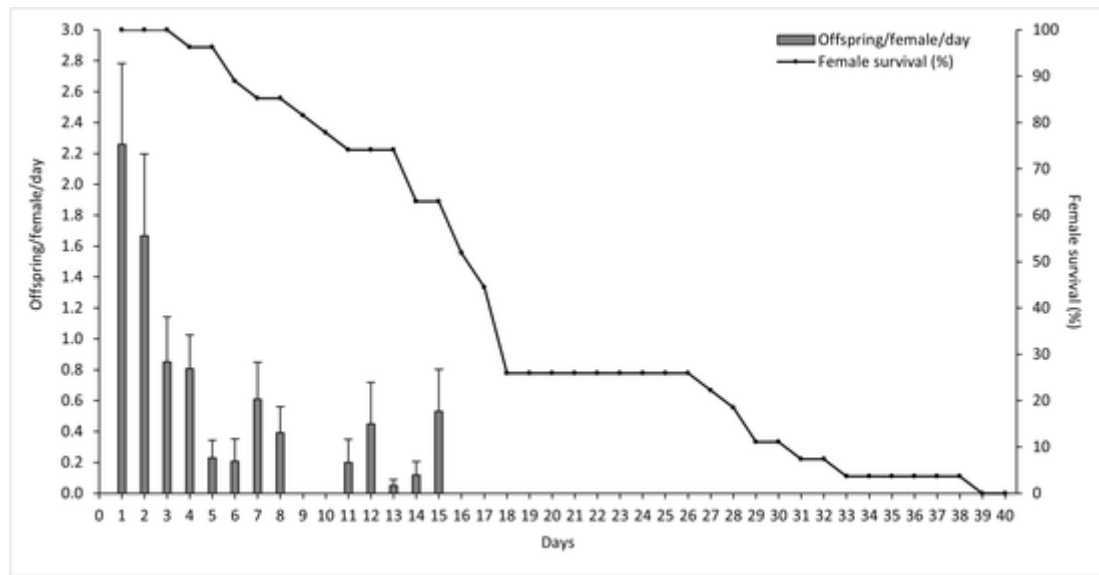
Fig. 2. Lifetime fecundity. Mean age-specific fecundity (bars) (+SE) and female survival (%) of *Ooencyrtus obscurus* reared on WCSB eggs under laboratory conditions (21 ± 2 ℃; RH 40 ± 10 %) and 16:8 L:D photoperiod and provided with honey *ad libitum* as food source. Third assessment data (n = 27).

Table 2

Demographic parameters. Mean parameters of *Ooencyrtus obscurus* reared on WCSB eggs under laboratory conditions (21 ± 2 ℃; RH 40 ± 10 %) and 16:8 L:D photoperiod and provided with honey *ad libitum* as food source. Third assessment data (n = 27).

Parameter	Mean value
Intrinsic rate of increase (r_m) (females/female/day)	0.05
Finite rate of increase (λ) (population multiplication/day)	1.05
Mean generation time (T) (days)	23.96
Doubling time (T_d) (days)	14.34
Net reproductive rate (R_0) (female progeny/female/generation)	3.19
Gross reproductive rate (GRR) (female progeny/female/generation)	3.55

Herrero, et al., 2022; Roversi et al., 2011a). Species of the genera *Anastatus* and *Ooencyrtus* have also been recorded on WCSB eggs in North America, however, the species *H. pennsylvanicus* has been considered the main WCSB egg parasitoid in that area (Bates and Borden, 2004; Maltese et al., 2012). This species has been proposed as having the po-tential to be a biological control agent for a classical biological control program against WCSB populations in Europe (Roversi et al., 2011b; Roversi et al., 2014; Sabbatini Peverieri et al., 2012; Sabbatini Peverieri et al., 2013). Nevertheless, biocontrol programs with native biological control agents cannot be discarded. Thus, in accordance with parasitoid surveys developed for three consecutive years, the parasitoid species *O. obscurus* has been found to parasitize from 6.2 % to 19.6 % of the eggs laid *in situ* and could therefore be a potential biological control agent in Europe (Ponce-Herrero et al., 2022). Assessments to determine its suit-ability have been conducted and the results have been compared with those obtained for the main WCSB natural enemy, *H. pennsylvanicus*.

Firstly, the egg cluster size did not affect the *parasitism rate* of *O. obscurus*, similarly to *O. pityocampae* reared on eggs of *Halyomorpha halys* (Stal) (Hemiptera: Pentatomidae) (Tunca et al. 2019). The *parasitism rate* recorded in both the First and Second assessments, in which one egg cluster was exposed in a single 96-hour exposure event to one paired (34.0 %) and one unpaired female (37.7 %), respectively, were higher to the findings obtained in the parasitoid surveys conducted in Central Spain. However, the *parasitism rate* of the Third assessment
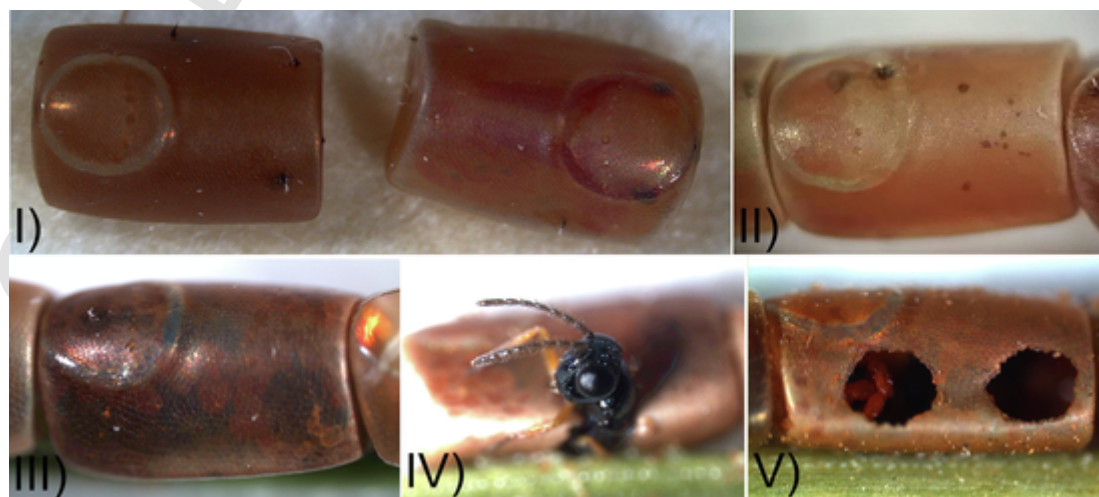

Fig. 3. External appearance of WCSB parasitized eggs by *Ooencyrtus obscurus*. External appearances: (I) stalks on the chorion that reveal the presence of par-asitoid eggs; (II) black dots and/or black lines under the chorion indicating parasitoid egg development; (III) detritus under the chorion that reveal the emer-gence of the larvae and its development; (IV) parasitoid emergence holes; and (V) emerging parasitoid. Third assessment data (n = 27).

(2.8 %) was much lower than expected, possibly because of the higher number of exposed eggs per female due to the replenishment of the egg clusters until female death, with the rate of ovipositing females (67.5 %) being higher than that recorded in both the First (35.2 %) and Second (30.0 %) assessments. The low proportions of ovipositing females, as compared with other egg parasitoid species (Giovannini et al., 2020; Sabbatini Peverieri et al., 2012), cannot be related to infertility because dissections were not conducted. Heimpel and Rosenheim (1998) associated the high proportion of *Aphytis aonidiae* (Mercet) (Hymenoptera: Aphelinidae) females without mature eggs at emergence under laboratory conditions with pathogens that prevent parasitoids from direct resources for egg maturation or that damages to eggs, for lack of essential components in host eggs for the parasitoid vitellogenesis, or for a polymorphism that allocated the resourced to other body parts rather than to oocytes. Martel et al., (2019) suggested that males can disrupted the female oviposition, however, in this study the rate of ovipositing females was lower for single females (Second assessment) than for paired females (First and Third assessments).

Despite the expected reduced impact of native natural enemies on exotic pest populations, (Cornell and Hawkins, 1993) when compared with the expected impact of the enemies from the same area as the pest, high *parasitism rates* have been recorded for the following native egg parasitoids reared on alien pests: *Ooencyrtus lucidus* Triapitsyn & Ganjisaffar (Hymenoptera: Encyrtidae) parasitized from 42.2 % to 89.9 % eggs of *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae) in life history assessments (Ganjisaffar and Perring, 2020). *Ooencyrtus gonoceri* Viggiani (Hymenoptera: Encyrtidae) parasitized a mean of 206.8 eggs per female of *H. halys* in life history assessments (Giovannini et al., 2020); with this value being much higher than that recorded for *O. obscurus* (4.5 parasitized eggs/female). *Ooencyrtus pityocampae* parasitized from 34.2 % to 62.7 % eggs of *H. halys* in a single 48 or 72-hour exposure event (Tunca et al., 2019), and the mean of parasitized eggs per female of this parasitoid reared on WCSB in a single 24-hour exposure event was between 2.5 and 5.7 (Binazzi et al., 2013), higher than the mean recorded for *O. obscurus* in the First and Second assessments, with 3.4 and 3.2 parasitized eggs per female, respectively.

In classical biological control programs, the introduction of natural enemies into invaded areas by an alien pest (Martel et al., 2019; Power, 2020; Sabbatini Peverieri et al., 2012) is proposed to reestablish host-natural enemy associations (Eilenberg et al., 2001). *Hadronotus pennsylvanicus* has been considered a suitable biological control agent of the WCSB because of its biological and reproductive parameters (Sabbatini Peverieri et al., 2012). The *parasitism rate* of *H. pennsylvanicus* (n = 40; 5–7 eggs; single 2-hour exposure event) was much higher (71.8 %) than the rate of *O. obscurus* (34.0 %) in the First assessment (n = 19; 5–13 eggs; single 96-hour exposure event). Regarding life history parameters, *H. pennsylvanicus* female longevity (40.2 days) was more than twice that of *O. obscurus* females (16.9 days), although honey was also provided. The mean *H. pennsylvanicus fecundity* rate (144.5 offspring/female) was almost twenty times higher that of *O. obscurus* (7.7 offspring/female), with this being influenced by the mean *oviposition period,* which was ten times longer for *H. pennsylvanicus* (23.0 days) than for *O. obscurus* (2.3 days). *Pre-imaginal survival* was high for both species (*H. pennsylvanicus*: 99.1 %; *O. obscurus:* 96.7 %), with the mean *pre-imaginal development time* being longer for females than for males (*H. pennsylvanicus* females: 18.2 days, males: 18.1 days; *O. obscurus* females: 21.6 days, males: 20.6 days), allowing them to have more mating events with the emerging females (Ganjisaffar and Perring, 2020; Martel et al., 2019). The *emergence ratio* was related to the gregarious and solitary character of each species. As is typical of *Ooencyrtus* spp. (Nechols et al., 1989), *O. obscurus* is gregarious (*emergence rate:* 1.7 offspring/egg), in contrast to *H. pennsylvanicus,* for which 1 offspring per egg was recorded on *Leptoglossus phyllopus* (L.) (Hemiptera: Coreidae) (Abudulai et al., 2001), and superparasitism was not found by Sabbatini Peverieri et al. (2012). This character allows *H. pennsylvanicus* to be more suitable to reduce the host population than *O. obscurus*, as it requires the highest number of host eggs to oviposit (Cornelius et al., 2018). The *sex ratio (% females)* was higher for *H. pennsylvanicus* (72.45 %) than for *O. obscurus* (41.2 %), while both species are capable of reproducing asexually by arrhenotokous (Nechols et al., 1989) and pseudo-arrhenotokous parthenogenesis, respectively, producing uniparental and haploid male offspring (n) from unfertilized eggs (Frank and Gillett-Kaufman, 2006; Heimpel and De Boer, 2008). In this regard, the use of the egg parasitoid *O. pityocampae* - which also found on WCSB eggs in fields in Europe (Roversi et al., 2011a;Lesieur and Farinha, 2021; Ponce-Herrero et al., 2022) -, as biological control agent has the advantage of producing only female offspring due to its thelytokous parthenogenesis reproduction (Tunca et al., 2019), which is characterized by diploid female offspring (Frank and Gillett-Kaufman, 2006).

Regarding lifetime fecundity, *H. pennsylvanicus* and *O. obscurus* began to oviposit after emergence and, in terms of their o*vigeny index* (*H. pennsylvanicus:* < 0.18 (Jervis et al. (2001); *O. obscurus*: 0.27) both species were defined as weakly synovigenic because most eggs are mature at emergence, but parasitoids need to feed to continue to mature their eggs (Jervis et al., 2001). Additionally, the *parasitism rate* and the lifetime fecundity being possibly reduced by the temporary egg limitation of this type of species (Heimpel and Rosenheim, 1998). However, the infertility of the females and the maturation dynamics of the eggs of *O. obscurus* must be confirmed through the dissection of eggs at different ages. *Fecundity* rapidly decreased over time for both species, with the *doubling time* (T_d) being lower for *O. obscurus* (T_d: 14.34 days) than for *H. pennsylvanicus* (T_d: 3.37 days). This parameter was reflected in the lifetime fecundity, because of 50 % of the eggs were laid on the 2nd day by *O. obscurus* and on the 6th by *H. pennsylvanicus*, while 90 % of eggs were laid on the 11th day and the 15th day, respectively. Both species also showed similar *generation time* (T), 23.96 days for *O. obscurus* and 22.48 days for *H. pennsylvanicus*. However, female progeny by female (R_0) was much higher for *H. pennsylvanicus* (greater than 100) than for *O. obscurus* (greater than 3), as it was influenced by their *intrinsic rate of increase* (*H. pennsylvanicus*: r_m: 0.203 females/female/day; *O. obscurus*: r_m: 0.05 females/female/day).

In conclusion, the exotic species *H. pennsylvanicus* is more suitable as a biological control agent for the WCSB in Europe than the native species *O. obscurus*. This is reinforced by the demographic parameters obtained for native *Ooencyrtus* species reared on invasive pests, as *O. lucidus* on *B. hilaris* (R_0: 103.8 female progeny/female/generation; r_m: 0.171 females/female/day; T: 27.1 days; T_d: 4.0 days; Ganjisaffar and Perring, 2020), and *O. gonoceri* on *H. halys* (R_0: 48.37 female progeny/female/generation; r_m: 0.168 females/female/day; T: 23.02 days; T_d: 4.11 days; Giovannini et al., 2020).

Additionally, it is necessary to evaluate the parasitism behavior of *H. pennsylvanicus* in European ecosystems (Sabbatini Peverieri et al., 2012;Cornelius et al., 2018; Martel et al., 2019) as well as all risks associated with the introduction of this exotic species (Van Lenteren et al., 2003), instead of its low negative effects on non-target European host species (Roversi et al., 2014).

Despite our results, it would be advisable to conduct parasitism assessments under the same conditions carried out by Sabbatini Peverieri et al. (2012). Moreover, it would be advisable not to discard the use of several native parasitoids as biological control agents in order to aggregate their parasitic advantages, given the weak synovigenic character of *O. obscurus* and the thelytokous character of *O. pityocampae*, together with the large distribution of its main host, *Thaumetopoea pityocampa* (Denis & Schiffermüller) (Lepidoptera: Notodontidae). As such, the biological and reproductive parameters of *O. pityocampae* must be defined in laboratory assessments to compare them with those obtained for *O. obscurus* and *H. pennsylvanicus*.

To conclude, regardless of the use of native or exotic biological control agents in Europe, it is necessary to define an effective monitoring

method for the WCSB populations, for example, based on chemical attraction (Millar et al., 2022), and improve the knowledge of synchronization (Stiling, 1993) and the density dependence relationship (Van den Bosch et al., 1982) between the egg parasitoid species and the WCSB populations within the scope of the biological control program.

Uncited references

.

CRediT authorship contribution statement

Laura Ponce-Herrero : Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – original draft, Visualization, Funding acquisition. **Valentín Pando Fernández :** Formal analysis, Writing – review & editing. **Fernando Manuel Alves Santos :** Writing – review & editing. **Juan Alberto Pajares Alonso :** Conceptualization, Methodology, Investigation, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

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

References

Abudulai, M., Shepard, B.M., Mitchell, P.L., 2001. Parasitism and predation on eggs of *Leptoglossus phyllopus* (L.) (Hemiptera: Coreidae) in cowpea: Impact of endosulfan sprays. J. Agric. Urban Entomol. 18, 105–115.

Anderson, J.F., Kaya, H.K., 1974. Diapause induction by photoperiod and temperature in the elm spanworm egg parasitoid, *Ooencyrtus* sp. Ann. Entomol. Soc. Am.. 67, 845–849. https://doi.org/10.1093/aesa/67.6.845.

Baker, B.P., Green, T.A., Loker, A.J., 2020. Biological control and integrated pest management in organic and conventional systems. Biol. Control 140, 104095. https://doi.org/10.1016/j.biocontrol.2019.104095.

Bates, S.L., Borden, J.H., 2004. Parasitoids of *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae) in British Columbia. J. Entomol. Soc. B. C. 101, 143–144.

Bates, S.L., Borden, J.H., 2005. Life table for *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) and prediction of damage in lodgepole pine seed orchards. Agric. Fore Entomol. 7, 145–151. https://doi.org/10.1111/j.1461-9555.2005.00254.x.

Beddington, J. R., Free, C. A., Lawton, J. H., 1978. Characteristics of successful natural enemies in models of biological control of insect pests. Nature. 273, 513–519.https://doi.org/10.1038/273513a0.

Binazzi, F., Benassai, D., Sabbatini Peverieri, G., Roversi, P.F., 2013. Effects of *Leptoglossus occidentalis* Heidemann (Heteroptera Coreidae) egg age on the indigenous parasitoid *Ooencyrtus pityocampae* Mercet (Hymenoptera Encyrtidae). Redia. 96, 79–84.

Blatt, S.E., Borden, J.H., 1996. Evidence for a male-produced aggregation pheromone in the western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae). Can. Entomol. 128, 777–778. https://doi.org/10.4039/ent12 8777-4.

Blatt, S.E., Borden, J.H., Pierce, H.D., Gries, R., Gries, G., 1998. Alarm pheromone system of the western conifer seed bug, *Leptoglossus occidentalis*. J. Chem. Ecol. 24, 1013–1031. https://doi.org/10.1023/A:1022350402535.

Calama, R., Gordo, J., Mutke, S., Conde, M., Madrigal, G., Garriga, E., Arias, M. J., Piqué,

M., Gandía, R., Montero, G., Pardos, M., 2020. Decline in commercial pine nut and kernel yield in Mediterranean stone pine (*Pinus pinea* L.) in Spain. iForest. 13, 251–260. 10.3832/ifor3 180-013.

Cornelius, M.L., Hu, J.S., Vinyard, B.T., 2018. Comparative study of egg parasitism by *Gryon pennsylvanicum* (Hymenoptera: Scelionidae) on two squash bug species *Anasa tristis* and *Anasa armigera* (Hemiptera: Coreidae). Environ. Entomol. 47, 1451–1458. https://doi.org/10.1093/ee/nvy145.

Cornell, H.V., Hawkins, B.A., 1993. Accumulation of native parasitoid species on introduced herbivores: A comparison of hosts as natives and hosts as invaders. Am. Nat. 141, 847–865. https://doi.org/10.1086/285512.

Eilenberg, J., Hajek, A., Lomer, C., 2001. Suggestions for unifying the terminology in biological control. BioControl 46, 387–400. https://doi.org/10.1023/A:1014193329979.

Farinha, A.C.O., Silva, J.E.P., Correia, A.C., Sousa, E.M.R., Roques, A., Branco, M., 2018. Is *Leptoglossus occidentalis* entirely responsible for the high damage observed on cones and seeds of *Pinus pinea*? Results from a Fertirrigation Trial in Portugal. For. Ecol. Manag. 429, 198–206. https://doi.org/10.1016/j.foreco.2018.07.014.

Fent, M., Kment, P., 2011. First record of the invasive western conifer seed bug *Leptoglossus occidentalis* (Heteroptera: Coreidae) in Turkey. North West. J. Zool. 7, 72–80.

Frank, J. H., Gillett-Kaufman, J. L., 2006. Glossary of Expressions in Biological Control: IPM-143/IN673, 8/2006. *EDIS*, 200615. https://doi.org/10.32473/edis-in673-2006.

Ganjisaffar, F., Perring, T.M., 2020. Life history evaluation of *Ooencyrtus lucidus*, a newly described egg parasitoid of *Bagrada hilaris*. Insects. 11, 292. https://doi.org/10.3390/insects11050292.

Giovannini, L., Mazza, G., Binazzi, F., Simom, S., Marianelli, L., Guerrieri, E., Roversi, P.F., Peverieri, G.S., 2020. Biological parameters of the egg parasitoid *Ooencyrtus gonoceri*. Bull. Insectol. 73, 313–319.

Heidemann, O., 1910. New species of Leptoglossus from North America (Hemipetera; Coreidae). Proc. Entomol. Soc. Wash. 12, 191–197.

Heimpel, G.E., De Boer, J.G., 2008. Sex determination in the Hymenoptera. Annu. Rev. Entomol. 53, 209–230. https://doi.org/10.1146/annurev.ento.53.103106.093441.

Heimpel, G.E., Rosenheim, J.A., 1998. Egg limitation in parasitoids: a review of the evidence and a case study. Biol. Control 11, 160–168. https://doi.org/10.1006/bcon.1997.0587.

Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A., Kidd, N.A.C., 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. J. Anim. Ecol. 70, 442–458. https://doi.org/10.1046/j.1365-2656.2001.00507.x.

Jervis, M.A., Copland, M.J., Harvey, J.A., 2007. The life-cycle. In: Jervis, M.A. (Ed.), Insects as Natural Enemies. Springer, The Netherlands, pp. 73–165.

Kim, D.E., Lee, H., Kim, M.J., Ban, Y.G., Kim, D., 2020. *Leptoglossus occidentalis* (Hemiptera: Coreidae) occurrence, potential habitats, and COI diversity in South Korea. J. Asia Pac. Biodivers. 13, 35–45. https://doi.org/10.1016/j.japb.2019.11.002.

Koerber, T.W., 1963. *Leptoglossus occidentalis* (Hemiptera, Coreidae), a newly discovered pest of coniferous seed. Ann. Entomol. Soc. Am. 56, 229–234. https://doi.org/10.1093/aesa/56.2.229.

Landis, T.D., Dumroese, R.K., 2014. Integrated pest management-an overview and update. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. Forest Nursery Notes. 34, 16–26.

Lesieur, V., Farinha, A.O., 2021. Responses of native egg parasitoids to the invasive seed bug *Leptoglossus occidentalis*. Agric. For. Entomol. 23, 323–333. https://doi.org/10.1111/afe.12434.

Maltese, M., Caleca, V., Guerrieri, E., Strong, W.B., 2012. Parasitoids of *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) recovered in western North America and first record of its egg parasitoid *Gryon pennsylvanicum* (Ashmead) (Hymenoptera: Platygastridae) in California. Pan-Pac. Entomol. 88, 347–355. https://doi.org/10.3956/2012-23.1.

Maple, J.D., 1947. The eggs and first instar larvae of Encyrtidae and their morphological adaptations for respiration. University of California Press. 8, 25–117.

Martel, G., Augé, M., Talamas, E., Roche, M., Smith, L., Sforza, R.F.H., 2019. First laboratory evaluation of *Gryon gonikopalense* (Hymenoptera: Scelionidae), as potential biological control agent of *Bagrada hilaris* (Hemiptera: Pentatomidae). Biol. Control 135, 48–56. https://doi.org/10.1016/j.biocontrol.2019.04.014.

Mercet, R. G., 1921. Fauna Ibérica. Himenópteros Fam. Encírtidos. Museo Nacional de Ciencias Naturales, Madrid, 732 pp. https://doi.org/10.5962/bhl.title.16110.

Millar, J.G., Zou, Y., Hall, D.R., Halloran, S., Pajares, J.A., Ponce-Herrero, L., Shates, T., Wilson, H., Daane, K.M., 2022. Identification and synthesis of Leptotriene, a unique sesquiterpene hydrocarbon from males of the Leaffooted Bugs *Leptoglossus zonatus* and *L. occidentalis*. J. Nat. Prod. 85, 2062–2070. https://doi.org/10.1021/acs.jnatprod.2c00470.

Mutke, S., Calama, R., Nasrallah Neaymeh, E., Roques, A., 2017. Impact of the Dry Cone Syndrome on commercial kernel yield of stone pine cones. Options Méditerr. Série A, Séminaires Méditerranéens 122, 79–84.

Nechols, J.R., Tracy, J.L., Vogt, E.A., 1989. Comparative ecological studies of indigenous egg parasitoids (Hymenoptera: Scelionidae; Encyrtidae) of the squash bug, *Anasa tristis* (Hemiptera: Coreidae). J. Kans. Entomol. Soc. 62, 177–188.

Niccoli, A., Benassai, D., Croci, F., Roversi, P.F., 2009. Anastatus Bifasciatus ooparassitoide di Leptoglossus occidentalis. Coppini Publishing, p. 337.

Olivera, L., Minghetti, E., Montemayor, S.I., 2020. Ecological niche modeling (ENM) of *Leptoglossus clypealis* a new potential global invader: Following in the footsteps of *Leptoglossus occidentalis*? Bull. Entomol. Res. 111, 1–12. https://doi.org/10.1017/S0007 48532 0000656.

Orr, D., 2009. Biological control and integrated pest management. In: Peshin, R., Dhawan, A.K. (Eds.), Integrated pest management: Innovation-development. Springer Science + Business Media BV, India, pp. 207–239. https://doi.org/10.1007/978-1-

4020-8992-3_9.

Parlak, S., 2017. An invasive species: *Leptoglossus occidentalis* (Heidemann) how does it affect forestry activities? Kastamonu University. Journal of Forestry Faculty 17, 531–542. https://doi.org/10.17475/kastorman.292220.

Pasek, J.E., Dix, E.M., 1988. Insect damage to conelets, second-year cones, and seeds of ponderosa pine in southeastern Nebraska. J. Econ. Entomol. 81, 1681–1690.

Ponce-Herrero, L., Farinha, A.O., Pando Fernández, V., Pajares Alonso, J.A., Alves Santos, F.M., 2022. Native egg parasitoids on *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae) in Spain: Potential biological control agents? J. Appl. Entomol. 146, 525–538. https://doi.org/10.1111/jen.12974.

Power, N. R., 2020. Evaluation of the Parasitoid *Ooencyrtus mirus* (Hymenoptera: Encyrtidae) as a Potential Biological Control Agent of *Bagrada hilaris* (Heteroptera: Pentatomidae). University of California, Riverside.

Roversi, P.F., Santini, L., Rossi, E., Loni, A., 2011a. Ooparassitoidi di Leptoglossus occidentalis in Toscana. In: Proceedings XXIII Congresso Nazionale Italiano di Entomologia. Erredi Publishing, pp. 13–16.

Roversi, P.F., Strong, W.B., Caleca, V., Maltese, M., Sabbatini Peverieri, G., Marianelli, L., Marziali, L., Strangi, A., 2011b. Introduction into Italy of *Gryon pennsylvanicum* (Ashmead), an egg parasitoid of the alien invasive bug *Leptoglossus occidentalis* Heidemann. EPPO Bulletin 41, 72–75. https://doi.org/10.1111/j.1365-2338.2011.02439.x.

Roversi, P.F., Sabbatini Peverieri, G., Maltese, M., Furlan, P., Strong, W.B., Caleca, V., 2014. Pre-release risk assessment of the egg-parasitoid *Gryon pennsylvanicum* for classical biological control of *Leptoglossus occidentalis*. J. Appl. Entomol. 138, 27–35. https://doi.org/10.1111/jen.12062.

Sabbatini Peverieri, G., Furlan, P., Simoni, S., Strong, W.B., Roversi, P.F., 2012. Laboratory evaluation of *Gryon pennsylvanicum* (Ashmead)(Hymenoptera, Platygastridae) as a biological control agent of *Leptoglossus occidentalis* Heidemann (Heteroptera, Coreidae). Biol. Control 61, 104–111. https://doi.org/10.1016/j.biocontrol.2012.01.005.

Sabbatini Peverieri, G., Furlan, P., Benassai, D., Caradonna, S., Strong, W.B., Roversi, P.F., 2013. Host egg age of *Leptoglossus occidentalis* (Heteroptera, Coreidae) and parasitism

by *Gryon pennsylvanicum* (Hymenoptera, Platygastridae). J. Econ. Entomol. 106, 633–640. https://doi.org/10.1603/ec12344.

Schowalter, T.D., 1994. Cone and seed insect phenology in a Douglas-Fir seed orchard during three years in Western Oregon. J. Econ. Entomol. 87, 758–765. https://doi.org/10.1093/jee/87.3.758.

Stiling, P., 1993. Why do natural enemies fail in classical biological control programs? Am. Entomol. 39, 31–37. https://doi.org/10.1093/ae/40.1.8.

Straser, R.K., Daane, K.M., Talamas, E., Wilson, H., 2022. Evaluation of egg parasitoid *Hadronotus pennsylvanicus* as a prospective biocontrol agent of the leaffooted bug *Leptoglossus zonatus*. BioControl 67, 123–133. https://doi.org/10.1007/s10526-022-10131-z.

Taylor, S.J., Tescari, G., Villa, M., 2001. A Nearctic pest of Pinaceae accidentally introduced into Europe: *Leptoglossus occidentalis* (Heteroptera: Coreidae) in northern Italy. Entomol. News 112, 101–103.

Tracy, J.L., Nechols, J.R., 1987. Comparisons between the squash bug egg parasitoids *Ooencyrtus anasae* and O. sp. (Hymenoptera: Encyrtidae): development, survival, and sex ratio in relation to temperature. Environ. Entomol. 16, 1324–1329. https://doi.org/10.1093/ee/16.6.1324.

Tunca, H., Venard, M., Colombel, E.A., Capelli, M., Tabone, E., 2019. Life history traits of *Ooencyrtus pityocampae* (Hymenoptera: Encyrtidae) reared on *Halyomorpha halys* eggs (Hemiptera: Pentatomidae). Entomol. Gen. 39, 93–101. https://doi.org/10.1127/entomologia/2019/0761.

Van den Bosch, R., Messenger, P. S., Gutierrez, A. P., 1982. An Introduction to Biological Control. Springer. New York. https://doi.org/10.1007/978-1-4757-9162-4_2.

Van Lenteren, J.C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H.M.T., Kuske, S., Loomans, A.J.M., Menzler-Hokkanen, I., Van Rijn, P.C.J., Thomas, M.B., Tommasini, M.G., Zeng, Q.-Q., 2003. Environmental risk assessment of exotic natural enemies used in inundative biological control. BioControl 48, 3–38. https://doi.org/10.1023/A:1021262931608.