Tunca Cosic *et al.*
Egyptian Journal of Biological Pest Control (2022) 32:117
https://doi.org/10.1186/s41938-022-00615-6

Egyptian Journal of Biological Pest Control

SCIENTIFIC (SHORT) NOTE

Open Access

Ooencyrtus pityocampae Mercet (Hymenoptera: Encyrtidae): a potential biocontrol agent of the seed bug, *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae)

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Abstract

Background: Western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae), is an invasive polyphagous pest of coniferous trees. It causes serious economic losses in pine kernel production. Biocontrol of *L. occidentalis* through the egg parasitoid *Ooencyrtus pityocampae* Mercet (Hymenoptera: Encyrtidae) seems to be sustainable solution to reduce economic losses of the pest. In this study, the biology of *O. pityocampae* was investigated on its natural host, *L. occidentalis*, under laboratory conditions.

Results: In this context, the effects of host age, female age, and temperature on parasitism rate, emergence rate, developmental time, and longevity were investigated. Female age had a great influence on the parasitism rate. Parasitism rates were 8.0, 17.0, and 37.3% in 1-, 3-, and 5-day-old females, respectively. There was no effect of host age or female age on emergence rates at the two different temperatures (25 and 30 °C). Emergence rate ranged from 74.0 to 88.3%. The longest developmental time (17.9 d) was obtained on 3-day-old hosts at 25 °C. The longevity, which was one of the important criteria of the parasitoid, was significantly affected by temperature.

Conclusion: The results of this study on the biology of *O. pityocampae* may contribute significantly to the biological control studies of *L. occidentalis*. This egg parasitoid can be mass-produced on lepidopteran hosts for field releases against *L. occidentalis.*

Keywords: *Leptoglossus occidentalis*, *Ooencyrtus pityocampae*, Biological control

Background

The Western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae), is an important conifer pest that feeds on the seeds' sap of coniferous trees, causing empty seeds. This pest is a North American origin. It was described in 1910 (Heidemann 1910). The pest is often found on pine trees (approximately 40 species) (Maltese et al. 2009) that feeds on young cones, sucking the sap from seeds containing protein and oil and causing empty seeds formation. Young cones fall prematurely due to the feeding. Regarding the economic damage of the species in Türkiye, the first detection was on *Pinus pinea* fields in İzmir-Bergama, causing economic losses in this region (Parlak 2017). For the control of harmful insects in forest areas, ecofriendly methods are preferred and one of them is biological control. Studies of the parasitoids of *L. occidentalis* are faunistic surveys. Ridge-O'Conner (2001) found the generalist adult parasitoid, *Trichopoda pennipes* (Fabricius 1781) (Diptera: Tachinidae), on the pest. Camponogara et al. (2003) reported the generalist egg parasitoid, *Anastatus*

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bifasciatus (Geoffroy) (Hymenoptera: Eupelmidae), on *L. occidentalis* egg masses. Bates and Borden (2004) reported another three egg parasitoids, *Gryon pennsylvanicum* Ashmead (Hymenoptera: Scelionidae), *Anastatus pearsalli* Ashmead (Hymenoptera: Eupelmidae), and *Ooencyrtus* spp. (Hymenoptera: Encyrtidae). Maltese et al. (2012) identified three egg parasitoids: *Gryon pennsylvanicum* Ashmead (Hymenoptera: Platygastridae), *Ooencyrtus johnsoni* Howard (Hymenoptera: Encyrtidae), and *Anastatus pearsalli* Ashmead (Hymenoptera: Eupelmidae). Oğuzoğlu ve Avcı (2020) reported two egg parasitoids: *Anastatus bifasciatus* (Geoffroy, 1785) (Hymenoptera: Eupelmidae) and *Ooencyrtus telenomicida* Vassiliev (Hymenoptera: Encyrtidae) on the *L. occidentalis* egg masses. Lesieur and Farinha (2021) recorded three egg parasitoids: *Anastatus bifasciatus*, *Ooencyrtus pityocampae*, and *O. telenomicida*. Ponce-Herrero et al. (2022) reported two native parasitoid species, *Ooencyrtus pityocampae* Mercet and *O. obscurus* Mercet (Hymenoptera: Encyrtidae), on the *L. occidentalis* eggs in Spain. As few biological studies have been carried out on the egg parasitoids of *L. occidentalis*, except of Binazzi et al. (2013), this study evaluated the effects of host and female ages on the potential of the parasitoid *O. pityocampae* at two different temperatures under laboratory conditions.

Methods

Rearing of *Leptoglossus occidentalis*

Leptoglossus occidentalis was collected from Pinus spp. fields around Bursa-Orhaneli province (39.751435° Lat-29.050089° Long and 996 m), in Türkiye. The collected adults were transferred to the biological control laboratory of Ankara University, Faculty of Agriculture, Department of Plant Protection. Adults were placed in the insect rearing box and kept under the climatic room (25 ± 1 °C, RH 65 ± 5% and L:D 16:8 h photoperiod). Pine nut seeds were provided for adults feeding, and *Pinus sylvestris* branches were placed in the box for females laying eggs. Moistened cotton with a solution honey water (10%) was placed inside a Petri dish to provide water and carbohydrates to the adults. In order to prevent adults from getting stressed, three Petri dishes with soil were also placed inside the rearing box.

Rearing of *Ooencyrtus pityocampae*

Eggs of the pine moth were collected from İzmir-Torbalı (38.178319° Lat-27.393717° Long and (94–227 m) province. Collected parasitized egg batches of pine processionary moth were transferred to a rearing room under controlled conditions of (25 ± 1 °C, RH 65 ± 5% and L:D 16:8 h photoperiod). The scales of the egg batches were removed and placed individually in test glass tubes closed by cotton and kept at the rearing room. The tubes were checked daily and the emerged parasitoids were separated, after they were identified under a stereomicroscope. Parasitoids *O. pityocampae* were placed into tubes separately and fed on honey water (10%) and maintained at a rearing room at (25 ± 1 °C, RH 65 ± 5% and L:D 16:8 h photoperiod). Daily collected *L. occidentalis* eggs were offered to the parasitoid, and their progeny were allowed to emerge. After their emergency, adult parasitoids were used in the experiments for the establishment of parasitoid cultures. Parasitoids were reared for over 10 generations on eggs of *L. occidentalis*.

Experimental procedures

To investigate the effect of female age, host egg age, and temperature, a 3 (1, 3, or 5 days old) × 2 (1 or 3 days) × 2 (25 or 30 °C) factorial design with 15 replicates was tested. In this experiment, 20 unparasitized *L. occidentalis* eggs (either 1 or 3 days old) were exposed in a test tube (1 × 7 cm) to one female of *O. pityocampae* (1, 3, or 5 days old) for 24-h parasitism at the two temperatures. The tested tubes were covered by cotton. Test tubes were checked daily until adult parasitoids emerged. Nymphs that hatched from unparasitized eggs were removed, and parasitism rate was calculated as a percentage. Number of the newly emerged *O. pityocampae* individuals was recorded daily. Emergence rate was calculated. To determine the longevity, honey water (10%) was given to adult parasitoids for feeding.

Statistical analysis

Parasitism rate, emergence rate, developmental time, and adult longevity were analyzed using a general linear model (GLM). Percentage data were normalized using an arcsine transformation (Zar 1999). The means were compared with Tukey's test at a significance level of $\alpha = 0.05$ (McKenzie and Goldman 2005; Minitab Release 14 2004; SAS Institute 2003).

Results

Statistical results are summarized in Tables 1, 2, 4, and 6. Only females' age had an effect on the parasitism rate (Table 1) ($F_2 = 128.51$, $P < 0.001$). The parasitism rate increased with the increase in the female age. The percentage of parasitism by *O. pityocampae* was nonsignificantly affected by host age ($F_1 = 2.17$, $P = 0.143$) and temperature ($F_1 = 0.95$, $P = 0.332$). The parasitism rate was the highest at the parasitoid age of 5 days (37. 3%). Parasitism rates were 8.0 and 17.0% in 1- and 3-day-old females, respectively. All observed factors and interactions were nonsignificant on the emergence rate (Table 2). All of the emergence rates ranged from 74.0 to 88.3% (Table 3). There was a significant interaction between the host age and temperature ($F_1 = 12.01$,

Table 1 Results of the GLM analysis of the parasitism rate of *Ooencyrtus pityocampae*

Source of variation	DF	SS	*F*	*P* value
Host age	1	69.21	2.17	0.143
Female age	2	8184.29	128.51	< 0.001
Temperature	1	30.15	0.95	0.332
Host age × Female age	2	43.12	0.68	0.510
Host age × Temperature	1	7.84	0.25	0.621
Female age × Temperature	2	25.36	0.40	0.672
Host age × Female age × Temperature	2	21.55	0.34	0.714
Error	138	4394.17		

Table 2 Results of the GLM analysis of the emergence rate of *Ooencyrtus pityocampae*

Source of variation	DF	SS	*F*	*P* value
Host age	1	281.1	0.45	0.501
Female age	2	84.3	0.07	0.934
Temperature	1	84.4	0.14	0.712
Host age × Female age	2	539.6	0.44	0.647
Host age × Temperature	1	190.6	0.31	0.580
Female age × Temperature	2	259.9	0.21	0.811
Host age × Female age × Temperature	2	252.5	0.20	0.816
Error	127	78,551.3		

Table 3 Results of the GLM analysis of the emergence rate of *Ooencyrtus pityocampae*

Female age	Emergence rate (%)			
	Temperature			
	25 °C		30 °C	
	Host age (1 day)	Host age (3 days)	Host age (1 day)	Host age (3 days)
1	79.16	83.33	74.07	81.25
3	81.03	78.0	82.43	86.70
5	86.63	84.83	88.34	85.50

There is no difference between the means

$P = 0.001$) on the developmental time of *O. pityocampae* (Table 4). The longest developmental time (17.9 days) was obtained from the 3-day-old *L. occidentalis* eggs at 25 °C, whereas the shortest development time (13.4) was obtained from the 1-day-old *L. occidentalis* eggs at 30 °C (Table 5). There was a significant two-way interaction between the female age and temperature on the longevity ($F_2 = 4.09$, $P = 0.018$) (Table 6). The longest longevity (34.8 days) was obtained from the 5-day-old female at

Table 4 Results of the GLM analysis of the development time of *Ooencyrtus pityocampae*

Source of variation	DF	SS	*F*	*P* value
Host age	1	114.268	138.93	< 0.001
Female age	2	0.295	0.18	0.836
Temperature	1	414.084	503.45	< 0.001
Host age × Female age	2	3.618	2.20	0.112
Host age × Temperature	1	9.876	12.01	0.001
Female age × Temperature	2	4.351	2.64	0.072
Host age × Female age × Temperature	2	0.091	0.06	0.946
Error	375	308.433		

Table 5 Development time of *Ooencyrtus pityocampae*

Development time (day)			
Temperature			
Host age (1 day)		Host age (3 days)	
25 °C	30 °C	25 °C	30 °C
15.94 ± 0.08B *n* = 111	13.44 ± 0.10D *n* = 96	17.90 ± 0.09A *n* = 82	14.52 ± 0.08C *n* = 98

Means in each row followed by the same capital letter do not differ statistically differences, $P \leq 0.05$

Table 6 Results of the GLM analysis of the longevity of *Ooencyrtus pityocampae*

Source of variation	DF	SS	F	*P* value
Host age	1	840.7	52.11	< 0.001
Female age	2	145.3	4.50	0.012
Temperature	1	6356.0	393.99	< 0.001
Host age × Female age	2	32.2	1.0	0.370
Host age × Temperature	1	14.8	0.92	0.339
Female age × Temperature	2	132	4.09	0.018
Host age × Female age × Temperature	2	26.1	0.81	0.446
Error	251	4049.2		

25 °C, while the shortest one (21.0 days) was obtained from the 5-day-old female at 30 °C (Table 7).

Discussion

The western conifer seed bug, *L. occidentalis*, is an economically damaging pest of stone pines (İpekdal et al. 2019). The habitat of the *L. occidentalis* is the forest areas; therefore, the use of insecticides for its control is extremely inconvenient. Biological control can be a key method for managing pests' populations in long-lived crops such as forestry plantations (Fischbein and

Table 7 Longevity of *Ooencyrtus pityocampae*

Longevity (day) **Temperature**					
Female age (1 day)		**Female age (3 days)**		**Female age (5 days)**	
25 °C	**30 °C**	**25 °C**	**30 °C**	**25 °C**	**30 °C**
33.74 ± 1.05B *n* = 19	22.50 ± 0.80E *n* = 17	31.77 ± 0.63C *n* = 53	21.24 ± 0.63D *n* = 50	34.87 ± 0.63A *n* = 62	21.03 ± 0.58D *n* = 62

Means in each row followed by the same capital letter do not differ statistically differences, $P \leq 0.05$

Corley 2015). In this study, some biological characteristics of the synovigenic egg parasitoid *O. pityocampae* were determined on its natural host, *L. occidentalis*, at two different temperatures. Results of the parasitism rate showed that female age of *O. pityocampae* was important for successful parasitism and had an effect on the parasitism rate. Highest parasitism rate (37.3%) was recorded in 5-day-old female parasitoids. The result was also supported by other researchers (Tunca et al. 2019). The effect of female age can vary depending on the parasitoid biological characteristics, such as synovigenic or pro-ovigenic.

All emergence rates were found above 74%. The emergence rate of *O. pityocampae* on the host *Philosamia ricini* Donovan (Lepidoptera: Saturniidae) differed according to the female age and host number. It ranged from 59.3 to 87.2% (Tunca et al. 2016). Tunca et al. (2019) reported that the emergence rate of *O. pityocampae* on the host *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) differed according to the host age and time of exposure.

The developmental time of the *O. pityocampae* was affected by temperature and host age. A shorter developmental time was obtained in one-day-old *L. occidentalis* eggs at 30 °C. The development of parasitoids was affected by the change of host species from which they developed (Jones et al. 2015) and temperature (Jerbi-Elayed et al 2021). Tunca et al. (2016) reported that the development time of *O. pityocampae* was influenced by the interaction of four factors (parasitoid age, parasitoid number, host number, and host egg age) on the host *P. ricini*. All of the developmental times ranged from 19.5 to 22.6 days. Also, three-way interactions among the host density, host age, and time of exposure on the *H. halys* were obtained. Developmental times ranged from 17.1 to 18.3 days (Tunca et al. 2019).

In the present study, it was found that the temperature significantly reduced the life span. In parasitoids, host species affects longevity; the life span of *O. pityocampae* was 46 days on *P. ricini* and 44 days on *H. halys* (Tunca et al. 2016 and Tunca et al. 2019).

Conclusion

The present study showed that the egg parasitoid *O. pityocampae* can be a potential biocontrol agent against *L. occidentalis* in pine trees, but further studies are needed.

Acknowledgements
All authors acknowledged the Bursa General Directorate of Forestry, for their support in providing necessary facilities for the study.

Author contributions
HTC contributed to investigations, writing, editing, and data analysis; ET, BC, ÖT, and AE helped in supervisions, methodology, and writing review. DÇ contributed to the rearing of insects. All authors have read and approved the final manuscript.

Funding
Funding information is not applicable.

Availability of data and materials
The datasets used and analyzed during this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 13 June 2022 Accepted: 5 October 2022
Published online: 14 October 2022

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