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EFFECTS OF *LEPTOGLOSSUS OCCIDENTALIS* HEIDEMANN (HETEROPTERA COREIDAE) EGG AGE ON THE INDIGENOUS PARASITOID *OOENCYRTUS PITYOCAMPAE* MERCET (HYMENOPTERA ENCYRTIDAE)

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

Leptoglossus occidentalis is a Nearctic polyphagous coreid capable to feed on cones and seeds of many coniferous plants. In Italy this pest threatens pine nut commercial production and represents a serious concern in protected areas where control by chemicals is restricted. For this reason, new strategies of biological control against *L. occidentalis* are under investigation. *Ooencyrtus pityocampae*, an indigenous egg parasitoid of the lepidopter *Thaumetopoea pityocampae* was recently found to parasitize *L. occidentalis* eggs. However low parasitization rates in the field were reported. Host egg age is often regarded as a key element in determining host acceptance and parasitoid capacity to exploit host eggs. Therefore, in the current work, the effects of host egg age on parasitization *O. pityocampae* were evaluated by exposing eggs ranging in age from < 24h to within a day of hatching. *O. pityocampae*. Results showed that number of parasitized eggs and sex ratio were not significantly influenced by the age of the host eggs. In contrast, female development time resulted to be longer in older eggs. *O. pityocampae* appeared to be not only flexible to adapt to the new host but also cable to efficiently exploit *L. occidentalis* eggs containing fully developed nymphs.

KEY WORDS: *Pinus pinea* L., Western Conifer Seed Bug, biological control, indigenous egg parasitoid.

INTRODUCTION

The Western Conifer Seed Bug (WCSB), *Leptoglossus occidentalis* Heidemann (Heteroptera Coreidae), is a polyphagous cone and seed feeder known to feed on several coniferous plant species (WERNER, 2011). This coreid is a pest native to Western North America that causes cone abortion and seed loss endangering seed production for reforestation of many pine species (KOERBER, 1963; HEDLIN *et al.*, 1981; CIBRIAN-TOVAR *et al.*, 1986; BATES *et al.*, 2000, 2002; STRONG *et al.*, 2001; STRONG, 2006). The pest, accidentally introduced into Europe, was firstly recorded in 1999 in Italy (TESCARI, 2001) where a few years later serious losses in commercial pine nut production due to feeding on *Pinus pinea* L. were reported (ROVERSI, 2009; SANTINI, 2010). The documented spreading history of *L. occidentalis* in Europe shows that this bug had a rapid expansion, moving from the mediterranean basin, to the United Kingdom, Scandinavia and Ukraine (EPPO, 2010; MJØS *et al.*, 2010; WERNER, 2011; HIZAL and INAN, 2012; GAPON, 2012; ZHU *et al.*, 2013).

In Italy pest control by chemicals is restricted because *P. pinea* woods are primarily located in protected areas along the coasts (parks, reserves and other natural habitats). Therefore, the use of natural biological control agents for targeting *L. occidentalis* is highly advisable. In North America, the platygastriid hymenopter *Gryon pennsylvanicum* (Ashmead) (Hymenoptera Platygastriidae) represents the predominant egg parasitoid of *L. occidentalis* (MASNER, 1983; BATES and BORDEN, 2004; MALTESE *et al.*, 2011). This egg parasitoid was introduced

in Italy in 2010 under quarantine conditions and its potential as a biocontrol agent is under investigation in laboratory conditions (ROVERSI *et al.*, 2011a; SABBATINI PEVERIERI *et al.*, 2012; SABBATINI PEVERIERI *et al.*, 2013; PAOLI *et al.*, 2013). Recent studies are particularly focusing on *G. pennsylvanicum* host range in order to detect potential negative effects of this hymenopter on the native fauna (ROVERSI *et al.*, 2013). Since *G. pennsylvanicum* introduction in the field is still a possibility under evaluation, an augmentative biological control program based on mass-production for field release of indigenous natural enemies may represent a valid alternative.

In Italy, two indigenous generalist egg parasitoids *Anastatus bifasciatus* (Geoffroy) (Hymenoptera Eupelmidae), and *Ooencyrtus pityocampae* (Mercet) (Hymenoptera Encyrtidae) were recently found to parasitize *L. occidentalis*. Nevertheless parasitization rates reported for these two hymenoptera were very low (CAMPONOVARA *et al.*, 2003; NICCOLI *et al.*, 2009; SANTINI, 2010; ROVERSI *et al.*, 2011b). Among these two hymenoptera, *O. pityocampae*, a parthenogenetic egg parasitoid of the lepidopter *Thaumetopoea pityocampae* (Den. & Schiff.) (Lepidoptera Thaumetopoeidae) deserves particular attention for its flexibility and capacity to adapt to different hosts including pentatomids and coreids (Heteroptera) (BATTISTI *et al.*, 1988).

Host egg age is often regarded as a key factor in the parasitization pattern. In fact changes in host quality related to egg age are reported as a consequence of modifications in host chemical composition, passing from simple stored nutrients to more developed embryonic tissues (see VINSON, 1998). These changes in the structure

of immature host may influence adult parasitoid preference and host acceptance. Host suitability for juvenile egg-parasitoid development may be affected as well with consequent variations in offspring biological traits like development time, adult size, mortality rates, and fitness of adult progeny (VINSON, 1998).

In some *Ooencyrtus* species younger host eggs are usually more suitable than older ones; nevertheless, even eggs close to hatching can be exploited by egg parasitoid females (NECHOLS *et al.*, 1989; TAKASU and HIROSE, 1993; HOFSTETTER and RAFFA, 1998).

In *L. occidentalis* eggs, host quality changes rapidly due to the short period of the embryonic development (SABBATINI PEVERIERI *et al.*, 2013); therefore in the current work the effects of WCSB egg age on *O. pityocampae* were investigated as a preliminary step in the evaluation of its potential for mass-rearing.

MATERIALS AND METHODS

INSECT ORIGIN

Parasitized egg batches of *T. pityocampa* hosting diapausing immature stages of *O. pityocampae* were initially collected from pine stands (300 m asl) in Gargano region (South East of Italy). Afterward batches were set into glass tubes (15 cm long and 2 cm diameter closed on both ends by a plastic net of 250 micron mesh) and placed in a rearing room at $30\pm1^{\circ}\text{C}$, $40\pm10\%$ RH and 16:8 L:D in order to interrupt parasitoid diapause (HALPERIN, 1990; BATTISTI *et al.*, 1990). After three weeks parasitoids hatched and a laboratory colony was established. Parasitoids were reared for over 15 generations on eggs of *L. occidentalis* in a climatic chamber (Binder KBWF 720, Tuttlingen, Germany) at standard condition of $26\pm1^{\circ}\text{C}$, $75\pm5\%$ RH and 16:8 L:D before the beginning of the experiments. Adult females were provided with pure honey drops *ad libitum* as food source. Honey drops were then refreshed three times per week.

L. occidentalis was originally collected in pine stands in Central Italy and reared in insect cages in rearing rooms at $26\pm1^{\circ}\text{C}$, $40\pm10\%$ RH and 16:8 L:D using young potted *P. pinea* as an oviposition substrate, and pine seeds of the European Black Pine (*Pinus nigra* Arnold *s.l.*) as a food source. In addition each colony cage was provided with moistened cotton as a water source. This method allowed egg production all year round.

HOST EGG AGING

L. occidentalis females lay their eggs in a single row on needles and clusters consist of about 3-20 eggs each. Because under laboratory conditions eggs of *L. occidentalis* are reported to hatch in a mean of 8.9 d., host eggs from seven age classes were employed to carry out the experiments: fresh eggs (< 24h) and 2, 3, 4, 5, 6, 7 days old (NECHOLS *et al.*, 1989; HIROSE *et al.*, 2003; SABBATINI PEVERIERI *et al.*, 2013).

EFFECTS OF THE HOST EGG AGE ON *O. PITYOCAMPAE* PARASITISM

The oogenesis of *O. pityocampae* is not complete at the emergence. In fact the quantity of mature eggs after the emergence is low and it is reported to increase for reaching its maximum only after several days (BATTISTI *et al.*, 1990). Our preliminary data on *O. pityocampae* biological traits are quite consistent with these findings showing that parasitization peak of females reared on *Graphosoma*

lineatum (L.) (Heteroptera Pentatomidae) eggs lies between days 4 and 7 of parasitoid life (BINAZZI and ROVERSI, unpublished data). Therefore in the current experiment newly hatched specimens were isolated one per each tube, maintained at standard rearing conditions and fed with honey *ad libitum* for 5 days before the trial. Eggs of *L. occidentalis* were daily collected from the colony by removing needles bearing an egg cluster from the pine plants. Batches of 15 eggs (*ad libitum*) were then aged in a climatic chamber at standard conditions and subsequently exposed for parasitization to *O. pityocampae* females, after tightening the egg-bearing pine needle by wire paper clips onto paper strips (BATES and BORDEN, 2004, 2005; SABBATINI PEVERIERI *et al.*, 2012).

A host egg batch of, all in one of the seven egg age classes, was offered to each female for a 24 h parasitization time lag. *L. occidentalis* eggs were considered parasitized when the presence of at least one egg stalk protruding from the host chorion was verified (MAPLE, 1947; BATTISTI *et al.*, 1988; NECHOLS *et al.*, 1989). After parasitization, host egg batches were removed and placed in a climatic chamber at standard conditions. Egg clusters were then daily checked until parasitoids emerged or non parasitized eggs hatched. All tests were replicated 13 times and the following parameters were recorded: no. of parasitized host eggs per batch, no. of egg stalks per batch, offspring production (no. of females emerged per batch), sex ratio (% females), and female development time.

MICROSCOPICAL OBSERVATIONS

After parasitoid emergence all parasitized host eggs were dissected under a stereomicroscope in order to describe the host egg content.

DATA ANALYSIS AND STATISTICS.

All data were tested for normality with the Shapiro-Wilk test. Transformations were applied but failed to normalize the data. Therefore One-way nonparametric ANOVA (Kruskal-Wallis test) followed by the Mann-Whitney U-test was used to compare the recorded parameters in the seven age classes. For multiple comparisons, the significance level ($\alpha = 0.05$) was adjusted with Bonferroni correction, α/n , where n was the number of pairs in the multiple comparison. Moreover the correlation of each parameter with host egg age was analyzed by non-parametric Spearman's rho test (ZAR, 2010). Statistical procedures were performed by the statistical software SPSS 20.0.0 (2011).

RESULTS

In laboratory tests *O. pityocampae* accepted and parasitized *L. occidentalis* host eggs of each age class and completed its development either in newly oviposited host eggs (<24h) or in eggs containing pharate nymphs close to hatching. No statistically significant differences among the seven age classes were observed in the no. of parasitized host eggs per batch (Kruskal-Wallis: $H = 9.21$; $df = 6$; $n = 89$; $P > 0.05$), no. of egg stalks per batch (Kruskal-Wallis: $H = 9.87$; $df = 6$; $n = 89$; $P > 0.05$), offspring production (Kruskal-Wallis: $H = 10.74$; $df = 6$; $n = 89$; $P > 0.05$) and sex ratio (Kruskal-Wallis: $H = 12.44$; $df = 6$; $n = 86$; $P > 0.05$). In contrast development time of immature *O. pityocampae* females was significantly affected by host egg age and increased in older *L. occidentalis* eggs from 14.85 ± 0.09 SE days on 24h eggs to 17.02 ± 1.25 SE days on 7-

days eggs (Kruskal-Wallis: $H = 15.24$; $df = 6$; $n = 62$; $P < 0.05$) (Table 1).

In addition host egg age was not significantly correlated with no. of parasitized host eggs per batch (Spearman's $\rho = -0.009$, $n = 89$ $P = 0.930$), no. of egg stalks per batch (Spearman's $\rho = 0.049$, $n = 89$, $P = 0.651$), offspring production per batch (Spearman's $\rho = 0.050$, $n = 89$ $P = 0.644$) and sex ratio (Spearman's $\rho = -0.161$, $n = 84$, $P = 0.142$). On the contrary a significant correlation was detected between host egg age and female development time (Spearman's $\rho = 0.301$, $n = 62$, $P = 0.017$).

After parasitoid hatching, the residues remaining inside the egg shell of *L. occidentalis* were analyzed and some relevant differences noticed (Fig. I). When the coreid eggs had been parasitized immediately after oviposition only few fecal pellets could be observed (Fig. I, 2). By contrast when parasitization had occurred at the end of embryo development a high quantity of fecal pellets could be detected (Fig. I, 4). In figure I,3, on a 7-days old dissected host egg, the remains of a *L. occidentalis* nymph adherent to the inner side of the chorion can be observed in transparency on the right side of an *O. pityocampae* hatching hole.

DISCUSSION

Egg-parasitoid strategies for exploiting variable host resources, including variation in the quality of a host egg over time, were described by VINSON (1998).

The parasitization pattern of encyrtids on old host eggs was investigated by TAKASU and HIROSE (1993) for *Ooencyrtus nezarae* Ishii. After exposing *Riptortus clavatus* Thunberg (Heteroptera Coreidae) eggs to females of *O. nezarae*, a decrease in number of laid eggs with increasing host age was observed. By contrast progeny survival, development time and size of emerged adults did not show significant differences among host ages except for a reduced *O. nezarae* survival rate in 7-days old eggs. The quantity of host material suitable for *O. nezarae* juvenile development seemed to decrease with increasing host age because larvae can not feed on sclerotized parts that remain in the host after adult hatching.

A similar parasitization pattern was also observed by NECHOLS *et al.* (1989) for another undetermined *Ooencyrtus* species and, to a lesser extent, for *Ooencyrtus anasae* Ashmead (Hymenoptera Encyrtidae), whose responses to *Anasa tristis* (DeGeer) (Heteroptera Coreidae) host egg age were more variable. Nevertheless in the oldest (9-days old) *A. tristis* host eggs, lower rates of parasitization, longer development times, increased immature mortality and female offspring reduction were recorded for both parasitoid species.

In other studies the combined effects of parasitoid age and host egg age on female performance were highlighted by HOFSTETTER and RAFFA (1998) who carried out a trial in which egg masses of different ages oviposited by the lepidopteran *Limantoria dispar* (L.) (Lepidoptera Limntriidae) were exposed for parasitization to females of *Ooencyrtus kuvanae* (Howard) (Hymenoptera Encyrtidae). As a result offspring production and proportions of females decreased when *O. kuvanae* immatures developed in older *L. dispar* eggs.

O. pityocampae showed a marked flexibility in the exploitation of the new host as most of the parameters considered were not influenced by the age of *L. occidentalis* eggs that ranged from fresh eggs to eggs

Table 1-Mean no. ($\pm SE$) of parasitized eggs/batch, egg stalks/batch (means $\pm SE$), offspring production (means $\pm SE$), female development time (days, means $\pm SE$) and sex ratio (%) of females $\pm SE$) of *Ooencyrtus pityocampae* on *Leptoglossus occidentalis* host eggs of different ages.

Parameter	Host egg age						
	<24h	2 d	3 d	4 d	5 d	6 d	7 d
No. of parasitized eggs	3.85 (± 0.79)a	3.20 (± 0.94)a	3.87 (± 1.01)a	3.27 (± 1.22)a	5.77 (± 0.77)a	4.55 (± 1.46)a	2.50 (± 1.04)a
No. of egg stalks	4.35 (± 1.05)a	4.33 (± 1.43)a	4.27 (± 1.32)a	4.45 (± 1.78)a	7.85 (± 1.22)a	5.64 (± 1.72)a	4.20 (± 2.04)a
Offspring production	4.35 (± 1.05)a	4.33 (± 1.43)a	3.87 (± 1.31)a	4.45 (± 1.78)a	7.77 (± 1.23)a	5.64 (± 1.72)a	3.80 (± 1.77)a
Female development time (d)	14.85 (± 0.09)a	14.67 (± 0.16)ab	15.01 (± 0.28)ab	15.85 (± 0.68)ab	14.79 (± 0.10)ab	15.20 (± 0.22)ab	17.02 (± 1.25)b
Sex ratio (% of females)	100a	100a	100a	84.53 (± 8.90)a	96.95 (± 2.57)a	100a	95.83 (± 4.16)a

Means in the same row followed by the same letters are not significantly different (Kruskal-Wallis Test; Mann-Whitney Test; $P < 0.05/21$).

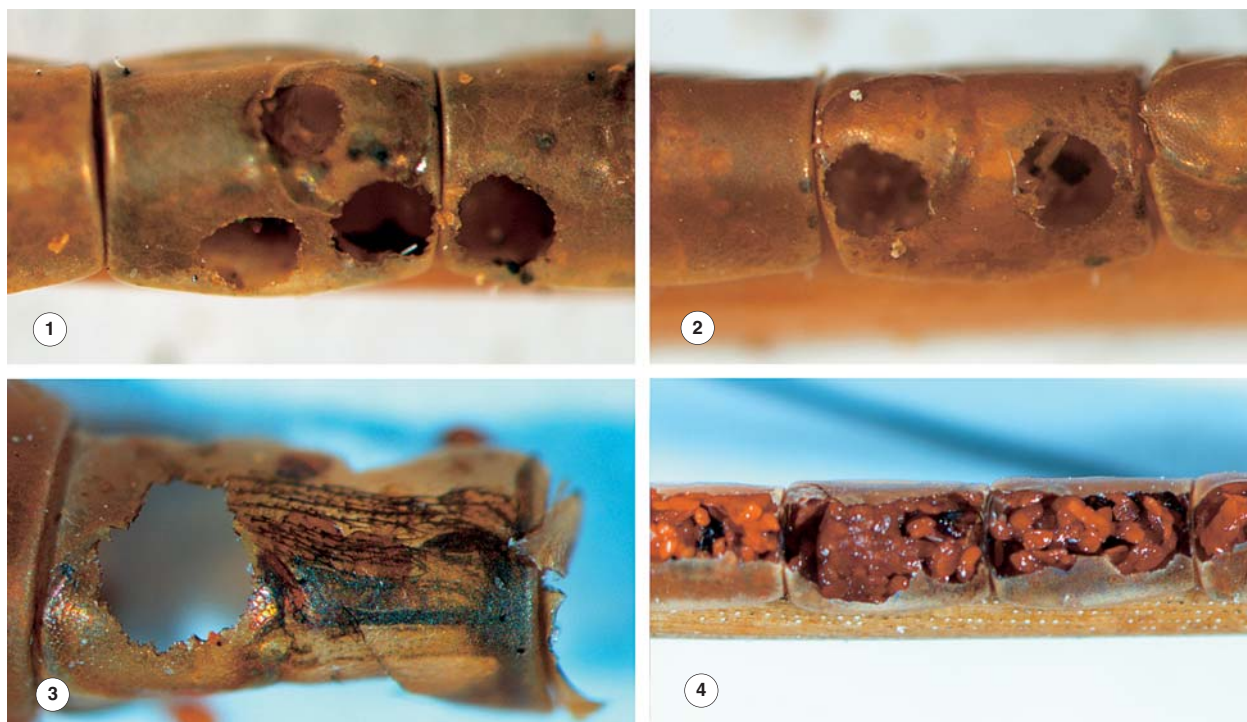


Fig. 1 – *Leptoglossus occidentalis* Heidemann eggs parasitized by *Ooencyrtus pityocampae* (Mercet): (1, 2) 1-day old parasitized eggs with parasitoid hatching holes and few fecal pellets visible inside the egg shell; (3) 7-days old parasitized eggs with sclerotized parts of a *L. occidentalis* nymph impressed on the inner side of the egg chorion; (4) 7-days old parasitized eggs dissected to show the several fecal pellets produced by developing parasitoids (parasitization occurred when host eggs contained pharate nymphs close to hatching).

presenting completely developed nymphs close to hatching. Infact data such as the number of parasitized eggs and sex ratio were not significantly affected by differences in host egg ages. Only *O. pityocampae* females developing in older eggs took longer in reaching adulthood, highlighting how in older host eggs the conversion of free nutrients into a more complex substrate (e.g., chitinated tissues) may partially impair both food quality and food availability (VINSON, 1998).

Our data on *O. pityocampae* parasitization of *L. occidentalis* eggs suggest a departure of this species from *Ooencyrtus* sp. general trend except for juvenile development time where results were more consistent with those recorded for *O. anasae*.

These findings may represent an adaptation to effective exploitation of older eggs in egg-parasitoids that target hosts whose eggs remain viable in nature only for a short period or show a rapid embryonic development followed by a more or less long lasting larval and pharate nymph stage. This phenomenon is evident in both *L. occidentalis* and *Thaumetopoea processionea* (L.) (Lepidoptera Thaumetopoeidae) (BIN and TIBERI, 1983).

In conclusion, recent studies on egg-parasitoids have focused on host egg age as a key factor for parasitization and in several works a reduced parasitoid acceptance of older batches and a decreased suitability of older eggs over fresh ones were often reported. This trend despite being widespread among many different egg-parasitoid families (see NECHOLS *et al.*, 1989; VINSON, 1998) did not apply to *O. pityocampae* reared on *L. occidentalis* at the conditions tested in our experiment. On the contrary *O. pityocampae* parasitization pattern was more consistent with the trend

described by SABBATINI PEVERIERI *et al.*, (2013) for *G. pennsylvanicum* parasitization of *L. occidentalis*.

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