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# Life History of the Invasive Bug *Oxycarenus lavaterae* (Heteroptera: Oxycarenidae) in Bulgaria

***Oldřich Nedvěd[1,2], Evgeni Chehlarov[3], Plamen Kalushkov[3]***

[1]Faculty of Biological Sciences, University of South Bohemia
[2] Institute of Entomology, Biology Center, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic
[3]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Blvd. Tzar Osvoboditel, 1000 Sofia, Bulgaria
E-mails: nedved@prf.jcu.cz; plamenkalushkov@yahoo.com

**Abstract:** Adult females of the Bulgarian population of true bug *Oxycarenus lavaterae* were 4.4 to 5.4 mm long. Their fresh weight in the end of September, at the beginning of diapause, ranged from 3.8 to 5.8 mg. The males were smaller, 4.25 to 4.95 mm long, with a fresh weight ranging from 2.2 to 4.1 mg. The longevities of adults transferred to the laboratory in October and reared at a long day (18L:6D) photoperiod and a temperature of 20 ± 2°C were 113 days. The pre-oviposition period of this group was 46 days and females laid 392 eggs in average. The longevities of adults transferred to the laboratory in November and reared at the same conditions were 79 days. The pre-oviposition period of this group was 46 days and females laid 331 eggs in average. A third group of adults was transferred to the laboratory in February. Their longevity was 63 days, the pre-oviposition period – 26 days and mean fecundity – 215 eggs. The mean (±S.D.) fecundity in the merged groups was 276 ± 150 eggs. The mating frequency (percentage of days with mating observed) was between 30 and 40% of the observations. Fecundity decreased, while the mating frequency and longevity increased with the time of overwintering.

**Keywords:** Size, weight, sexual dimorphism, pre-oviposition period, longevity, fecundity, mating

## Introduction

True bug *Oxycarenus lavaterae* (Fabricius, 1787) (Heteroptera: Lygaeiodea: Oxycarenidae) is distributed in the Palaearctic region; in Europe it has earlier been known from the Mediterranean (Pericart 2001). During the last 20 years *O. lavaterae* spread northwards and was found in Montenegro (1985), Hungary (1994), Slovakia (1995), Serbia (1996), Bulgaria (1998), Northern France (1999), Austria (2001), Northern Switzerland (2002), Finland (2003), Czech Republic (2004), Germany (2004), and Romania (2009) (Rabitsch 2008, 2010).

This alien species is included among pest insects in some countries (Velimirović *et al.* 1992). In Spain, the bug was the most numerous among heteropteran species in a citrus grove but no damages were detected (Ribes *et al.* 2004). In Paris it caused no damage or nuisance as well (Reynaud 2000). The bug has extended its geographical range northerly – to colder regions – probably with the rise of the temperature on the Earth, and it is likely to become an agricultural (Velimirovic *et al.* 1992) or a nuisance (Wermelinger *et al.* 2005) pest in the future. During its expansion, it seems to switch from herbaceous Malvaceae host plants to utilisation of the linden trees (*Tilia*), similarly to the firebug *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae) that is also known to breed on herbaceous and shrubby Malvaceae in south Europe and on *Tilia* trees in central Europe (Hauznerová 2003). The reported host plants of *O. lavaterae* extend outside the order Malvales (Wermelinger *et al.* 2005, Kalushkov, Nedvěd 2010).

Even in warmer countries such as Spain, *O. lavaterae* hibernates as adults, which have a balanced sex ratio. In Basel, Switzerland, it survives the long winter with temperatures as low as –10°C without significant mortality (Wermelinger *et al.* 2005). The species form large exposed overwintering aggregations on trunks and branches of *Tilia* or, in lower numbers, on the herbaceous plants *Lavatera* sp. In Sofia, Bulgaria, when the winter temperatures fall below –15°C for a several days, the mortality in the winter aggregations reach up to 99% (Kalushkov *et al.* 2007b). In Bulgaria the individuals start to disperse from winter aggregations in May, which coincides with the beginning of flowering of lindens (Simov *et al.* 2012). Passive dispersal by human-mediated translocations via clothes, cars, etc., is the most probable cause of spread of this species in Bulgaria (Simov *et al.* 2012). Kalushkov *et al.* (2007a, 2007b) published some data about biology of this bug. Nevertheless, to date there have been no in-depth investigations on the life history of *O. lavaterae*.

The aim of the present study is to record some selected biological parameters of the Bulgarian population of *O. lavaterae*.

# Material and Methods

### Weight and size

The fresh weight of individual adult specimens (40 males and 40 females) was measured on Sartorius analytic balances (sensitivity 0.1 mg), while the size (length of body and width of pronotum) under a stereomicroscope equipped with an eyepiece scale that provided an accuracy of 0.05 mm, at the beginning of diapauses at the end of September. Power function regression (not linear regression of log-transformed data, see Packard *et al.* 2011) between the body weight and product of the two linear dimensions representing the size allometry in *O. lavaterae* was used for calculating the body mass index (BMI) as the ratio between fresh weight (Fw) and the 1.28 power of product of length (L) and width (W):

$$\mathrm{BMI} = \frac{100\ \mathrm{Fw}}{(\mathrm{L\ W})^{1.28}}$$

### Fecundity, longevity, mating frequency

About 500 larvae of the fourth and fifth instars of *O. lavaterae* were collected in late September 2004 near Sofia on linden trees and reared under laboratory conditions, fed with linden seeds from *Tilia cordata* Miller, and supplied with water. After emergence on the 5th October the adults were stored outdoors and only 15 pairs were selected and kept in laboratory, each pair in a separate Petri dish (7 x 1.2 cm), with zigzag filter paper inside. The bugs were reared at a long day (18L:6D) photoperiod and a temperature of 20 ± 2°C. Each pair was provided with about 10 linden seeds and water in glass vials (3 cm x 1 cm) supplied with a cotton plug.

The seeds were without coat (= shell) because females often lay eggs on the inner side of coat, making them inaccessible to counting. Daily we recorded the number of mating pairs and the number of eggs. We also recorded the pre-oviposition period and longevity of each female. The variable mating frequency analysed correspond to the percentage of days when the pairs of *O. lavaterae* were observed to mate. Eggs were removed regularly and life-span fecundity was counted. Another group of 15 pairs were included into the experiment on the 20th November after 45 days of rest outdoors, and a third group of 15 pairs was included on the 2nd February after 122 days outdoors. Four females from October, four from November and two from February died before starting to lay eggs.

# Results

### Body size

The adult females were 4.4 to 5.4 mm long (average 5.11 ± 0.23, median 5.15 mm, distribution negatively skewed ), and 1.5 to 1.75 mm wide (average 1.60 ± 0.06, median 1.60 mm). The fresh weight at the end of September, when adults are with most energetic reserves, ranged from 3.8 to 5.8 mg, with an average of 4.5 ± 0.5 mg, median of 4.4 mg, and distribution was slightly positively skewed.

The adult males were smaller, 4.25 to 4.95 mm long (average 4.54 ± 0.18, median 4.53 mm, distribution positively skewed), and 1.30 to 1.50 mm wide (average 1.44 ± 0.05, median 1.45 mm). The fresh weight ranged from 2.2 to 4.1 mg, with an average of 3.0 ± 0.4 mg, median of 3.1 mg, and distribution symmetric. The length/width ratio was 3.2:1 in both sexes.

The length of females was 1.13 times greater than that of males (Mann-Whitney U test: $Z=7.0$, $p<10^{-6}$). The weight of females was 1.5 times higher than that of males (Mann-Whitney U test: $Z=7.6$, $p<10^{-6}$). The body mass index was 29 in females and 27 in males, which means that females were more robust.

The correlation between the fresh weight and the product of length and width was not significantly close in either sex, meaning that the mass index was highly variable, almost independent of the size of body in either male or female. Only when the two sexes were merged, the weight/size relationship became visible (Fig. 1). The exponent parameter of the power function was 1.28 ± 0.13, meaning that mass was dependent on size with an exponent of 2.56.

### Fecundity

Lifetime fecundity of *O. lavaterae* ranged from 22 to 589 eggs per female. The median fecundity of the first (October) group was 392 eggs per female, of the second (November) group 331 eggs, and of the third (February) group 215. There was a slight negative correlation between fecundity and the time of outdoor storage before starting the particular part of experiment ($F=6.9$, $p=0.013$), and the storage time

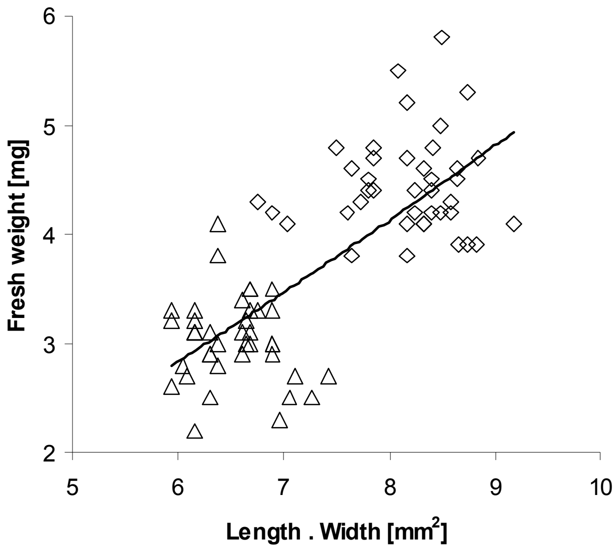


**Fig. 1**. Weight-size relationship in *O. lavaterae* collected in the field in late September. Triangles represent males, diamonds females. Trend is a power function: Fw=0.29·(L·W)$^{1.28}$.

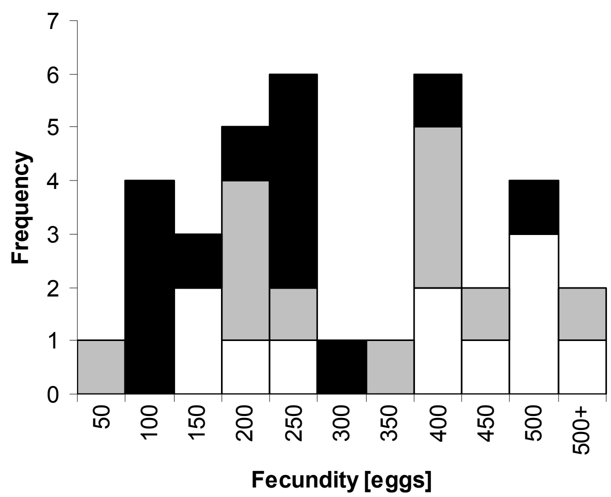


**Fig. 2**. Distribution of life-span fecundity (number of eggs) in *O. lavaterae* kept at 20°C and 18L: 6D photoperiod. White bars: October group; grey bars: November group; black bars: February group. Scale labels indicate upper limits of individual fecundity classes.

explained 15% of variability in fecundity. However, Kruskal-Wallis test of homogeneity showed no significant difference among these three groups (chi-square=2.81, p=0.25) and that is why we merged the groups. The mean (±S.D.) fecundity was 276±150 eggs, and the median 230 eggs per female. The distribution of the merged fecundity values found in *O. lavaterae* was rather bimodal (Fig. 2).

### Longevity

The distribution of all the values of longevity resembled normal distribution. There was a gradual decrease in the longevity with storage time (Kruskal-Wallis test chi-square=7.2, p=0.03). The median longevities measured under laboratory conditions for the particular groups were 113 days (October), 79 days (November), and 63 days (February) (Fig. 3).

The correlation between longevity and fecundity was significantly close, positive (Fig. 4), (F=6.4, p=0.016), with longevity explaining 14% of the variability in fecundity.

### Pre-oviposition period

The pre-oviposition period decreased with time of overwintering, but the difference between the three groups was marginal (Kruskal-Wallis test: chi-square=6.1, p=0.05). The median times of the groups were 46, 43, and 26 days, respectively, the common median was 39 days, and the distribution rather equitable (Fig. 5). There was no relationship between the pre-oviposition period and fecundity (F=0.16, p=0.69).

### Mating frequency

Large proportion of the pairs displayed mating frequency between 30 and 40% of observations (Fig. 6). This behaviour was stable among the three groups (Kruskal-Wallis test H=1.6, p=0.46). The median mating frequency for all 35 ovipositing pairs was 36%. Those pairs that did not oviposit did not mate or mated at low frequency.

The correlation between the mating frequency and fecundity was significantly close, positive and parallel for the three separate groups and for the three groups together (Fig. 7) (F1.33=7.6, p=0.009), with mating frequency explaining 17% of the variability in fecundity. There was no correlation between the longevity and mating frequency (r=0.04, F=0.06, p=0.81).

### Multiple correlations

Because we have got several explaining variables for the total fecundity, which were inter-correlated, we set up a multivariate model: linear regression of fecundity dependent on pre-oviposition period, longevity, storage time before including into experiment (0, 45, 122 days), and mating frequency. The complete multiple linear regression was very significant (F=7.75, p=0.0002), the total explained variability was 44%. Significant partial correlations were found with the variables storage time (F=8.22, p=0.008), mating frequency (F=6.05, p=0.02) and longevity (F=4.42, p=0.04). The pre-oviposition period was not significantly correlated with fecundity.

## Discussion

According to Péricart (1999), the body length of the adults in *O. lavaterae* is: 4.7 – 5.1 mm in males and 5.5 – 6.0 mm in females. In our sample of *O. lavaterae,* the males and females overlapped in their body

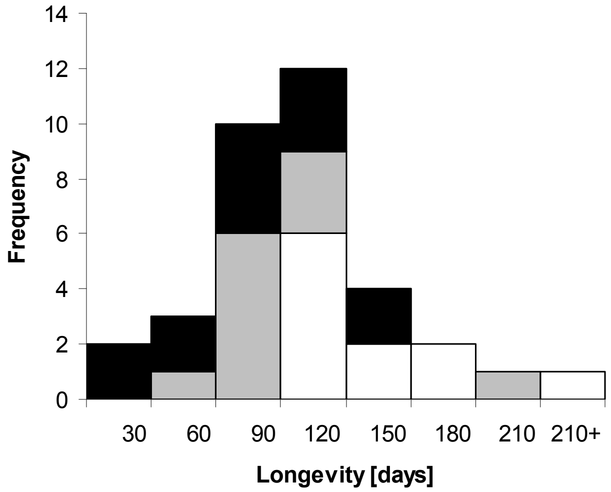


**Fig. 3**. Distribution of longevity values in females of *O. lavaterae* kept at 20°C and 18L: 6D photoperiod. White bars: October group; grey bars: November group; black bars: February group. Scale labels indicate upper limits of individual longevity classes.

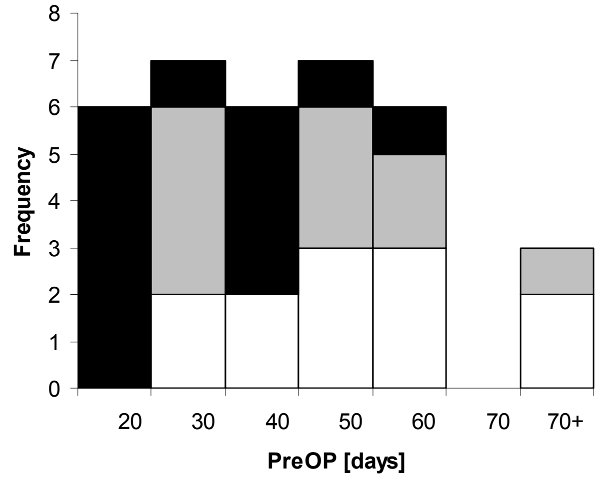


**Fig. 5**. Distribution of pre-oviposition period (days) in females of *O. lavaterae* kept at 20°C and 18L: 6D photoperiod. White bars: October group; grey bars: November group; black bars: February group. Scale labels indicate upper limits of individual longevity classes.

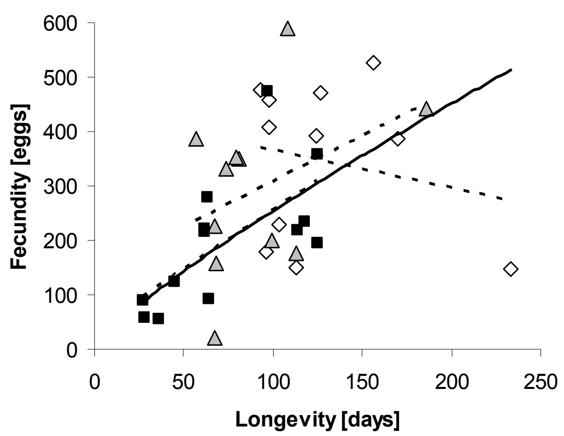


**Fig. 4**. Relationship between longevity and life-span fecundity in *O. lavaterae* transferred into the laboratory (20°C and 18L: 6D photoperiod ) from outdoors in October (white diamonds), November (grey triangles), and February (black squares). Common trend (solid curve) is an arbitrary power function fit, partial fits (dotted) are linear.

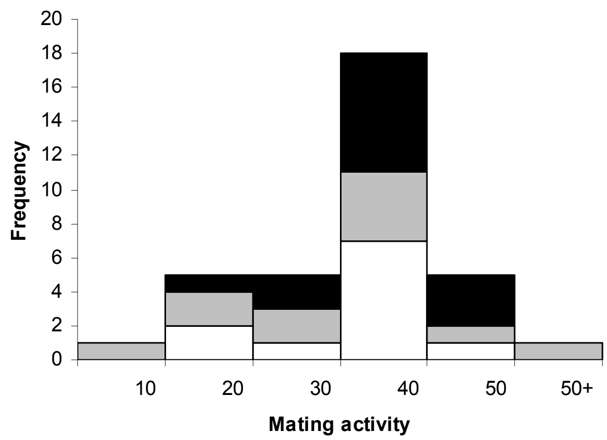


**Fig. 6**. Distribution of mating frequency (percentage of days with mating observed) in pairs of *O. lavaterae* kept at 20°C and 18L: 6D photoperiod. White bars: October group; grey bars: November group; black bars: February group. Scale labels indicate upper limits of individual longevity classes.

length, but more than a half of females were larger than the largest male. Overlap in the fresh weight between the two sexes was negligible (two heavy males). If the two size characteristics were combined, the result would distinguish the two sexes unambiguously (see Fig. 3). Such a phenomenon was used for sexing the handsome fungus beetle, *Stenotarsus subtilis* (Nedvěd, Widsor 1994). Sexual size dimorphism expressed as a ratio of the mean fresh weight of females to that of males of *O. lavaterae* was 1.5 times, which is much higher than is usual in beetles (1.1–1.2 times, Nedvěd, Honěk 2012). The weight-size relationship value is close to usual values in insects (Rogers *et al.* 1976).

The fecundity of *O. lavaterae* decreased slightly with the time spent outdoors before the experiment (from October to February). In an experiment from the autumn 2005, at a higher temperature the mean fecundity was approximately the same – 291 eggs (Kalushkov *et al.* 2007a). In some insects, the natural (horotelic) diapause development at natural outdoor conditions during autumn and early winter increases their performance including subsequent fecundity (*P. apterus,* Hodek 1974). However, the prolonged cold storage at 3 and 6°C has no an adverse effect on fecundity of the ladybird beetle *Harmonia*

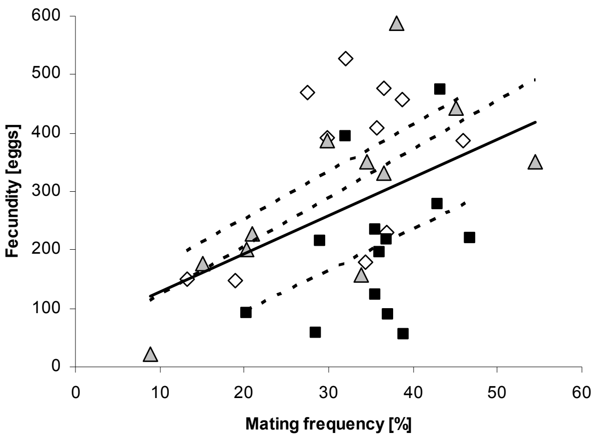


**Fig. 7**. Relationship between mating frequency and life-span fecundity in *O. lavaterae* transferred into the laboratory (20°C and 18L:6D photoperiod ) from outdoors in October (white diamonds), November (grey triangles), and February (black squares). Plotted are partial trends for separate groups (dashed lines) and the common trend (solid line).

*axyridis* as compared to that of fresh females. Those females that experience moderately long (90 day) storage at 0°C have the largest reproductive capacity (Ruan *et al.* 2012). Fecundity of braconid wasp *Habrobracon hebetor* decreases after more than 20 days of storage at 5°C (Chen *et al.* 2011).

Hodek, Iperti (1983) suppose that the fast intensive activation by long days (by tachytelic processes) may be less suitable for reproduction than the slower horotelic processes at short days. In our case, the insects that had not undergone natural diapause development were more fecund. However, the duration of the exposure outdoors before the experiment explained only 15% of variability in fecundity. Longevity positively influenced the life-span fecundity, but again explained only 14% of the variability. Unfortunately, we failed to measure the body size in those females included in the fecundity experiment in order to see whether the size was responsible for variability in fecundity.

Distribution of fecundity values in insects is often positively skewed (many small values, few very large values, *e.g.* in *Dolycoris baccarum* under 12L:12D (Hodek, Hodková 1993), while other types of distribution, such as the bimodal distribution, found here in *O. lavaterae* (Fig. 4) or polymodal distribution in *Dolycoris baccarum* under 18L:6D (Hodek, Hodková 1993) are rare.

Repeated and multiple mating is widespread even in insect species where nuptial gifts are not involved. Mating twice within a 10-day span causes a 16% increase in fecundity in *Lygus hesperus*, mating more than twice during the same period does not cause additional increases in the egg number (Brent *et al.* 2011). However, fecundity is reduced in females of *Nezara viridula* mated more times, although those females have an increased longevity (Fortes, Consoli 2011). The positive effect of very high mating frequency in our *O. lavaterae* was rather unexpected.

Diapauses of different intensity can be induced in individual adults hatching at the end of September and thus different pairs might need differently strong stimuli for activation in laboratory. In the ladybird *Ceratomegilla undecimnotata*, females activated only by temperature increase at 12L:12D photoperiod, live longer and have higher daily oviposition rate than the individuals artificially activated at 18L:6D photoperiod (Hodek, Iperti 1983). The regression between longevity and fecundity (Fig. 6) of our bugs supports the observation of constant oviposition rate. The longevity is shorter in *Oxycarenus gossypinus* males (44–59 days) and females (33–41 days) than in our *O. lavaterae* females (63–113 days), but the former species was investigated at temperatures fluctuating between 25 and 33°C (Evete, Osisanya 1985) while the latter at 20°C.

The fecundity in *Pyrrhocoris apterus* with 360–550 eggs per female (Hodek 1988) is well comparable to that of *O. lavaterae* (276 egg per female) if we take into account the size of the two species. The oviposition rate of *O. lavaterae* is thus about 4 eggs per day, while it ranges, depending on food quality, from 5 to 15 eggs per day in *O. gossypinus* (Evete, Osisanya 1985), which gives lifetime fecundity of about 160–600 eggs per female. Higher oviposition rate was measured in *Oxycarenus hyalinipennis* (7–25 eggs per day, depending on food quality (Ananthakrishnan *et al.* 1982), but its lifetime fecundity and conditions of rearing were not reported.

Since *Oxycarenus hyalinipennis* is an important cotton pest worldwide, feeding on diverse plants of families Malvaceae, Tiliaceae and Sterculiaceae (Slater, Baranowski 1994, Smith, Brambilla 2008), *Oxycarenus laetus* is one of the most common and polyphagous pests in Southern Pakistan (Awan, Qureshi 1996), and *Oxycarenus palens* and *Oxycarenus hyalinipennis* are pests of safflower fields in Iran (Saeidi, Adam 2011), we assume that *O. lavaterae* with its similar diet range (Kalushkov, Nedvěd 2010) and recent expansion of area might also become an important pest.

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