

Biological parameters of *Xylotrechus arvicola* females, an insect pest in Iberian Peninsula vineyards

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Abstract

Aims: *Xylotrechus arvicola* is an important pest in vineyards of the Iberian Peninsula. The action of *X. arvicola* larvae, associated to the spread of fungi, causes direct and indirect damage in the vineyard. Biological parameters from wild (captured in the field) and laboratory females (reared in the laboratory) were investigated to provide more information about the pest-control measures.

Methods and results: The pre-laying period, post-laying period, longevity and egg laying parameters (fecundity, viability and number) were evaluated in wild and laboratory females. Both female groups (wild and laboratory) needed a short pre-laying period, which was longer in wild females. Laboratory females, whose larvae were reared on artificial diet, had the greatest fecundity during the 1st two egg layings. Wild females showed the greatest fecundity and viability of eggs during the 1st egg laying; these fecundity and viability rates decreased over time with the next egg layings, whereas in laboratory females, fecundity and viability decreased faster. Wild females had the greatest percentage of viable eggs in the 1st six egg layings (44.11% in the 1st and 11.15% in the 6th), reaching a maximum number of 18 egg layings in laboratory.

Conclusions: These results suggest that the diet satisfies larval nutritional requirements, increasing production of laboratory females' eggs (greatest fecundity in the 1st two egg layings). Nevertheless, this artificial diet may lack certain essential nutrients that would increase the viability of eggs.

Significance and impact of the study: The host, a woody plant, would provide these essential nutrients when the larvae of wild females are developing in the field, these wild females being able to perform successive egg layings in laboratory with a high viability of eggs.

Key words: insect pest, cerambycids, *Vitis vinifera*, artificial diet, egg laying, behavior

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Introduction

Xylotrechus arvicola (Coleoptera: Cerambycidae) is a polyphagous xylophagous insect which has become an important pest in vineyards (*Vitis vinifera*) of the Iberian Peninsula with Protected Designation of Origin (PDO). This insect attacks vineyards with different training systems (Rodríguez-González *et al.*, 2016), in the main wine-producing regions, for instance, La Rioja Alta and Alavesa (Ocete and Del Tío, 1996; Ocete and López, 1999), Navarra (Ocete *et al.*, 2002), Castilla - La Mancha (Rodríguez-Pérez *et al.*, 1997) and Castilla y León (Ocete and López, 1999; Peláez *et al.*, 2001). It has also been reported in *Prunus spinosa* L. orchards (Biurrun *et al.*, 2007).

After mating *X. arvicola* females lay the eggs in cracks or under the rhytidome of vine wood. The location of eggs enables the emerging larvae to get into the wood without any difficulty, making galleries inside the plant. The most susceptible stages of the species are adults, eggs and neonate larvae, although eggs are usually protected by the rhytidome or the wood cracks. The larvae, once inserted in the wood, are inaccessible to chemical compounds (Peláez *et al.*, 2002). The pattern of emergence of *X. arvicola* adults is very staggered in time. Thus, this behavior supposes another problem for their treatment (García-Ruiz, 2009).

The action of the larvae, associated with the spread of wood fungi, causes two types of damage: 1) direct damage, when the larvae dig galleries that diminish the plant's capacity to transport sap by reducing the vascular area; and 2) indirect damage from fungal attack (García-Ruiz *et al.*, 2012), especially in main grape varieties in Spain such as Tempranillo or Cabernet-Sauvignon (Ocete *et al.*, 2002; García-Benavides *et al.*, 2013).

Linsley (1959) described that the phenology of cerambycid insects is difficult to study due to the fact that their larvae are internal feeders of the hosts. Some authors such as Keena (2005) and Keena and Moore (2010) have also attempted to estimate cerambycid phenologies. Efforts have been made to establish the exact biological cycle of this cerambycid species, as reflected in previous studies by García-Ruiz *et al.* (2012), who studied *X. arvicola* adult females captured from infested grapevine wood and reared in laboratory using an artificial diet.

Rearing cerambycid beetles in laboratory is also difficult to handle due to their long life cycles and high mortality during larval stages (Cannon and Robinson, 1982; Linit, 1985; Hanks *et al.*, 1993; García-Ruiz *et al.*, 2012). Hanks *et al.* (1993) listed

three drawbacks for rearing cerambycids on an artificial diet: 1) difficulty in finding a diet that provides all the necessary nutrients; 2) intensive work, as larvae have to be transferred periodically; and 3) alteration of the physiology and behavior of the adults.

Knowledge of the reproductive traits of insect pests is essential in pest risk analysis, monitoring, and management (Lu *et al.*, 2013). Some authors such as Hanks (1999), Keena (2002), Naves *et al.* (2006) and Lu *et al.* (2011) have described that cerambycid adults live from 4 to 140 days. Cerambycid adults that feed during their adult stage usually live significantly longer than those that do not (Hanks, 1999). The life spans of species in the subfamily Cerambycinae (to which *X. arvicola* belongs) vary between 21 d for *Enaphalodes rufulus* (Coleoptera: Cerambycidae) adults (Galford, 1985) and 29 d for *X. quadripes* (Coleoptera: Cerambycidae) adults (Visitpanich, 1994).

The aim of this work was to explore different biological parameters of *X. arvicola* females in order to determine whether different environmental conditions during larval development and adult stage could affect the fecundity, viability and number of egg layings after pairing. The information produced in this study is important for the development of pest-control measures and in-depth knowledge of the biology of this insect pest.

Materials and methods

These experiments were designed to record biological parameters of *X. arvicola* females, such as pre-laying period (period from pairing until the 1st egg laying), post-laying period (period from the last egg laying until the female died) longevity (life span in days of females in laboratory) and egg laying parameters, such as fecundity (percentage of total eggs in one egg laying), viability (percentage of total viable eggs in one egg laying) and number of egg layings (number of egg layings in laboratory).

1. Insect groups and rearing diet

Two groups of *X. arvicola* females were used in this study (Figure 1):

1) Wild *X. arvicola* adult females were captured in vineyards during 2011 using an interception trap (CROSSTRAP®) in two important PDO wine-producing regions of the Iberian Peninsula. PDO is a certification that distinguishes quality food products of a particular region (EU Reg. No. 1151/2012

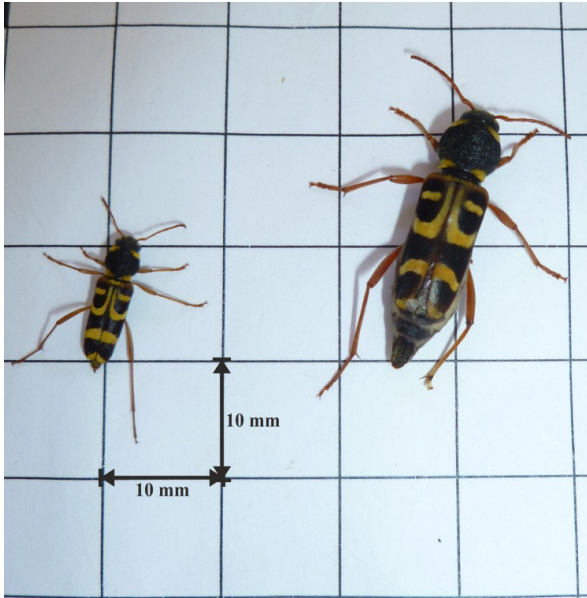


Figure 1. Wild (left) and laboratory (right) *X. arvicola* females from which the biological parameters have been studied.

published on 21 November 2012), in our case “Ribera Del Duero” and “Toro”.

2) Laboratory *X. arvicola* adult females were obtained in laboratory during 2011 from a population of *X. arvicola* larvae which were reared in laboratory (for 9 months) using the Semi Synthetic of Iglesias (SSI) diet (Iglesias *et al.*, 1989) from wild adults captured during 2010. To rear every larvae hatched by the SSI diet, the methodology described by García-Ruiz *et al.* (2012) was used. Once the fatty abdominal reserves were reabsorbed, it was possible

to distinguish body colors between the males and the females as described by Moreno (2005). In order to assess the biological parameters of these females obtained in the laboratory, they were paired with males obtained and fed with the same diet. If a male died, another was added to allow females to continue laying eggs. More details about the origin vineyards of wild females and laboratory females (first adult capture in 2010) are shown in Table 1.

2. Environmental conditions

For evaluating biological parameters of *X. arvicola* adult females (wild and laboratory), the care/rearing of insect stages and environmental conditions were similar. The adults were paired (one female and one male) and introduced in glass jars (80 mm in diameter and 100 mm high); the bottoms of the jars were covered with filter paper, and substrates for oviposition (corrugated cardboard nets 120 x 40 mm) and drinking bowls (cotton soaked in 10.0% organic honey in distilled water) were placed on the filter paper. The *X. arvicola* stages (eggs, larvae and adults) were kept in a chamber with controlled temperature ($24 \pm 1^\circ\text{C}$) and humidity ($60 \pm 5\%$), and adults were subjected to 16 hours of light photoperiod (luminous intensity of 1000 lux).

3. Assessment of adults' biological parameters

Biological parameters were determined as follows: 28 females captured from the field were paired individually with 28 males also captured from the field, and 36 females obtained in the laboratory were paired with 36 males also obtained in the laboratory.

Table 1. Details of experimental vineyards with PDO, captures and years of wild *X. arvicola* females used in the experiments.

Vineyards	“PDO Ribera Del Duero”		“PDO Toro”
Location (Province)	Peñafiel (Valladolid)		El Pego (Zamora)
Coordinates	41°35'39.1"N		41°20'26.4"N
	4°05'19.1"W		5°25'51.8"W
Height above sea level (m)	754		697
Annual average temperature (° C)	11		12.5
Average rainfall (mm)	450		375
Training system of vines	“Bilateral Cordon”		“Bush Vines”
Training system characteristics	Spur pruning over two arms per trunk (1 m)		Spur pruning over 4-5 branches per trunk (0.5 m)
<i>Vitis vinifera</i> variety	“Tempranillo”		“Tempranillo”
Vine age (years)	25		50
Insects			
Year of grape field capture	2010 ^a	2011	2011
Number of males	5 ^a	13	7
Number of females	6 ^a	18	10

^aInitial catches obtained in field during 2010. A larval population from these captures was reared in laboratory. In 2011, laboratory adults (28 males and 63 females) were obtained.

Table 2. Biological parameters (mean ± SE) of wild and laboratory females.

Parameter	Wild females	Laboratory females
Number of females	28	36
Pre-laying period (days) ^a	4.93 ± 0.68a	2.53 ± 0.31b
Maximum number of egg layings	18	16
Mean egg laying ^a	5.29 ± 0.94a	3.58 ± 0.51b
Post-laying period (days) ^a	22.18 ± 4.37a	25.06 ± 2.60a
Longevity (days) ^a	35.50 ± 4.19a	39.50 ± 3.34a

^aMeans within a row followed by the same lowercase letter are not significantly different; Tukey's test, $p = 0.05$.

The oviposition substrates were replaced daily and the numbers of eggs laid were recorded. The eggs were extracted and placed in 55-mm diameter Petri dishes. The plates were covered with aluminum foil with the aim of ensuring hatching in complete darkness. Egg laying dates were noted on the aluminum foil in order to know the eggs' age. Eggs hatched 7-8 days after oviposition, and the neonate larvae were extracted daily with the help of a brush and transferred to cylindrical plastic containers with the diet. If a *X. arvicola* male died, another male was added to allow females to continue laying eggs. The glass jars were checked daily until the last female died (wild and laboratory).

4. Statistical analyses

Statistical analyses were performed using SAS software, version 9.1.2 (SAS Institute Inc., 2004, Cary, NC, USA). Mean comparisons were performed using analysis of variance (Tukey's test, considered significant at $p \leq 0.05$) to evaluate the biological parameters and egg laying parameters between wild and laboratory females (for the same number of egg layings) and among number of egg layings (for the same female group).

Results

1. Biological parameters of *X. arvicola* females

Wild females needed significantly more days than laboratory females for the pre-laying period ($F_{3,21} = 11.85$, $P < 0.001$; Table 2). The mean egg laying of wild females (5.29 ± 0.94 with a maximum of 18 egg layings) was significantly different from that of laboratory females (3.58 ± 0.51 with a maximum of 16 egg layings; Table 2).

2. Egg layings of *X. arvicola* females

No significant differences were found for fecundity between wild and laboratory females within the same number of egg layings (lowercase letters, Figure 2A).

However, significant differences were found for fecundity among number of egg layings within the same female group (capital letters, Figure 2A). Laboratory females showed the highest fecundity in the 1st egg laying, significantly different ($F_{17,630} = 44.82$, $P < 0.001$) from the 2nd egg laying, the latter being significantly different from the 3rd and 4th egg laying. Wild females showed the highest fecundity in the 1st egg laying, significantly different ($F_{17,486} = 26.47$, $P < 0.001$) from the 2nd, 3rd and 4th egg laying, the latter not being significantly different from one another.

Significant differences in viability were found during the 1st six egg layings between wild and laboratory females within the same number of egg layings (lowercase letters, Figure 2B). Wild females showed greatest viability in the 1st ($F_{1,29} = 8.12$, $P = 0.008$), 2nd ($F_{1,43} = 4.07$, $P = 0.05$), 3rd ($F_{1,52} = 6.58$, $P = 0.01$), 4th ($F_{1,52} = 4.10$, $P = 0.05$), 5th ($F_{1,55} = 4.02$, $P = 0.05$), and 6th ($F_{1,62} = 4.03$, $P = 0.05$) egg laying, significantly different than laboratory females within the same number of egg layings. No significant differences were found between wild and laboratory females from the 7th until the last egg laying. It is remarkable that the viability of eggs was always greater in wild females. Wild females showed the last egg laying with viable eggs in the 15th egg laying, while laboratory females showed viability of eggs up to the 6th egg laying.

Significant differences were found for viability among number of egg layings within the same female group (capital letters, Figure 2B). Laboratory females did not show significant differences among the 1st four egg layings. Wild females had the greatest viability in the 1st egg laying, significantly different ($F_{17,486} = 10.41$, $P < 0.001$) from the other egg layings.

Different lowercase letters indicate significant differences between wild and laboratory females within the same number of egg layings (Tukey's test, $p \leq 0.05$). Different capital letters indicate significant

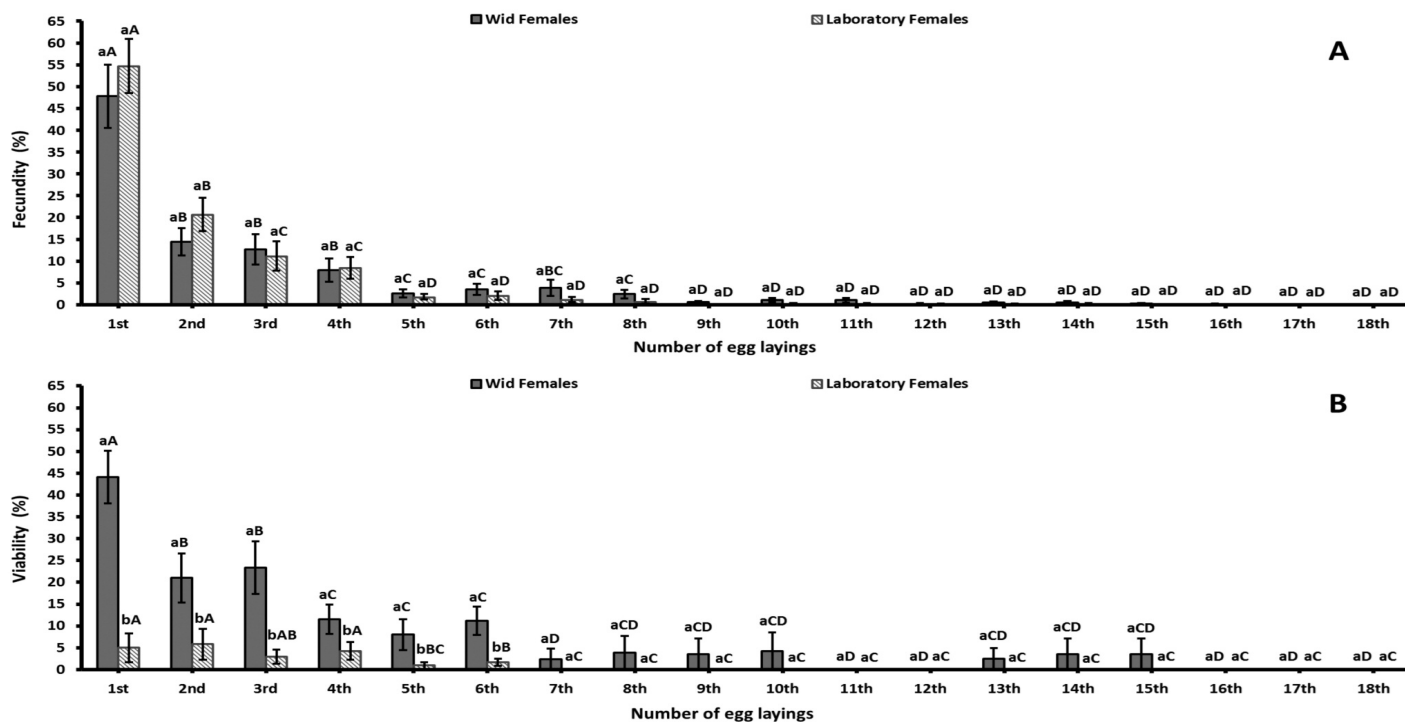


Figure 2. Fecundity (A) and viability (B) of egg layings of wild and laboratory *X. arvicola* females.

differences among number of egg layings within wild (left side) and laboratory (right side) females (Tukey's test, $p \leq 0.05$).

Discussion

The pre-laying periods in both female groups were very short (from 2 to 5 days). Wild females needed a longer pre-laying period (4.93 days) than laboratory females (2.53 days). The results of *X. arvicola* females' laying behavior are quite similar to those of Visitpanich (1994) for *X. quadripes* (Coleoptera: Cerambycidae) females, whose 1st egg laying occurs during the 1st week of life. In the subfamily Cerambycinae, *Enaphalodes rufulus* (Coleoptera: Cerambycidae) (Galford, 1985) and *Aeolesthes sarta* (Coleoptera: Cerambycidae) needed a longer pre-laying period, with 8 and 4 days, respectively (Mazaheri *et al.*, 2007). Knowing the distribution of eggs laid during the entire life of *X. arvicola* females allows designing more efficient control strategies against this pest in vineyards (García-Ruiz *et al.*, 2012).

Laboratory females needed more days for the post-laying period (25.06 days) than wild females (22.18 days). If this result is compared with the García-Ruiz *et al.* (2012) assay (the authors used the same species of *Xylotrechus* females), we could see that their post-laying period (9.95 days) was shorter. The longevity

obtained in wild females (35.50 days, even not knowing the days of life before being caught by interception traps) is higher than reported by García-Ruiz *et al.* (2012) for females captured from infested wood (23.64 days). The longevity obtained in laboratory females (39.50 days) is similar to that reported by García-Ruiz *et al.* (2012) for females reared with the same diet (37.42 days). Cerambycid adults, which feed during the adult stage, generally live between 1 and 2 months, depending on the sex and the rearing method employed (Hanks, 1999). The longest life spans have been documented for species in the subfamily Lamiinae (Linsley, 1959), with *Anoplophora glabripennis* (Coleoptera: Cerambycidae) adults living up to 112 days (Keena, 2005). Adult stages from the subfamily Cerambycinae, to which *X. arvicola* belongs, have shown life spans of 21 days for *Enaphalodes rufulus* (Coleoptera: Cerambycidae) adults (Galford, 1985) and 29 days for *Xylotrechus quadripes* (Coleoptera: Cerambycidae) adults (Visitpanich, 1994).

The fecundity of all *X. arvicola* females was higher during the 1st egg laying (54.73% eggs/laboratory female and 47.81% eggs/wild female) and decreased during successive egg layings. A similar behavior was described by Dojnov *et al.* (2012) on *Morimus funereus* (Coleoptera: Cerambycidae) females, where fecundity between the 1st and 2nd egg laying decreased from 477 to 88 eggs/female in wild

females and from 80 to 56 eggs/female in reared females. The greatest viability of eggs in all egg layings was higher in wild females. On the one hand, the viability of eggs laid by laboratory females decreased faster with time, having viable eggs until the 6th egg laying (0.79% total viable eggs). On the other hand, wild females had viable eggs until the 15th egg laying (3.57% total viable eggs).

Conclusion

The results showed that wild and laboratory females needed a short pre-laying period, so these females started to lay eggs during the 1st week of life. Laboratory females, whose larvae were fed with the SSI diet, showed the highest fecundity during the 1st two egg layings in laboratory. The 1st egg laying of wild females showed the highest fecundity and viability, decreasing during the next egg layings, with this decrease being faster in laboratory females. Wild females - even not knowing their age at the time of field capture - showed the greatest viabilities of eggs in laboratory during the 1st six egg layings (44.11% in the 1st and 11.15% in the 6th). Wild females were also able to make a maximum number of 18 egg layings. These results suggest that the diet satisfies larval nutritional requirements of laboratory females, favoring the production of eggs (greatest fecundity in the 1st two egg layings), but that this diet may lack certain essential nutrients that would increase the viability of eggs in the egg laying. The woody plant host would provide these essential nutrients when the larvae of wild females are developing in the field, these wild females being able to perform successive egg layings in laboratory with a great viability of eggs. The information provided in this study is important for advancing the knowledge of the biology of this insect pest.

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