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Biometric traits of *Xylotrechus arvicola* adults from laboratory and grape fields

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Summary

***Xylotrechus arvicola* is a pest in vineyards on the Iberian Peninsula. The action of the larvae, associated to the spread of wood fungi, causes a direct and indirect damage in the crop. This article presents biometric traits of *X. arvicola* population adults captured in three grape fields with Protected Designation of Origin (PDO) from the Iberian Peninsula and one population of this beetle obtained in the laboratory. The aim of the study was to observe the influence of sex and environment on the size of adults. The adults showed intersexual differences for the length and width of the pronotum, the length and width of the elytra and total length. The wild females were larger than wild males and the males from grape fields were slender than wild females. The environment affected the size of the adults. The laboratory adults reached the greatest body size. The size of the adult reflects the volume of material that had been used as host where the insect had developed. The captured adults in PDO Toro, with Bush Vines Training System (BVTS) were larger than the other PDOs, leading to bigger galleries and exit holes, which could affect the structural resistance of the plant and increase pathogenic fungi infection. However, BVTS facilitates the renovation of attacked branches, which is more difficult and slower in the Bilateral Cordon Training System (BCTS).**

Key words: *Xylotrechus arvicola*; biometric traits; body size; grape field; laboratory; *Vitis vinifera.*

Introduction

Xylotrechus arvicola (Coleoptera: Cerambycidae) is a xylophagous polyphagous insect native to riverside trees but also has been associated with the genus *Quercus*, *Carpinus*, *Castanea*, *Fagus*, *Populus*, *Salix*, *Tilia*, *Morus*, *Sorbus*, *Crataegus*, *Malus*, and *Cydonia* (Bahillo 1996, Vives 2000, Moreno 2005). This cerambycid has become a pest in vineyard, associated with *Vitis vinifera* in the main Iberian Peninsula wine-producing regions, as for example, La Rioja Alta and Alavesa (Ocete and Del Tío 1996, Ocete and López 1999), Navarra (Ocete *et al.* 2002), Castilla - La Mancha (Rodríguez and Ocaña 1997) and Castilla y León (Ocete and López 1999, Peláez *et al.* 2001). However, it has also been reported as a larval host on *Prunus spinosa* L. plantations (Biurrun *et al.* 2007), which indicates an extension of the species' niche. The developing of the larvae, associated to the spreading of wood fungi, cause direct (by *X. arvicola*) and indirect damage (for fungal attack) especially in main grape varieties in Spain such us 'Tempranillo' or 'Cabernet-Sauvignon' (Ocete *et al.* 2002, García-Benavides *et al.* 2013).

X. arvicola develops in different environments of wine-producing regions with different training system of the vines. Laboratory studies can reduce environmental variation allowing researchers to focus on biological variation within and among populations due to abundant nutritional resources and controlled conditions. Often insects are collected from the field and brought into the laboratory at different times for rearing specific populations (Li *et al.* 2014). In addition, an advantage of laboratory-reared insects is that generational effects can be standardized to laboratory conditions, *i.e.*, laboratory rearing can eliminate differential responses specific to the environment in the different fields. However, this carries the risk of laboratory adaptation, *i.e.* genetic changes in traits over generations may appear (Rössler 1975, Miyatake and Yamagishi 1999, Spurgeon 2012).

Traits of pest insects are specifically investigated to more fully understand questions in applied insect ecology (Huettel 1976), usually with the goal of improving pest management strategies (Richerson and Cameron 1974 Prokopy *et al.* 1975). The biometric traits of xylophagous insects can be a valuable source of information on a population inhabiting a given territory (Michalcewicz and Ciach 2012) and these traits can inform about the sizes of galleries and exit holes. The body size of insects may depend on the host plant species, the size/volume of breeding material and the conservation status of a habitat (Starzyk and Strojny 1985, Gutowski 1986, Hanks *et al.* 1993, 2005, Naves *et al.* 2006). Consequently, body size can be used as a potential parameter determining the attractiveness of the breeding material and the quality of a habitat (Michalcewicz and Ciach 2012). The aim of the study is to observe the influence of sex and environment in the size of *X. arvicola* adults. This study analyzes the biometric traits of beetles to determine their potential influence on the size of galleries and exit holes, what will affect to the structural resistance of the vines and

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to the pathogenic fungi infection. Rearing the adults in the laboratory is a unique opportunity to determine the potential size that the species can reach.

Material and Methods

Insect populations origin: On the one hand, wild adults were captured in vineyards through interception trap (CROSSTRAP®) in three grape fields with Protected Designation of Origin (PDO). The traps were visited three times a week and the adults were taken to the laboratory. In the three fields, the grape variety was 'Tempranillo', but with two different training systems. Bilateral Cordon Training System (BCTS), whose vines have a short trunk above ground level with two branches or canes trained by wire along the two sides of the vine and this hardened wood is never cut away. Bush Vines Training Systems (BVTS), whose vines trunks are maintained very short so that there is little more than ½ meter of the vine above ground level. The three grape fields (Tab. 1) were located in: PDO Ribera del Duero (BCTS with a trunk 0.6 meter high and two wires at 1 meter, 25 years old, 450 mm average rainfall and 11 °C annual average temperature), PDO Toro (BVTS with four branches and 0.5 high, 50 years old, 375 mm average rainfall and 12.5 °C annual average temperature) and PDO Tierra de León (BCTS with a trunk 0.6 meter high and two wires at 1 meter, 18 years old, 500 mm average rainfall and 11.7 °C annual average temperature).

On the other hand, the laboratory population was obtained from adults captured in the field through interception trap, CROSSTRAP®. The laboratory insects were reared through Semi Synthetic Diet of Iglesias (Iglesias *et al.* 1989), following the methodology described by García-Ruiz *et al.* (2012). Each neonate larva (< 24 h) was transferred to the diet cage with the help of a camel hairbrush. In each cage, a piece of 20 mm diameter paper was placed over the larva and the diet to retain excess humidity and to recreate the natural conditions under the bark. These papers were removed after a week. The diet was introduced in plastic cylindrical cages where each larva was reared individually. During the first month, plastic cylindrical cages (20 mm diameter and 40 mm high) filled with the respectively tested diet were used, and one month later, developed *X. arvicola* larvae were transferred to plastic cylindrical containers (40 mm diameter and 65 mm high) filled with the same diet to half their capacity. The diet in these cages was changed monthly to avoid diet contamination and desiccation. About nine months later, the larvae stopped eating. As proposed by many other authors, diapausing larvae were kept 45 d inside a cold chamber at 8 ± 1°C in complete darkness to break the diapause (Dubois *et al.* 2002, Hodoková and Hodek 2004, Keena 2005). Larvae were then transferred to containers with fresh diet under the same conditions they had before the cold treatment, and they started to pupate about one month later. Sex identification was performed after the complete formation, esclerotisation and melanisation of adults. Once the fatty abdominal reserves were reabsorbed, it was possible to distinguish body colours between males and females (Moreno 2005).

Standard rearing conditions: The wild adults were paired with individuals of the same origin (one female and one male) and introduced in glass jars (80 mm in diameter and 100 mm high) having covered the bottom with filter paper; the substrate for oviposition (corrugated cardboard nets 120 x 40 mm) and the drinking bowls (cotton soaked in a solution of organic honey to 10 % in distilled water) were then placed on top. *X. arvicola* stages (eggs, larvae rearing and adults), were kept in a chamber with controlled temperature (24 ± 1 °C), humidity (60 ± 5 %), and subjected to a 16 h of light photoperiod (luminous intensity of 1000 lux).

Table 1

X. arvicola adults populations captured in grape fields and obtained in laboratory through artificial diet

Populations	Captured in grape field (Protected Designation of Origin)			Obtained in laboratory
	Ribera del Duero (Valladolid)	Toro (Zamora)	Tierra de León (León)	
	Training system of vines			Host material
	Bilateral cordon	Bush vines	Bilateral cordon	SS Diet
Coordinates	41º35'39.1''N 4°05'19.1''W	41°20'26.4''N 5°25'51.8''W	42°08'14.9''N 5°25'41.6''W	-LAB
Year of grape field captured	2011	2011	-	-
	2012	2012	2012	2012
No. of adults evaluated	126	103	101	101
Males	55	53	51	51
Females	71	50	50	50

SS Diet: Semi Synthetic Diet of Iglesias (Iglesias *et al.* 1989).

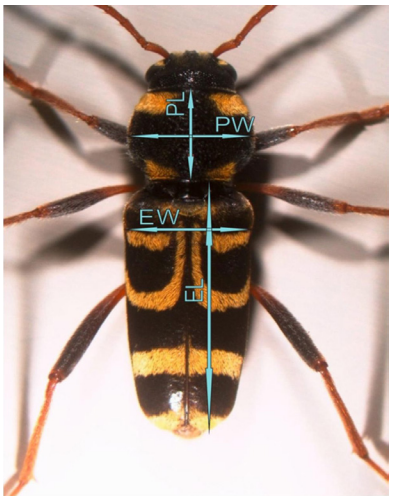

Figure: Biometric traits studied in different body parts of *X. arvicola* adult*:* Pronotum Length (PL), Pronotum Width (PW), Elytra Length (EL), Elytra Width (EW). Other biometric relationships also were conducted from the measures listed: Total Length (TL) = (EL + PL), Pronotum Width / Pronotum Length (PW/PL) and Elytra Width / Elytra Length (EW/EL).

Biometric traits studied: Once an adult insect died, the biometric study was conducted. The studied biometric traits (Figure) were Pronotum Length (PL), Pronotum Width (PW), Elytra Length (EL) and Elytra Width (EW) to perform a biometric characterization of different *X. arvicola* populations. Biometric indices were also studied from the four basic measures previously performed on each adult: Total Length (TL, is obtained from the sum of EL and PL), Pronotum Width/Pronotum Length ratio (PW/PL) and Elytra Width/Elytra Length ratio (EW/EL), to have a better knowledge in the indices of body shape of this insect (length, slender or thick). The biometric traits were evaluated on 431 adults (with the help of a magnifying glass and Motic software, version 2.0).

Statistical analyses: Combined analysis of variance (sex and environment) was performed. Mean comparisons were performed using the LSD test to examine differences ($p < 0.05$) among environments and between sexes. Statistical analyses were performed using SAS software, version 9.1.2 (SAS Institute Inc., 2004, Cary, NC, USA).

Results

Combined analysis of variance showed significant differences in all analyzed traits among environments and between sexes. Environment*sex interaction was not significant in none of studied traits (Tab. 2).

PL: By environments, laboratory adults had the greatest PL, showing significant differences with wild adults. By sexes, the laboratory adults did not show significant differences for PL; however, the wild females significantly had larger PL than wild males in all PDOs (Tab. 3).

PW: By environments, laboratory adults had the greatest PW, showing significant differences with those captured in the grape fields. Adults of PDO Toro had the greatest PW among field captures, significantly different from Ribera del Duero and Tierra de León wild adults By sexes, the wild females showed significantly larger PW than wild males in all PDOs (Tab. 3).

EL: By environments, laboratory adults had the greatest EL, showing significant differences with wild adults. By sexes, the wild females significantly had larger EL than males in all PDOs (Tab. 3).

EW: By environments, laboratory adults had the greatest EW, showing significant differences with those captured in the grape fields. Adults of PDO Toro showed the greatest EW among field captures, significantly different from Ribera del Duero and Tierra de León wild adults. By sexes, the wild females showed significantly larger EW than wild males in all PDOs (Tab. 3).

Table 2

Mean squares of the combined analyses of variance for biometric traits

Biomentric traits	Source of variation					
	d.f.	Sex (mean square)	d.f.	Environment (mean square)	d.f.	Sex*Environment (mean square)
PL (mm)	1	3.01837***	3	48.04250***	3	*NS*
PW (mm)	1	6.44036***	3	57.16528***	3	*NS*
EL (mm)	1	39.75956***	3	230.297980***	3	*NS*
EW (mm)	1	12.61595***	3	78.62689***	3	*NS*
TL (mm)	1	65.5503***	3	487.0789***	3	*NS*
PW/PL	1	0.037055***	3	0.00736*	3	*NS*
EW/EL	1	0.004795**	3	0.032684***	3	*NS*

Mean squares followed by (*), (**) and (***) were significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ respectively; d.f.: degrees of freedom; (*NS*), not significant.

TL: By environments, laboratory adults had the greatest TL, showing significant differences with wild adults. By sexes, the laboratory adults did not show significant differences for TL; however, the wild females significantly had larger TL than wild males in all PDOs (Tab. 3).

PW/PL: By environments, Ribera del Duero females had the lowest PW/PL showing Tierra de León males higher values than others PDOs. By sexes, the relation PW/PL only was higher in females than wild males in laboratory and Toro (Tab. 3).

EW/EL: By environments, laboratory adults had the greatest EW/EL, showing significant differences with wild adults. By sexes, only the laboratory adults had significant differences for EW/EL, showing lower values males than females for this relationship (Tab. 3).

Table 3

Mean comparisons (LSD test) for biometric traits of *X. arvicola* adults among environments and between sexes

Biometric traits	Females (Mean ± SD)				Males (Mean ± SD)			
	PDO Ribera del Duero	PDO Toro	PDO Tierra de León	Laboratory	PDO Ribera del Duero	PDO Toro	PDO Tierra de León	Laboratory
PL (mm)	3.051 ± 0.19aB	3.234 ± 0.10bA	3.065 ± 0.16bA	4.353 ± 0.13aA	2.835 ± 0.18bB	3.013 ± 0.13bB	2.823 ± 0.13bB	4.358 ± 0.19aA
PW (mm)	3.259 ± 0.06cA	3.511 ± 0.08bA	3.337 ± 0.04cA	4.745 ± 0.11aA	2.993 ± 0.09cB	3.204 ± 0.07bB	3.031 ± 0.08cB	4.641 ± 0.15aA
EL (mm)	7.866 ± 1.33bA	8.322 ± 1.58bA	8.065 ± 1.00bA	10.735 ± 1.61aA	7.194 ± 0.93bB	7.571 ± 1.04bB	7.193 ± 0.84bB	10.585 ± 1.35aA
EW (mm)	3.569 ± 0.07cA	3.805 ± 0.11bA	3.636 ± 0.10cA	5.337 ± 0.17aA	3.227 ± 0.05cB	3.406 ± 0.08bB	3.245 ± 0.06cB	5.092 ± 0.18aA
TL (mm)	10.940 ± 1.81bA	11.556 ± 2.17bA	11.130 ± 1.35bA	15.088 ± 2.30aA	10.030 ± 1.29bB	10.584 ± 1.46bB	10.017 ± 1.15bB	14.943 ± 1.92aA
PW/PL	1.067 ± 0.04bA	1.086 ± 0.04aA	1.088 ± 0.04aA	1.090 ± 0.04aA	1.056 ± 0.04bA	1.063 ± 0.03bB	1.072 ± 0.04aA	1.064 ± 0.04abB
EW/EL	0.453 ± 0.01bA	0.457 ± 0.01bA	0.450 ± 0.01bA	0.495 ± 0.03aA	0.448 ± 0.02bA	0.449 ± 0.02bA	0.451 ± 0.02bA	0.479 ± 0.02aB

Different lowercase letters indicate significant differences among different environments for the same sex. Different capital letters indicate significant differences ($p \leq 0.05$) between sexes for the same biometric trait and environment; SD = standard deviation.

Discussion

Concerning environment, the reared adults in the laboratory reached greater size than the captured adults in grape fields, determining the potential size that this species can reach. According to origin of grape field, captured adults in the PDO Toro had higher values in biometric traits relative to width. The PDO Toro vineyard, with BVTS, had greater volume of wood (older and higher diameter) where larvae were developed. This training system could favour the largest body size in this PDO, leading to bigger galleries and exit holes what could affect the structural resistance of the plant and increase the pathogenic fungi infection. These size variations in insect body may also occur depending on host genera plants, species and variety.

The total length measures for *X. arvicola* adults in *Prunus spinosa* trees described by Biurrun *et al.* (2007), varied between 11.15 mm in females and 13.00 mm in males. *X. arvicola* adults captured in *Vitis vinifera,* with an average total length of 10.58 mm in males and 11.55 mm in females, were smaller than those described by Moreno (2005) with 11.52 mm and 13.44 mm in males and females, respectively. However, the adults can potentially reach 15.08 mm in females and 14.94 in males. So, the variation of environmental and nutritional parameters where insects were developed can affect body size. Gutowski (1986) described different body sizes in *Alosterna tabacicolor* De Geer (Coleoptera: Cerambycidae) from insects collected in natural forests and managed forests, being larger in the natural forest. Starzyk and Strojny (1985) described increased body size and weight of adult insects for *Cerambyx cerdo* L. (Coleoptera: Cerambycidae) collected in trees that grew in circumference and diameter compared to those which were raised or collected in smaller trees three times.

Concerning sex, wild females always had the pronotum wider than males. Bahillo (1997) described that female's pronotum width was larger than males in *Iberodorcadion fuliginator* Linnaeus 1758 (Coleoptera: Cerambycidae). While Vives (1983) in *Iberodorcadion fuliginator* Linnaeus 1758 (Coleoptera: Cerambycidae) and Ciach and Michalcewicz (2013) in *Rosalia alpina* (L.) (Coleoptera: Cerambycidae) observed an average pronotum width higher in males than females. On the other hand, González *et al.* (2001) described a pronotum width similar in both sexes of *Iberodorcadion* (Hispanodorcadion) *pseudomolitor* and *Iberodorcadion* (Hispanodorcadion) *mosqueruelense*; in laboratory adults we have not detected differences between sexes in *X. arvicola* for this biometric trait.

Wild females showed the pronotum larger than wild males in all studied wine PDOs. The largest size in the PL of

X. arvicola females matched as referred by Moreno (2005) who also cited for this species a greater pronotum length in wild females (3.20 mm) than in wild males (2.80 mm). This biometric trait has also been described in other cerambycids, as for example Bahillo (1997) in *Iberodorcadion fuliginator* Linnaeus 1758 (Coleoptera: Cerambycidae) who also described an average length greater in females than males; however, Vives (1983) described for this species a pronotum length in males slightly larger than females.

The elytra width of wild females was greater than wild males in all studied wine PDOs. The elytra width was also measured in other cerambycids, showing also wider elytra in females than in males, as it is evidenced by the works done by Vives (1983) and Bahillo (1997) in *Iberodorcadion fuliginator* Linnaeus 1758 (Coleoptera: Cerambycidae) or by Ciach and Michalcewicz (2013) in *Rosalia alpina* (L.) (Coleoptera: Cerambycidae). However, González *et al.* (2001) described a similar elytra width in both sexes of *Iberodorcadion* (Hispanodorcadion) *pseudomolitor*, and *Iberodorcadion* (Hispanodorcadion) *mosqueruelense*. In our study, wild females also had greater elytra than wild males in all studied wine PDOs, however there were no significant differences between males and females obtained in laboratory. Higher EL in wild females than in wild males, captured in grape field, was also described by Moreno (2005) with EL of 9.1 mm in females and 7.6 mm in males. This biometric trait and its sexual dimorphism was also described by Vives (1983) and Bahillo (1997) in *Iberodorcadion fuliginator* Linnaeus 1758 (Coleoptera: Cerambycidae) and Ciach and Michalcewicz (2013) in *Rosalia alpina* (L.) (Coleoptera: Cerambycidae). While González *et al.* (2001) described a similar length of the elytra in both sexes of *Iberodorcadion* (Hispanodorcadion) *pseudomolitor*, and *Iberodorcadion* (Hispanodorcadion) *mosqueruelense.* Captured females had a greater TL than captured males in all studied wine PDOs. Similar relationship between sexes was obtained by Moreno (2005) in captured *X. arvicola* adults from grape field. By environment, laboratory adults showed greater total length than wild adults in both sexes. Laboratory conditions have favoured larger sizes in all stages of *X. arvicola* development. The greatest total length of females was also cited in other species of Cerambycidae, as *Iberodorcadion fuliginator* Linnaeus 1758 (Coleoptera: Cerambycidae) by Bahillo (1997) or *Iberodorcadion* (Hispanodorcadion) *pseudomolitor* and *Iberodorcadion* (Hispanodorcadion) *mosqueruelense* by González *et al.* (2001) and in the most insect species, as for example *Harmonia axyridis* (Coleoptera: Coccinellidae) (Barbehenn *et al.* 2015).

The analysed indices (PW/PL and EW/EL) give a quantitative parameter about the body shape of the males and females. The PW/PL ratio, describes the slenderness of *X. arvicola* adults. By sexes, the laboratory females had higher PW/PL ratios than laboratory males, *i.e.* the laboratory males, having lower values in this ratio of pronotum, were more slender than laboratory females. In wild adults, only in PDO Toro, the males were more slender than females. These results are agreed with Moreno (2005) that described *X. arvicola* males as more slender than females, obtaining PW/PL ratios higher in females than males. These results would confirm that males are more slender than females. PW/PL and EW/EL ratios are biometric traits that can be used as quantitative traits to estimate the slenderness of adults.

The most appreciable difference in wild adults, according to sex, was TL of the body of the insect, most notable in adults from grape field than adults from laboratory. The final size of emerged insect may reflect the volume of material that has been used as host or the state of habitat conservation where it has been developed (Michalcewicz and Ciach 2012). The controlled conditions of development in the laboratory in which larvae were kept in greater volume with artificial diet could result in laboratory adults being greater than wild adults.

Environment and sex affect the biometric traits of *X. arvicola* adults. By sexes, wild females are greater than wild males. Male adults are more slender than female adults. By environments, the reared adults in laboratory are greater than wild adults from all studied wine PDO. The adult body sizes reflect the volume of material that had been used as host where the insect had been developed. The captured adults in PDO Toro (with BVTS) were larger than other PDOs (with BCTS), leading to bigger galleries and exit holes in PDO Toro, what could affect the structural resistance of the plant. However, BVTS facilitates the renovation of attacked branches, which is more difficult and slower in BCTS.

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