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EFFECTS OF *XYLOTRECHUS ARVICOLA* (OLIVIER, 1795) (COLEOPTERA CERAMBYCIDAE) INFESTATION ON SOME PARAMETERS OF GRAPEVINE PRODUCTION IN SPAIN

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Ocete R., Ocete C.A., Rubio-Casal A.E., López M.A., Soria F.J., Maistrello L., Arru L., Armendariz I. – Effects of *Xylotrechus arvicola* (Olivier, 1795) (Coleoptera Cerambycidae) infestation on some parameters of grapevine production in Spain.

The wood borer *Xylotrechus arvicola* has recently become a pest of vineyards in most of the productive winegrowing areas of Spain. The activity of its larvae has important consequences on grapevine production. Different production parameters such as bunch length and weight, number of flowers, wood weight, leaf pigments and Baumé degree of the must were registered in Viura and Tempranillo varieties in La Rioja, separating *X. arvicola* affected and not affected branches on the individual plants.

All the considered parameters were influenced by the wood borer activity, except the Baumé degree. Important differences detected in some of the parameters, indicate that Viura cultivar is more damaged than Tempranillo, despite the fact that the last one shows a higher attack by the borer in the field. Considering the increasing infestation by *X. arvicola* in Spanish grape production areas, our findings allowed to understand the borer action on the plants and indicated that Tempranillo has a higher tolerance to the attack of this borer, with possible consequences in orienting the choices of varieties for winemaking.

Key Words: La Rioja, Tempranillo, Viura, Cerambycidae, damage, leaf pigments.

INTRODUCTION

Xylophagous cerambycids constitute the main pests affecting the wood plantations all over the world, especially when, due to the wood trading, these boring beetles are introduced in countries outside of their origin areas (Cocquempot, 2006; Grebennikov *et al.*, 2010). Wood damage by these borers is mostly related to timber degradation by larval feeding [i.e. *Neoclytus acuminatus acuminatus* (Fabricius, 1775) on hardwood tree species (Lacey *et al.*, 2004)], adult feeding [i.e. *Hylobius abietis* (Linnaeus, 1758) on pinewood (Dillon *et al.*, 2006)] and by facilitation to fungal diseases and/or inoculation by nematodes [i.e. inoculation of *Bursaphelenchus xylophilus* (Nickle, 1981) (Jurc *et al.*, 2012)]. Serious damage caused by long-horned beetles in vineyards was reported in Europe (Galet, 1982), in the Asia-Pacific region (Azam, 1979; Ashihara, 1982; FAO, 2001) and in Australia (Goodwin & Pettit, 1994; Goodwin, 2005).

Xylotrechus arvicola (Olivier, 1795) (Coleoptera, Cerambycidae) is a polyphagous borer with a holo-mediterranean distribution (Vives, 2000). In Spain it occurs on several wild shrubs and trees, in natural habitats of the Basque country, Andalusia, Murcia (Bahillo, 1995; Vives, 2000; Lucas, 2008) and also in the Balearic Islands (Compte, 1963; Vives, 1984).

X. arvicola was first detected in a vineyard in 1970 in La Rioja, one of the most important Spanish Designation of Origin wine districts, when, at pruning time, large and numerous larval galleries were discovered inside grapevine trunks and branches (Ocete *et al.*, 2009). The first affected vineyard is very close to the Rioja river-bank forest, where the typical vegetation includes also willows (*Salix* spp.) and poplar (*Populus* spp.) trees, that are typical host plants for this coleopteran (Bahillo & Iturrondobeitia, 1996; Vives, 2000).

Similarly affected vineyards were later observed also along the Tirón and Ebro rivers, in the same region (Ocete & Del Tío, 1996). Attacks by this borer on grapevines became more generalized and grew alarmingly in La Rioja (Ocete & Del Tío, 1996) and were later recorded also in other Spanish territories producing designation of origin wines, such as Castilla y León (Moreno *et al.*, 2003), La Mancha and Valdepeñas (Rodríguez *et al.*, 1997), Navarra and Aragón regions (Ocete *et al.*, 2010).

Among the hypotheses to explain this outbreak, García-Calleja (2004b) suggests the intensification of grapevine cultivation, the age of the plantations, the import of planting material from different European countries, the prohibition to use sodium arsenate as winter biocide treatment. Other authors indicate the pruning system and lack of wound seal practices (Peláez *et al.*, 2001; Ocete *et al.*, 2002a, 2002b) as well as the absence of long periods of frost, possibly related to the climatic changes (Álvarez & Villarías,

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2003). The presence of wood pathogenic fungi also might constitute an additive factor (PELÁEZ *et al.*, 2001; OCETE *et al.*, 2002a, 2002b). We can assume that the existence of galleries would facilitate the fungus activity, but this is still a matter of debate.

X. arvicola is a typical monovoltine species, with a single flight peak that occurs between late June and mid-July, and a period of adult emergence that can last until mid August (SORIA *et al.*, 2013). Females lay about 200 eggs (GARCÍA-RUIZ *et al.*, 2012) in the cracks, crevices or wounds of the plant, in proximity of the pruning cuts or underneath the crust rhytidome (PELÁEZ *et al.*, 2002; ORTEGA & SÁEZ, 2010). Larvae feed inside the trunk forming galleries, and the egg-to-adult development in the field lasts about two years (GARCÍA-RUIZ *et al.*, 2012). The most evident symptoms of infestation by this borer are the emergency holes, of about 4 mm in diameter, made by the adults in the trunk and in the arms of the vine.

In the vineyards, the distribution of the infestation is not uniform and shows several aggregation centres with different levels of infection and a long-term spatio-temporal stability (OCETE *et al.*, 2010). Infested vine stocks exhibit rachitic shoots with low vigour, very similar to the appearance caused by the fungal diseases Eutypa dieback, as described by MOLLER & KASIMATIS (1980). Moreover, an association between *X. arvicola* galleries and wood fungi, such as those associated with Petri disease, Esca disease, Black Dead Arm and Eutypa dieback causing vine decline was first observed in 2002 (OCETE *et al.* 2002) and has been recently confirmed (GARCÍA-BENAVIDES *et al.*, 2013). The combined effect of the borer and the wood fungi results in the progressive weakening of the affected vines, which become extremely fragile and more susceptible to breaking during wind episodes or crop management using machinery (ARMENDÁRIZ *et al.*, 2008). Replacement of dead vines results in plots with plants at different ages and different pruning requirements which make vineyard management more cumbersome each year (GARCÍA-CALLEJA, 2004a).

Regarding management, due to the endophytic development and the extremely scattered emergence period, chemical treatments are an unfeasible/unsustainable control strategy (SORIA *et al.*, 2013). So far, control measures rely mainly on the use of preventive cultural methods, such as the removal of cut branches during pruning and wounds covering with sealing paste. In cases of heavy infestations, drastic and expensive sanitary measures must be taken, consisting in cutting back the trunk of the vines to enable re-growth (OCETE *et al.*, 2004).

Presently, it is estimated that almost half of the Spanish winegrowing area (about 550,000 ha) shows important infestation nuclei affected by this borer (LUCAS, 2008; OCETE *et al.*, 2010). On the other hand, among the international cultivars introduced in Spain in the last 20 years, Cabernet Sauvignon and Sauvignon blanche have shown a high susceptibility to this pest (OCETE *et al.*, 2004). In La Rioja region, Tempranillo, which is the main traditional cultivar, shows the highest level of infestation by this coleopteran, whereas Viura, that is the most planted white variety, shows a lower infestation by this pest (OCETE & LÓPEZ, 1999). It has been hypothesized that the different wood composition of the two cultivars, in terms of dry matter weight, percentages of holocellulose and lignine, and total nitrogen values could have a specific role on larval feeding and might therefore explain the different levels of infestation (MORENO, 2005).

In a previous study it was found that branches affected by this longhorn beetle produce smaller bunches with less flowers than healthy ones, resulting in considerably less and lower quality production (OCETE *et al.*, 2004, 2010). This work aimed to evaluate more deeply the impact of this rapidly spreading pest on different parameters of grapevine production, focusing on the most important varieties cultivated in La Rioja region, Tempranillo and Viura.

MATERIAL AND METHODS

Sampling was performed in a vineyard situated in the Rioja Alta sub region, in Tirgo municipality, situated inside the following vertices 42º 33´32.5´´/ 2º 57´17.7´´; 42º 33´29.7´´/ 2º 57´23´´, 42º 33´28.6´´/ 2º 57´22.8´´; 42º 33´31.7´´/ 2º 57´17.7´´. The cultivars were Tempranillo (83%) and Viura (17%) grafted on Richter 110 rootstock, with a planting framework of 3.20 x 1.20 m. In this area, the first exit holes caused by *X. arvicola,* affecting 7% of the vines, were observed in 1998 (12 years after the vineyard plantation). The percentage of infestation grew up to 81.3% in 2012, the year in which sampling took place.

In 2011, at pruning time (December) one hundred Tempranillo vines and 55 Viura vines were selected and marked, choosing the plants that had at least one branch with the borer's exit holes and one branch apparently not affected (not showing the beetle's exit holes).

In 2012, a two-stage sampling was performed: a) at flowering time (3rd week of June), when the phenological development of the vines was in the stage "I" (BAGGIOLINI, 1952), and b) at harvesting time (2nd week of October). For each marked vine, two bunches were selected (secondary units): the longest of those situated on a seemingly not affected branch (not showing holes or galleries, no stunted development), and the longest of those hanging from an infested branch (with visible exit holes of adults and stunted shoots), recording the inflorescence length and the number of flowers per bunch. During harvesting time, for each marked vine, sampling was performed collecting and weighting the two selected bunches. Later, each sample of must, obtained by either *X. arvicola* affected or not affected bunches, was analysed with a refractometer to determine the Baumé degree. In December, both the affected and not affected branches (between 65 and 110 cm each) were cut and their weights recorded.

In summer 2013, fresh leaves were collected from both affected and not affected branches of both cultivars from all marked plants. Photosynthetic pigments were extracted using 0.1 g of fresh leaves material in 10 ml of 80% aqueous acetone. The samples were centrifuged at 12.000 rpm during 2 min. The concentrations of chlorophyll a (chl a), chlorophyll b (chl b), chlorophyll a+b (chl a+b) and carotenoids were determined using a *Hitachi U-2001* spectrophotometer (Hitachi Ltd., Tokyo, Japan) at three wavelengths (663.2, 646.8, and 470.0 nm). Concentrations of pigments, expressed as mg per fresh weight (mg gPF^{-1}) were obtained through calculation (LICHTENTHALER, 1987).

Statistical methods were used to prove the normality of data (Shapiro Wilks test), the significant differences between affected and not affected bunches (Fisher-Snedecor distribution) and the dependence between variables (Pearson product-moment correlation coefficient). All data were proved normal (Shapiro Wilks test) except for bunch length of the affected Tempranillo, numbers of flowers and bunch weight in not affected Viura, wood weight and Baumé in affected Viura, and chl a, chl b, chl a+b in both Tempranillo situations. These data were normalized applying normal scores. Statistical analyses were performed with InfoStat2013 (National University of Cordoba, Argentina).

RESULTS

For both cultivars, on all agronomic and physiological parameters (Table 1), the values related to branches not affected by *X. arvicola* are higher than those of the affected ones, with the only exception of carotenoids in Tempranillo cultivar, where values are slightly higher in the affected branches. Baumé degree was the less affected parameter, showing no significant differences for both cultivars in F test. Beside this, comparisons between affected and not affected branches were significant for all parameters in Tempranillo cultivar, with the exception of Chl_{a+b}, whereas in Viura no significant differences were detected in bunch length and Chl_b. (Table 1).

Number of flowers, bunch weight and wood weight are drastically reduced in the affected branches. Nevertheless, the relationship between reduction in number of flowers and bunch weight is favourable to Tempranillo, that is: in Tempranillo the percentage reduction of the bunch weight is smaller than that recorded in Viura (52.44% versus 64.00%), in spite of a higher reduction in flowering (81.16% versus 72.56%), as has been indicated by the proportion between affected and not affected branches (Table 2).

Chlorophyll pigments on average show a 24% reduction in Tempranillo (except carotenoids that are increased) and a stronger reduction in Viura (77% on average) in all chlorophyll pigments; carotenoids pigments are now reduced in a 35.88%. Calculating the proportion between affected and not affected branches for both grapevine cultivars (Table 2) the obtained values are usually higher in Tempranillo (64.36% as average) than in Viura (44.74% as average), especially for bunches and wood weight, and for content of chlorophylls and carotenoids. Lower values indicate that this cultivar is more damaged by the borer than Tempranillo.

Pearson product-moment correlations (Table 3 and 4) show that in Tempranillo cultivar most of all considered parameters are correlated, indicating that they are varying on the same way, whereas in Viura only half of the correlations were significant. Baumé degree and bunch weight did not show any significant correlation with any other parameter.

Considering the photosynthetic pigment contents (Tables 1, 2 and 3) from leaves collected in *X. arvicola* affected and not affected branches, the variation in the content of chlorophylls and carotenoids is more pronounced in Viura than in Tempranillo.

The ratio between chlorophyll a and b is reduced in affected branches in Viura (45% of decreased) but not in Tempranillo (only a 2% of decreased), whereas, considering the carotenoids/chlorophyll ratio, this is higher in the affected branches, showing an increase of 37% in Tempranillo and 109% in Viura (Table 5).

DISCUSSION

The results show that infestation by *X. arvicola* considerably affected production parameters in both varieties, and that Tempranillo, which is the mostly attacked variety in the field (Ocete & López, 1999), is the one whose production parameters are less affected by this pest. According to our findings, branches attacked by this borer have inflorescences of lesser length (about 50% shorter in Tempranillo and 45% in Viura), a heavily reduced number of flowers (about 81% less flowers in Tempranillo and 73% in Viura) and a lower bunch weight (a reduction of about 52% Tempranillo and 64% in Viura).

Similar results were observed previously by Ocete *et al.* (2004, 2008, 2009), that verified also that affected Tempranillo grapevines had a lower compactness of the

Table 1 – Values (average ± standard deviation) and results of the F test (*= p ≤ 0.05; **= p ≤ 0.001, n.s.= not significant) obtained for each considered parameter considering branches (Aff.= affected by *X. arvicola;* Not Aff.= showing no exit holes of the borer) of the two grapevine cultivars (Viura; Temp.= Tempranillo). Chl_a, Chl_b, and Chl_{a+b} indicate the concentration of photosynthetic pigments measured at different wavelengths (mg/g of fresh weight).

Parameters	Parameter values				F test	
	Tempranillo		Viura		Temp.	Viura
	Not Aff.	Aff.	Not Aff.	Aff.	Not Aff. vs Aff.	Not Aff. vs Aff.
Bunches length (cm)	19.61 ± 1.82	10.02± 1.09	19.53 ± 1.65	10.80 ± 1.11	0.36*	0.45 (n.s.)
Number of flowers	213.88 ± 40.18	40.32 ± 9.43	188.8 ± 41.14	51.76 ± 11.03	0.06**	0.07**
Bunches weight (g)	386.88 ± 95.69	184.02 ± 49.82	719.98 ± 236.93	259.24 ± 65.20	0.27**	0.08**
Wood weight (g)	504.60 ± 110.11	168.60 ± 60.24	611.20 ± 126.87	156.80 ± 51.45	0.27**	0.30**
Baumé degree	12.24 ± 0.62	11.63 ± 0.59	11.5 ± 0.44	10.88 ± 0.48	0.91 (n.s.)	1.18 (n.s.)
Chl_a	0.206 ±0.130	0.155± 0.087	0.229± 0.087	0.056± 0.025	0.34*	0.08**
Chl_b	0.167± 0.134	0.129± 0.109	0.106± 0.036	0.047± 0.026	0.05**	0.55 (n.s.)
Chl_{a+b}	0.373± 0.242	0.284± 0.190	0.335± 0.117	0.103± 0.046	0.46 (n.s.)	0.16**
Carotenoids	0.133± 0.047	0.139± 0.028	0.170 ± 0.028	0.109 ± 0.018	0.06**	0.39*

Table 2 – Proportion (%) between affected and not affected branches for each considered parameter for the two grapevine cultivars. Chl_a, Chl_b, and Chl_{a+b} indicate the concentration of photosynthetic pigments (mg/g of fresh weight) measured at different wavelengths.

Parameters	Tempranillo	Viura
Bunches length (cm)	51.10	55.30
Number of flowers	18.84	27.44
Bunches weight (g)	47.56	36.00
Wood weight (g)	33.41	25.65
Baumé	95.02	94.61
Chl_a	75.24	24.45
Chl_b	77.25	44.34
Chl_{a+b}	76.14	30.75
Carotenoids	104.51	64.12
Average	64.34	44.74

bunch (OCETE *et al.,* 2009). In a study that compared unhealthy (= attacked by *X. arvicola)* and healthy (not attacked by the borer) whole plants of different varieties, MORENO (2005) reported that even if the total production was always higher in healthy vines, the production and physiological parameters varied according to the cultivar. Specifically, content of chlorophylls, foliar area and oenological parameters were not affected in Garnacha, Viura, and Tinta de Toro varieties, whereas in both Verdejo and Tempranillo the foliar area was significantly lower in unhealthy vines, and in Verdejo the content of chlorophylls was significantly lower in attacked vines (MORENO, 2005).

In our study, the branches of both Tempranillo and Viura attacked by the borer showed a significant loss of flowers (Table 1). Although the proportion of affected branches (and the consequent loss of flowers) was higher in Tempranillo than in Viura, the weight of the bunches was higher in Tempranillo, and this apparently suggest a higher tolerance of this variety to *X. arvicola* attacks.

In spite of significant differences for other parameters, we found that the Baumé degree of the must obtained from both varieties, and therefore the alcoholic content of the

Table 3 – Pearson product-moment correlation coefficients obtained for the different parameters in Tempranillo cultivar. Values higher than 0.79 are considered significantly correlated.

Parameters	Bunches lenght	Wood weight	Flowers number	Chl_a	Chl_b	Chl_{a+b}
Wood weight	0.82					
Flowers number	0.99	0.82				
Chl_a	1,00	0.82	0.99			
Chl_b	0.99	0.82	1.00	0.99		
Chl_{a+b}	0.92	0.84	0.90	0.92	0.89	
Carotenoids	0.87	0.80	0.84	0.87	0.84	0.98

Table 4 – Pearson product-moment correlation coefficients obtained for the different parameters in Viura cultivar. Values higher than 0.79 are considered significantly correlated.

Parameters	Bunches length	Wood weight	Flowers number	Chl_a	Chl_b	Chl_{a+b}
Wood weight	0.88					
Flowers number	0.98	0.84				
Chl_a	0.81	0.68 n.s.	0.78 n.s.			
Chl_b	0.68 n.s.	0.59 n.s.	0.69 n.s.	0.87		
Chl_{a+b}	0.85	0.74 n.s.	0.83	0.99	0.88	
Carotenoids	0.77 n.s.	0.72 n.s.	0.75 n.s.	0.85	0.76 n.s.	0.86

Table 5 – Ratio between pigments in affected and not affected branches for both cultivars.

	Tempranillo		Viura	
	Not affected	Affected	Not affected	Affected
chl_a / chl_b	1.23	1.20	2.16	1.19
Car/Chl_{a+b}	0.36	0.49	0.51	1.06

wine, was not significantly affected by the borer infestation. This is in contrast with a former study by OCETE *et al.* (2009) who showed that in Tempranillo cultivar the wine obtained from affected branches had a lower alcoholic content and a considerably higher content of malic, citric and tartaric acids. These organic acids are typical of unripe grapes and are thought to be responsible for an unbalanced, excessively sharp "greenish" taste. The locations of our study and of the former one are quite close one to each other (Labastida and Cuzcurrita del Río Tirón, 16 km away). Some hypotheses to explain this difference could be related to specific micro-climatic features and different vine clones. In a similar study performed by MORENO (2005) in Castilla y León Region, the alcoholic degree, malic acid and total acidity of the must obtained in Tempranillo, Garnacha, Viura, Verdejo and Tinta de Toro varieties were not affected by the borer infestation. However, in that case data were obtained from the whole plant, either affected or not affected branches by the borer, whereas in our study, data were obtained from single affected/not-affected branches, and this could explain why no significant differences were detected in the considered parameters. In any case, Baumé degree can be influenced by many different factors, including the harvest period, so that the importance of this parameter in terms of the effects due to the borer should be downsized.

The attack of this longhorn beetle caused a significant loss of the wood weight in the affected shoots at pruning (66.59% in Tempranillo cultivar and 74.35% in Viura cultivar). ARMENDÁRIZ *et al.* (2008) in Tempranillo and Cabernet Sauvignon vines found a relation between *X. arvicola* infestation and the loss of vegetative units, due to the break of affected branches. Considering the higher wood loss recorded in Viura than in Tempranillo, the occurrence of branch breaking should be more likely in this cultivar, confirming the higher vulnerability of Viura to the borer activity.

The appearance of *X. arvicola* affected branches is similar to the ones affected by Eutypa dieback (OCETE *et al.*, 2010), with rachitic shoots and reduction of leaf surface, and our findings show that this can be reflected also in the content of chlorophyll pigments, especially chlorophyll a, that was significantly lower in affected vines of both varieties. In plant-pathogen interactions, downregulation of photosynthesis is one of the mechanisms that allows plants to save energies that can be diverted to activate pathways useful for defence responses (ROJAS *et al.*, 2014). It is possible that a xilophagous pest such as *X. arvicola* can induce this type of response also in this case.

Grapevines have a strong capacity of compensation for pests, diseases and unsuitable weather conditions, which are all phenomena, related to presence and accumulation of pigments contents (CANDOLFÍ-VASCONCELOS & KOBLET, 1991). However, in this case, the attack by the borer reduces the chlorophyll contents, although without defoliation. As a result, fruit production was strongly reduced.

Considering the pigment relationships, in the case of the Tempranillo variety, there is not any significant variation in leaves affected and not affected by the borer, apparently suggesting the observed tolerance to *X. arvicola* attack. Conversely, a strong decrease of the chlorophyll a/b ratio was detected in the leaves of the affected Viura branches. Our observations agree with those described by FERNÁNDEZ-MARTÍNEZ *et al.* (2014) in *Populus* sp. It can also be speculated a positive relation between photosynthetic activity and chlorophyll content (GRATANI *et al.*, 1998), leading in some cases to a reduction in yield even if, again, other authors reported an increase in chlorophyll content as a rapid local resistance mechanism to pest attack (CÁRDENAS & GALLARDO, 2016).

Otherwise in Viura, an increase in the carotenoids/chlorophyll relationship was observed indicating high chlorophyll degradation, because the level of carotenoids levels starts to decline when chlorophylls are fully degraded (DANI *et al.*, 2016). Similar findings were obtained by BERTAMINI *et al.* (2002) in grapevines infected by Phytoplasmas.

The present study for the first time highlighted important differences in some production parameters due to the effects of attacks by the borer *X. arvicola* in two grapevine varieties used in Spain, Tempranillo and Viura. Tempranillo a widely used variety, admitted for wine production in many Spanish denomination of origin areas. No data are available on the susceptibility of other Rioja red varieties like Graciano and Maturana. Considering the increasing infestation by this longhorn beetle in Spanish vineyards, our findings indicating that Tempranillo shows a higher tolerance to the attacks of this pest, may be useful to growers in the implantation phase of new vineyards.

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