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FAILURE UNDER STRESS OF GRAPEVINE WOOD: THE EFFECTS OF THE CERAMBYCID *Xylotrechus arvicola* ON THE BIOMECHANICS PROPERTIES OF *Vitis vinifera*

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ABSTRACT

Xylotrechus arvicola is an insect pest on *Vitis vinifera* in the main wine-producing regions of Spain. *X. arvicola* larvae bore inside grapevine wood, which cause structural damages in the plants' biomechanical properties. Grapevine wood affected and unaffected by larvae, were collected from vineyards. Compression and flexural tests were used to quantify biomechanical wood properties. Affected wood broke more quickly and endured a lower supported force than unaffected wood in both varieties and moisture states. Tempranillo was the most resistant variety on trunks, while Cabernet-Sauvignon was the most resistant variety on branches, where all infested varieties showed a lower rate of bending. Grapevine wood affected by *X. arvicola* larvae shows an important decrease in its resistance in both moisture states - dry and wet wood - and it is observed due to the faster break in time and a lower supported force. These damages give the affected wood greater sensitivity to external mechanical factors in the vineyards, such as strong winds, harvest weight and the vibration exerted by harvesting machines. The aspect of stress-time curves in all cases follow similar patterns, so in future studies might be possible to stablish relationships between both wet and dry samples and different infestation levels.

Keywords: Cerambycid, compressive test, flexural test, insect borer, vineyard.

INTRODUCTION

Xylotrechus arvicola (Coleoptera: Cerambycidae) is a xylophagous polyphagous insect native to riverside trees, being the genus *Quercus* spp., *Carpinus* spp., *Castanea* spp., *Fagus* spp., *Populus* spp., *Salix* spp., *Tilia* spp., *Morus* spp., *Sorbus* spp., *Crataegus* spp., *Malus* spp., *Cydonia* spp. and *Prunus* spp., the hosts reported (Bahillo 1996, Vives 2000, Moreno 2005, Biurrun *et al.* 2007). Its geographical distribution is mediterranean, spreading around Europe, Minor Asia and northern Africa (Villiers 1978, Nikitsky *et al.* 2016). This insect has become a pest of grapevines (*Vitis vinifera*) in the main wine-producing regions of the Iberian Peninsula (Ocete and del Tío 1996, Rodríguez and Ocaña 1997, Ocete and López 1999, Pelaez *et al.* 2001, Ocete *et al.* 2002a). *Trogoxylon impressum* Comolli (Coleoptera: Lyctidae), *Xyloperthodes incertus* Lesne (Coleoptera: Bostrichidae) and *Acalolepta vastator* Newman (Coleoptera: Cerambycidae) are three species of wood borers that have been described as grapevine pests in Israel and wine-growing areas of Switzerland, Germany and Austria, South Africa and Australia, respectively (Goodwin and Pettit 1994, Halperin and Geis 1999, Allsopp and Knipe 2004).

Plant wood can be degraded by fungi and termites due to its biological origin (Syofuna *et al.* 2012). One of the most important causes of wood damage in plants are insects (Sen *et al.* 2017) that produce technical and economic losses (Ssemaganda *et al.* 2011). Sen *et al.* (2017) reported that the most important borer insects

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are those belonging to the Cerambycidae, Anobidae and Lyctidae families. A wood-damaging insect type is *Xylotrechus arvicola*, which belongs to the Cerambycidae family from the Coleoptera class. This insect is most commonly observed in the main wine producing regions of the Iberian Peninsula, Spain, damaging wood of different varieties of vine (*Vitis vinifera*).

Adults of *Xylotrechus arvicola* measures between 8 mm and 20 mm in length. The average size of the female is larger than the male, their coloration is brown or blackish and the pronotal and elytral bands are usually yellow (Moreno *et al.* 2003). After mating, *X. arvicola* females lay eggs, concentrated in cracks or under the rhytidome in the vine wood (Pelaez *et al.* 2002). The fecundity and viability of eggs laid by *X. arvicola* females are extended over a long period of time (Rodríguez-González *et al.* 2016a). The location of the eggs enables the emerging larvae to move into the wood, making galleries inside the plant (Garcia-Ruiz 2009). The most susceptible states of the specie are adults, eggs and neonate larvae, although eggs are usually protected by the rhytidome or crack. The larvae, once inserted in the wood, are inaccessible when treated with traditional chemicals compounds (Pelaez *et al.* 2002) that do not have penetrative attributes (Rodríguez-González *et al.* 2017). Another problem is the treatment on *X. arvicola* adults, because it has an emergence pattern which is very staggered over time (Garcia-Ruiz 2009).

As was briefly commented in the previous paragraph, the damage to grapevines is caused by *X. arvicola* larvae which bore into grapevine plants to feed on wood making galleries within the plant for up to two years (Moreno 2005). Also, indirect damaged is caused by the formation of adult emerging holes which are the entry point for fungal diseases (Garcia-Benavides *et al.* 2013) such as *Diplodia seriata* (De Not), *Eutypa lata* (Tul and Tul), *Phaeoacremonium minimum* (Tul. and C. Tul), *Phaeomoniella chlamydospora* (Crous and Gams), and *Formitiporia mediterranea* (Fisch). Consequently, infection of fungal diseases on grapevines will cause a serious impact on host plants, e.g. stunting growth, low quality grape..., specifically on main grape varieties in Spain such as Tempranillo or Cabernet-Sauvignon (Ocete *et al.* 2002b, Garcia-Benavides *et al.* 2013). In severe attacks lasting years, plants die due to damaged plant vascular tissue, making it more difficult for sap to be transported along the plant, thus needing the plant to be replaced with the corresponding long lasting economic losses (Ocete *et al.* 2002a). A typical vineyard affected by *X. arvicola* will show numerous broken branches due to the larvae galleries weakening the wood structure of the plant (Garcia-Ruiz 2009). Cultural measures to control *X. arvicola* consist of removing the rhytidome of the grapevines (Pelaez *et al.* 2006) or pruning affected branches below the area of galleries (Ocete *et al.* 2004), to the grapevine structure to grow again, which could be time consuming and financially draining, as these techniques are expensive and not sustainable (Pelaez *et al.* 2006). The renovation of attacked branches in grapevines is easier with the bush vines training system than in grapevines with bilateral cordon training systems (Rodríguez-González *et al.* 2016b).

Other borers such as *Spondylis buprestoides* (Coleoptera: Cerambycidae) have been observed to cause damage in coniferous forests, especially in Scots pine, and other types of pine, spruce and fir in Australia, Siberia, Korea, Japan, and China (Kolk and Starzyk 1996). Moreover, the Esmerald Ash Borer (EAB) *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) has become an important pest, which is decimating white ash populations in North America nowadays (Persad *et al.* 2013, Persad and Tobin 2015, Finley *et al.* 2016).

Returning to this insect pest (*X. arvicola*) in this crop (*V. vinifera*), the grapevine wood structure resistance is of paramount importance owing to the many functions it performs. There is no information on grapevine wood material resistance once it has been affected by *X. arvicola* larvae. This is the argument for this study, in order to provide more information in how wood-borers' galleries affect structure and material properties of woody plants and all its implications. This information will be important for measuring how this pest could affect grapevine wood resistance. Consequently, having this information would enable vinicultors to estimate the wood resistance under those physical challenges present in vineyard (e.g. wind, grape carrying capacity, bunch carrying capacity, mechanical harvest). The present study would enable an estimate of the wood resistance loss in a vineyard affected by *X. arvicola*.

The aim of this study was to investigate the biomechanical properties of wood branches and trunks of grapevines, affected and unaffected by *X. arvicola* larvae, simulating the stresses (compression and flexural) to which they are subjected in the field.

MATERIAL AND METHODS

Vineyards of study

Two vineyards where severe pruning was taking place to rebuild the plant structure and avoid *X. arvicola* spreading were sampled during 2017 and 2018. The vineyards were located in a Protected Denomination of Origin (PDO), which is a certification to distinguish quality food products of a particular region (UE Reg. No. 1151/2012 published on 21 November 2012), called 'Ribera Del Duero', located in Peñafiel (Valladolid; Spain). These vineyards were planted uniformly with the same *V. vinifera* variety ('Tempranillo' and 'Cabernet-Sauvignon'), planted in soils with Franco-Sandy texture, each variety being 26 and 29 years old, respectively). Vines were spaced 3 m × 1,5 m and were surrounded by other vineyards. The training system of vines was 'Espaldera' (Double Cord or Royat), formed by two branches (1,0 m) and a trunk (0,7m) in length.

Wood samples and experimental conditions

Wood trunks and branches from different grapevines, which were previously exposed to *X. arvicola* larvae attack a number of years, were collected to study the cumulative effects of larvae feeding in the vascular tissues of grapevine wood over time. Wood samples were randomly selected from affected and unaffected trunks and branches per grapevine and variety ('Tempranillo' and 'Cabernet-Sauvignon') in order to make a comparison between affected and unaffected woods within the same variety.

Grapevine wood varieties were labelled as affected and unaffected according to external observable symptoms described by Pelaez *et al.* (2006) (such as exit holes of adults and/or galleries in pruning cuts) and taken to laboratory. The European Standard EN 14251(2004) (Structural Round Timber, Test Methods) was used to calculate the necessary measures on wood samples before testing. The diameter and length of the sample trunks and branches were measured according to the European Standard cited. The wood samples that did not fulfil the requirements of the EN 14251(2004) standard were not considered for testing.

Part of the wood samples from both varieties were evaluated in fresh conditions (to avoid drying and to ensure that the moisture content was similar to that in the field). The rest of wood samples, to ensure the elimination of all moisture content, were air-dried for 30 days in laboratory ambient temperature ($26^{\circ} \pm 1^{\circ}\text{C}$) before mechanical testing. According to the European Standard EN 14251(2004) to calculate the moisture content of the wood samples, 10 specimens of each variety and part of the plant (trunks and branches with a diameter greater than 75 mm and without knots) were dried in the aforementioned laboratory conditions and were weighed at the beginning and end of the drying process. The following equation (Equation 1) was used to calculate the moisture percentage of the wet wood samples:

$$MS = \frac{WS - DS}{DS} \times 100 \quad (1)$$

Where, MS (Moisture Samples) in percentage; WS (Wet Sample) in grams; DS (Dry Sample) in grams.

Mechanical testing of wood samples

The effects of *X. arvicola* larvae on the biomechanical properties of the plant in trunks and branches of *V. vinifera* varieties were evaluated using two standard experiments: a compression strength test for wood trunks and flexural strength test for wood branches.

All trunks and branches (wet and dry) were tested with a hydraulic press (i.e. a universal press of compression and bending) of the EIC (Engineering, Instrumentation and Control) brand of maximum load of 2000 kN. This apparatus applies the load by using a pump that generates oleohydraulic pressure and collects the data with a data logger. The EIC software takes the total applied load and voltage data. Performed data collection and processing was carried out with EIC software, in which the loading rate was the same for all the experiments, 200 N/s. The trunks were placed vertically to mimic the compression strength in the field, placing the surfaces of both ends which had been cut perpendicular to the longitudinal axis of the sample (Figure 1A). The branches were placed horizontally, trying to load branches topside in down position, to mimic downward

bending in the field with a support span of 30 cm (at 18 times the branch nominal diameter to minimize shear stresses), and applied the central load (Figure 1B). Trunks and branches that twisted or slipped during the run were omitted from the analyses.

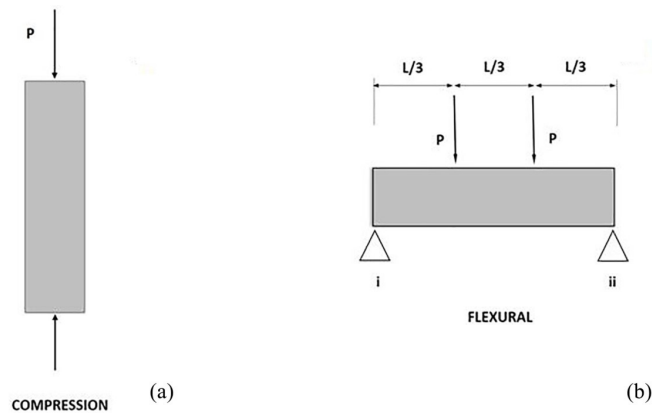


Figure 1: (a) Schemes of tests in trunks (P axial force applied until breakage). (b) Schemes of the test in branches (the forces P were applied in central third of the specimen until breakage, 'i', and 'ii' were the supporting points in the extremes of wood samples).

Experiment 1: Mechanical testing of grapevine wood trunks (break in time)

In the compression test, different dimensions from each sample (wet and dry) were measured: 3 measurements of minimum diameter, 3 measurements of maximum diameter, and total sample length.

For the affected wood trunks samples, measurements of the number of adult exit holes and presence or absence of galleries appearing on both ends of the sample were also registered. The compressive test of samples was calculated according to the European Standard EN 14251(2004) and the following Equation 2, Equation 3, Equation 4, Equation 5, Equation 6:

$$\lambda = \frac{lk}{i} \quad (2)$$

$$i = \sqrt{\frac{I}{A}} \quad (3)$$

$$I = \frac{\pi r^4}{4} \quad (4)$$

$$A = \pi r^2 \quad (5)$$

$$\sigma = \frac{N}{\pi r^2} \quad (6)$$

Where, λ : mechanical slenderness (dimensionless); lk : buckling length or length of the test piece (mm); i : turning radius (mm); I : moment of inertia (mm⁴); A : transversal section area (mm²); r : radius (mm); σ : normal

tension (MPa=N/mm²); N: strength (Newtons).

In each mechanical testing, two break times were measured, time “T1”, the time when the force applied by the hydraulic press to the wood sample remains constant, and time “T2”, the time where the wood sample is completely broken.

Experiment 2: Mechanical testing of grapevine wood branches (break in time)

In the flexural test different dimensions from each sample (wet and dry) were measured: 3 measurements of minimum diameter, 3 measurements of maximum diameter, 2 measurements at the midpoint where the load is exerted (one in the direction of the load and another one in the perpendicular direction to the load) and total sample length.

For the affected wood branches, the number of adult exit holes and presence or absence of galleries appearing on both ends of the sample were also measured. The flexural test of samples was calculated according to the European Standard EN 14251(2004) and the following Equation 3, Equation 4, Equation 7, Equation 8:

$$A = \pi r^2 \quad (7)$$

$$\sigma = \frac{M_z}{W_z} = \left(\frac{M_z}{I} \right) r \quad (8)$$

Where, I: moment of inertia (mm⁴); A: transversal section area (mm²); r: radio (mm); σ : normal tension (flexural) (MPa = N/mm²); M_z: bending moment (N·mm); W_z: resistant module (mm³).

In each mechanical testing, two break times were measured ‘time T1’, the time when the force applied by the hydraulic press to the wood sample remains constant, and ‘time T2’, the time where the wood sample is completely broken.

Statistical analysis

Statistical analyses were performed using the SAS software version 9.1.2 (SAS Institute Inc. 2004). Means parameters (supported force and time) evaluated on trunks and branches were performed using analysis of variance (Fisher’s LSD, considered significant at $p \leq 0,05$) to evaluate the biomechanics properties of affected and unaffected grapevine wood.

RESULTS

Experiment 1: Mechanical testing of grapevine wood trunks (break in time)

The moisture content obtained in wet trunks samples was 63,8 % in Tempranillo and 57,0 % in Cabernet-Sauvignon.

Concerning the time needed to break wet trunks, unaffected Tempranillo and Cabernet-Sauvignon showed the highest time of resistance under compression test, which was significantly greater than their respective affected samples in the two measurement times described (Figure 2A). Affected varieties presented an average of 2,0 holes (5,6 mm in diameter) and 1,0 gallery (10,6 mm in diameter) in Tempranillo and, an average of 3,0 holes (5,3 mm in diameter) and 1,0 gallery (11,4 mm in diameter) in Cabernet-Sauvignon. After being dried, only Cabernet-Sauvignon (unaffected), showed a break in time (363,6 s in T1 and 472,0 s in T2) significantly greater than its respective affected samples in time T1 ($F=10,41$; d.f.=3,16; $P \leq 0,001$) and T2 ($F=7,77$; d.f.=3,16; $P \leq 0,001$) (Figure 2B). Affected Cabernet-Sauvignon presented an average of 1,3 holes (4,7 mm in diameter) and an average of 7,7 galleries (4,6 mm in diameter).

Concerning the supported force in wet trunks, unaffected Tempranillo and Cabernet-Sauvignon varieties showed the highest resistance under compression test (8,2 MPa and 6,7 MPa, in Tempranillo and Cabernet-Sauvignon respectively), significantly greater than their respective affected samples (Figure 2A). The

behavior of the dry varieties was similar, in these conditions unaffected Tempranillo and Cabernet-Sauvignon showed the highest resistance under compressive test, 11,3 MPa in Tempranillo and 7,7 MPa in Cabernet-Sauvignon, significantly greater ($F=6,78$; d.f.=3,16; $P=0,004$) than their respective affected samples (Figure 2B).

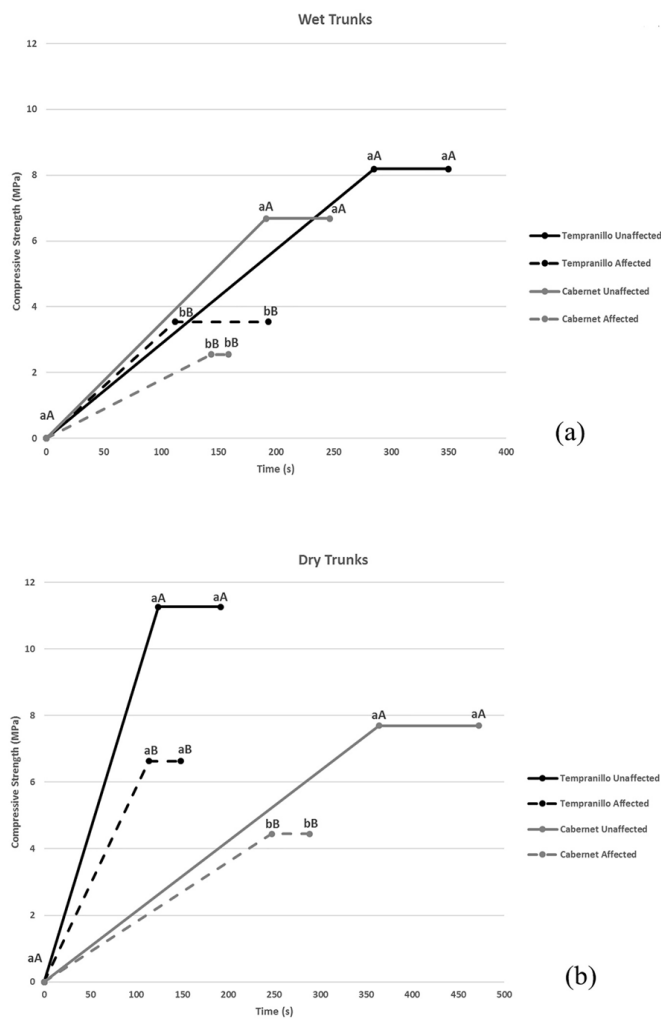


Figure 2: Diagram of compression test in grapevine trunks (a), wet wood; (b), dry wood), depicting the stress–time curve. All samples were loaded at the same rate, and load (represented as MPa, y-axis) as well as breakage in time (x-axis) were measured. ‘Dashed line’ represents wood samples infected by *X. arvicola* larvae, with higher stress and time at breakage; ‘Continuous line’ represents unaffected wood samples, with higher stress and time at breakage. Different lowercase letters indicate significant differences between grapevine trunks of each variety (affected and unaffected) in the same breakage in time. Different capital letters indicate significant differences between grapevine trunks of each variety (affected and unaffected) in the supported force.

Experiment 2: Mechanical testing of grapevine wood branches (break in time)

The moisture content obtained was 62,5 % in Tempranillo and 59,6 % in Cabernet-Sauvignon in samples of wet branches.

In relation to break in time of wet branches, Cabernet-Sauvignon and Tempranillo unaffected showed the highest time of resistance under flexural test, which was significantly greater than their respective affected samples in the time T1 ($F=3,43$; d.f.=3,16; $P=0,042$) y T2 ($F=5,98$; d.f.=3,16; $P=0,006$) measured (Figure 3A). Affected varieties presented an average of 3,0 holes (6,6 mm diameter) and 3,7 galleries (8,85 mm in diameter)

in Cabernet-Sauvignon and, an average of 2,0 holes (4,7 mm in diameter) and 1,7 galleries (4,7 mm in diameter) in Tempranillo. After being dried, only Cabernet-Sauvignon (unaffected), showed a break in time (56,0 s in T1 and 73,5 s in T2) significantly greater than its respectively affected samples in both times measured (Figure 3B). Affected variety (Cabernet-Sauvignon) presented an average of 1,0 hole per sample (4,7 mm in diameter) and 2,0 galleries (5,3 mm in diameter).

Regarding the supported force in wet branches, unaffected Cabernet-Sauvignon and Tempranillo, showed the highest resistance under flexural test (75,3 MPa and 66,4 MPa, in Cabernet-Sauvignon and Tempranillo respectively), significantly greater than their respectively affected samples (Figure 3A). In the same dried branches, only Cabernet-Sauvignon (unaffected), showed the highest resistance under flexural test, 57,8 MPa, significantly greater ($F=8,71$; $d.f.=3,16$; $P\leq0,001$) than its respectively affected samples (Figure 3B).

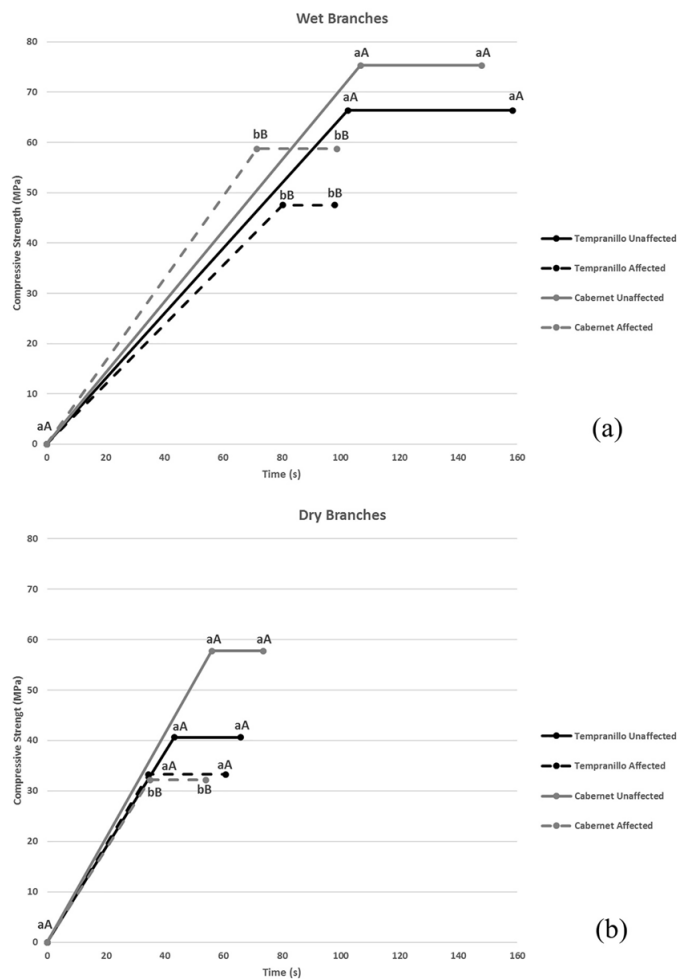


Figure 3: Diagram of flexural test in grapevine branches (a), wet wood; (b), dry wood), depicting the stress–time curve. All samples were loaded at the same rate, and load (represented as MPa, y-axis) as well as breakage in time (x-axis) were measured. ‘Dashed line’, represents wood samples infected by *X. arvicola* larvae, with higher stress and time at breakage; ‘Continuous line’ represents unaffected wood samples, with higher stress and time at breakage. Different lowercase letters indicate significant differences between grapevine branches of each variety (affected and unaffected) in the same breakage in time. Different capital letters indicate significant differences between grapevine branches of each variety (affected and unaffected) in the supported force.

DISCUSSION

The tests carried out show *X. arvicola* larvae produce numerous damages on the biomechanics properties of *V. vinifera* wood in the varieties studied, such as a fast failure under stress or a decrease in its resistance (no matter whether the wood is wet or dry, or it is compressed or flexed).

Based on these results, damage caused by *X. arvicola* larvae can produce, in extreme situations, a break in vine branches affected (because the wood of the arms, both wet and dry, broke faster and withstood less force than their respective unaffected samples). This phenomenon has also occurred in other woody species such as hemlock woolly adelgid HWA *Adelges tsugae* Annand (Homoptera: Adelgidae) on *Tsuga canadensis* Carrière (Pinales: Pinaceae) (McClure 1991, Stadler *et al.* 2005). Also, Biurrun *et al.* (2007) have observed *X. arvicola* in *Prunus pisardi* Carrière, Koehne (Rosales: Rosaceae) trees causing the weakening of the affected trees and/or the death or breakage of the affected branches for several years.

The lower supported force observed in the grapevine wood affected by *X. arvicola* larvae can be the indirect damage caused by the propagation of wood diseases that kill the vascular tissues of the wood and/or the direct damage caused by the reduction of vascular tissue of wood ingested by larvae.

Wood pathogens or diseases affect the biomechanical properties of other woody species, as it has been described in other genera, such as *Pinus spp.* (Kurkela *et al.* 2005, Drenkhan *et al.* 2006), *Pseudotsuga spp.* (Hansen *et al.* 2000) and *Larix spp.* (Krause and Raffa 1992). The action of *X. arvicola* larvae inside the grapevine wood favors the propagation of grapevine trunk diseases (described above in the introduction) through the emergence holes in the wood created by *X. arvicola* adults on their way out, leading to the death of plant vascular tissue (Ocete *et al.* 2002a, Garcia-Benavides *et al.* 2013). Tempranillo and Cabernet-Sauvignon varieties, which are two of the main varieties of Spanish vineyards, are the varieties where the fungal attack is more severe (Ocete *et al.* 2002a). Varieties affected by wood diseases accumulate a higher amount of dead wood that renders it to have a greater fragility, producing a gradual death of the affected areas, as also has been described by Hauer *et al.* (1993) in other woody species. James and Kane (2008) and Detters *et al.* (2008) have described that an accumulation of dead wood by the attack of pathogens on branches or trunks predisposes woody species affected to damage or breakage when they are subjected to external agents, such as snow, wind and static charges. Or static loads, such as weights of grapes (in harvest conditions) which grapevine wood is exposed at the time of production.

Ingestion of vascular tissue by insect larvae affects physical properties of wood species. *X. arvicola* larvae feeding modify the physical properties of trunks and branches of grapevine wood varieties, due to the decreased of break in time and supported force. The continuous years of infestation by these larvae in woody tissues pronounced changes in wood will be identified. Hence, leaf development becomes scarce and shoots are not very vigorous and productive (Moreno *et al.* 2004). Moreover, clusters are smaller, flowers are fewer and shorter in length, and they drop more easily (Ocete *et al.* 2002b). Soltis *et al.* (2014) described that branches breakage due to physical factors can reduce plant fitness because of biomass and meristem loss, showing the same results of biomechanics effects on *Tsuga canadensis* produced by *Adelges tsugae*. If woody species have suffered previous damage, several years of strong gusts of wind can cause mechanical instability (Spatz and Bruechert 2000).

Affected wood trunks and branches evaluated (wet and dry) supported a lower force and they broke more quickly, respect to uninfested wood. These wood varieties (trunks and branches) could become more sensitive to external mechanical factors, as is the case in other woody species (Soltis *et al.* 2014), such as the weight of the grape harvest, and/or vibration exerted by harvesting machines in those vineyards that use this technique. The aspect of the stress-time curves in all cases follow similar patterns, so in future studies it may be possible to establish relationships between both wet and dry samples and different infestation levels.

CONCLUSIONS

This work shows the failure under stress (compression and flexural tests) of grapevine wood (trunks and branches) bored by *X. arvicola* larvae. Affected trunks broke more quickly, 112,0 s and 143,0 s in Tempranillo and Cabernet Sauvignon, respectively, and endured a lower force, not exceeding 6,6 MPa in both varieties and conditions of the wood, in the compression test. In addition, affected branches broke more quickly, 71,5 s and 34,5 s in Cabernet Sauvignon and Tempranillo, respectively, and endured a lower force, not exceeding

58,7 MPa in both varieties and conditions of the wood, in the flexural test. Tempranillo was the most resistant variety on wood trunks and Cabernet-Sauvignon was the most resistant variety on wood branches, all infested varieties showing a lower bending moment. Attending to results, it cannot be ruled out that in an extreme situation, the damage caused by larvae can produce a break (by bending) in branches of grapevines over time. The infestation of grapevine wood trunks and branches by *X. arvicola* larvae produces numerous damages in biomechanics properties of vascular tissue and causes an important decrease in its resistance, in both moisture conditions (wet and dry), and it is observed through the speed of break in time and a lower supported force. These damages give the affected wood a greater sensitivity to external mechanical factors in the vineyards such as strong winds, harvest weight and the vibration exerted by harvesting machines. The aspect of the stress-time curves in all cases follow similar patterns, so in future studies it may be possible to establish relationships between both wet and dry samples and different infestation levels.

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