



Article

Impact of Wood-Boring Larvae of *Xylotrechus arvicola* (Coleoptera: Cerambycidae) on Mechanical Properties of *Vitis vinifera* Plants

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Abstract: *Xylotrechus arvicola* represents a significant insect pest impacting *Vitis vinifera* within the principal wine-producing territories of the Iberian Peninsula. The larvae of this species bore into grapevine wood, resulting in significant structural and biomechanical deterioration to the plant. Compressive and flexural tests were conducted to assess the mechanical properties of wood affected by *X. arvicola*. Compressive and flexural strength exhibited a decline with the escalation of the Total Damaged Surface Area (TDSA) of the samples, ranging from 0.31% to 0.73% in trunks and from 0.04 to 0.76% in branches, irrespective of the wood moisture content (fresh and dry). The most significant reduction in resistance occurred in affected dry trunks and branches. Notably, the deflection at break for dry samples was lower compared to fresh samples (65.00 and 97.85 mm, respectively). Moreover, the deflection at break for affected fresh samples (164.37 mm) significantly surpassed that of unaffected fresh samples (72.58 mm) and affected dry samples (37.50 mm). It is noteworthy that a higher percentage of TDSA coincided with diminished wood resistance. The percentage of fungal growth symptoms observed in affected wood samples was 66.66% for dry trunks, 75.00% for fresh branches, and 60.00% for dry branches. The damage inflicted by larvae facilitated the spread of grapevine diseases via emergence of holes created by insects upon exiting the wood and through the larval galleries connected to them. This damage also altered the mechanical properties of grapevine plants, with fresh branches exhibiting the most pronounced effects.

Keywords: xylophagous insect; vineyard; damaged surface; compressive strength; flexural strength; deflection



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1. Introduction

The degradation of wood by fungi and termites is well documented [1]. However, among the significant contributors to wood damage, insects stand out as paramount. Insects from the families Cerambycidae, Anobidae, and Lyctidae assume a pivotal role in the deterioration of wood materials, leading to substantial economic losses [2]. These insect families cause irreversible damage to forests, crops, and timber due to their impact on the transport of sap and nutrients in stems [3,4].

Grapevine, a woody species, is also sensitive to be attacked by borer insects. There are insects among the European species, polyphagous and monophagous, attacking different

woody species [5,6]. *Xylotrechus arvicola* Olivier 1795 (Coleoptera: Cerambycidae) is one example of a wood-damaging insect that is affecting in the main wine-producing regions of the Iberian Peninsula, especially in Spain, attacking wood from different vine varieties (*Vitis vinifera*).

Xylotrechus arvicola is a xylophagous polyphagous wood-boring insect from riverside trees (*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.) [7–10]. Since the late 90 s, this insect has become an important insect pest of *Vitis vinifera* in the main wine-producing areas of the Iberian Peninsula [11–15]. *Xylotrechus arvicola* adults measure between 8 and 20 mm in length, with insect females larger than insect males. Its coloration is brown or blackish, and the pronotal and elytral bands are yellow [16]. *X. arvicola* females lay eggs concentrated in cracks or under the vine rhytidome [17]. The oviposition is extended over a long period of time in which the viability and number of eggs laid by a female *X. arvicola* could vary [18,19]. About eight days after egg laying, the larvae emerge [20]. The larvae move into the wood, boring galleries inside the plant [21]. The most fragile stages of this insect are adults, eggs, and neonate larvae. A treatment against eggs and larvae is not effective because the eggs are usually protected by the rhytidome, and the larvae, once inserted in the wood, are inaccessible when applying traditional chemicals, which do not have penetrative attributes [17,22]. The control of *X. arvicola* adults is also difficult because their pattern of emergence is very staggered over time [21] and is highly dependent on weather conditions (rain and temperature increases) [23].

As a wood-borer insect, the direct damage in grapevine wood is caused by larvae, which bore into grapevine plants to feed on wood tissue, making galleries within the plant for one year or a couple of years [9]. The indirect damage is produced by adults emerging from holes providing a means to facilitate fungal growth, among which *Diplodia seriata* (De Not), *Eutypa lata* (Tul and Tul), *Phaeoacremonium minimum* (Gams, Crous, Wingf., Mugnai), *Phaeomoniella clamydospore* (Crous and Gams), and *Formitiporia mediterranea* (Fisch) could be highlighted [24]. This fungal growth on grapevine wood causes a serious impact on the crop, such as reducing plant growth or negatively affecting grape quality. Fungi's impact is especially important in 'Tempranillo' and 'Cabernet-Sauvignon' varieties, which are two of the main varieties grown in the most important wine-producing areas of Spain. These two varieties are also known to have a greater sensitivity to attack by *X. arvicola* [15,24]. After severe attacks over years, plants die due to damages in vascular tissues, causing important economic losses [25]. In a vineyard affected by *X. arvicola*, broken branches could be observed due to weakened wood structure caused by the galleries bored by larvae [21]. The only current cultural techniques consist of removing the rhytidome of the grapevines [26] or/and pruning branches below the affected area [27], so the plant structure could rebuild again, but these techniques are expensive and not sustainable at a large scale [26]. The renovation of branches affected in grapevines is easier to perform in the 'bush/gobelet vine' training system in comparison to the 'bilateral cordon' training system [18,28].

Previous studies carried out with wood samples simulating the load conditions carried by the grapevine on the field have demonstrated that the wood affected is more sensitive to breakage in comparison to unaffected wood [29]. In addition, the affected wood breaks faster than the unaffected wood [30], regardless of the cross-sectional area of the part of the plant [23].

The aim of this study was to investigate the resistance of branches and trunks of grapevine wood in relation to the external surface observed, previously damaged by *X. arvicola* larvae. Additionally, other parameters such as the deflection at break of the wood samples during the mechanical tests and the incidence of fungal diseases in relation to the damages caused by larvae in wood samples were measured.

2. Materials and Methods

2.1. Plant Material (Vineyards)

Plant material was obtained from vineyards during the 2017 and 2018 seasons from plants pruned in winter in order to avoid *X. arvicola* spreading in the wood during the next years. Vineyards were located in the P.D.O. (Protected Designation of Origin) named 'Ribera Del Duero', in the Peñafiel location, Valladolid province, Castilla y Leon community, Spain (country). Sampled vineyards consisted of 28-year-old plants of the Tempranillo variety growing in loam-sandy soils. The vines were spaced 3×1.5 m, and they were surrounded by other vineyards. The vines trained into 'Trellis' system (Double Cord or Royat) were formed by two branches with each one 1.0 m in length and a trunk with 0.7 m in height. Tillage is performed in rows and also in the alleys using ploughing machines (cultivator chisel and inter-vine cultivator, respectively) so that no cover crops are in between vine rows in this type of soil management. For fertilization of the plant, chemical fertilizer is not applied, but sheep manure is applied every four years. In terms of plant protection, sulfur, copper, and two systemic fungicides are sprayed as active ingredients per campaign.

2.2. Grapevine Wood Samples and Experimental Conditions before Mechanical Tests Were Performed

Wood samples were selected from trunks and branches. Grapevine wood samples previously classified were then sorted into affected or unaffected material according to external observable symptoms described by Peláez et al. [26], as for example, exit holes of insect adults and/or larva galleries in pruning cuts. Locating a vineyard affected by this pest is a challenge and must also coincide with the exact moment in which a vineyard that is more than 20 years old is attacked by this pest, hence the small number of samples in some cases.

Before tests were performed, standards from the European Standard (EN 14251; Structural round wood. Test methods; Madrid, Spain, 2003), developed by the Spanish Association for Standardization and Certification (AENOR) [31], were used to choose those samples with the appropriate lengths to be analyzed. Diameter and length of both trunks and branches were measured for all the samples according to the European Standard-cited norms. Grapevine wood samples that did not fulfil the UNE 14251-2004 standard requirements were not used for the subsequent strength tests.

Twenty-one wood samples were evaluated in fresh conditions, 11 samples of trunks and 20 samples of branches, to ensure that the moisture content was similar to that in the field. To ensure the elimination of moisture from the wood samples, the rest of the wood samples, 24 samples of trunks and 22 samples of branches, were dried at room temperature (26 ± 1 °C) for 30 days before the mechanical tests were performed. In order to calculate the moisture content of the wood samples according to the European Standard UNE 14251-2004 [31], wood samples were dried in the aforementioned room conditions and weighed at the beginning and at the end of the drying process. The moisture content of the fresh wood samples in percentage was calculated as described in Equation (1):

$$MC = \frac{FS - DS}{DS} \times 100 \quad (1)$$

where MC (moisture content) in percentage (%); FS (fresh sample) in grams (g); and DS (dry sample) in grams (g).

2.3. Mechanical Strength of Grapevine Wood Samples

The effects of *X. arvicola* larvae on the mechanical properties of the grapevine wood in trunks and branches of *V. vinifera* were evaluated using two standard experiments to determine the strength of the wood: compression and flexural tests for trunks and branches, respectively.

All trunks and branches, unaffected or affected, fresh or dry, were tested with a hydraulic press (EIC—Engineering, Instrumentation and Control) that works with a maximum load of 2000 kN. This device applies loads by using a pump that generates oleohydraulic pressure. Data collection (total applied load), recorded with a data logger, and processing was carried out with EIC software (UTMNet version). All the tests were performed by a constant load speed up to failure (200 N/s). Grapevine wood trunks and branches that twisted or slipped away during the test were discarded for the data.

2.3.1. Experiment 1: Compressive Strength (CS) of Grapevine Wood Trunks in Relation to the Total Damaged Surface Area (TDSA) of Samples

Before the test was performed and according to the European Standard (EN 14251; Structural round wood. Test methods. Madrid, Spain, 2003), trunk samples should have 10 times the length of their diameter. The following dimensions were measured in each of the trunk samples (fresh and dry): minimum diameter (3 measurements of different diameters obtained by connecting their nearest points), maximum diameter (3 measurements of different diameters obtained by joining their farthest points), and total length of the trunk. Both the External Surface Area (ESA) (mm²) and the number of adult exit holes and galleries (on the sides and both ends of the samples) were registered for the affected wood trunk samples. Groups based on the percentage of damaged external total surface were created (Total Damaged Surface Area, TDSA) (Figure 1A). Numeric values in orange and green represent the TDSA (%) of wood samples (Class 1: <0.40%, Class 2: ≥0.40 ≤ 0.50%, and Class 3: >0.50%).

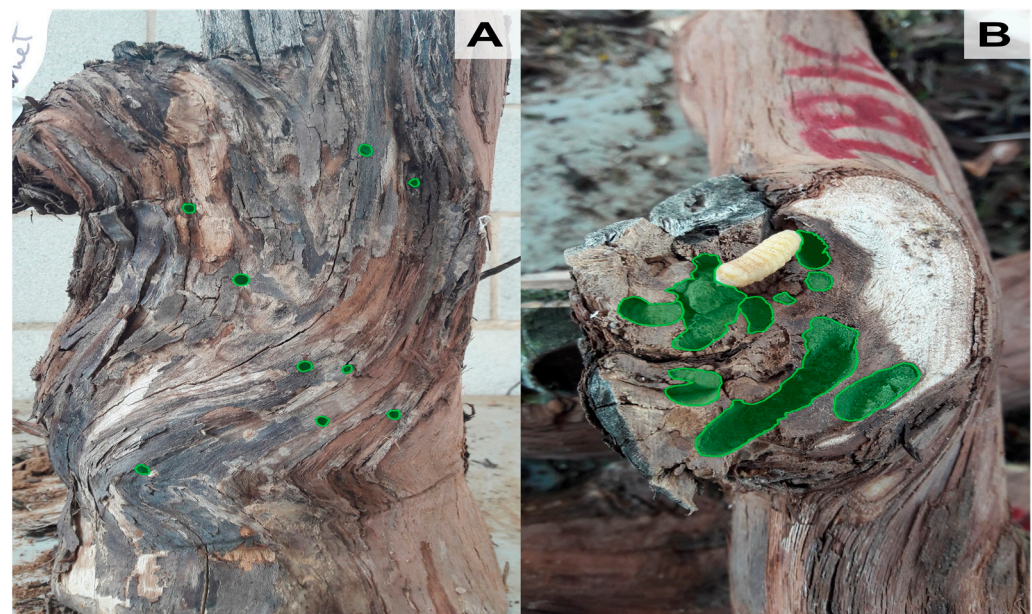


Figure 1. Effects of larval infestation. (A) Adult exit holes on a *Vitis vinifera* wood trunk. (B) Larval galleries with accumulation of sawdust and waste material on a *Vitis vinifera* wood branch. Where green color is shaded, the surface damaged by the insect in the wood samples appears.

The trunks were placed vertically to mimic the compression strength suffered by the plant trunks in field conditions, with both end surfaces cut perpendicularly to the longitudinal axis of the sample. The compressive test was performed according to the European Standard (EN 14251; Structural round wood. Test methods; Madrid, Spain, 2003), and the following Equations (2)–(6) were considered for the analysis [32]:

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

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

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

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

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

where λ : slenderness ratio (dimensionless); lk : buckling length or length of the sample (mm); i : radius of gyration (mm); I : area moment of inertia (mm⁴); A : cross-sectional area (mm²); r : radius (mm); σ : compression normal stress or compressive strength (MPa = N/mm²); and N : normal force (N).

2.3.2. Experiment 2: Flexural Strength (FS) of Grapevine Wood Branches in Relation to the TDSA of Samples

Before the mechanical strength was performed and according to the European Standard (EN 14251; Structural round wood. Test methods; Madrid, Spain, 2003), branch samples should have 18 times the length of their diameter. The following dimensions were measured in each of the branch samples (fresh and dry): minimum diameter (3 measurements of different diameters obtained by connecting their nearest points), maximum diameter (3 measurements of different diameters obtained by joining their farthest points), diameter at the point of the branch where the load was applied (one measurement in the direction of the load and another one in the perpendicular direction to the applied load), and total length of the branch.

Both the ESA (mm²) and the number of adult exit holes and galleries (on the side and both ends of the samples) were registered for the affected wood branches. Groups based on the percentage of damaged external total surface were created (Total Damaged Surface Area, TDSA) (Figure 1B). Numeric values in orange and green represent the TDSA (%) of wood samples (Class 1: <0.40%, Class 2: $\geq 0.40 \leq 0.50\%$, and Class 3: >0.50%).

The branches, placed horizontally, rested in two roller supports 30 cm apart (18 times the branch nominal diameter). Two concentrated loads were applied from the top side of the wood sample to mimic the downward bending suffered by the plant branches in field conditions. The flexural test was performed according to the European Standard EN 14251:2003 and the following Equations (7)–(10) were considered for the analysis [32]:

$$i = \sqrt{\frac{I}{A}} \quad (7)$$

$$I = \frac{\pi r^4}{4} \quad (8)$$

$$A = \pi r^2 \quad (9)$$

$$\sigma = \frac{M_Z}{W_Z} = \left(\frac{M_Z}{I} \right) r \quad (10)$$

where i : radius of gyration (mm); I : area moment of inertia (mm⁴); A : cross-sectional area (mm²); r : radius (mm); σ : normal stress from bending or flexural strength (MPa = N/mm²); M_Z : bending moment (N·mm); and W_Z : section modulus (mm³).

2.4. Experiment 3: Deflection at Break of Grapevine Wood Samples

Figure 2A,B show the deflection at break ('d' in mm) after both compressive and flexural tests were measured in grapevine wood samples. This value defines the maximum distance in which the sample has deflected from the original position. In order to make sample measurements easier, before and after the tests, the wood samples were placed on a grid of cells, 1 cm high × 1 cm long.

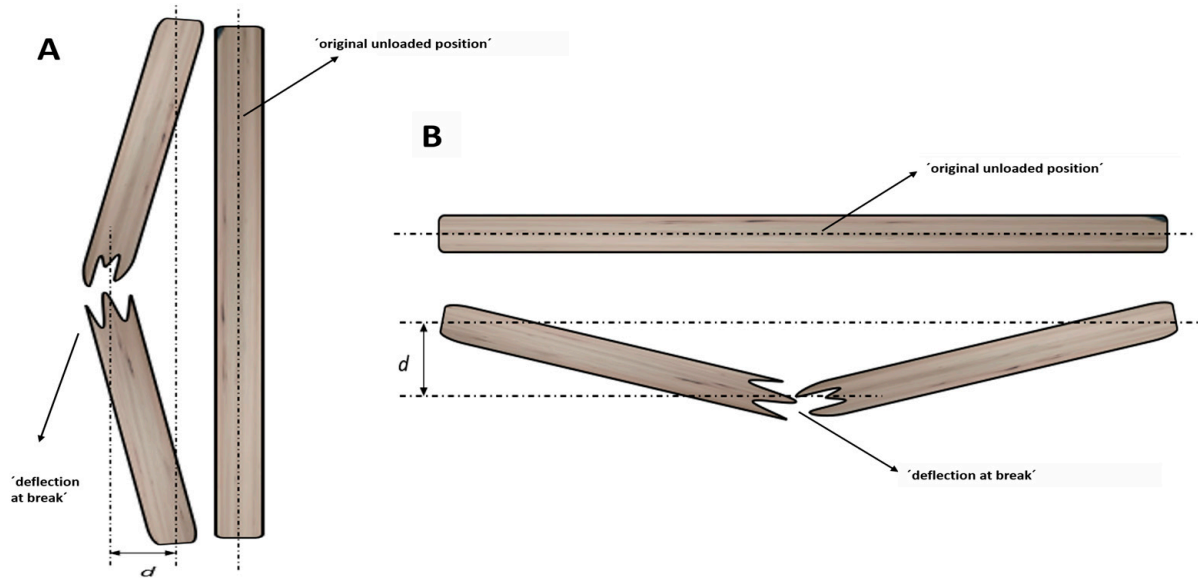


Figure 2. (A) Load diagram for compressive test in grapevine wood trunks; (B) load diagram for flexural test in grapevine wood branches. ‘Straight dashed line’ represents original unloaded position. ‘d’ represents deflection at break from the original unloaded position of wood samples.

2.5. Experiment 4: Grapevine Wood Samples with Fungal Growth

The fungal growth on the surface of each grapevine wood sample (trunks and branches) was registered. The symptomatology (wood diseases) of each sample was identified visually (dark, necrotic, or soft wood) according to the pathology described in grapevine wood by *D. seriata* (De Not), *E. lata* (Tul and Tul), *P. minimum* (Gams, Crous, Wingf., Mugnai), *P. chlamydospora* (Crous and Gams), and *F. mediterranea* (Fisch) [24]. If vine plants presented symptomatology as necrotic tissues (usually cankers), dry or soft wood with a silver appearance and a coloration that could develop a white to yellow soft rot, those plants were marked as affected [33]. And samples without symptoms were identified as unaffected. For affected samples, analyses were performed to analyze the main diseases in wood vines. A classic microbiological analysis consisted of taking small chips of affected wood and putting them on media growth (Potato Dextrose Agar medium) and incubating them in controlled conditions (temperature 24 ± 1 °C; humidity $60 \pm 5\%$; and photoperiod of 16 h of light, luminous intensity of 1000 lux, and 8 h of darkness) for 7–10 days in order to isolate and identify main species associated to fungal grapevine diseases.

2.6. Statistical Analysis

Analysis of covariance (ANCOVA) was used to examine the effect of the TDSA of grapevine wood samples (fixed factor) on compressive strength (CS) or flexural strength (FS) as a covariate. The linear regression coefficients of the interaction $CS \times TDSA$ and $FS \times TDSA$ were tested using an F-test. Deflection at break of wood samples (unaffected and affected) was evaluated using analysis of variance (ANOVA) followed by a Fisher’s LSD test (significance at $p \leq 0.05$). The percentage of wood samples (unaffected and affected) with fungal growth in the wood was evaluated using analysis of variance (ANOVA) followed by a Fisher’s LSD test (significance at $p \leq 0.05$). Analyses were conducted using SPSS software, version 24 software (IBM SPSS Statistics, 1968, Armonk, NY, USA).

3. Results

3.1. Experiment 1: Mechanical Strength of Grapevine Wood Trunks (CS in Relation to TDSA)

The moisture content of fresh trunks from the ‘Tempranillo’ grapevine variety was 60.83%.

The four fresh wood samples with damages were classified into three groups of TDSA based on the percentage of TDSA area. One sample was classified as Class 1 (0.31%), another one was classified as Class 2 (0.41%), and two samples were classified as Class 3 (0.51 and 0.55%) (Table 1).

Table 1. TDSA in relation to the External Surface Area (ESA) of wood grapevine trunks. Each group was arranged according to the external percentage of damage: Class 1: <0.40%, Class 2: $\geq 0.40 \leq 0.50\%$, Class 3: >0.50% of tissue damage.

	Grapevine Trunks	ESA of Sample (mm ²)	ESA of Damages (mm ²)		TDSA (Holes + Galleries) (mm ²)	TDSA (%)	Class	
			Holes (n) *	Galleries (v) **				
Fresh samples	1	99,665.75	-	-	-	-	-	
	2	171,378.63	-	-	-	-	-	
	3	100,192.53	-	-	-	-	-	
	4	104,709.14	-	-	-	-	-	
	5	101,257.14	-	-	-	-	-	
	6	98,208.14	-	-	-	-	-	
	7	99,592.39	-	-	-	-	-	
	1	114,633.97	17.60 (1)	570.25 (2)	587.85	0.51	3	
	2	94,995.80	19.79 (1)	272.80 (2)	292.59	0.31	1	
	3	135,591.07	- (0)	558.84 (14)	558.84	0.41	2	
	4	169,054.48	144.72 (14)	793.45 (9)	938.17	0.55	3	
Dry samples	1	92,907.60	-	-	-	-	-	
	2	70,285.55	-	-	-	-	-	
	3	40,898.55	-	-	-	-	-	
	4	38,466.37	-	-	-	-	-	
	5	32,664.39	-	-	-	-	-	
	6	50,357.97	-	-	-	-	-	
	7	88,423.09	-	-	-	-	-	
	8	64,874.71	-	-	-	-	-	
	9	67,044.42	-	-	-	-	-	
	10	145,707.45	-	-	-	-	-	
	11	159,414.31	-	-	-	-	-	
	12	145,493.83	-	-	-	-	-	
		1	98,835.75	29.19 (2)	439.49 (4)	468.68	0.47	2
		2	37,335.64	34.73 (2)	167.97 (5)	204.70	0.54	3
		3	68,825.39	61.12 (4)	168.70 (4)	229.82	0.33	1
		4	77,075.59	25.53 (2)	428.37 (3)	453.90	0.58	3
		5	120,266.59	59.02 (3)	820.20 (6)	879.22	0.73	3
		6	28,099.99	- (0)	117.53 (3)	117.53	0.41	2
		7	97,994.55	57.68 (3)	594.51 (4)	652.19	0.66	3
	8	61,750.37	78.31 (3)	315.31 (2)	393.62	0.63	3	
	9	206,440.81	71.88 (4)	1131.92 (2)	1203.80	0.58	3	
	10	160,910.17	34.11 (2)	814.95 (1)	849.06	0.53	3	
	11	136,689.25	- (0)	716.92 (4)	716.92	0.52	3	
	12	122,840.49	- (0)	713.05 (2)	713.05	0.58	3	

* n: number of exit holes; ** v: number of galleries.

The twelve dry wood samples affected by *X. arvicola* were classified into three groups of TDSA based on the percentage of TDSA. One sample was classified as Class 1 (0.33%), two samples were classified as Class 2 (0.41 and 0.47%), and nine samples were classified as Class 3 (from 0.52 to 0.73%) (Table 1).

Concerning affected trunk wood, the relationship between CS and TDSA did not exhibit significant differences between fresh and dry wood trunks. However, the linear regression coefficients of the CS \times TDSA interaction varied significantly depending on the moisture content condition ($F = 51.292$; d.f. = 1.16; $p \leq 0.001$) between fresh and dry wood trunks. Regardless of the moisture content, the CS achieved by affected wood trunks decreased with the increase in the TDSA in both fresh and dry wood trunk samples, decreasing faster in dry wood trunks (Figure 3).

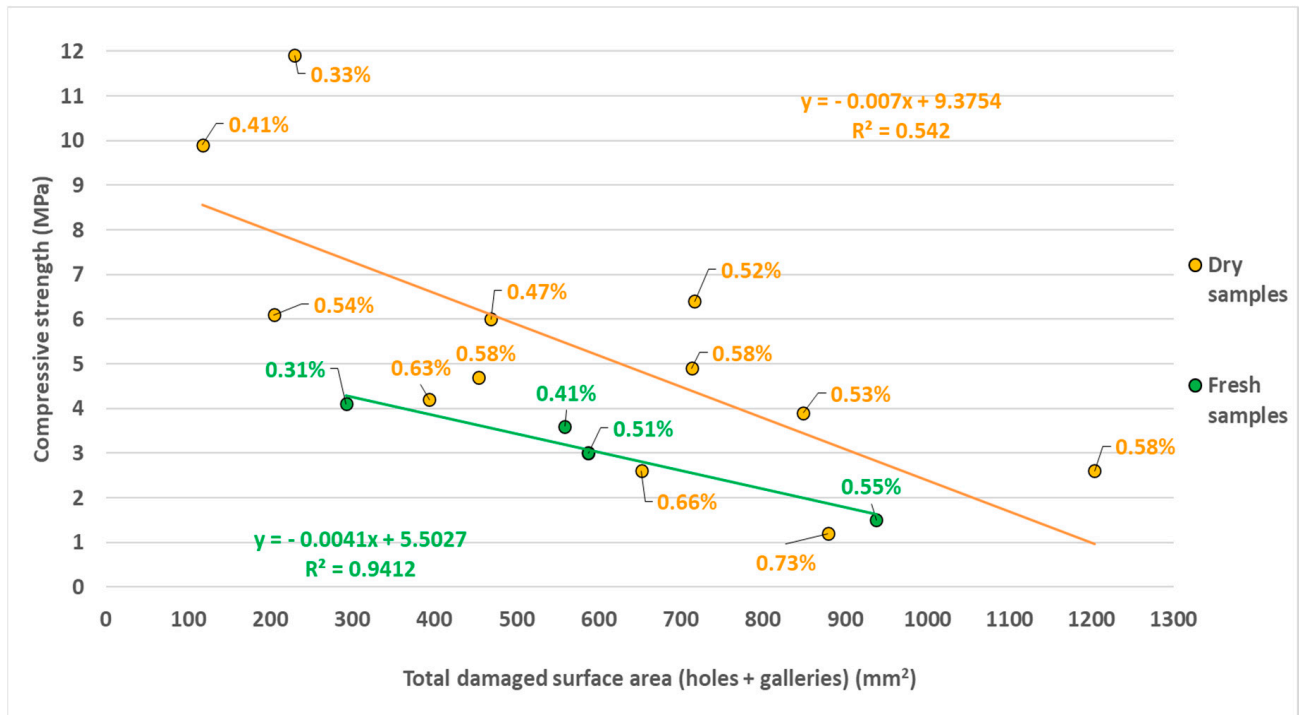


Figure 3. Linear regression of compressive strength (MPa, y -axis) in relation to TDSA (mm^2 , x -axis) in grapevine wood branches. ‘Green points’ represent values for fresh trunk samples damaged by *X. arvicola* larvae; ‘orange points’ represent values for dry trunk samples damaged by *X. arvicola* larvae. ‘Green line’ is the trendline for ‘green points’; ‘orange line’ is the trendline for ‘orange points’.

3.2. Experiment 2: Mechanical Strength of Grapevine Wood Branches (FS in Relation to TDSA)

The moisture content of fresh branches from the ‘Tempranillo’ grapevine variety turned out to be 61.77%.

The eight fresh wood samples affected by *X. arvicola* were classified into two groups of TDSA based on the percentage of TDSA. Five samples were classified as Class 1 (from 0.12% to 0.39%), and three samples were classified as Class 3 (from 0.53 to 0.61%) (Table 2).

The ten dry wood samples affected by *X. arvicola* were classified into three groups of TDSA based on the percentage of TDSA. Five samples were classified as Class 1 (from 0.04 to 0.35%), two samples were classified as Class 2 (0.42 and 0.45%), and three samples were classified as Class 3 (from 0.53 to 0.76%) (Table 2).

Regarding affected branch wood, FS as a function of the TDSA was significantly different ($F = 6.191$; d.f. = 1.16; $p = 0.025$) between fresh and dry wood branches. However, the linear regression coefficients of the FS \times TDSA interaction with regard to moisture content condition were significantly different ($F = 221.893$; d.f. = 1.16; $p \leq 0.001$) between fresh and dry wood branches. Regardless of the moisture content, the FS achieved by affected wood branches decreased with the increase in the TDSA in both fresh and dry wood samples, decreasing faster in dry wood branches (Figure 4).

Table 2. TDSA in relation to the External Surface Area (ESA) of wood grapevine branches. Each group was arranged according to the external percentage of damage: Class 1: <0.40%, Class 2: $\geq 0.40 \leq 0.50\%$, Class 3: >0.50% of tissue damage.

	Grapevine Branches	ESA of Sample (mm ²)	ESA of Damages (mm ²)		TDSA (Holes + Galleries) (mm ²)	TDSA (%)	Class	
			Holes (n) *	Galleries (v) **				
Fresh samples	1	66,380.61	-	-	-	-	-	
	2	59,697.05	-	-	-	-	-	
	3	77,196.38	-	-	-	-	-	
	4	46,292.06	-	-	-	-	-	
	5	41,982.93	-	-	-	-	-	
	6	54,320.83	-	-	-	-	-	
	7	53,935.75	-	-	-	-	-	
	8	73,918.05	-	-	-	-	-	
	9	37,208.91	-	-	-	-	-	
	10	45,797.81	-	-	-	-	-	
	11	66,177.12	-	-	-	-	-	
	12	45,024.32	-	-	-	-	-	
		1	34,765.54	36.17 (2)	94.48 (2)	130.65	0.37	1
		2	43,831.52	53.67 (3)	118.41 (3)	172.08	0.39	1
		3	64,609.07	56.34 (3)	98.61 (1)	154.95	0.23	1
		4	76,528.70	53.42 (3)	108.60 (1)	162.02	0.21	1
	5	56,372.96	31.82 (2)	310.79 (4)	342.61	0.61	3	
	6	33,846.14	42.01 (3)	- (0)	42.01	0.12	1	
	7	46,355.11	70.20 (5)	178.93 (3)	249.13	0.53	3	
	8	56,433.62	43.65 (4)	274.46 (3)	318.11	0.56	3	
Dry samples	1	46,231.24	-	-	-	-	-	
	2	64,228.78	-	-	-	-	-	
	3	67,981.04	-	-	-	-	-	
	4	67,059.61	-	-	-	-	-	
	5	68,540.18	-	-	-	-	-	
	6	54,579.34	-	-	-	-	-	
	7	53,807.26	-	-	-	-	-	
	8	46,149.63	-	-	-	-	-	
	9	83,990.55	-	-	-	-	-	
	10	72,089.69	-	-	-	-	-	
	11	72,451.35	-	-	-	-	-	
	12	84,694.19	-	-	-	-	-	
		1	59,525.32	19.01 (1)	254.40 (2)	273.41	0.45	2
		2	38,104.89	33.70 (2)	259.06 (5)	292.76	0.76	3
		3	57,272.18	- (0)	325.56 (2)	225.56	0.56	3
		4	51,040.10	- (0)	181.19 (2)	181.19	0.35	1
	5	62,042.15	32.20 (2)	- (0)	32.20	0.05	1	
	6	63,107.76	18.70 (1)	155.02 (1)	173.72	0.27	1	
	7	74,550.08	19.24 (1)	379.51 (2)	398.75	0.53	3	
	8	70,947.80	18.62 (1)	279.71 (4)	298.33	0.42	2	
	9	44,752.36	16.54 (1)	- (0)	16.54	0.04	1	
	10	45,376.31	- (0)	51.59 (1)	51.95	0.11	1	

* n: number of exit holes; ** v: number of galleries.

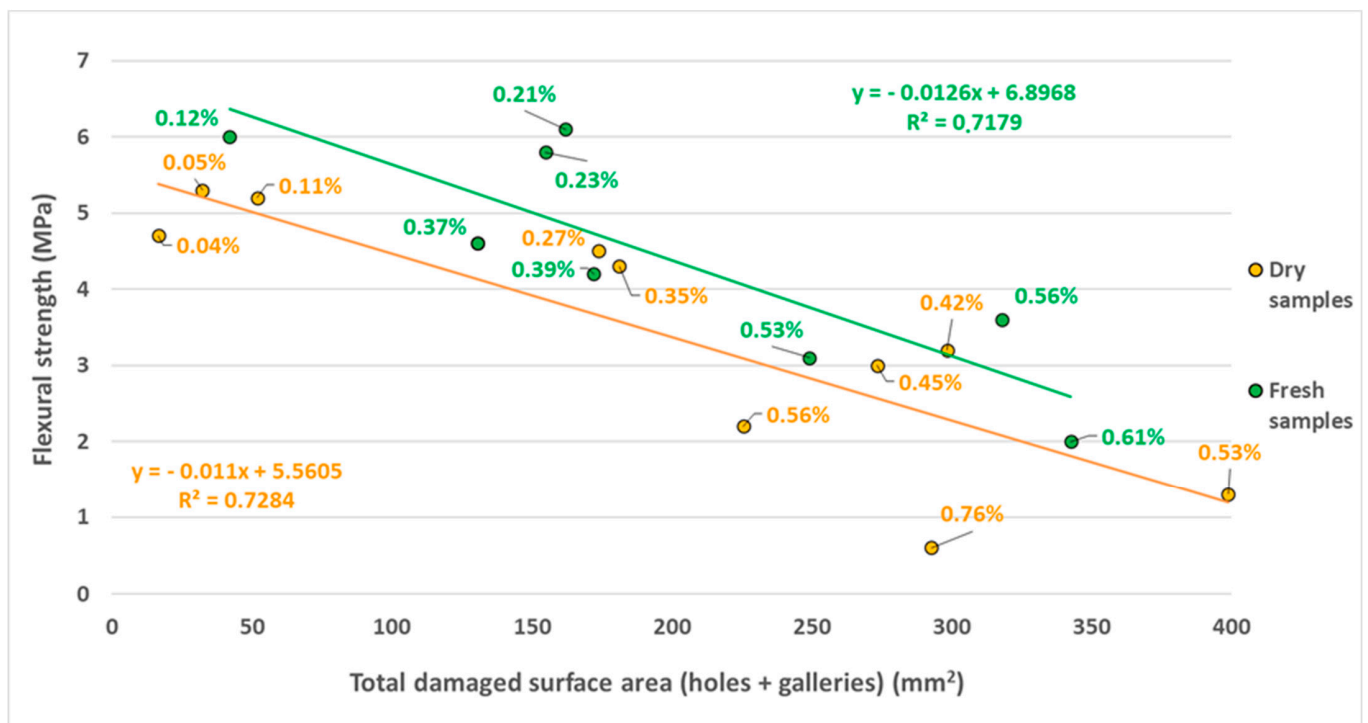


Figure 4. Linear regression of flexural strength (MPa, *y*-axis) in relation to TDSA (mm², *x*-axis) in grapevine wood trunks. ‘Green points’ represent values for fresh trunk samples damaged by *X. arvicola* larvae; ‘orange points’ represent values for dry trunk samples damaged by *X. arvicola* larvae. ‘Green line’ is the trendline for ‘green points’; ‘orange line’ is the trendline for ‘orange points’.

3.3. Experiment 3: Deflection at Break of Grapevine Wood Samples

In relation to trunks under CS, unaffected fresh samples showed a lower deflection at break (97.85 ± 7.47 mm), a value significantly higher than that in unaffected dry samples (65.00 ± 8.18 mm) (Table 3).

Table 3. Deflection at break of grapevine under mechanical strength tests (compressive strength on trunks and flexural strength on branches).

	Compressive Strength			Flexural Strength		
	Unaffected Wood (n) (s)	Affected Wood (n) (s)		Unaffected Wood (n) (s)	Affected Wood (n) (s)	
Fresh samples	97.85 ± 7.47 aA (7 samples) (7.3 MPa)	83.75 ± 21.92 aA (4 samples) (3.0 Mpa)	F = 0.562 df = 1.9 p = 0.473	Fresh samples (12 samples) (72.33 MPa)	164.37 ± 48.71 aA (8 samples) (76.52 MPa)	F = 4.859 df = 1.18 p = 0.041
Dry samples	65.00 ± 8.18 aB (12 samples) (9.8 MPa)	86.67 ± 7.16 aA (12 samples) (5.4 MPa)	F = 3.969 df = 1.22 p = 0.059	Dry samples (12 samples) (43.65 MPa)	37.50 ± 8.37 bB (10 samples) (30.45 MPa)	F = 16.835 df = 1.20 p ≤ 0.001
F=	7.249	0.029		F=	0.177	8.221
df=	1.17	1.14		df=	1.22	1.16
p=	0.015	0.868		p=	0.678	0.011

n = number of samples; s = strength (MPa); different lowercase letters mean significant differences between unaffected and affected grapevine wood within the same wood moisture content (fresh or dry), part of the grapevine (trunk or branch), and mechanical test (compression or flexural) (Fisher’s LSD test, *p* ≤ 0.05). Different capital letters mean significant differences between fresh and dry wood within the same damage condition (unaffected or affected by *X. arvicola* larvae), part of the grapevine (trunk or branch), and mechanical test (compression or flexural) (Fisher’s LSD test, *p* ≤ 0.05).

With regard to branches under FS, unaffected fresh samples showed the lowest deflection at break (72.58 ± 11.31 mm), a value significantly lower than that for affected fresh samples (164.37 ± 48.71 mm), whereas unaffected dry samples showed a significantly higher deflection at break (77.92 ± 5.69 mm) than affected dry samples (37.50 ± 8.37 mm). Regarding the moisture content of the grapevine, fresh affected wood samples showed the greatest deflection at break (164.37 ± 48.71 mm), significantly greater than the respective ones in dry samples (37.50 ± 8.37 mm) (Table 3).

3.4. Experiment 4: Deflection at Break of Grapevine Wood Samples

In relation to the fungal growth on the surface of the grapevine wood trunks, affected dry samples showed the greatest number of fungal diseases ($66.66 \pm 49.23\%$), a value significantly higher ($F = 7.615$; d.f. = 1.22; $p = 0.011$) than that for unaffected dry samples ($16.66 \pm 16.66\%$).

With regard to the fungal growth on the surface of the grapevine wood branches, the percentage of samples that showed fungal growth was higher for both affected fresh and dry samples ($75.00 \pm 36.29\%$ and $60.00 \pm 31.63\%$, respectively), significantly higher ($F = 15.890$; d.f. = 1.18; $p \leq 0.001$ in fresh samples; $F = 8.780$; d.f. = 1.20; $p = 0.008$, in dry samples) than that for the respective unaffected fresh ($8.33 \pm 8.33\%$) and dry ($8.33 \pm 8.33\%$) samples (Figure 5).

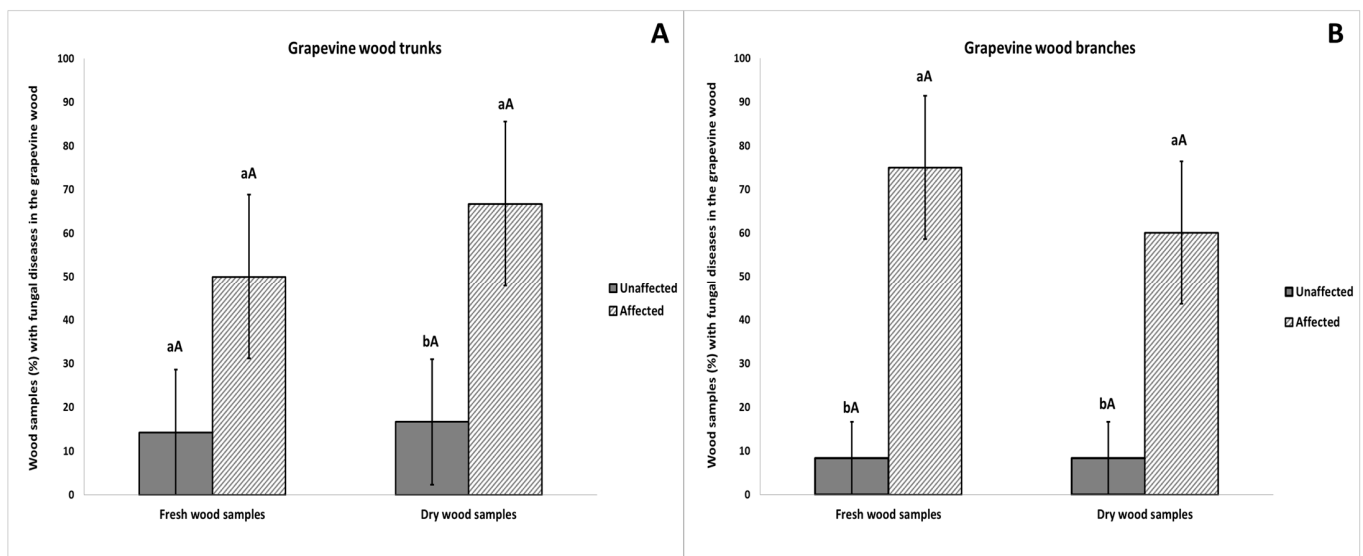


Figure 5. Percentage of wood samples showing fungal growth in grapevine wood after mechanical tests: (A) trunks; (B) branches. Different lowercase letters mean significant differences between unaffected and affected grapevine wood within the same wood moisture content (fresh or dry) and part of the grapevine wood (trunk or branch). Different capital letters mean significant differences between fresh and dry wood within the same damage condition (unaffected or affected by *X. arvicola* larvae) and part of the grapevine (trunk or branch).

4. Discussion

X. arvicola larvae negatively affected the strength of *V. vinifera* wood. Both strengths, CS and FS, decreased with the increase of TDSA, which varied from 0.31% to 0.73% in trunks and from 0.04 to 0.76% in branches, irrespective of the moisture content of the wood sample (fresh or dry). The loss of resistance was more evident in affected dry wood trunks and branches. Significant differences between fresh and dry grapevine wood samples were also verified through the linear regression coefficients of the CS \times TDSA and FS \times TDSA interactions. Furthermore, it should also be noted that the affected dry wood samples reached very different resistance values in samples classified as Class 2 or Class 3 (the dry and large sections of wood from these samples had great resistance); hence, the adjustment

of the regression was only around 54.20%, compared to the 94.12% that was obtained in the fresh wood samples of trunks.

The higher the percentage of external damages (holes and galleries), the lower the resistance of the wood. It has to be stated that the aforementioned external damage (holes and galleries) represents only the visible part of the damage caused by the larvae, but apart from that there is also internal damage (that could only be assessed with 'X rays') caused during the long larval cycles of *X. arvicola* inside the samples.

A similar pattern of hoist damage is observed in the case of the long life cycle of the larva of *Torneutes pallidipennis* Reich (1837) (Coleoptera: Cerambycidae) on *Prosopis flexuosa* (Fabales: Fabaceae), resulting in significant harm to the tree. Adult females have a tendency to lay eggs on previously infested trees, thereby increasing reinfestation rates [34]. Numerous studies [35,36] suggest that larvae residing in the deep wood with low nutritional value and minimal predation pressure have a prolonged development period, up to several years. Given that multiple larvae of *T. pallidipennis* can inhabit a single tree, successive infestations intensify the pressure on the tree, leading to its weakening. Hanks [37] noted that plant resistance is partly attributed to high bark or sapwood moisture, and most cerambycid species require host plants to be weakened in some manner [37], as evidenced by comparisons of sapwood thickness between 'healthy' and 'infested' branches. The consecutive subcortical galleries created by larvae directly impact the active tissues of the sapwood and cambium, responsible for water and nutrient transport and growth-ring formation. The resultant stress from these losses diminishes plant vigor, facilitating reinfestations and leading to reduced growth in heavily infested trees [38].

In trunk samples, unaffected dry wood had a lower deflection at break than unaffected fresh wood (65.00 and 97.85 mm, respectively). This shows that unaffected wood fibers with a high moisture content (fresh) are more flexible and less resistant than the dry samples. The deflection at break in affected and fresh branch samples was significantly greater (164.37 mm) than unaffected fresh (72.58 mm) and affected dry samples (37.50 mm). This demonstrates that fibers of affected wood branches with a high moisture content are more flexible and less resistant than the unaffected fresh or affected dry samples.

Throughout the growth period, grapevine wood (and especially its branches) undergoes rigorous pruning to establish specific training systems (unilateral, bilateral, . . .). This pruning practice induces non-uniform growth of wood fibers in branches, leading to the development of knots and cracks that directly impact its strength and resistance. Consequently, this phenomenon elucidates the greater deflection observed in affected fresh samples, as older wood in branches has undergone more pruning cuts compared to the trunk, which has not been subjected to any pruning at all. Studies by Rodríguez-González et al. [30] have indicated that vine wood affected by these larvae exhibits lower resistance and higher breaking speeds compared to unaffected wood, irrespective of whether the samples are fresh or dry. Additionally, Rodríguez-González et al. [29] demonstrated that affected wood of the 'Cabernet-Sauvignon' variety could experience a reduction in structural capacity of up to 62% when subjected to typical crop loads associated with this variety. Both the weight of grapes and the vibration generated by harvesting machines in *V. vinifera* may influence the wood's resistance and structural capacity in grapevines affected by larvae. This phenomenon has also been observed in other woody species, such as *Prunus pisardi* Carrière, Koehne (Rosales: Rosaceae), where *X. arvicola* larvae attacks can result in branch death or breakage over several years or weaken the affected *P. pisardi* trees [10].

Ingestion of vascular tissues by cerambycid insect larvae affects the physical properties of woody species. It was also demonstrated in this study how *X. arvicola* larvae, likewise the larvae of other cerambycids, modify the mechanical properties of grapevine plants, fresh wood branches being the most affected part of the plant. The effects of a continuous infestation of the grapevines by these larvae result in deeper changes in the plants, such as leaf development becoming scarce and the shoots not being very vigorous or productive [39]; clusters being smaller; and flowers being less numerous, diminishing their length and coming off more easily [25]. Soltis et al. [40] described that branches'

breakage due to physical factors can reduce plant fitness because of biomass and meristem loss, when it comes to biomechanic effects on *Tsuga canadensis* Carrière (Pinales: Pinaceae) produced by the hemlock woolly adelgid *Adelges tsugae* Annand (Homoptera: Adelgidae) [41,42], *Monochamus galloprovincialis* (Coleoptera: Cerambycidae), and *Acanthocinus aedilis* (Coleoptera: Cerambycidae) on *Pinus sylvestris* [43] or the cerambycid *T. pallidipennis* on *P. flexuosa* (Fabales: Fabaceae) [38]. Spatz and Bruechert [44] described mechanical instability when woody species have suffered damages from strong gusts of wind for several years, which lead to their fracture. Another cerambycid insect pests is the red oak borer, *Enaphalodes rufulus* (Haldeman), which is an important pest of living oaks [45,46].

It was described in the introduction that damages by *X. arvicola* in grapevine wood can be direct, meaning a reduction of vascular tissues of the plant, which are ingested by larvae, or indirect, due to the propagation of wood diseases in affected wood that killed the vascular tissues of the wood. In our study, wood samples affected by *X. arvicola* larvae had a higher percentage of symptoms of fungal growth, this percentage being particularly high in dry wood trunks (66.66%), fresh wood branches (75.00%), and dry wood branches (60.00%), in comparison to the respective unaffected samples. All this confirms that *X. arvicola* larvae damage to grapevine wood favors the propagation of grapevine diseases (described in the Introduction and Materials and Methods sections) through the emergence holes originated by *X. arvicola* adults on their way out of the wood and through the larval galleries that are connected to them, leading to the death of plant vascular tissue [15,21]. Ocete et al. [15] cited that the fungal attack is more severe in 'Tempranillo' and 'Cabernet-Sauvignon' varieties than in other varieties cultivated in the main wine-growing regions of the Iberian Peninsula.

The fungal symbionts associated with cerambycid beetles are endosymbiotic fungi, playing a vital role as producers of enzymes for the degradation of organic matter, particularly wood [47–49]. The impact of wood pathogens or diseases on the biomechanical properties of woody species is well documented across various genera, including *Pinus* spp. [43,50,51], *Pseudotsuga* spp. [52], and *Larix* spp. [53]. Species affected by wood diseases tend to accumulate a higher volume of dead wood, resulting in increased fragility and eventual progressive deterioration of the affected areas [54]. Other authors [55,56] found that an accumulation of dead wood caused by the attack of fungal growth on different parts of the host (trunks or branches) predisposes affected species to damage or breakage when exposed to external agents, including wind, or as in our particular case, the static loads that the weight of the grapes apply to the grapevine wood during several weeks a year at the time of harvesting.

5. Conclusions

This study demonstrates the heightened susceptibility of grapevine wood samples (trunks and branches) to fragility when affected by *X. arvicola* larvae.

- Both CS and FS declined as the TDSA of the wood samples increased, which varied from 0.31% to 0.73% in trunks and from 0.04 to 0.76% in branches, irrespective of the moisture content (fresh or dry). Significant differences between affected grapevine wood samples (fresh and dry) were also confirmed by examining the linear regression coefficients of the interactions CS × TDSA and FS × TDSA.
- Regarding unaffected trunk wood, deflection at break for dry wood samples was lower than that for fresh wood samples (65.00 and 97.85 mm, respectively). With regard to branch wood, deflection at break for affected fresh samples (164.37 mm) was significantly greater than that for both unaffected fresh samples (72.58 mm) and for affected dry samples (37.50 mm).
- Considering the external damages present in wood samples (holes and galleries), a higher percentage of TDSA meant a lower wood resistance.
- Larvae damages on grapevine wood facilitated the spread of grapevine diseases via the emergence of holes created by adult *X. arvicola* as they exit the wood, as well as through the larval galleries that are connected to them. The percentage of symptoms

of fungal growth for affected wood samples were 66.66% for dry wood trunks, 75.00% for fresh wood branches, and 60.00% for dry wood branches.

In this study, it has been shown that *X. arvicola* larvae modified the mechanical properties of grapevine plants in this variety, fresh branches being the most affected wood. To further advance the knowledge of this pest on its host, more studies should be carried out with other grape varieties and vineyard training systems.

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Data Availability Statement: The data supporting the results of this study are included in the present article.

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