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ORIGINAL RESEARCH ARTICLE

Xylem water transport is influenced by age and winter pruning characteristics in grapevine (*Vitis vinifera*)

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ABSTRACT

In order to investigate the effect of age and pruning characteristics on grapevine hydraulic conduction, a study was carried out between 2017 and 2020. Two pruning regimes (respecting or not sap flow pathways) and two vine age levels (older and younger vine plants) were considered and compared on two different vineyard plots located in French north-east Jura region and in south-west Bordeaux one. The assessment of pruning characteristics in relation to sap flow pathway was based on a visual characterization of the external wood aspect of the trunk and arms and consisted of a set of criteria involving the number, size and position of pruning wounds.

Sap flow measurements of entire vine plants were carried out using the Xyl'em® tool, as well as an assessment of the necrotized, living and conductive xylem area in the trunks and arms after Phloxine staining. The biomass of the vegetation was also assessed.

Results showed that vines pruned without considering the sap flow pathways had a 40-to-50 % less conductive sap flow than vines pruned taking into account the sap pathway. No difference was observed with vine age within each pruning regime. However, for the vineyard plot where the amount of conductive xylem area was assessed, older vines that were not pruned to respect the sap pathways showed a smaller area of living wood as well as conductive wood than the older ones pruned to respect the sap flows. The amount of living and conductive areas of these vines was equivalent to that of younger vines pruned to respect sap pathways. These older vines also showed less vegetative biomass.

These results show that pruning without taking into account the sap pathways has a negative impact on the conduction of xylem sap pathways in grapevines, both in terms of hydraulic efficiency and quantity of living and conducting tissues. However, the possible consequences of these reductions on grapevine physiological functions still need to be further investigated.

KEYWORDS: grapevine, pruning, sap flow, conductivity, xylem, decline

INTRODUCTION

Grapevine is a liana which is pruned each year in winter in most cultivated areas, at least in France. Winter pruning is aimed at controlling the vegetative development and the size of plants as well as regulating crop load thanks to the number of buds per vine left after pruning.

Apart from these cultural concerns, a pruning method referred to as "respectful of sap flows pathways" is dedicated to the principles of sap conduction. It proposes additional criteria to preserve the sap flow pathways in grapevines. A sap flow pathway can be defined as a pathway most likely to be used by xylem sap flow during sap ascent from roots to the different parts of the canopy. The objective of this pruning method is to minimize redirections of sap flow paths from one year to another because of disturbing pruning wounds occurring on these paths and subsequent desiccation cones underneath acting as flow blockers. In practice, these rules consist in gathering most of the degraded tissues resulting from pruning wounds on the same side of the trunk or arms in order to maintain undisturbed sap flow pathways on the opposite side (Lafon, 1921). With 'Guyot' pruning type, the pathway most likely to be used by sap flow is located under the arms.

In addition to potentially hampering sap conduction in the trunks and arms, necrosis deriving from pruning wounds are also unwanted because they are considered the first step in the development of grapevine trunk diseases, as pruning provide dead and desiccated wood as well as entry points for pathogens (Gramaje *et al.,* 2018; Mugnai *et al.,* 1999). For these two reasons, it is assumed that pruning may be a key factor in the process of vine decline. Vine decline can be defined as a recurrent and unplanned loss of vineyards productivity due to both a decrease in crop load and an increase in vine mortality due to a combination of factors of various nature (pathogenic, climatic, cultural practices). The observation of a serious and widespread decline among the different French wine producing areas led to the creation of a National Plan to better understand and contend Vineyard Decline (named "PNDV"). This present work is part of this approach.

Xylem sap conduction is poorly documented in grapevine compared to other physiological functions like growth or photosynthesis for instance. Existing research is generally focused on specific issues such as water uptake and resistance to drought (Schultz and Matthews, 1988; Zufferey, 2013) - in that case mainly concerning vegetative organs (shoots, petioles and leaves) -, soil salinity and mineral uptake (Shani *et al.,* 1993) or deals with vascular pathogens such as bacteria (Chatelet *et al.,* 2006) or fungi like grapevine trunk disease agents (Bortolami *et al.,* 2019; Pouzoulet *et al.,* 2019). Conversely, sap conduction in forest trees is better documented, as are the tools available to study it. The relationship between sap conduction and tree ecophysiology is also better understood. For instance, a strong relation has been assessed between the hydraulic efficiency of a conducting tissue and the photosynthetic capacities of the leaves connected to it (Brodribb *et al.,* 2005). On a larger scale, there is also a relationship between xylem hydraulic efficiency and tree growth rate (Tyree, 2003).

The first principles of pruning method 'respecting the sap flow pathways' were described on grapevine a century ago. There are based on observations made by Eugène Poussard, a winegrower from Charentes region. They are mostly based on empirical knowledge, field observations and "common sense" of their authors but are hardly based on experimental or scientific data. Knowledge on the impacts of pruning wounds exists for woody trees (Eisner *et al.,* 2002) and fruit trees (Grosclaude, 1993) whereas for grapevine, knowledge remains scarce and mostly ancient (Dezeimeris, 1891; Lafon, 1921) although recent results emerged in the last two decades. Indeed, some authors have particularly worked on defining righteous pruning principles and training systems with respect to their impact on trunk diseases and vine decline (Dal *et al.,* 2008; Lecomte *et al.,* 2011; Lecomte *et al.,* 2015; Lecomte *et al.,* 2020; Simonit, 2018). Some experiments have been conducted to identify what characteristics of pruning are best, such as the size and localization of the wounds, and the pruning quality in terms of short or high way to cut, to reduce the internal consequences of pruning on dead and desiccated wood (Bruez *et al.,* 2022; Cholet *et al.,* 2017; Delorme, 2015; Faúndez-López *et al.,* 2021; Lecomte *et al.,* 2020). Finally fundamental research also exist, for example to better understand xylem vascular occlusion mechanisms after pruning or investigate the sectoring of xylem flow pathways (McElrone *et al.,* 2021; Sun *et al.,* 2006; Sun *et al.,* 2008).

The objective of our work was therefore to better understand the physiological basis of this pruning method 'respecting the sap flow pathways' (Lecomte *et al.,* 2020) on grapevine, by comparing, under the same environmental conditions of the vineyard, two contrasting pruning regimes on several indicators of xylem sap conduction. The age of the vine is a determinant of growth, particularly visible on xylem thickness, but a higher age also directly increases the number of winter pruning episodes that impact the number of wounds. For these reasons, age has also been included as a factor in this study.

MATERIALS AND METHODS

1. Techniques and measurements used: wood staining, sap flow measurements, vegetative biomass assessment

1.1. Vegetative biomass assessment

Vegetative biomass was assessed by counting and measuring the diameter of each shoot of the canopy. Measurements were done just before assessing conductance with Xyl'em® apparatus (see chapter 1.3). Shoots were excised at the level of the second internode, and their diameter was measured with a calliper (precision 0.1 mm).

Only on vineyard "B" in Bordeaux, the detached shoots and their bunches were placed to dry in an oven and weighed once dried.

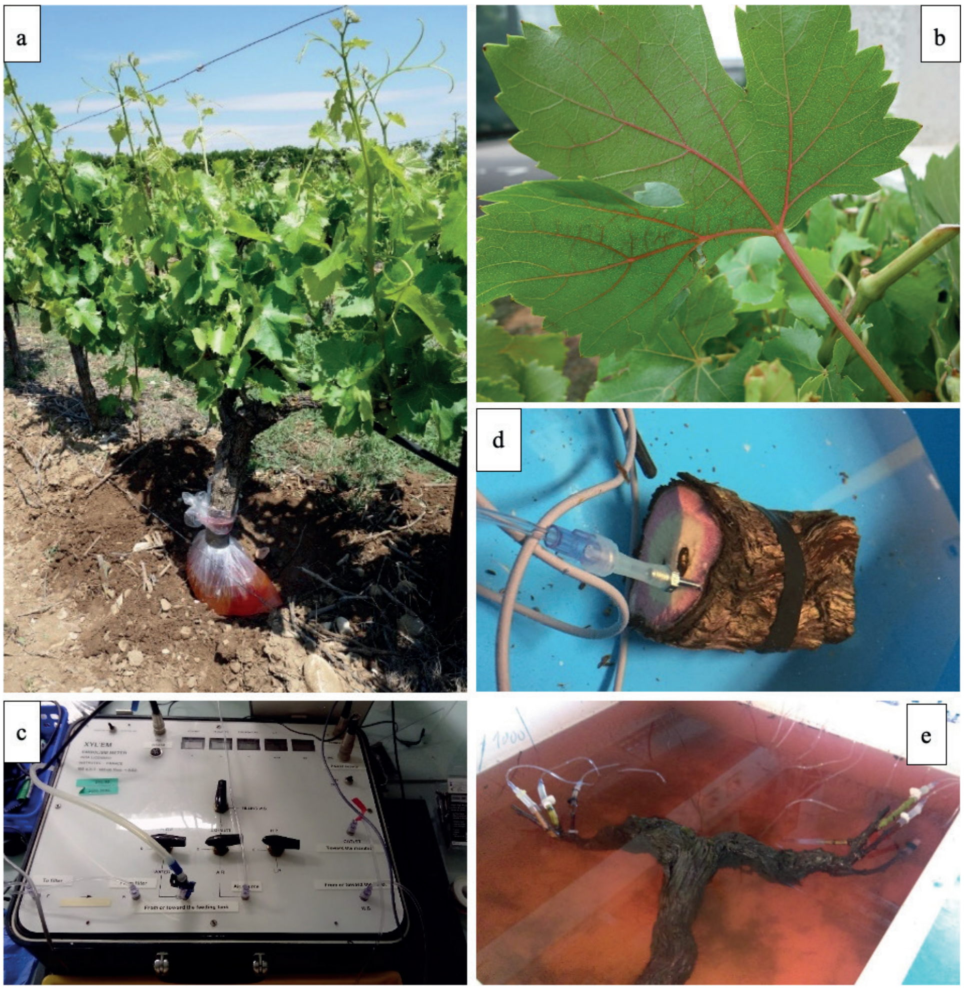


FIGURE 1. Overview of the different tools and techniques used in this study: staining of vine wood in field and conductance measurements using Xyl'em® apparatus.

(a) Phloxine staining of a vine in progress, (b) leaf showing typical Phloxine pink staining, (c) Xyl'em® apparatus main device, (d) piece of vine trunk connected to the Xyl'em® steel canula outlet and (e) whole-vine connected to the Xyl'em® pipe outlets ready for a sap flow measurement.

In this vineyard, the experiment was conducted on 3 week period between the end of June and mid-July, i.e., during an active period of vegetative growth, and a positive correlation was therefore observed between the date of measurement and the vegetative biomass. Although special attention was paid to balance the distribution of vines of the different pruning and age factors over the period, a normalized average weight index was calculated and therefore used for data analysis.

1.2. Wood staining and stained area measurements

In order to visualize the parts of the xylem that are active in the conduction of sap, a staining technique was used. The dye Phloxine was used (Phloxine B, Sigma Aldrich, Co, St Louis, MO, USA) for its good staining capacity, as previously assessed by Hietz *et al.* (2008).

Vine plants were cut below the graft union and immediately immersed in a 1 g.L^{-1} Phloxine solution. The Phloxine dye rises thanks to the evapotranspiration flow until leaf veins show the typical pink colour of Phloxine a few hours later.

In order to measure the surface area of the different types of tissues (stained, unstained, necrotized), the trunk and arms of the vine were transversely cut at several points: 3 to 4 points along the trunk, i.e., generally at the bottom, middle and top, and 1 to 3 points along the arm, depending on its length, always ending with current year cane cross-section. Before cutting, each point was numbered and pictured. After cutting, each piece was also numbered on the side section and photographed again before proceeding to image analysis.

Image analysis was processed using ImageJ® software (Schneider *et al.,* 2012). On each cross-section, pink stained wood, non-coloured white but visually healthy wood and necrotic wood were each manually delineated so that the area could be calculated by the software. Each section of wood can then be characterized by 4 variables: the sap conducting area, the non-conducting area, the necrotized area, and the total area corresponding to sum of the previous ones.

Necrotized areas were observed but, due to probable previous deterioration of this type of wood, the corresponding areas were probably underestimated, especially for vines with advanced level of wood deterioration. Conversely, the sap conducting area was a relevant variable as it corresponded to a functional reality. For these reasons, absolute area measurements were considered, rather than relative rates of each wood type.

1.3. Xyl'em® device and sap flow measurements

The Xyl'em® device (Bronkhorst, Montigny les Cormeilles, France) is a flowmeter tool that measures the water flow rate in a portion of pipe in response to a gradient of potential applied between its two end points (Figure 1c). This tool was originally developed by the PIAF team in Clermont-Ferrand to assess cavitation in the xylem (Cochard *et al.,* 2015). The principle of the cavitation measurement is to first assess the conductance of a small portion of wood before injecting degassed pressurized water to expel or dissolve air bubbles trapped in the xylem conduits. The device thus performs a series of measurements: an initial measurement of the conductance K at "low pressure" (6 kPa), then a flush at "higher pressure" (200 kPa) to expel the air from the vessels before a second "low pressure" measurement giving Kmax conductance.

In our case, we used the Xyl'em® technique to assess the conductance of whole vine sap flow paths. Sampling tests performed in 2017 prior to the main experiment showed that the "low pressure" measurement was most suitable for our purpose. Indeed, "higher pressure" measurements showed that apart from a few observations where Kmax drops drastically after water flushing (suggesting possible plugging of the vessels with gels), there is only small variation in Kmax compared to the initial K. Therefore, we only considered the "low pressure" measurements in this study.

Two kinds of measurements were carried out in the course of our work:

- Measurements on whole vines (Figure 1e): in this case, the Xyl'em® device was connected to one or several shoots; the shoots were previously cut at a few cm length (like spurs) to connect them to the Xyl'em® pipes. After checking that the pipes were watertight, the measurements could be taken. The conductance of each shoot was assessed separately, shoot by shoot, for all the shoots of the vine. This measurement provided the absolute value of the conductance of the shoot (unit $mmol.s^{-1}.MPa^{-1}$), but it could also be normalized by the diameter of the shoot to give the value of specific conductance of the shoot (i.e., conductance per unit area of the section, unit $mmol.s^{-1}.cm^{-2}.MPa^{-1}$). The conductance measurements were not normalized by the length of the vine (to give then conductivity) as this parameter is difficult to obtain and, besides, part of our investigation.

- Measurements on portions of trunk and arms (Figure 1d): in that case, the Xyl'em® device was connected to a 10 cm portion of trunk or arm by mean of a steel canula of constant diameter (1.2 mm) inserted into it. The measurements could therefore be normalized per unit of section and then be replicated at different points of the section.

2. Experimental description and progress

2.1. Field trials: age and pruning factors comparison

2.1.1. Experimental design

Measurements have been replicated in two distinct vineyard plots, one located in the Jura wine region, Bourgogne-Franche-Comté, France (Jura vineyard) and the other in the Bordeaux Wine region, Nouvelle-Aquitaine, France (Bordeaux vineyard). Measurements were carried out over a few days for Jura vineyard (from July 15th to 18th 2019) and over a 3-week period for Bordeaux vineyard (from June 25th to July 18th 2019).

The experimental sites were selected because they both combined in the same vineyard plot:

- Vines with distinct favourable vs unfavourable rating levels of pruning relative to sap flow pathways (see Table 1)

- Younger and older vines; 'older' being the mature originally planted vines and 'younger', the replaced plants replanted after the death of a previous one.

Based on these characteristics, 4 treatment levels were identified: "old / respecting sap flow pathways" (O+), "old / not respecting sap flow pathways" (O-), "young / respecting sap flow pathways" (Y+), "young / not respecting sap flow pathways" (Y-).

For Jura vineyard, it was possible to find both levels of pruning notation Y+ and Y- on young vines but not for Bordeaux vineyard, where only young vines respecting the sap flow pathways (Y+) were present. Bordeaux vineyard therefore had only 3 treatment levels (O+, O- and Y+) whereas Jura vineyard had all 4 treatments (O+, O-, Y+ and Y-).

Of the 30 to 40 vines that were scored for pruning evaluation, 22 and 24 vines were finally selected for the experiment on Bordeaux and Jura vineyards, respectively.

On the day of the measurement, the vines were cut below the graft union and immerged in the Phloxine solution until the dye was driven up to the leaves. The vines were then transported (with the cut section kept immerged in water) by groups of 2 to 3 plants to the site where sap flow measurements were performed. The measurement sites were both located close to the vineyard, at most a few kms away.

The conductance measurement set-up consisted of a tank with a capacity of about 1 cubic meter filled halfway with water, so that the vine could fit totally immerged in it. Once inside, the shoots of the vine were excised under water, measured for diameter and length, and then connected to the Xyl'em® pipe outlets following the protocol described in chapter 1.3.

Once conductance measurement was completed and vines were removed from the water, the trunks and arms were cut transversely and the sections were photographed following the protocol in chapter 1.2.

In Jura vineyard, for an undetermined reason but possibly related to high air temperatures, Phloxine staining of vine wood did not occur properly (no or too little pink staining of leaf veins was osbserved after a sufficient time period) and gave erratic results from one vine to another. Thus, information on wood conducting area was not possible in Jura vineyard and only Bordeaux vineyard showed the complete sequence of measurements.

2.1.2. Sites description

Jura vineyard (46.909270, 5.760842) was planted in 1975 with the typical Jura cultivar 'Savagnin'. The oldest vines were thus 44 years old at the time of the experiment. The plant density was 6666 vines per ha. The younger replaced vines were about 10-15 years old. The rootstock is not known.

Bordeaux vineyard was located in the Bordeaux region (44.790150, -0.576459), planted in Sauvignon on 101-14MGT in 1991 (i.e., 28 years old at the time of the experiment). Planting density was 5681 vines per ha. The age of younger replaced vines was not known precisely, but they were less than 15 years old.

The training system was similar for both vineyards: 'espalier' with two-cane "Guyot" type of pruning.

Both vineyards were managed with a permanent natural cover cropping between rows (between all rows in Bordeaux vineyard, and half the rows in Jura vineyard).

2.1.3. Pruning evaluation method and criteria

The scoring grid used in this study to evaluate the characteristics of the type of pruning in relation to the respect of the sap flow pathways comes from a method elaborated by Chambre d'Agriculture du Jura (unpublished). This method was based on general rules previously established by Eugène Poussard (Lafon, 1921) and summarized in a collaborative work paper co-signed by several experts (Dal *et al.,* 2017).

The idea of this method is "to assess from the outside what is probably happening in the inside" using relevant rating criteria. These criteria were based on external observation of the trunk and arms. They considered (i) the number of main visible sap pathways of the vine, (ii) the importance of wounds located on these pathways, as well as (iii) the patent redirections of the pathways that may have occurred during the past pruning seasons. These three criteria were integrated into a global vine rating, ranging from 0: 'very good' to 5: "highly deteriorated" (Table 1 and supplementary data Figure S1).

Among the treatments compared, those that respect the sap flow pathways (O+ and Y+) generally show grades 1 and 2, occasionally 0 for some young vines in Jura vineyard. On the contrary, vines belonging to treatments "not respecting the sap flow pathways" (O- and Y-) most often have scores of 4 or 5.

As these criteria require a certain amount of expertise, the evaluation of the vines in this study has been done by experts that were familiar with the method.

2.2. Laboratory experiment: conductivity of portions of trunks and arms

These measurements were carried out on in May 2018 on vines from a third vineyard plot located in Côtes-du-Rhône wine region. Six vines of cultivar 'Grenache' aged 20 years-old, were used. Five of them were trained in 'cordon', one in 'goblet'.

After staining with Phloxine and cross-sectioning of the trunk and arms, conductance measurements were made on portions of the wood (Figure 1d) to validate the ability of the differentially coloured parts of the wood to conduct sap. As wounds were sometimes present on the wood portions (from previous pruning wounds), the measurement also took into account the factor "close to a wound" or not (Figure 2).

For each vine, 10 cm long sections of trunks and arms were cut under water, each vine being characterized by at least one trunk and one arm section, sometimes more. After refreshing the section surface with a razor blade, a 1.2 mm wide steel canula was inserted in the wood at on three locations: unstained (white) wood, pink coloured wood close

TABLE 1. Description of criteria associated to each rating grade of vine pruning condition relatively to sap flow pathways and correspondence with experimental treatments.

Pruning grade	Criteria relatively to respect of sap flow pathways	Treatments Bordeaux v.	Treatments Jura v.
0	Perfect		6 vines Y+
1	Two pathways on the trunk, 1 or 2 sap flow reorientations but no major wound on the arms	8 vines Y+ 7 vines O+	6 vines O+
2	Two pathways on the trunk, 2 or 3 sap flow reorientations or a marked area of dead wood on the top of the trunk		
3	Two pathways on the trunk, many sap flow reorientations and/or a marked area of dead wood on the top of the trunk or single pathway on the trunk with no major dysfunction elsewhere		
4	Single pathway on the trunk, 1 to 3 sap flow reorientations or a marked area of dead wood on the top of the trunk	7 vines O-	6 vines Y-
5	Single pathway on the trunk, many sap flow reorientations and/or a marked area of dead wood on the top of the trunk		6 vines O-

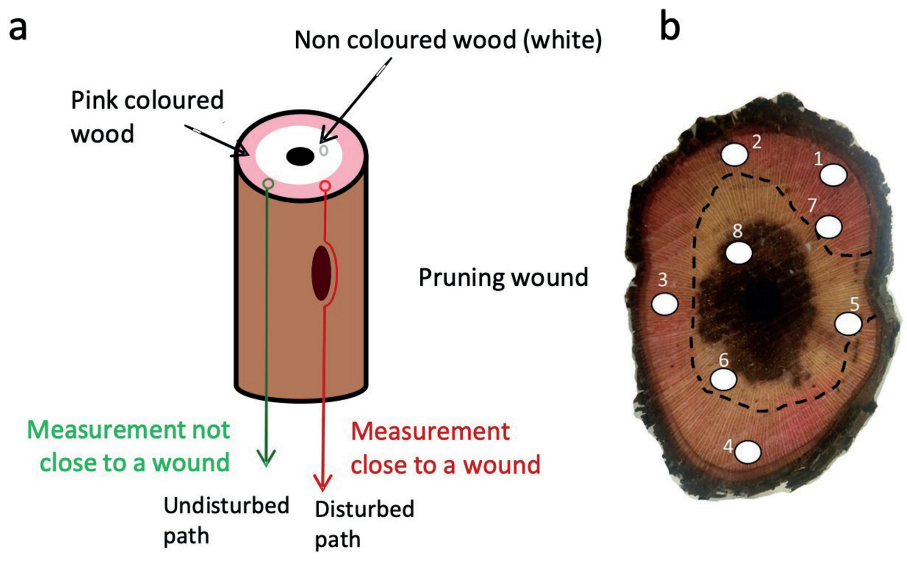


FIGURE 2. Description of conductivity measurements on portions of wood from vine trunk and arms.

(a) description of treatments et (b) examples of insertion points of the steel canula on a section of vine.

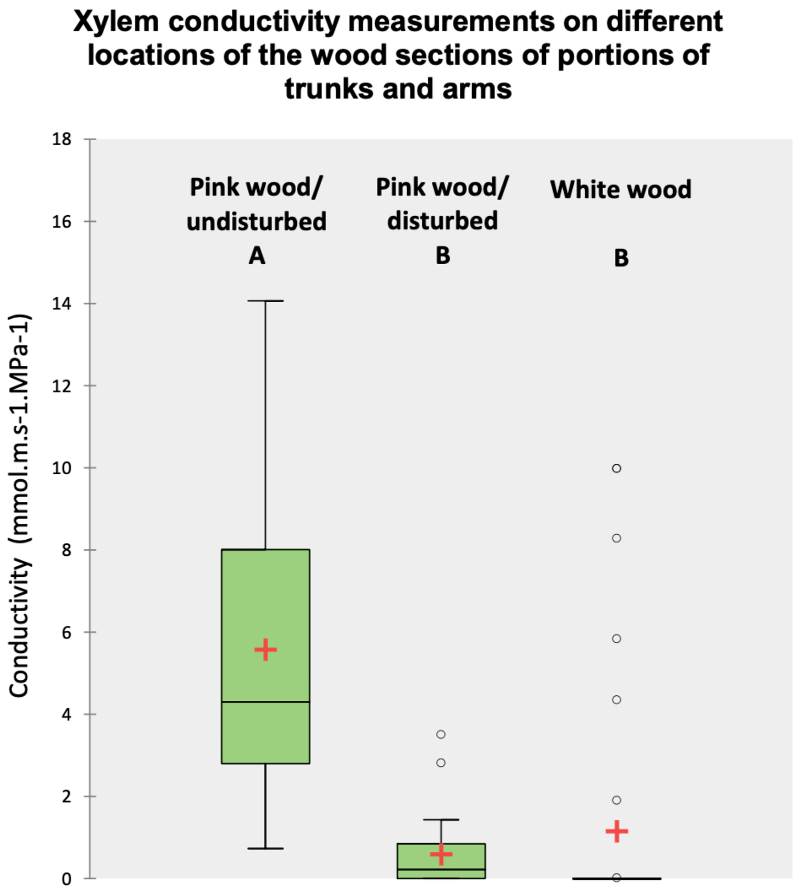


FIGURE 3. Conductivity (conductance/unit length of section) on portions of trunks and arms depending on the type of coloured area (pink coloured wood or uncoloured "white" wood) and the vicinity of a pruning wound (undisturbed or disturbed pathway).

Côtes-du-Rhône vineyard, June 2018, 6 vines, 15 sections of trunk and arms, 104 measurement points (45 for 'pink'/undisturbed wood, 24 for 'pink'/disturbed wood and 35 for white wood). Letters: Dunn test p = 0.05 from Kruskal-Wallis test. Top and down horizontal bars = maximum and minimum, box lines = quartile 1, median and quartile 3 values, red cross = mean value, circles = extreme values.

to a wound downstream and pink coloured wood without any wound downstream (see Figure 2a). For each section, 3 to 9 measurements were made. A total of 15 sections were cut from 6 different vines.

3. Statistical analysis

All statistical analyses (variance analysis, non-parametric tests of sample comparison, generalized mixed models and principal component analysis) as well as 'box-plot' charts were performed using XLSTAT software (Addinsoft, XLSTAT statistical and data analysis solution. Paris, France.).

Treatment comparisons at the vine level (vegetative biomass) or for the conductivity of portions of trunks were performed using variance analysis, except when the conditions for the ANOVA were not properly fulfilled (normal distribution of residues). In that case, non-parametric Kruskal-Wallis tests were used. Post-hoc tests for pairwise comparisons are specified in the legend of each Figure.

When comparisons were made at the shoot level, but taking into account the vine they belonged to, we used generalized mixed model with random effect, where random factor was the vine and the fixed factors could be, depending on the situation, site (when two sites were present i.e., for conductance analysis), age and pruning (for Jura vineyard) or age/pruning treatments (for Bordeaux vineyard, as missing Y- treatment did not allow us to separate the two factors) and organs (trunk or arms). The analysis was carried out first with all factors main effects and their interactions. When the effect of the interaction was not significant, the analysis has been repeated without the interaction. The different effects finally considered are indicated in the legend of the Figures.

RESULTS

1. Conductivity of portions of trunks and arms with or without wound in the vicinity

The results from the conductivity measurements realized in 2018 on portions of wood are shown on Figure 3.

First of all, the results confirm that pink coloured area corresponds to conductive xylem sap tissues, whereas "white" uncoloured one does not. Nevertheless, in the latter, some conductance values reach the same level as the "pink undisturbed" ones (5 measurements out of 35). Further investigation would be useful to determine if these points consist of conductive xylem not connected to the rest of the network, or mere experimental artifacts.

With regard to the vicinity of a pre-existing wound, the conductivity of pink coloured wood measured close to a wound (i.e., on a 'disturbed pathway') was significantly lower than the one measured on 'undisturbed pathways' (i.e., without any proximal wound) and about the same range as the white non-conductive wood level.

2. Vegetative biomass

In both vineyards, vines "not respecting sap flow pathways" showed a significantly reduced vegetative biomass, as shown in Figure 4 on indicator "sum of shoot sections" and for Bordeaux vineyard, on the total dry mass of vegetative parts. This reduced biomass was probably due to a conjunction of a reduction in the number of shoots par vine and reduction in diameter, although each variable was not significantly reduced *per se*.

3. External and internal impact of treatments on the wood

Examples of external appearance of entire vines as well as cross-sections of trunks and arms are shown on Figure 5 and 6 for the two vineyards according to each age and pruning treatment.

3.1. External appearance of vines between treatments

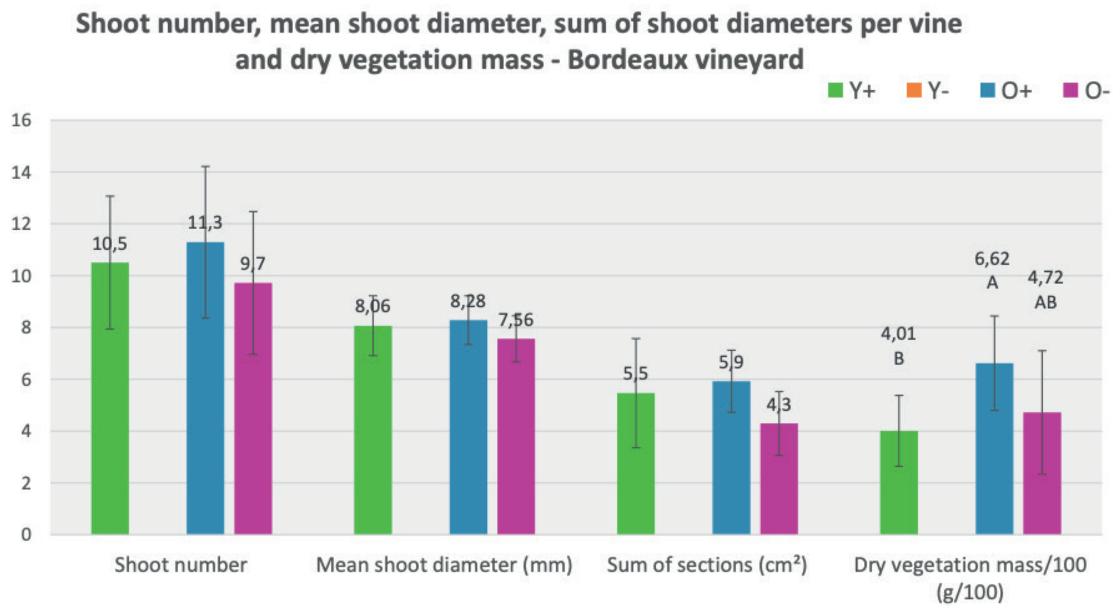
The older vines from Jura vineyard were bigger than the ones from Bordeaux vineyard, which can be, to a certain extent, explained by the age difference between the two vineyards.

Even though both vineyards were double Guyot pruned, vines showed distinct types of architectures: vines from Bordeaux vineyard mostly showed vertical trunk and horizontal arms supporting the canes (Figure 5a to d), while Jura vineyard, on the contrary, showed this type of architecture only on the O+ treatment, whereas O- and Y+ vines showed only vertical trunk and canes directly connected onto it (Figure 5j, 5h, 5i, 5j, 5m and 5n). This latter type can be due to mortality on the arms (probably the cause of single armed vine on Figure 5n for example) leading to arm renewal (Figure 5e where we can see younger arms reformed above the oldest dead one) but this can also reflect different pruning strategies between the two vineyards and growers' pratices, with the grower in Jura vineyard being probably keener to contain the development of the vines and the elongation of the arms. Vines O- from Bordeaux vineyard also have a slightly different architecture than the O+ ones: they display younger arms (probably due to renewal after death of an arm) that are consequently thinner (Figure 5d vs 5e for example).

3.2. Internal aspect of sections of trunk and arms

With the exception of the youngest vines, which often showed visually fully functional and poorly necrotized sections, the oldest ones in contrast showed degraded sections with a much greater presence of necrosis inside, especially in the arms (when the arms were present) or at the top of the trunk (Figure 6o and 6p vs 6q and 6r for example), the latter being probably a consequence of Guyot type of pruning.

Necrosis can be restricted to the centre of the trunk or affect the periphery. In that latter case, especially on the older vines, the trunks are no longer circular and living wood is not present on the entire circumference circle. Trunks sometimes show a circular section, but arms never do, showing large zones of dead and decayed wood (Figure 6o vs 6s to 6v, the latter showing only a small chip of living wood). Indeed, in the ideal view of Guyot pruning type, trunk of a mature vine is fully circular, and arms are only half-circular as pruning wounds are supposed to be localized on the top of the arms, in order to maintain the underneath portion unharmed.



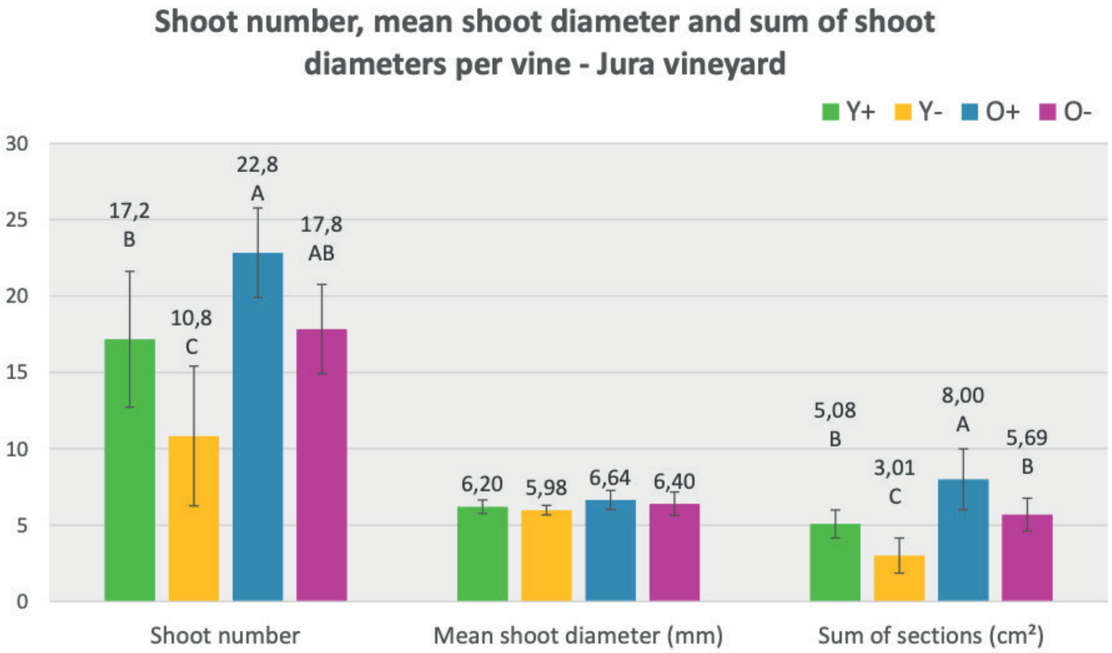


FIGURE 4. Vegetative biomass indicators of the different age and pruning treatments on Bordeaux vineyard (top) and Jura vineyard (down). Bars = standard deviation. Letters: ANOVA and Tukey test ($p = 0.05$) except for Dry vegetation mass (Kruskal-Wallis test $p = 0.05$). For Jura vineyard, ANOVA with 2 factors (age and pruning); for Bordeaux vineyard, ANOVA with 1 factor (age/pruning).

To illustrate that, supplementary data Table S1 summarizes, for each age and pruning treatment, the number of vines with 'correct architecture' for a Guyot type, i.e., for which living wood is present on the entire circumference of the trunks (Figure 6a and 6o vs 6i, 6j, 6m, 6s and 6t) and on at least half the circumference of the arms, when arms are present (Figure 6q and 6r vs 6u and 6v).

Age and pruning have an impact on the aspect of the sections of trunks: almost all Y vines, at least when pruning is respecting sap flows, show complete circumference of living wood. When not respecting sap flows (Y- on Jura vineyard), the rate of living wood decreases: 3 vines out of 6 look like Y+ and the remaining 3 like older ones.

For older vines, the O+ trunk sections were generally mostly circular whereas the O- ones were much less so. On the arms at the contrary, pruning doesn't seem to be influent, both treatments showing poorer percentages of living wood circumference.

3.3. Measurements of living and conductive wood areas

In Bordeaux vineyard where Phloxine staining worked well, the area of the different types of wood (pink conductive,

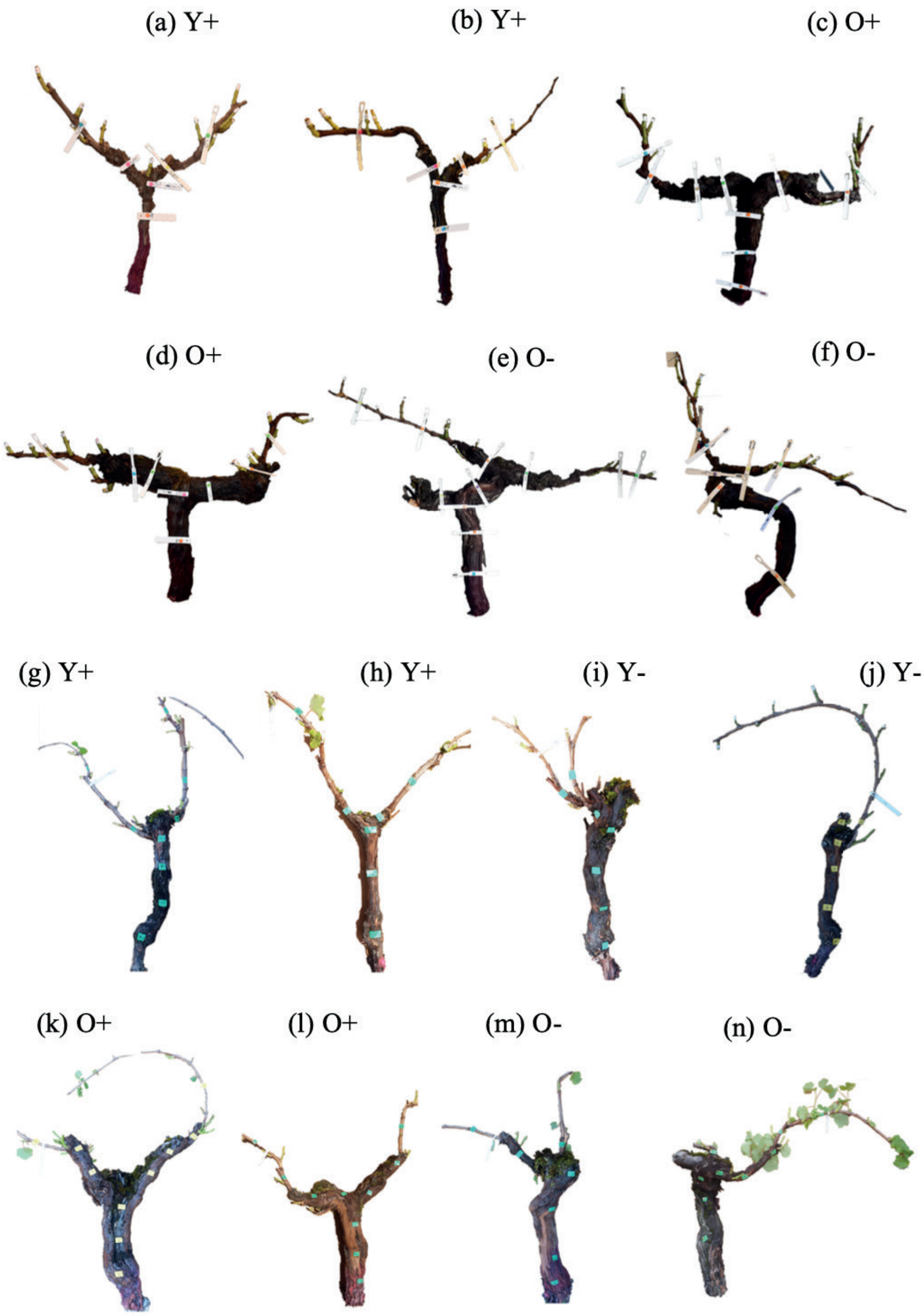


FIGURE 5. Examples of vines from the different vineyards and treatments: (a) to (f) Bordeaux vineyard (a, b = Y+, c, d = O+, e, f = O- and (g) to (n) Jura vineyard (g, h = Y+, i, j = Y-, k, l = O+ and m, n = O-).

white unstained non-conductive and necrotized wood) was measured by image analysis. Amounts of each type of wood (and consequently total area of trunk and arms sections deriving from sum of all) are shown in supplementary data Figure S2. As already mentioned above and visible to the naked eye, Y+ vines show lower total areas of wood (due to their age) and little presence of necrosis. On the opposite, older O+ and O- vines are characterized by high amounts of necrotized tissues which counts for more than half the total section area in average. O- vines show less total area and necrotized area than O+ vines, especially on arms. This may be due, at least for some vines, to arm renewal after death of the original one. It can also be due to lesser thickness growth (visible for example on Figure 6 (t) where only one part of the trunk has continued to grow where the right one part has not) but above all to degradation of necrotized wood over the years (visible for example on Figure 6 (v) picture where necrotized portion is no longer circular due to probable degradation of necrotized tissues over time).

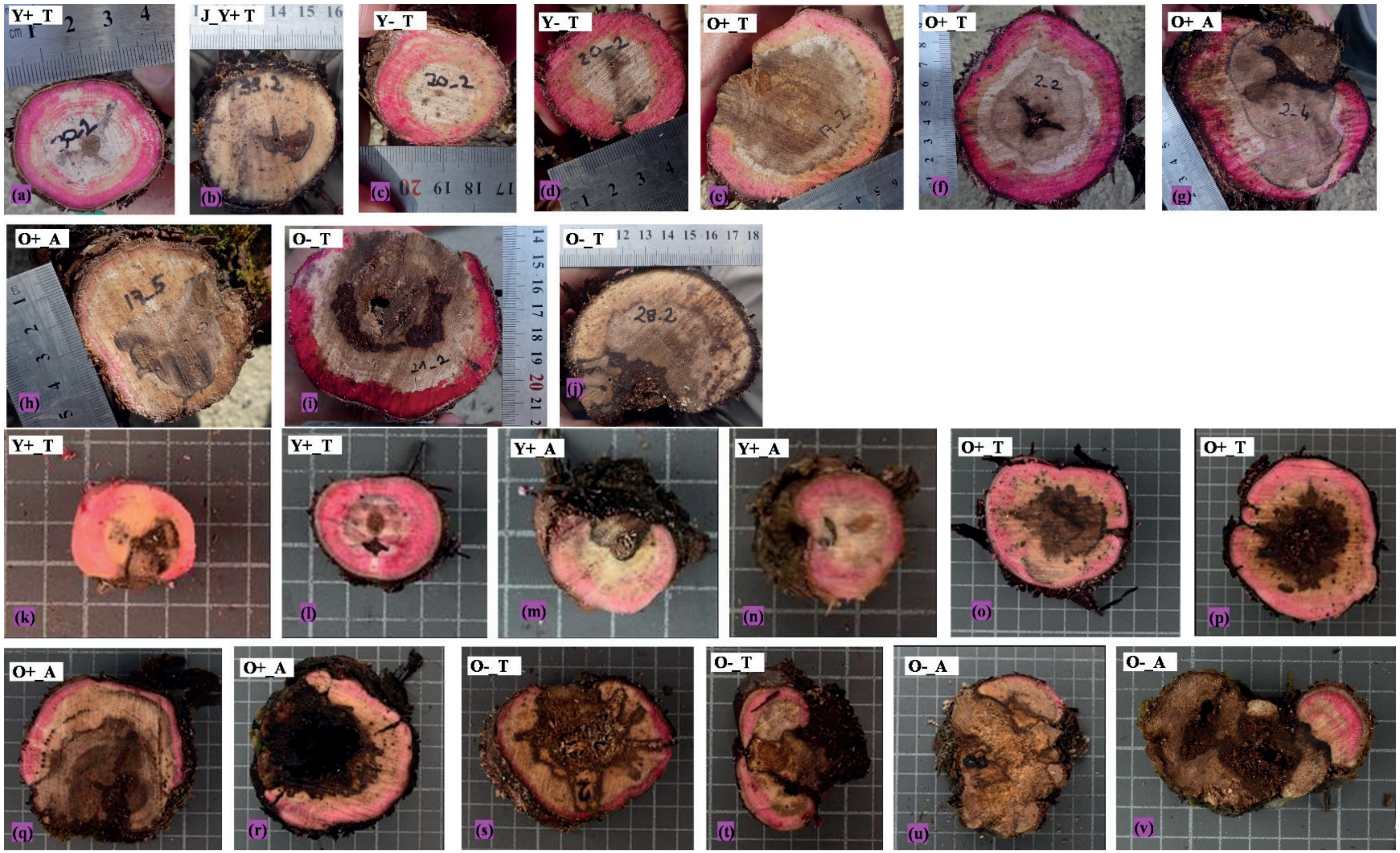


FIGURE 6. Examples of transverse sections of trunk or arms from the different vineyards and treatments: (a) to (j) Jura vineyard (a and b = Y+ Trunk , c and d = Y- Trunk, e and f = O+ Trunk, g and h = O+ Arms and i and j = O- Trunk) and (k) to (v) Bordeaux vineyard (k and l = Y+ Trunk, m and n = Y+ Arms, o and p = O+ Trunk, q and r = O+ Arms, s and t = O- Trunk and u and v = O- Arms). In Jura vineyard, Phloxine staining has been erratic. Treatments without arms section pictures: no arms present on the vines. Scale = 1-to-1 cm graduated ruler (Jura vineyard) and 1 cm^2 grid (Bordeaux vineyard).

Figure 7 specifies results on living and conductive wood.

O+ vines had significantly higher values of living and conductive sap area than Y+ and O-, especially on the trunks where it was about twice as high. The conductive sap area of trunks from older vines pruned without respecting sap flow pathways was markedly lower than the one of older vines pruned respecting the sap flow pathways, and approximately the same range as the younger vines. There is no significant difference between O+ and O- neither for living nor conductive areas in the arms.

Interestingly, the area of living as well as conductive sap decreased significantly between the trunk and the arms (around half the active area for O+ and O- and a quarter for Y+ in the arms compared to the trunk) suggesting that arms are acting like a bottleneck for conductive wood. Again, this was not surprising considering the location of the wounds on the arms for this Guyot pruning type.

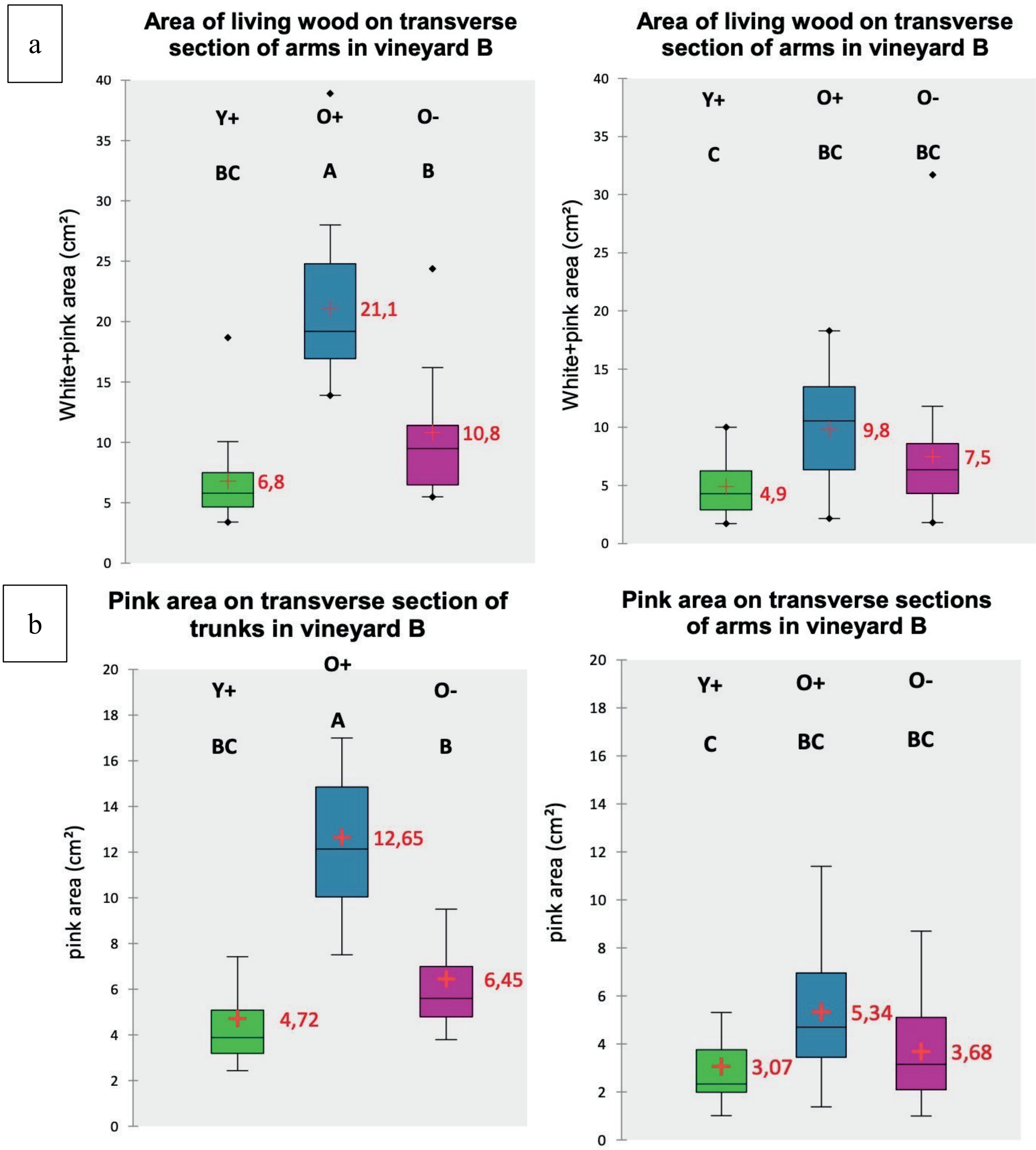


FIGURE 7. (a) area of living wood (pink + white stained wood) and (b) area of conductive wood (pink stained wood) on the transverse sections of the different treatments of age and pruning, for trunks (left) and arms (right) for Bordeaux vineyard.

18, 19 and 21 measured sections for trunks and 18, 28 and 22 sections of arms of respectively Y+, O+ and O- treatments. Letters: Tukey test significant groups from mixed model with pruning treatments, organ (trunk or arms) and interaction treatment*organ as fixed effects and vines as random effect. Top and down horizontal bars = maximum and minimum, box lines = quartile 1, median and quartile 3 values, red cross = mean value.

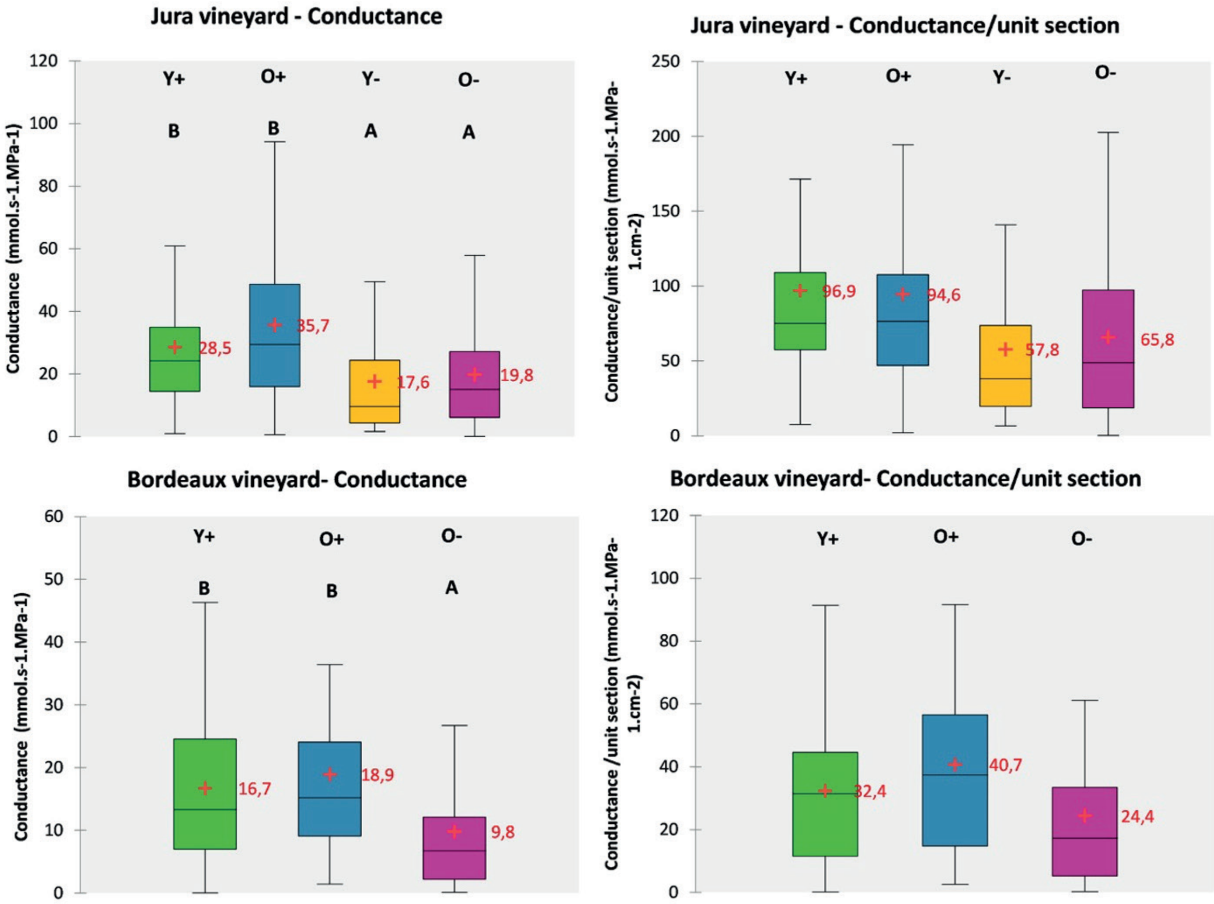


FIGURE 8. Comparison between age/pruning treatments for conductance and conductivity measurements for the 2 vineyards Jura (top) and Bordeaux (down).

Bordeaux vineyard: 84, 79 and 68 shoots measured respectively for Y+, O+ and O- treatments. Jura vineyard: 103, 137, 65 and 107 shoots measured respectively for Y+, O+, Y- and O- treatments. Letters: Tukey test significant groups from mixed model with pruning treatments, age as fixed effects and vines as random effect. Top and down horizontal bars = maximum and minimum, box lines = quartile 1, median and quartile 3 values, red cross = mean value.

4. Whole vine conductance measurements

The results for the entire vine conductance measurements are shown in Figure 8, where the treatments are compared at the shoot level, but taking into account the vine they belong to (thanks to mixed models statistical design).

Conductance was significantly lower on pruning treatments "not respecting sap flow pathways", 35 % and 40 % lower for Jura and Bordeaux vineyard respectively. Conductance per unit section is less significant, but still show the same trend in tendency (p value respectively 0.07 and 0.12 for Jura and Bordeaux vineyard).

Conductance was not different between age treatments for a given pruning treatment.

These differences were not significant at the vine level (i.e., when the average values per vine are compared) probably due to the lower number of vines for each treatment (6 to 8 depending on the treatment and the vineyard, cf. Table 1) and to a certain heterogeneity between the vines of a same treatment (supplementary data Figure S3). Similarly, shoot conductance values for a same vine were also highly variable.

5. Relationships between Xyl'em® conductance, conductive wood area and vegetative biomass on Bordeaux vineyard in association with pruning and age treatments

In Bordeaux vineyard, where the full experimental design was successfully carried out and the data set complete, a PCA was realized at the vine level to relate the different variables together and explore their inter relations in association with age and pruning treatments. For this latter reason, pruning score were included in the PCA as a supplementary variable. To ensure a clear reading of the results, only values of conductive area per vine were included in the PCA; detail for the trunk and arms, highly correlated to values per vine, were not included. In addition to the conductive sap area, the necrotized area of the wood sections was included as well. The conductive plus the necrotized area of the wood sections gave the total area of the section.

Two interesting significant relationships can be pointed out from the correlation matrix (Figure 9a): the first one between the area of the different kinds of tissues (necrotized, conductive, and total) and the vegetative biomass, and second one between the pruning score and the average conductance per vine. The first principal component (F1 axis) described the vegetative biomass and the conductive sap area and second one the conductance and the pruning score (F2 axis, Figure 9b). The cumulated variance of these first two axes was 62 % of total variance.

Differences between treatments are also well represented on the first two axis of the PCA: O+ vines are most likely located on the side of F1 axis corresponding to high biomass and conductive area on and on the higher conductance side on axis F2, and in accordance with their lower pruning score. In contrast, younger vines Y+ show less vegetative biomass and conductive area, but as much conductance as older O+ vines. O- vines are intermediate in terms of vegetative biomass and conductive area, but they display a lower conductance in accordance with their higher pruning score level (Figure 9c).

DISCUSSION

Conductance measurements carried out in 2018 on portions of trunk and arms and in 2019 on entire vines resulted in a consistent parallel: the former showed a lower xylem conductivity in the vicinity of a wound when the latter showed that shoots from vines pruned 'without respecting the sap flow pathways' (i.e., characterized, among other impediments, by wounds on the trunk and arms) had an average 40 % reduced conductance and conductivity compared to vines pruned 'respecting the sap flow pathways'. The fact that the reduced conductance is maintained even after normalization per unit area of the shoot section suggests that it is due to modifications in the very structure of the xylem (and not only to a reduced number of shoots or shoot sections). Besides the presence of air bubbles in the vessels, these modifications may be due to the anatomy of the downstream xylem (number and diameter of vessels) or to its spatial arrangement (diversions of the path or in the arrangement between vessels). This latter hypothesis is supported by some observations made with X Ray microtomography (supplementary data Figure S3). From these pictures, the spatial arrangement of the vessels in the vicinity of a wound appears to be disturbed, as some planes show both transversely and longitudinally cut vessels, suggesting a clear disorganization of the xylem in relation with the presence of the wound.

(a) Variables	Conductance/ vine (mmol.s^{-1}.Mpa^{-1})	Conductivity/ vine (mmol.s^{-1}.Mpa^{-1}.mm^{-2})	Shoot number	Shoot diameter (mm)	Sum shoot sections (cm²)	Vegetative biomass (g)	Total section area (cm²)	Conductive area (cm²)	Necrotized area (cm²)	Pruning rate
Conductance/ vine (mmol.s^{-1}.Mpa^{-1})	**1**	**0,913**	0,285	-0,012	0,188	0,028	-0,089	0,133	-0,134	**-0,453**
Conductivity/ vine (mmol.s-1.Mpa-1.mm^{-2})	**0,913**	**1**	0,398	-0,312	0,040	-0,122	-0,056	0,108	-0,080	-0,350
Shoot number	0,285	0,398	**1**	-0,263	**0,614**	0,085	0,158	0,254	0,139	-0,103
Shoot diameter (mm)	-0,012	-0,312	-0,263	**1**	**0,579**	**0,553**	0,155	0,420	0,072	-0,242
Sum shoot sections (cm²)	0,188	0,040	**0,614**	**0,579**	**1**	**0,457**	0,235	**0,523**	0,151	-0,253
Vegetative biomass (g)	0,028	-0,122	0,085	**0,553**	**0,457**	**1**	**0,488**	**0,579**	**0,479**	-0,010
Total section area (cm²)	-0,089	-0,056	0,158	0,155	0,235	**0,488**	**1**	**0,807**	**0,977**	0,157
Conductive area (cm²)	0,133	0,108	0,254	0,420	**0,523**	**0,579**	**0,807**	**1**	**0,700**	-0,193
Necrotized area (cm²)	-0,134	-0,080	0,139	0,072	0,151	**0,479**	**0,977**	**0,700**	**1**	0,261
Pruning rate	**-0,453**	-0,350	-0,103	-0,242	-0,253	-0,010	0,157	-0,193	0,261	**1**

Bold type values significantly differ from 0 with a confidence level of 0.05

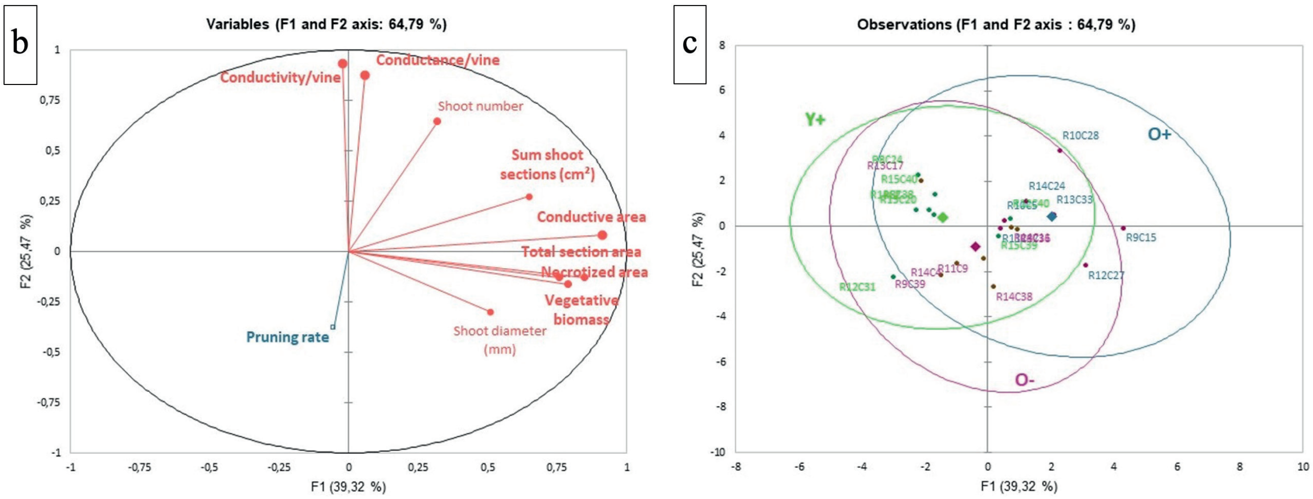


FIGURE 9. PCA of complete data set from Bordeaux vineyard: conductance, conductive area and vegetative biomass at the vine level in relation with age/pruning treatments.

(a) correlation matrix between initial variables, (b) representation of initial variables on the first 2 components of the PCA (size of the plot reflects the level of contribution of the variable to the component, in bold if significative) and (c) representation of vines and treatments (with 95 % confidence circles).

For all age/pruning treatments, the conductance values showed significant level of heterogeneity between vines within a same treatment and also between shoots of the same vine, suggesting substantial variability in the efficiency of the conducting sap flow pathways within a vine. To account for that heterogeneity, it is possible to suggest different hypothesis. First, it is interesting to note that the pruning score from which the treatment levels (+) and (-) are derived is a visual global evaluation given for an entire vine despite the fact that it is highly probable that infra-vine differences occur between portions of this vine, as attested by experts that assessed the vines scores: within a same vine, some parts may be properly supplied when others show disturbed pathways. In addition, regarding the lowest conductance values, apart from the cause previously mentioned, it is interesting to consider the impact of gels that were frequently observed during the cross-section of the trunks and arms. These gels, generally translucid and clear, exuded spontaneously and instantaneously from the vessels after cutting at several different locations on the section, suggesting that these vessels might have been plugged previously with those gels and that they might thus be non-functional. Conversely, particularly high values of conductance (about the same range as a blank measurement, i.e., without connecting the tube of the apparatus to a sample) were also noted during the experiment. Indeed, grapevine is known for its long and wide vessels, which may explain such results (Jacobsen *et al.,* 2015; Zimmermann and Jeje, 1981).

Finally, it may be interesting to add that this study focused on the trunk and arms part of the vine wood, because it is the place where pruning takes place. The results on entire vine conductance might be different if graft union and root system were included, for instance if the conductance measurements had been done in situ (without cutting off the vines), because of the internal structure of the graft junction or the spatial arrangement of the root system for water uptake. It could be interesting to further study the evolution of the system under real field conditions.

With regard to the conductive xylem, conductance measurements on portions of wood confirmed that the pink stained zone of the xylem is the one that supports conduction, in contrast with the non-coloured ‘white’ one, that does not. This result is in accordance with other published work (McElrone *et al.,* 2021).

In the Bordeaux vineyard where Phloxine staining worked well enough to allow interpretations of conductive areas, the results showed as expected an impact of the age of the vine on the volume of conductive wood, with the Y+ treatment showing lower volume than O+ relatively to its younger age, especially on the trunk. Given the absence of a significant amount necrosis in the wood, this can only be explained by the difference in radial increment due to growth. For older vines, pruning characteristics had an impact on the volume of living and conductive wood, both results showing a parallel trend: the O- treatment ‘not respecting the sap flow pathways’ is associated with lower amounts of living and conductive wood, as low as the ones of a younger Y+ vine. This is significant in trunks, but in the arms, only a tendency is visible. In addition to greater wood degradation visible to the naked eye and by image analysis particularly on trunks, it is possible to assume that this lower amount is due to destruction of living wood, taken over by necrotized one. This result suggests that pruning choices have an impact on the remaining volume of living and conductive wood and its location on the outer rings, and specifies the extent of this impact. Unfortunately, the results on conductive tissues from Jura vineyard did not allow us to verify the convergence with the observations from Bordeaux vineyard.

Finally, less vegetative biomass seemed to be observed on vines pruned ‘without respecting the sap flow pathways’, due to a reduction in the number of shoots, but also perhaps to the presence of thinner ones, although confirmation on more vineyards would be necessary to reinforce this observation. A first possible explanation is that it may be more difficult for the pruner to find a constant number of structures (canes and spurs) on a vine showing a bigger amount of necrosis and dead portions of wood, but this may also be due to a reduced vegetative expression of theses vines, as the decrease of shoot number is not compensated *in fine* by an increase of the diameter of the remaining shoots. It is also interesting to note the significant correlation in Bordeaux vineyard between the volume of conductive wood and the vegetative biomass, which evokes Shinozaki’s “Pipe model” theory (Shinozaki *et al.,* 1964), that postulates that there is a relationship between the volume of vegetation and the volume of wood connected to it. However, further ecophysiological studies are needed to understand the relationships between vegetative biomass, xylem conductive volume and conductance.

Finally, during the 2019 experiment, vines O- from treatment ‘not respecting sap flow pathways’ were purposely chosen from those with the most unfavourable pruning criteria, so that the differences with treatment O+ were maximized in order to investigate the “proof of concept”. In this context, a relationship between pruning characteristics and the two descriptors of sap conduction, namely the volume of living/conductive wood and sap flow conductance, has been evidenced. Choosing to prune each year ‘without respecting the sap flow pathways’ has indeed led to a decrease in conductive wood with a lower conductivity efficiency and the vines concerned have less vegetative biomass.

These results call for two important comments. First, that it is impossible to know which one of these observations is a cause or a consequence; we are unable to reconstruct the sequence of events triggered by pruning between the different variables: reduction in conductance, volume of conductive wood and vegetative biomass. Second, given this reduction, we are unable to assess the physiological consequences of these reductions in conductance and volume of conductive wood on functioning of the vine and its possible involvement in decline process. Finally, due to a lack of knowledge on the relationship between sap conduction and vine physiology, we are not able to understand the implications of the different values of conductance observed between the two vineyards (factor 2 between the two vineyards). If these values are indeed in the range of the conductances previously measured in 2017 and 2018 on different vineyards and regions (not shown), we are unable to understand their significance in terms of vine functioning and explanatory factors. It is likely that, in addition to pruning and age, factors like genotype of cultivar and rootstock, vigour of the vine (and all its driving factors) and sanitary status of the wood (in particular relative to trunk diseases) might play a role.

To make things easier, we have used an average value for pruning scoring at the vine level, but it is much more likely that pruning characteristics would be best assessed at an infra vine level, as strongly suggested by the variability of the performances between vines and shoots of the same vine. It is therefore reasonable to postulate that a vine could be a sum of sap flow pathways each more or less efficient, functional and durable. In addition to this ecophysiological point of view, it is important to consider a pathological point of view, given the widespread exposure of grapevine to trunk disease pathogens. These fungi, considered as the causal agents of grapevine trunk diseases, enter the vine through pruning wounds (Gramaje *et al.,* 2018), which probably contribute to aggravate the vine status in terms of the volume of functional wood (causing necrosis and dead wood) as well as on its efficiency (gels, tyloses).

One might then be tempted to ask about the resilience of such an endangered system, namely a pruned vine. And in particular, what is the capacity of one part of the vine to supply water to another damaged and less functional part? On this point, recently published work (McElrone *et al.,* 2021) suggests that, even if xylem sap preferentially flows in discrete portions of the xylem, because of low radial resistance due to long xylem vessels of this liana, interconnectivity exists that can supply another non connected part of the canopy if needed. This suggests a certain plasticity of grapevine conduction that is consistent with the practice of trunk renewal, where an entire vine can be rebuilt thanks to a lateral portion of living wood, or with the existence of very old vines displaying only a thin strip of living xylem to supply the aerial parts. However, despite this favourable result concerning the impact of wounding and plasticity of grapevine, it must be challenged in the scope of a succession of pruning events, with cumulative annual wounding that can cause multiple reorientations of the sap pathways year after year. In this context, it is critical that successive pruning events preserve enough undisturbed wood to ensure good conduction or, at least, to allow new structures to be rebuilt from undisturbed starting points.

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