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Short Notes – 12th Special issue on Grapevine Trunk Diseases

Grapevine histological responses to pruning: the influence of basal buds on tissue defence reactions

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Summary. Grapevines require pruning procedures to maintain plant morphology and ensure productivity, and these procedures cause wounds that induce physical and biological host defence mechanisms. Grapevine tissue reactions to wounding resulting from four different pruning methods were assessed. Rapid (immediate) defence reactions were detected in 1-year-old canes with preserved basal buds. Formation of tyloses (≈ 90% of xylem vessels) was observed 1 month later on canes where the basal buds were maintained and no short stubs were left (i.e. the pruning cuts preserved the buds). At 2 months after pruning, lignin was slightly increased in cortical parenchyma after pruning of 3-year-old grapevine wood. Neither callose nor suberin production was observed in healing wounds, as is known in other fruit or broadleaf trees. In 3-year-old canes, fungal hyphae were observed in the non-active wood below the pruning cut surfaces. Preliminary observations of desiccation cones within canes confirmed that the basal buds preserved the canes from desiccation, after comparing different pruning procedures on canes of the same age. After 9 months, the desiccation cones were greater in 3- than 1-year-old wounds.

Keywords. Pruning, wood anatomy, tyloses, early tissue defence reactions.

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INTRODUCTION

High-quality grapevine production depends on the phytosanitary state of vineyards, which includes the management of pruning strategies (Palliotti *et al.*, 2014). Pruning is required to maintain the vine size and shape, through control of shoot numbers and positions (Deloire, 2012), and to remove necrotic plant parts. Reduction of excessive and tangled shoots leads to improved sunlight exposure and air circulation (Palliotti *et al.*, 2014).

Adjusting bud numbers also regulates crop production (Keller *et al*., 2005; Keller, 2020), creating a balance between vegetative growth and grape yields. When optimum balance is achieved, grape quality is enhanced (Kliewer and Dokoozlian, 2005).

Cane pruning and removal produces wounds, and the amount of exposed surface is related to the diameter of the pruning cuts and the pruning method (Dal *et al*., 2008; Dal, 2013). The size of the wounds is related to the age of the cane; pruning an older cane (e.g., more than 2-years-old) creates larger wounds compared with a young cane cut at the same distance from the cane base. In canes of the same age, wound size depends on where the pruning cut is made relative to the shoot base (Sun *et al*., 2006); wound size is smaller if the cuts are made close to the cane apex compared to cuts near the cane base. Faúndez-López *et al*. (2021) and Henderson *et al*. (2021) demonstrated that cutting at distance from cane bases exposes wounds to potential airborne pathogens.

In the last 30 years reports of damage due to fungal wood pathogens in grapevines, i.e. Grapevine Trunk Diseases (GTDs) (Bertsch *et al*., 2013; Guérin-Dubrana *et al*., 2019; Mondello *et al*., 2018) have increased, and research has shown that wounds are their main infection sites for wood pathogens (Úrbez-Torres, 2011; Úrbez-Torres *et al*., 2013; Travadon *et al*., 2015, 2016; Lecomte *et al*., 2018). This has raised concerns about the roles of training systems and pruning methods, which may increase wood exposure to pathogen colonization, infection, and wood degradation by GTD pathogens (Sicavac, 2022).

There is little information on grapevine wood histological reactions to pruning, but this could be important for understanding wound colonization by pathogens. Wound sealing reactions in grapevines consist of tyloses development in xylem vessels. During each growing season, tyloses are early tissue responses near the cut surfaces, but when plants are dormant, wounds induce gel formation that will partially occlude vessels (Sun *et al*., 2006, 2008). Tyloses observed in 1-year-old canes pruned on active vines appeared under the cut surfaces 1 day after pruning, and developed rapidly, occluding the vessels up to 10 mm from the cuts (Sun *et al*., 2006). Following tyloses occlusion, the regions below the cut surfaces showed reduced water flow in vessels, and sap flow rate was negatively correlated with increased tyloses that limited pathogen entrance and impaired xylem function (Zhao *et al*., 2014). As a result, dehydration from reduced water translocation induced formation of necrotic dry areas just below the cut surfaces. These areas have been described as "desiccation cones" (Faúndez-López *et al*., 2021), due to the tapered shapes of the dry wood from the cut surfaces to the inner central wood. The desiccation area, with low tissue water content, is a physical barrier discouraging proliferation of invading microorganisms. The extent of the affected regions is variable (Faúndez-López *et al*., 2021), depending on the diameter, age or location of the removed part, or the grapevine cultivar (Bruez *et al*., 2022).

In summary, grapevines display specific reactions to wounds made during the growing season, consisting of tyloses occlusions in xylem vessels and formation of 'desiccation cones', as consequences of natural dehydration due to vessel deactivation (Faúndez-López *et al*., 2021). As well, "summer pruning wounds" (in actively growing grapevines) do not induce callus production or resin secretion to seal the cut surfaces, as occurs in fruit trees (Brown, 1995).

Considerable research and a recent metadata study (Rosace *et al*., 2023) on effects of winter pruning have focused on factors that most affect the period of grapevine pruning wound susceptibility to fungal colonization, especially timing to reduce wound infections. Late pruning may reduce susceptibility to colonization by increasing defence response in relation to pathogen activity in spring, based on the occurrence of rain, as has been reported in Italy, Spain, and California (Larignon and Dubos, 2000; Serra *et al*., 2008; Rolshausen *et al*., 2010; Úrbez-Torres and Gubler, 2011; Elena and Luque, 2016).

Histological research is required to investigate grapevine reactions in woody tissues to different pruning practices. This could assist selection of efficacious techniques for reducing pathogen infections and increase host defence reactions, and, therefore, wound protection efficacy (Martínez-Diz *et al*., 2021; Di Marco *et al*., 2022) to minimize damage and losses following infections. As a practice to protect pruning wounds, up to now technical operators report that pruning cuts made over the basal buds on canes prevent wood necroses, especially if a stub ("legno di rispetto" in Italian, or "chicot" in French) is left at each pruning site (Simonit, 2013). However, no histological observations have been made on grapevine tissues react to produce physical barriers or active defence substances (i.e., tannins and phenols; Falsini *et al*., 2022), that potentially prevent pathogen entry and colonization.

The present study focused on the early response to pruning wounds in the *V. vinifera* L. 'Trebbiano Toscano', a white grape variety that is widely planted in central and southern Italy. This cultivar displays moderate to very high susceptibility to the Esca complex of diseases (Mugnai *et al*., 1999; Andreini *et al*., 2013; Borgo *et al*., 2016). This study investigated how grapevine tissues reacted during the 9 months after wounding in late pruning (March) on 1-year-old canes. The purpose was

to consider histological reactions within one host phenological cycle, without interfering with the possible interfering carry-over effects into the following season. The main parameters compared were removal or retention of cane basal buds, and the basal portions of the canes (i.e. leaving cane stubs). A preliminary investigation was carried out on the different tissue reactions depending on cane age, comparing 1- and 3-year-old canes.

The aims of this study were: (i) to examine responses in grapevine vessels with tyloses within the first month after pruning; (ii) to document synthesis of defence compounds at the end of the second month after pruning; and (iii) to make preliminary observations of desiccation areas induced 9 months after pruning with four different procedures.

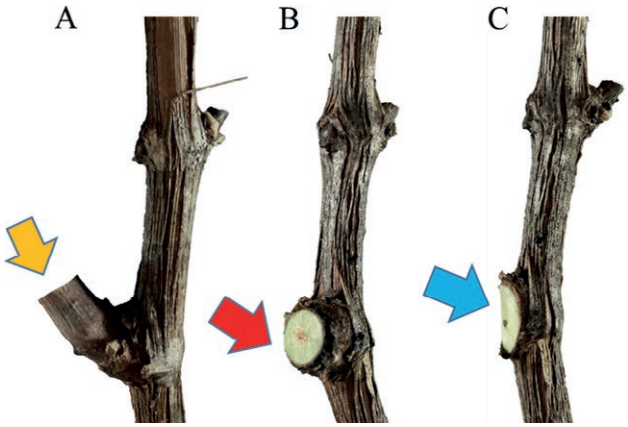

Figure 1. A) Cut on a 1-year-old grapevine cane preserving a short stub (treatment designated 1ySS+BB; see text); B) cut on a 1-year-old cane taking off the short stub (designated 1yNoSS+BB); C) cut on a 1-year-old cane removing the basal bud (designated 1yNoSS-NoBB).

MATERIALS AND METHODS

The vineyard

An experiment was conducted between March and December 2019, in a vineyard of the Azienda Agricola Montepaldi s.r.l., located in the northern part of the Chianti Classico production area of Tuscany (San Casciano in Val di Pesa, Florence, Italy) (43°39'46.8"N, 11°09'16.0"E). The vineyard has a plant density of 5200 plants ha^{-1}, the soil is medium textured, and vineyard was managed under integrated agricultural practices with no artificial irrigation. The selected vines were *V. vinifera* 'Trebbiano Toscano', and were 18-years-old and trained to cordons with spur pruning.

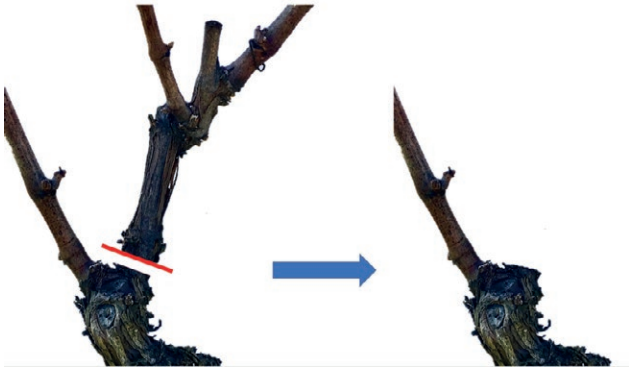

Figure 2. Cut on a 3-year-old grapevine spur taking off the short stub but leaving the basal bud (treatment designated 3yNoSS+BB).

Pruning methods, sampling and trial set-up

To obtain information about how wounds react to pruning, four different pruning methods were applied to grapevines in the trial. These were: i) cuts on 1-year-old canes preserving short stubs of lengths twice the their diameters, and therefore preserving the basal buds, (designated 1ySS+BB; Figure 1A); ii) cuts on 1-year-old canes taking off the short stubs but leaving the basal buds (1yNoSS+BB; Figure 1B); iii) cuts on 1-year-old canes removing short stubs and basal buds (1yNoSS-NoBB; Figure 1C); and iv) cuts on 3-year-old spurs taking off the short stubs but leaving the basal buds (3yNoSS+BB; Figure 2). The mean diameters of 1-year-old canes were 0.8 ± 0.2 cm (wound area ≈ 0.5 ± 0.2 cm^2), and of 3-year-old canes were 2.1 ± 0.1 cm (wound area ≈ 3.5 ± 0.3 cm^2).

Pruning was carried out in March 2019, at the end of the winter season, when the average temperature was 10.5°C. Samplings were carried out either on the same day as pruning (experimental control, T0); or after 1 (T1), 2 (T2), or 9 (T9) months after pruning. Three replicates from different plants (biologically independent) were examined at each sampling time (n = 3), following the methods described by Battiston *et al.* (2022). For each of the four pruning methods (described above), at T0 and T1 the percentages of stem vessels occluded by tyloses were recorded, at T2, light microscopy observations of histological responses were carried out, and at T9, the desiccation areas below the wounds were described.

Histological analyses: sample preparation, chemicals and data collection

Histological studies were carried out on transverse or longitudinal sections (thickness 30–40 μm) made

with a cryo-microtome (Cryo-cut, American Optical), from 1 cm long cane samples. The sections were then mounted on glass microscope slides and stained, following several protocols. Toluidine blue O 0.5% (w/v) in distilled water added sodium carbonate (to give pH = 11.1; Feder and O'Brien, 1968) was used to resolve tissue sections into cell-type components by different colour gradations. Sudan III and IV (Backer, 1946) were used to detect the presence of suberin. Phloroglucinol-HCl (Johansen, 1940) was used to indicate presence of lignin. Tannic and phenolic compounds were stained with Vanillin-HCl (Gardner, 1975). Cellulose and chitin of fungal hyphae were revealed using fluorescence of Calcofluor white staining (Hughes and McCully, 1975).

A Zeiss stereomicroscope equipped with an Optika digital camera and a Leitz D.M.-R.B. Fluo Optic microscope (Wetzler, Germany) equipped with a Nikon DS-Fi3 digital camera were used for qualitative and quantitative analyses of stained tissue samples. Percentage (%) of xylem vessels partially or totally occluded by tyloses (per mm^2 of each tissue section) were determined. For this purpose, three 0.5 cm length grapevine canes were sampled from the different plants receiving each of the four pruning methods. The cut surfaces were observed using a Zeiss stereomicroscope equipped with an Optika digital camera.

Tyloses formation was measured at two different times, either immediately after pruning (T0) or 1 month after pruning (T1), and the data obtained were analyzed as indicated below. Histological observations of defence compounds in the tissues immediately under the cut surfaces were made 2 months after pruning (T2), using light microscopy.

To evaluate the morphological features of the desiccation areas resulting from each pruning method, three 2.5 cm long cane cuttings were sampled at T9. The longitudinal section of each short cutting was photographed using a Canon Power Shot SX100 IS camera.

Trial design and statistical analyses

A completely randomized design was used for the field experiment. For each histological observation, three biologically independent replicates were considered (n = 3). Data distributions were checked using the Kolmogorov-Smirnov test, and homoscedasticity was determined using the Brown-Forsythe test. The percentages of xylem vessels occluded with tyloses were analyzed using one-way ANOVA followed by Tukey's multiple-comparison test ($P < 0.05$) to separate groups of means. Diameters of xylem vessels (occluded and non-occluded) underwent Kruskal-Wallis nonparametric analyses, followed by Dunn's multiple-comparison test ($P < 0.05$) to separate mean ranks. The nonparametric test was chosen because the data distribution was not normal (Kolmogorov-Smirnov test, $P \leq 0.05$). Statistical analyses were carried out using Prism8 (GraphPad Software).

RESULTS

Histological analyses

The overall anatomical regions under the wounded tissue were investigated to describe tissue reaction at different times (T0, T1, T2 and T9) after pruning.

In general, tyloses appeared rapidly within 1 month after pruning cuts had been made, and the tyloses extended up to several mm from the cuts, but there were differences among the pruning methods. Other common responses to multiple stresses such as callose production and cell wall suberification were not detected.

Figure 3 shows transverse sections of the four cut types immediately after cutting (T0) and 1 month later (T1), stained with Toluidine blue O. At T0, in all types of pruning cuts, some vessels already had tyloses at different stages of development. Most of the vessels were partially occluded, and only a small number were totally occluded (Figure 3, A, C, and G). Only vessels from treatment 1yNoSS-NoBB were mostly free from tyloses (Figure 3E).

Plant defence responses included differences in tyloses formation among the four treatments after 1 month. Tyloses development increased particularly from treatment 1yNoSS+BB, where this was increased at T1 (Figure 3D) compared to T0 (Figure 3C). In the 1ySS+BB (Figure 3B) and 3yNoSS+BB (Figure 3H) treatments, the increases in tyloses were less evident compared to 1yNoSS+BB. At T1 from 1yNoSS-NoBB, no appreciable differences were detected compared to T0 (Figure 3F). To support the histological observations, a statistical analysis regarding the numbers of xylem vessels that were partially and totally occluded by tyloses were also assessed, as described below.

Observations of histological responses carried out after 2 months (T2) showed that thin necrotized layers had started to develop below the stem cut surfaces in all the four pruning methods. At the edges of these areas, the host defence responses were investigated using different staining procedures, as shown in Table 1 and in the representative images in Figure 4. Sudan III-IV positive stained tissues were observed in all samples (Figure 4 A), showing suberin deposition only on the cell walls of the cork tissues, but never on the wound surfaces, to protect the living tissues from the exter-

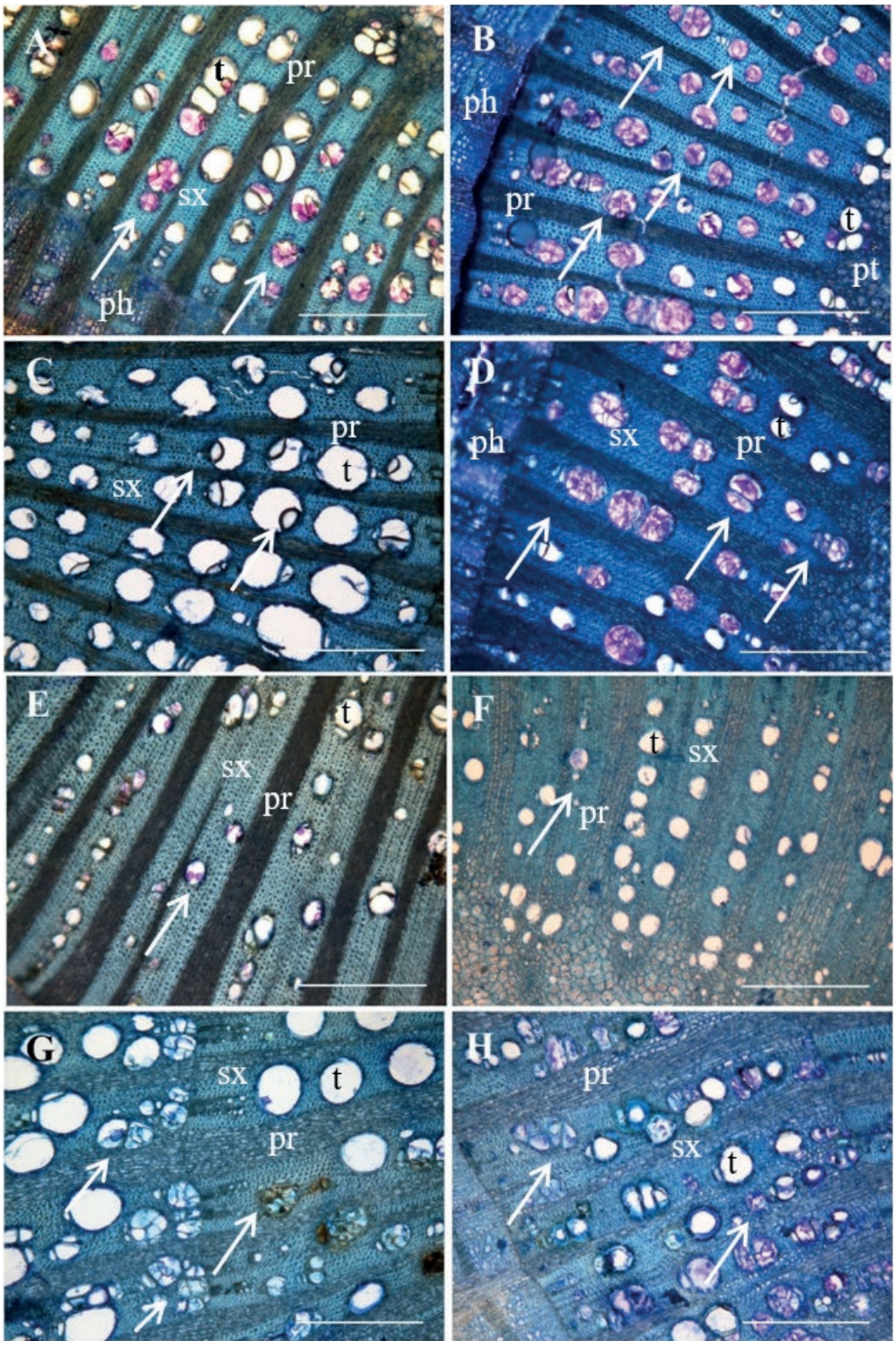

Figure 3. Micrographs of pruned grapevine cane cross sections observed after different pruning methods at different times (T, months) after pruning. (A) is for the pruning method designated (see text) as 1ySS+BB at T0, (C) designated 1yNoSS+BB, (E) designated 1yNoSS-NoBB, and (G) designated 3yNoSS+BB; at T1: (B) is from treatment 1ySS+BB, (D) from treatment 1yNoSS+BB, (F) from treatment 1yNoSS-NoBB, and (H) from treatment 3yNoSS+BB at T1. Sections were stained with Toluidine blue. Scale bars: (A, B, D, E, F, G, H) = 250 μm; (C) = 200 μm. White arrows indicate vessels, t trachea, sx secondary xylem, pt pith, ph phloem, and pr parenchyma rays.

Table 1. Results from different staining methods to show defence mechanisms of defence observed in the four pruning techniques, including (see text) 1ySS+BB, 1yNoSS+BB, 1yNoSS-NoBB or 3yNoSS+BB, and presence of living fungal hyphae in the wounded tissues.

Pruning techniques (see text)	Sudan III-IV for suberin	Phloroglucinol HCl for lignin		Vanillin for tannin	Calcofluor for cellulose and chitin	
		Cortical parenchyma	Tyloses		Plant cell walls	Fungal hyphae
1ySS+BB	-*	++	-	+	+++	+
1yNoSS+BB	-	++	-	+	+++	+
1yNoSS-NoBB	-	++	-	+	+++	+
3yNoSS+BB	-	+++	+	+	++	+

* - = not detected; +, ++, +++ = presence at different levels.

nal stress factors. Although suberin deposition was not detected, other protection mechanisms were observed as physical barriers (Danti *et al*., 2018). For example, lignin deposition was observed onto the cellulose frameworks of the primary walls in the cortical parenchyma. This, in addition to tyloses, produced physical obstacles to pathogen penetration (Sun *et al*., 2006), and is an early response that appears soon after the pruning cuts are made (at T0 and T1). At the boundaries of necrotized regions, lignification (shown from Phloroglucinol-HCl staining), involved longitudinal, continuous layers of parenchyma cells in the cortical cylinders. Thickness of the lignified tissues differed for the different pruning methods (Figure 4, B and C). The most extended and deepest parenchymatic lignified tissues close to the necrotic areas were those formed in treatment 3yNoSS+BB (Figure 4B), and a representative example of a pruning wound in a 1-year-old cane is shown in Figure 4C. The treatment 3yNoSS+BB gave cellular walls modified by lignin deposition in parenchyma, but this thickening process was also observed in tylosis walls (Figure 4D).

In the cortical regions at the borders between dead and living tissues, another defence response was represented by tannin biosynthesis, as a biochemical mechanism for host cell protection in addition to physical barriers created through lignification. Vanillin staining (Figure 4E) showed that tannin accumulation regions were more extended than the lignified areas in parenchyma. Tannin compounds accumulated either in the cell walls or in the vacuoles. Similar tannin production was detected from all the four pruning techniques applied.

Calcofluor reactions confirmed what was shown from Phloroglucinol-HCl treatment, since these are complementary stains. The cellulose components (e.g. in the tylosis walls) were detected in the living cell whereas chitin of fungal hyphae was found in living and dead tissues (Table 1). The experimental treatment 3yNoSS+BB showed dead wood colonized by fungal hyphae (Figure 4F). Thus, in large pruning cuts, the superficial wound tissues (> 2-years-old) underwent dehydration to form large dead areas.

Descriptions of wood anatomy traits

The numbers of partially and totally occluded vessels after the four different pruning cut treatments at T0 were compared to the numbers at T1 (Figure 5). In general, the proportions of occluded vessels increased within 1 month after treatment, for all the types of cuts (Figure 5). Presence of basal buds influenced the plant defence reactions, as these were activated more efficiently in 1yNoSS+BB treatment compared to 1yNoSS-NoBB. The proportions of xylem vessels with tyloses at T1 was close to 93% for 1yNoSS+BB compared to 5% for 1yNoSS-NoBB, which was similar to the proportion (2 %) recorded at T0.

Regardless of time after application of treatments (T0 and T1), 3-year-old grapevine cane samples receiving the 3yNoSS+BB pruning method always had xylem vessels with the greatest diameters (Figure 6).

At T0, mean diameters of occluded and non-occluded vessels followed similar trends, i.e. the vessels showed large diameters in the 3yNoSS+BB treatment, but these were smaller for 1yNoSS-NoBB, where the cuts were at the cane nodes and the basal bud were removed. At T1, influence of the different cane cutting methods was apparent. Among all the 1-year-old canes, more occluded vessels were of bigger sizes. Only in treatment 3yNoSS+BB were the non-occluded (mean = 104 μm) and occluded (mean = 93 μm) vessel diameters closely similar (Figure 6).

Desiccation cones

The stem desiccation cones were evaluated at the end of the experiment (T9), as indicated by necrotized dry zones at the edges of the cut surfaces of the sampled grapevine canes. The means cone sizes were not significantly different ($P > 0.05$), but these indicated deeper necrotic zones were formed in larger than smaller wounds (3yNoSS+BB), and in wounds with no basal buds and no short stubs. Differences were detected in the sizes and morphologies of the desiccation areas, depending on the pruning method. The necrot-

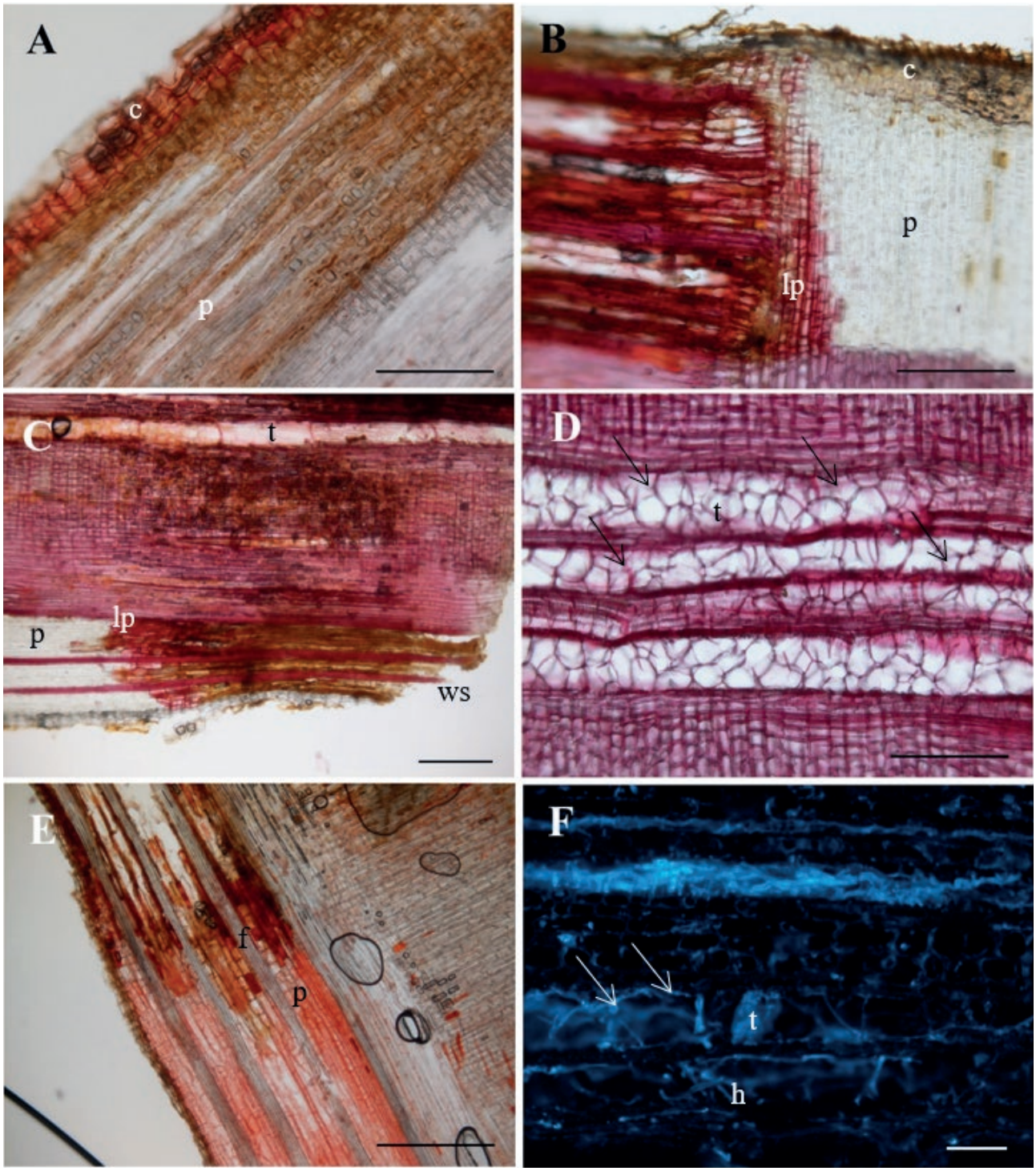

Figure 4. Wound tissues of the pruned grapevine canes, from the cork to the depth of vascular cambium at T2 (see text), as representative images of the four different pruning techniques used in this study. The sections were stained with: (A) Sudan III-IV for suberin; (B) Phloroglucinol-HCl for lignin observed after experimental treatment (see text) in 3yNoSS+BB, and (C) in a 1-year-old cane; (D) Phloroglucinol-HCl staining for lignified cell walls of tyloses; (E) Vanillin staining for tannins as a representative image for all four pruning techniques; (F) Calcofluor staining for cellulose and chitin after treatment 3yNoSS+BB; c = cork tissue; p = parenchymatic tissue; lp = lignified parenchymatic tissue; t = trachea; ws = wound surface; white or black arrows = lignified tyloses; h = fungal hyphae.

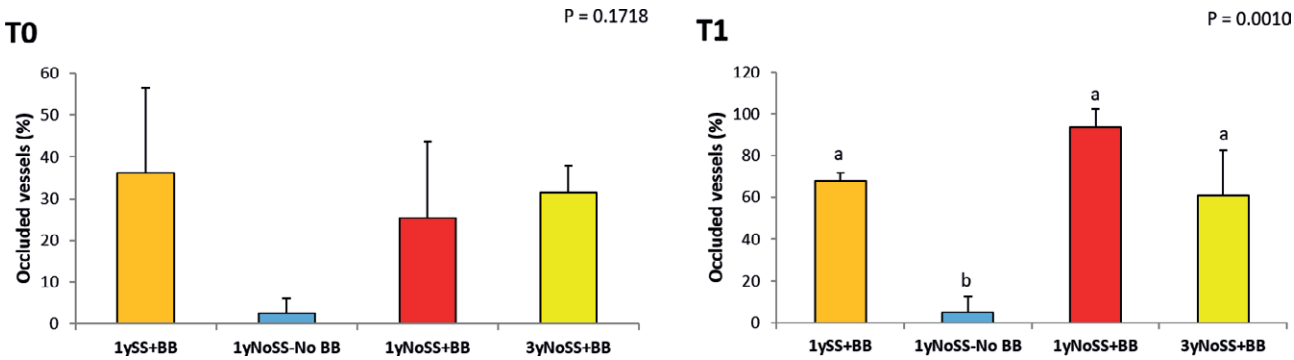

Figure 5. Mean percentages of partially and totally occluded grapevine stem vessels found after four different pruning techniques: 1ySS+BB, 1yNoSS-NoBB, 1yNoSS+BB or 3yNoSS+BB at T0 and T1 (see text). The values are means + standard deviations (n = 3). Results for each time point were analyzed using one-way ANOVA, and groups of means were separated by Tukey's multiple comparison test. Different letters indicate differences (P < 0.05) indicated by post-hoc tests.

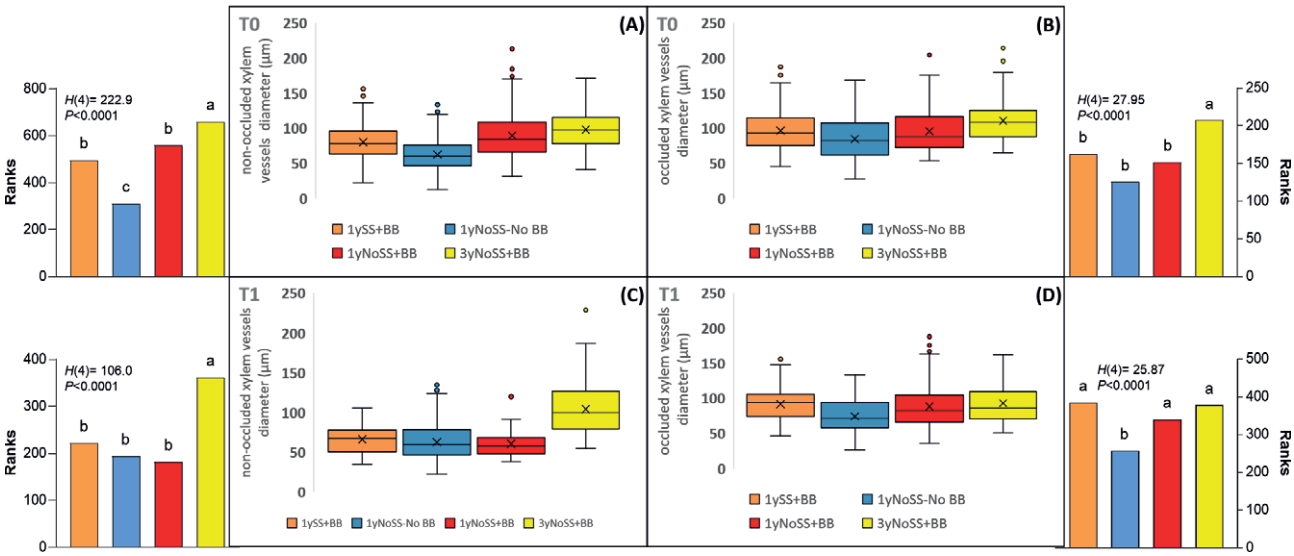

Figure 6. Boxplots (A, B, C and D), and respective mean rank analyses (barchart external panels) of mean diameters (µm) of non-occluded and occluded grapevine xylem vessels. Analyses were conducted before (T0) and one month after pruning (T1), using four different pruning techniques: 1ySS+BB, 1yNoSS-NoBB, 1yNoSS+BB or 3yNoSS+BB. In each boxplot, the Tukey whiskers represent maximum and minimum values (n = 3) excluding outliers (represented by dots). Horizontal black lines inside the boxes are the median values, while the crosses represent the treatment means. Results for each time point for both conditions (occluded and non-occluded vessels) were analyzed using Kruskal-Wallis tests, and mean ranks were separated according to Dunn's multiple comparison tests (P < 0.05). Results of mean ranks are plotted on the barchart external panels. Different letters indicate differences (P < 0.05) indicated by post-hoc tests.

ic areas from treatment 3yNoSS+BB had overall mean depth of 4.1 mm, while treatment 1yNoSS-NoBB gave mean depth of 1.2 mm. In contrast in the 1-year-old canes where the basal buds were maintained (treatments 1ySS+BB and 1yNoSS+BB), the necrotized zones developed a few millimeters below the cut surfaces without producing deep desiccation regions in the tissues around the pith, as was observed from treatments 3yNoSS+BB and 1yNoSS-NoBB.

DISCUSSION AND CONCLUSIONS

In *V. vinifera*, a species with creeping habit, pruning wounds do not heal as in fruit trees, where the stem cambium usually develops new tissues such as callose (Nakashima *et al.*, 2003; Câmpu, 2009; Grünwald *et al.*, 2002; Battiston *et al.*, 2022). Neither does the plant produce suberin in comparison to other trees (Rittinger *et al.*, 1987; Hawkins and Boudet, 1996) to protect the internal living tissues against the external environment.

Different defence mechanisms were adopted by grapevines to seal wounds to prevent the entry of pathogens. The grapevine survival strategy is based on producing new substitution basal shoots, rather than allocating energy to wound healing. The histochemical analysis carried out in the present study has shown the formation of physical barriers to external stressors, consisting of tylosis development in xylem vessels and lignin deposition in living tissues immediately below cut surfaces in grapevine canes.

Biochemical responses of tannins and suberin have been studied as important factors in protecting living tissues (Danti *et al*., 2018; Falsini *et al*., 2022). In the present trials, tannins and suberin highlighted by specific staining reactions were found to be similar in the different pruning cut methods examined. The production of tannins was widely extended in living tissues under the necrotized regions as discussed by other authors also for histopathological studies (Al-Saadoon *et al*., 2012).

The present study has demonstrated that, in 'Trebbiano Toscano' grapevines, winter pruning, applied on 1- or 3-year-old canes at the end of winter (in March) but close to commencement of vegetative growth, caused tissue activation and the rapid development of tyloses, as previously shown by Sun *et al*. (2006) in current year shoots during the growing season. The present study has shown that tylosis initiation was clearly visible 1 month after wounding, with the exception of canes where no basal buds were preserved (treatment 1yNoSS-NoBB). It was anticipated at the onset of this research that, under field conditions, some tyloses were already present at T0 in each type of pruning method, but, at T1, tylosis presence rapidly increased, especially in the samples where the basal buds were retained. Thus, this study allows development an hypothesis that the basal buds are involved in activating the processes of tylosis occlusion of xylem vessels. At T1, the occlusion process increased as from the 1yNoSS+BB treatment where up to 90% of the vessels contained tyloses.

Results from the present study also support, with histological data, observations from previous studies. Faúndez-López *et al*. (2021) showed that pruning cuts over nodes can preserve the basal buds and diaphragms and prevent wood necrosis in the permanent wood structures. Bruez *et al*. (2022) demonstrated that leaving 2–3 cm pruning stubs stopped desiccation cone at the diaphragm, leaving unaffected sap flow. These results were confirmed also by preliminary observations from treatment 1yNoSS-NoBB, where the desiccation areas were deeper than from the 1yNoSS+BB and 1ySS+BB treatments, both of which retained the basal buds. In treatment 3yNoSS+BB, the desiccation cones were even deeper than for 1yNoSS-NoBB, confirming that wounds in older and larger diameter wood than in young canes produces large necrotic areas, as from treatment 1yNoSS+BB, as described by Faúndez-López *et al*. (2021). Thus, comparing 1-year-old samples, we hypothesize that the basal buds on grapevine canes prevent extensive wood necroses. Moreover, comparing the 1yNoSS+BB samples with those from the 3yNoSS+BB treatment (both with basal buds but with different cane ages), the necrotic area depths were influenced by cane age, and thus by wound size.

The light microscopy observations indicated that the more occluded vessels by tyloses were the larger vessels, but no gels were found. Gels entangled between tyloses may increase wound susceptibility to pathogens, by providing substrate for growth and routes to escape occluded vessels (Pouzoulet *et al*., 2017).

Grapevine pruning needs to start with appropriate management of pruning wounds. Even if wound protection reduces pathogen infections (Mounier *et al*., 2016; Di Marco *et al*., 2022), the cutting methods can also influence vine reactions to wounding, and these differences will influence the efficiency and amount of fungal colonization by wood pathogens (Pouzoulet *et al*., 2020, 2022).

Future research should include confirmation of the relationship between fungal colonization from artificial and natural infections in grapevine cuts, applied with different methods, and host histological defensive reactions that impact pathogen colonization and activity. This information could then be used to provide specific guidelines to growers for reducing pathogen entry to, and infection of, their grapevine hosts.

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