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Effect of pruning strategy on 'Syrah' bud necrosis and fruitfulness in Brazilian subtropical Southeast

O. DE ALMEIDA JUNIOR¹), C. R. DE SOUZA²), F. ALCÂNTARA NOVELLI DIAS²), F. DE PAULA FERNANDES²), L. TORREGROSA⁴), C. NORONHA FERNANDES-BRUM³), A. CHALFUN-JUNIOR³), R. VIEIRA DA MOTA²), I. PEREGRINO²) and M. DE ALBUQUERQUE REGINA²)

¹) Coordination of Integral Technical Assistance of São Paulo (CATI), Cajuru, SP, Brazil

²) Agronomical Research Institute of Minas Gerais (EPAMIG), Technological Center of Grape and Wine Research, Caldas, MG, Brazil

³) Federal University of Lavras, Biology Department, Lavras, MG, Brazil

⁴) AGAP, Montpellier University, CIRAD, INRA, Montpellier SupAgro, Montpellier, France

Summary

The change of wine grape harvest from wet season (summer) to dry season (winter) by changing the pruning management has improved quality of wines produced in the Brazilian Southeast. However, the vines need to be spur pruned twice a year, *i.e.* with a 1st pruning in August (winter pruning) for a vegetative cycle during the hot and wet summer, and a 2nd pruning in January (summer pruning) for a productive cycle during the cold and dry season. This double pruning strategy is made necessary by the fact that latent buds developed during the dry season cycle are not fruitful to support a productive cycle in the following year. This histological study, performed in the South of Minas Gerais State (Brazil), showed that annual single pruning done in the wet season (in January) displayed a high rate of necrosis on primary and secondary buds (bud necrosis – BN). In April, 99 days after summer pruning (DASP), the rates of BN were 40 % and 50 % at basal and apical node positions, respectively, reaching 80 % of BN in December (322 DASP). As a consequence of BN, bud potential fertility was drastically reduced from 0.5 inflorescence primordial (IP) per bud (in July) to 0.06 (in December) and bud burst in the next cycle from secondary and tertiary bud axes. Vines managed by double pruning system (submitted to summer and winter pruning) displayed a much higher fruitfulness potential, *i.e.* 1.46 IP per bud in December (112 days after winter pruning) and limited BN occurrence (20 %). On single pruned vines, we also observed a significant decrease of starch content in canes, trunks and roots. Internal bud anatomy showed that a random cell breakdown started 70 days DASP. At 211 DASP, all buds showed a large starch granule concentration, raphides and crystals of calcium oxalate inside idioblasts of leaf primordia and also in cortical parenchyma of the vegetative axis. The bud starch content was increased and a positive correlation between necrosis and starch accumulation was observed. The impact of carbohydrate availability on bud necrosis development was discussed.

This study showed that the necrosis development towards secondary and tertiary axis of the dry season buds is the main reason of unfruitfulness in the vineyards managed by single pruning in the wet season, making the double pruning compulsory.

Key words: *Vitis vinifera*; bud necrosis; starch; pruning management; fruitfulness; bud anatomy.

Introduction

In the Southeast of Brazil, the winter grape harvest has improved the wine quality. The synthesis of sugar and phenolic compounds in berries are increased by sunny days, low rainfall and high thermal amplitude during the dry season as compared to grape ripening under rainy summer cycle (MOTA *et al.* 2010, FAVERO *et al.* 2011). As illustrated in Fig. 1, to change the date of harvest from rainy summer

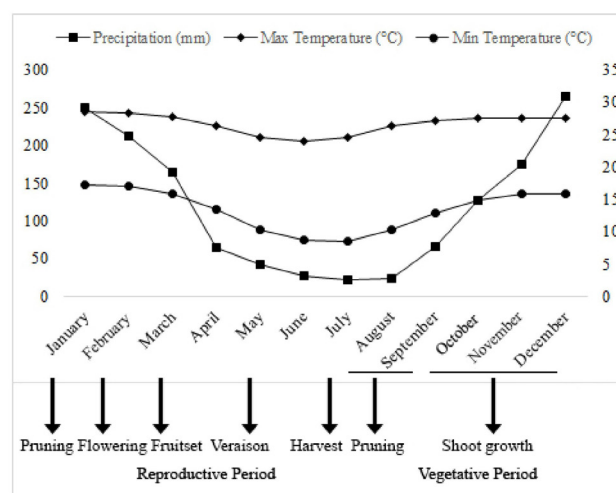


Fig. 1: Annual rainfall, maximum and minimum temperatures and main phenological stages of 'Syrah' growing under double pruning management in Brazilian Southeast. Meteorological data are 30-year average (Source: www.climate-data.org).

Correspondence to: Dr. C. R. DE SOUZA, Agronomical Research Institute of Minas Gerais (EPAMIG), Technological Center of Grape and Wine Research, C.P. 33, 37780000 Caldas, MG, Brazil. E-mail: crsouza@epamig.br

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(January) to dry winter (July and August), vine growers perform two annual prunings, a management so called double pruning method (REGINA *et al.* 2011, FAVERO *et al.* 2011). The productive cycle starts with a pruning done in January (or February) to allow grape ripening during the autumn – winter season (from May to August), then vines are spur pruned again one month after winter harvest (August or September) to perform a vegetative cycle where all clusters are removed (REGINA *et al.* 2011). As also shown in Fig. 1, although the flowering stage (February) and fruit set (March) occurs during high precipitation and temperature, the grape ripening period occurs during autumn – winter under dry and cool conditions.

This vineyard management has considerably improved the grape quality for wine production under tropical and subtropical climatic conditions of the Brazilian Southeast (MOTA *et al.* 2011, REGINA *et al.* 2011, DIAS *et al.* 2012, 2017). Although the winter harvest has opened a new possibility for high quality wine production in Brazil (CARBONNEAU 2010) the vineyards production costs have increased due to the two growing cycles performed per year for only one harvest. First attempts to lighten the management by removing winter pruning were unsuccessful due the low fertility of shoots arising from buds set up during the previous first semester (DE Souza *et al.* 2015). This unexpected bud infertility was attributed to a possible reduction in starch contents in canes, trunks and roots, due to apical shoots development at spring.

In other climatic contexts, bud fruitfulness variations have been sometimes associated to the occurrence of a physiological disorder known as primary bud necrosis (BN). Primary bud necrosis corresponds to the browning of the primary axis of the latent bud (COLLINS *et al.* 2006, VASCONCELOS *et al.* 2009, COX *et al.* 2012). The necrosis can also extend to secondary axes of the latent buds (COLLINS *et al.* 2006, KAVOOSI *et al.* 2013). The reasons of BN remain unclear. Several studies suggested that BN could result from high scion vigor and yield (DRY and COOMBE 1994, VASUDEVAN *et al.* 1998, COLLINS and RAWNSLEY 2005, VASCONCELOS *et al.* 2009, KAVOOSI *et al.* 2013), unbalance between growth inhibitors and promoters (NAITO *et al.* 1985, 1987, 1989, WOLF and WARREN 1995, ZIV *et al.* 1981, COLLINS and RAWNSLEY 2008), high temperatures and vigor rootstocks (COX *et al.* 2012), bad microclimate (PEREZ and KLIEWER 1990, VASUDEVAN *et al.* 1998, COX *et al.* 2012) or low carbohydrate levels (VASUDEVAN *et al.* 1998). However, the direct effect of carbohydrates on BN development is poorly documented. Although there is a positive correlation between starch content in the wood at bud break and grapevine fruitfulness (BENNETT *et al.* 2002, VASCONCELOS *et al.* 2009), the relationship between necrosis incidence and carbohydrate levels during latent bud development has not been much investigated. Carbohydrates are the main source of energy for bud metabolism (LEBON *et al.* 2008) therefore they might be related to necrosis development under bud low sugar availability as suggested by VASUDEVAN *et al.* (1998). Sucrose reduction in buds of Japanese pear was also found related to the incidence of floral primordia necrosis (HORIKOSHI *et al.* 2017). In Brazil, the occurrence of grapevine bud necrosis and its potential relationship with carbohydrate accumulation and bud fruitfulness was never

demonstrated. This present study investigated the reasons for grapevine bud unfruitfulness managed by annual single summer pruning as previously observed by DE SOUZA *et al.* (2015). In the first part of the study, a comparative investigation was undertaken in a 'Syrah' vineyard under double and single pruning management to examine the occurrence of bud necrosis and fruitfulness. The second part shows a detailed anatomical analysis of latent buds development during the autumn-winter season in order to describe the progression of this physiological disorder. Furthermore, the relationship between starch levels in latent bud and necrosis incidence was also investigated and discussed.

Material and Methods

Experimental site and pruning management: The study was carried out during two years (2015 and 2017) in a commercial vineyard located in Três Corações, in the south of Minas Gerais State (21°41'S e 45°15'W, 900 m altitude) in the Southeast of Brazil. According to ALVARES *et al.* (2013) the climate is classified as Cwa (rainy and hot summer and mild and dry winter). The vineyard was planted in 2003 with 'Syrah' (clone 174 ENTAV-INRA) grafted onto '1103 Paulsen'. Vines were spaced 1.5 m between vines and 2.5 m between rows, trained on a vertical shoot position (VSP), north-south oriented and spur pruned with three regular buds.

In order to investigate the histological reasons of grape clusters absence in vineyard under single pruning management two independent experiments, described below, were carried out in the same vineyard, but in different years. In 2015, the single pruning was compared to double pruning management. The relationship between necrosis development and starch accumulation in latent buds of 'Syrah' growing during the dry season was investigated in 2017.

Experiment 1. Effect of pruning on 'Syrah' bud necrosis and fruitfulness: In the single pruning treatment (SP), the vines were pruned only in the wet season, in January 2015. In the double pruning treatment (DP), the vines were pruned in the wet season (January 2015) and dry season (August 2015). In January 2016, both treatments were submitted again to yield summer pruning to induce the productive cycle during the dry season. In the SP treatment, the anatomical analyses of latent buds were performed monthly performed from March, during the fruitset (45 d after summer pruning – DASP) at stage E-L 27 (COOMBE 1995), to December (335 DASP). At each sampling date, latent buds (rank 1 to 10) from 6 shoots were observed with a stereomicroscope (Stemi 2000-C; Zeiss, Jena, Germany) to quantify the number of inflorescence primordia (IP) per primary bud axis and to score necrosis presence on primary and secondary buds (buds necrosis – BN). Each shoot was divided into three sections according to bud position: a basal section (1 to 3), medium section (4 to 6) and apical section (7 to 10). In DP treatment, anatomical analyses were only done in December 112 d after winter pruning (DAWP). In March 2016 (49 DASP), the axis origin (primary, secondary or tertiary bud axes) of the developed shoots were recorded according to the phyllotaxis

of the previous year (GERRATH and POSLUSZNY 2007). Shoots originated from primary axis show plan of phyllotaxy similar to the previous year. Shoots from secondary axis show a perpendicular plan of phyllotaxy to the previous year. Although shoots from tertiary axis also show similar plan of phyllotaxy to the previous year, they are unfruitfulness and bifurcated.

The starch concentration was assessed on dried and powdered samples of shoot, trunk and root taken from six grapevines only from single pruning treatment after winter harvest (August 2015; 200 DASP) and before yield pruning in the wet season (January 2016; 370 DASP). Shoot samples were collected from the first internode per vine (two samples per vine). The trunk samples were collected using a 5-mm drill bit. For each vine, holes were drilled at three positions per vine (one per arm and one from the middle of the trunk). The lateral root samples (around 2-5mm diameter) were taken at 20 cm distance from the trunk of each vine (around 30 cm depth). Samples of five roots per plant were collected from six grapevines. All samples were oven dried and stored until analysis. The starch was extracted from 100 mg sample with 80 % (v/v) ethanol (80 °C, 20 min) and centrifuged (9,160 x g, 15 min). This process was repeated three times. The extracted pellet was dried overnight at room temperature and was hydrolyzed through incubation at 75 °C for 1 h with Termamyl® 120 L (diluted 1:500 in water), followed by incubation at 50 °C for 1 hour with Amyloglucosidase 300 L (28 unit·mL⁻¹, in sodium acetate buffer, pH 4.8). The starch content was quantified from released glucose by colorimetry at 450 nm using glucose oxidase/peroxidase/ABTS assay (BERGMEYER 1974). Starch content was calculated as glucose multiplied by conversion factor of 0.9 (CORDENUNSI and LAJOLO 1995). The data collected were tabulated and the results were presented with mean incidence and standard deviation.

Experiment 2. Necrosis development and starch accumulation in latent buds of 'Syrah' growing during the dry season (autumn-winter seasons): In 2017, a detailed bud anatomy analysis in light microscopy was done during autumn – winter cycle in the same vineyard submitted to double pruning management as usually done in this region. Latent buds of ranks 1 to 3 were randomly collected and fixed in 70 % ethanol (v/v). Bud samples were submitted to alcohol dehydration series (80, 90, and 99.5 %) during 2 h in each ethanol concentration (JOHANSEN 1940). Samples were infiltrated in a 1:1 mixture of historesin (Leica, Heidelberg) and ethanol (v/v in 50 % historesin and 50 % ethanol) during 24 h, followed by infiltration in pure historesin during 48 h according to manufacturer's instructions. After infiltration, samples were put in molds containing resin hardener. Embedded buds were longitudinally sectioned in 7 µm of slices using a Lupetec microtome. The sections were stained with 1 % toluidine blue pH 4.7 (FEDER and O'BRIEN 1968) and the micrographs were taken using a Canon PowerShot A620 8.0 megapixels attached to light microscopy (Ken a Vision TT118 at 40, 100 and 200x magnifications).

The starch concentration was also quantified for equivalent buds in order to investigate the correlation between carbohydrate and necrosis development during the dry

season. In each vine six buds were sampled (three buds per shoot). Nine vines were monthly sampled from March to August on the same day of the sampling used for histological analyses. The starch extraction was done in dried and powdered buds following the same procedure as detailed in experiment 1. The statistical analyses were performed using SISVAR software (FERREIRA 2011). The necrosis incidence data were transformed ($Y + 1.0 - \text{SQRT}(Y + 1.0)$) to meet the ANOVA assumptions. Significant differences ($p < 0.05$) were analyzed by linear regression or Tukey's HSD according to the variables.

Results and Discussion

Experiment 1. Unfruitfulness and necrosis of latent buds under annual single pruning: DE SOUZA *et al* (2015) showed that to harvest wine grape during the dry winter season the vineyards need to be submitted to annual double pruning management. According to these authors, there was no grape production during the dry season if yield pruning in the wet season (January) is done on one-year-old shoots originating from the previous wet season. In the present study, the histological bud analysis showed that bud necrosis (BN) is the main reason for bud unfruitfulness of vines managed by annual single pruning (Fig. 2). The BN appeared from 89 DASP with 40 % and 50 % necrosis at basal and apical node positions, respectively (Fig. 3A). The necrosis also reached the secondary bud axis where 20 % and 12 % at the basal and apical node position, respectively, were also found necrotic (data not shown).

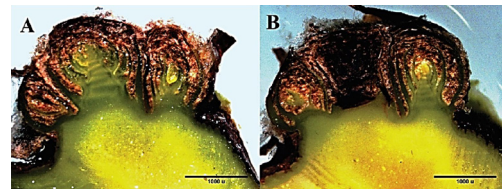


Fig. 2: Cross section of latent bud of 'Syrah' grapevines. Healthy bud (A) and primary bud necrosis (BN) (B). Scale in µm.

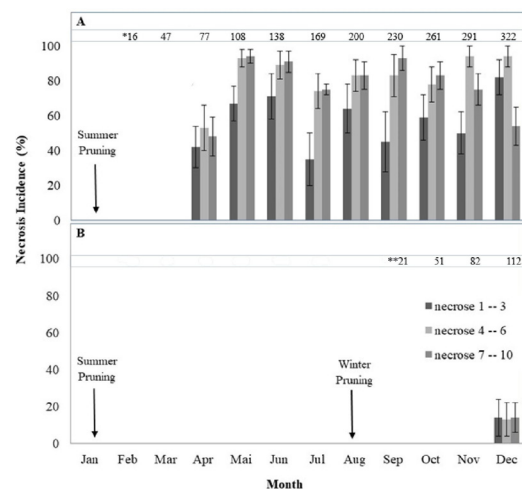


Fig. 3: Bud necrosis incidence (%) in primary axis (according to the node position) in single pruning (A) and double pruning treatment (B) during 2015. *Days after summer pruning; **Days after winter pruning.

Although BN was firstly observed at the beginning of berry development of autumn-winter cycle, the highest BN incidence (higher than 80 % in first six node positions) occurred at the end of the year (December, 322 DASP) in the annual single pruned vines (with no dry pruning). In contrast, on the same date, the BN incidence in double pruned vines (submitted to wet and dry pruning) was lower than 20 % in primary axis from all node positions whereas the secondary axis did not show any significant necrosis (Fig. 3B).

Despite the high level of BN in single pruned vines, in the middle of the year (July), there was still some bud fruitfulness potential (0.5 IP per primary bud axis) estimated by inflorescence primordia per primary axis (Tab. 1). This is in agreement with previous observations by FAVERO *et al.* (2011) who reported a real fruitfulness (number of grape cluster per shoot) of 0.9 for 'Syrah' pruned in winter. This explains why when a second growing cycle is launched by winter pruning, grapes develop during the summer. However, if winter pruning is not performed bud necrosis evolves during the rest period of the second semester. We have shown that in December, just before summer pruning, the potential bud fruitfulness was drastically reduced (0.1 IP per primary bud axis) in single pruned vines due to the high level of BN (Tab. 1). When DP is performed, the new developed buds did not display a significant BN and exhibited a regular bud potential fruitfulness (1.3 IP per primary bud axis).

Table 1

Potential fruitfulness of 'Syrah' grapevines under single and double pruning management in July and December of 2015. Values are the mean ± SE.

	Potencial Fruiffulness	
	July	December
Single Pruning	0.5 ± 0.12	0.1 ± 0.06
Double Pruning	-	1.3 ± 0.23

It is well documented that most grapes of proleptic shoots originate from inflorescence primordia of primary bud axis with a marginal contribution of secondary and tertiary bud axes (SRINIVASAN and MULLINS 1981, VASCONCELOS *et al.* 2009, LI-MALLET *et al.* 2016). In 2016, 42 DASP it was observed that most proleptic shoots of annual SP vines originated from secondary (48 %) and tertiary axes (30 %) whereas 17 % of sampled latent buds were fully necrotic (Fig. 4). Furthermore, most of the shoots originating from tertiary axes showed bifurcation indicating an abnormal growth pattern.

According to these results, the high necrosis incidence on cells of primary and secondary axis explains why a second pruning in winter is needed to regenerate fruitful buds

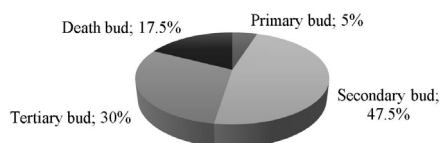


Fig. 4: Origin of the main shoots according to the plan of phyllotaxy of grapevines under annual single pruning. The evaluation was done during bloom time (49 DASP) in 2016.

as shown by DE SOUZA *et al.* (2015). This study showed for the first time the complete unfruitfulness of latent buds due to the necrosis on one-year-old shoots. Several studies have shown only a partial reduction in fruitfulness caused by death of the primary axis from grapevines submitted to annual dry season pruning, as usually done under temperate climates. The primary bud necrosis has been studied in a range of varieties such as 'Riesling' and 'Viognier' in Virginia, USA (WOLF and WARREN 1995, 2000), 'Shiraz' in Australia (COLLINS *et al.* 2006), 'Askari' in Iran (KAVOOSI *et al.* 2013), 'Riesling', 'Viognier', 'Shiraz' and 'Chardonnay' in New York USA (VASUDEVAN *et al.* 1998), 'Queen of Vineyard' in Israel (LAVEE *et al.* 1981) 'Thompson Seedless' in Chile (PEREZ and KLIEWER 1990) and 'Kyoho' in Japan (NAITO *et al.* 1986). In temperate climate, early bud development is prevented by the low temperatures (ecodormancy) which also reduce the respiration of carbohydrates. In tropical and subtropical climates, vines managed by single summer pruning resumed growth from apical buds in the next spring. Due to acrotony, basal buds are inhibited which makes it possible to postpone their development to the next year. On the other hand, the DP vines resumed their normal growing cycle from spur pruned shoots as usually occurs in traditional viticulture. In SP vines, the growth of apical shoots in spring and summer which can represent around 2.5 kg of fresh weight per vine (data not shown) corresponds to a huge cost in term of vegetative biomass loss. The growth of these shoots is largely at the expense of starch from permanent vegetative structure (Tab. 2). Indeed, we have observed a significant reduction of root and shoot starch from August 2015 to January 2016 whereas the trunk starch was unaltered. Furthermore, the SP management reduces the starch content in grapevines as compared to double pruned vines as already shown by DE SOUZA *et al.* (2015). This SP effect on starch decrease could be also attributed to carbon assimilation reduction due to leaf senescence. On the other hand, the new leaf area and cluster removal practice in grapevines under DP management may have favored the starch accumulation during the vegetative cycle in the wet season. It is well known, that carbohydrate levels play a key role in the grapevine reproduction (LEBON *et al.* 2008, VASCONCELOS *et al.* 2009) and it may be a contributing factor for necrosis incidence (VADUSEVAN *et al.* 1998, HORIKOSHI *et al.* 2017). Probably the starch reduction on permanent structures induced by resuming vegetative growth of apical shoots and leaf senescence may have contributed to increase the necrosis severity at 322

Table 2

Starch content in shoot, trunk and roots of 'Syrah' submitted to single pruning management. The sample tissues were sampled in August 2015 and January 2016. Values are the mean ± SE. Different letter suffixes in the lines show statistically significant differences ($p < 0.05$). Each point is the mean of six replicates.

Sample	Starch (mg·g DW ⁻¹)	
	28/05/2015	21/01/2016
Trunk	126.01 ± 10.21 a	102.43 ± 8.69 a
Root	183.67 ± 24.50 a	129.62 ± 24.51 b
Shoots	67.81 ± 1.19 a	49.40 ± 2.27 b

DASP in latent buds of vines managed by SP in the wet season. Based on these results, a second study was carried out during the 2017 growing period in the dry season, in order to have a better understanding of bud necrosis development and its correlation with carbohydrate contents.

Experiment 2. Histological study of necrosis progression and starch storage in latent buds during dry season (autumn – winter): In 2017, the longitudinal bud sections from developed shoots after summer pruning revealed that the beginning of necrotic zones could be found at apical meristems and also at the basal zone between the second and fourth prophylls (Fig. 5A). The necrosis location appeared to be related to the bud age. Buds sampled in March and April (data not shown) showed a beginning of necrosis at apical and basal regions whereas buds collected from May onward showed tissue distortion at the central zone of the preformed bud axis, at the base of leaf primordia. Several authors also have revealed that cell breakdown due to necrosis appeared to have a random distribution (VASUDEVAN *et al.* 1998, COLLINS *et al.* 2006, KAVOOSI *et al.* 2013).

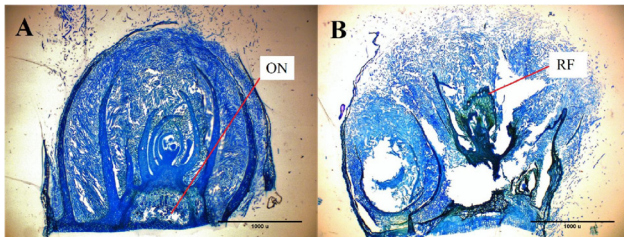


Fig. 5: Longitudinal section of dormant buds showing early development of primary bud necrosis (BN) on 70 DASP in 2017. Onset of necrosis (ON) at the basal zone and healthy tissues at the upper layers (A) and advanced necrosis stage with presence of raphides (RF) (B). Scale in μm .

At 70 DASP, the necrosis reached the cortical parenchyma at the base of the vegetative axis creating a scission between the base and the apex of the primary latent axis (Fig. 5A) as observed in previous studies (WOLF and WARREN 1995). Under high magnification, it was possible to observe some layers of cells surrounding the necrotic area (Fig. 6A and B). Healthy tissues showed approximately isodiametric cells, typical of undifferentiated young tissues. The first cell layer had abnormal features with small, deformed and compressed cells that presented thicker cell walls, suggesting a type of protection zone against the expansion of the necrotic area. The cell lysis area was involved by the zone of irregular cells containing rests of distorted cell membranes with remaining walls, similar to cellular apoptosis usually observed in programmed cell death (PCD) (LEVINE *et al.* 1996). However, it was not possible to confirm this hypothesis because these slides were prepared per section, and the widespread and extensive distribution in the tissues confirms the occurrence of necrosis rather than PCD. Fig. 6B shows the necrotic zone with complete cell breakdown containing distorted and broken cell walls as also observed by some authors (COLLINS *et al.* 2006, KAVOOSI *et al.* 2013). In some cases, the necrosis occurred at leaf primordia (Fig. 6C) as also related by COLLINS *et al.* (2006). When necrosis began in the cortical parenchyma at the base of the leaf primordia,

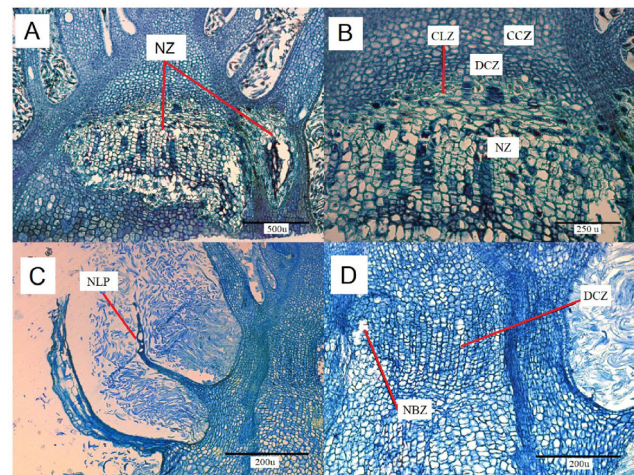


Fig. 6: Longitudinal section of latent bud at 117 DASP in 2017. Beginning of the necrotic zones in the cortical parenchyma and at the leaflet base. (A) Necrosis zone inside bud cortex parenchyma (NZ). (B) Cell lysis zones (CLZ), deformed cell zone (DCZ), compressed cell zone (CCZ), necrotic zone (NZ). (C) Necrosis on leaf primordial (NLP). (D) Onset of necrosis and formation of the zone of deformed cells throughout the internode (NBZ), deformed cell zone (DCZ). Scale in μm .

the deformed zone progressed towards the internode forming a transverse band of cells with irregular walls (Fig. 6D).

Fig. 7A and B show a layer of healing cells used to isolate the damaged tissue from the basal part of the bud. These healing cells were formed after cellular death, in contrast with abscission layer during leaf senescence. At 211 DASP, all analyzed dissected buds showed large starch granules concentration, raphides and crystals of calcium oxalate confined inside of idioblasts (Fig. 8), located in leaf primordia and also in cortical parenchyma of the vegetative axis, as also demonstrated by COLLINS *et al.* (2006). These raphides were also easily found in the necrotic zones, because they are hardly degraded (Fig. 5B).

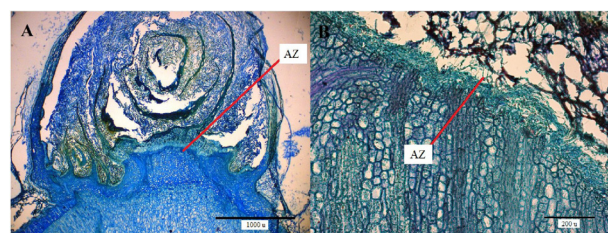


Fig. 7: Longitudinal section of latent bud at 211 DASP in 2017. Necrotic meristematic apex and formation of the abscission zone (AZ) (A). Abscission zone composed by compressed cells and thick cell walls (B). Scale in μm .

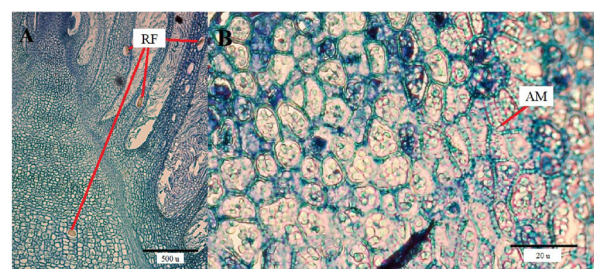


Fig. 8: Cross section of the vegetative axis of the primary bud at 211 DASP in 2017. Idioblasts with raphide (RF) (A); and amyloplasts (AM) (B).

At the basal part of the bud, there was a formation of tissues with striated appearance due to thick cell walls (Fig. 9). The absence of vascular bundles suggests that all transport of water and nutrients occurs by symplast and apoplast pathways.

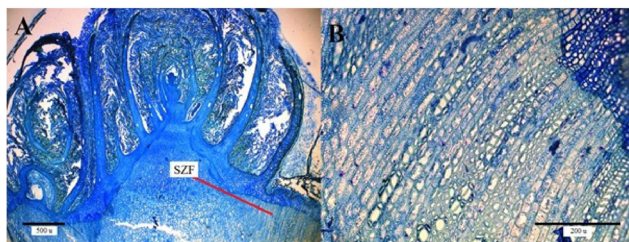


Fig. 9: Cross section of the vegetative axis of the primary bud at 211 DASP in 2017. Striated zone formation (SZF) (A) and (B); Scale in μm .

During the flowering period (70 DASP) 10 % of sampled buds showed the necrosis only in the primary axis (Fig. 10B). In August, at the end of the experiment, the primary axis was necrotic in 54 % of sampled buds in the 1-3 node position sampled. Stored carbohydrates are thought to have a strong influence on the differentiation of inflorescences in young buds (LEBON *et al.* 2008). Although the necrosis incidence has been correlated to low carbohydrate availability in the buds (VASUDEVAN *et al.* 1998) and in the permanent structure (COLLINS and RAWNSLEY 2005, COX *et al.* 2012), our results showed an increase in bud starch (Fig. 10A) throughout time and a high positive correlation ($R = 0.72$) between necrosis and starch storage was found (Fig. 11).

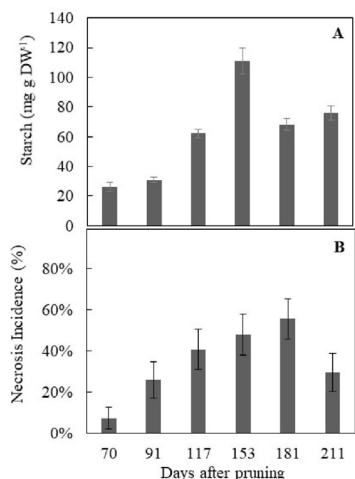


Fig. 10: Seasonal evolution of starch content (A) and necrosis incidence (B) and in 'Syrah' latent buds during reproductive cycle of 2017.

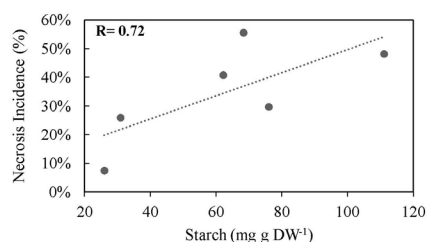


Fig. 11: Relationship between starch content and necrosis incidence in latent buds of 'Syrah' grapevines during reproductive cycle of 2017.

In addition, the histological sections also showed a large amount of starch granules in buds collected at 211 DASP (Fig. 8B). Probably the bud starch content was increased due to dormancy entrance induced by low temperatures, which is common in this region during autumn – winter season (ripening period). In this region, the average of minimum temperature frequently recorded from June to August is around 8 °C (Fig. 1).

Usually, the development of the endodormancy in grapevine buds is associated with the accumulation of starch inside buds, probably due to low respiratory activity (LAVEE and MAY 1997). Recently, in Chile, some authors have also observed an intense starch accumulation in 'Thompson Seedless' buds sampled from the end of summer until winter (RUBIO *et al.* 2015, 2019). In Spain, FADON *et al.* (2018) also observed that an increase of starch content in sweet cherry flower buds occurred concomitantly with the beginning of chilling accumulation. In our study, the observed starch increment in dormant buds could be associated to a possible reduction in soluble sugar availability. Restriction on soluble sugar availability to be used as source of carbon and energy for bud metabolic activity could lead to cell breakdown and necrosis. Low soluble sugar supply in latent buds could have decreased the cell wall deposition leading its hydrolysis as also suggested by COLLINS *et al.* (2006). HORIKOSHI *et al.* (2017) also associated low sucrose concentration in buds to incidence of floral primordia necrosis in Japanese pear flower buds. Furthermore, low soluble sugar and high starch concentration could be observed during the onset of endodormancy in buds of Superior Seedless (BEN MOHAMED *et al.* 2010 and 2012).

It is important to take into account that low availability of soluble sugar induced by endodormancy probably was more accentuated due to the presence of grape clusters during the winter growing season. During the ripening period, grapes are stronger sinks for photoassimilates than latent buds (VASCONCELOS *et al.* 2009). The high photoassimilates competition between buds and grape clusters could have contributed to increase the necrosis incidence during the ripening period in winter. Unfortunately, whether latent bud necrosis is triggered by low soluble sugar could not be confirmed in this present study. However, this hypothesis may be supported by the fact that, even under winter freezing temperatures in another vineyards in the same region, there is no bud necrosis occurrence in vineyard submitted to double pruning management but with no grape production (unfruitfulness vineyard) during the dry winter season (unpubl. data). These data suggest that absence of strong sinks such as grape clusters during latent bud development decreases photoassimilates competition and increases the sugar availability for bud cell metabolism. The soluble sugar availability hypothesis might also be used to explain the necrosis occurrence at the beginning of bud development even under low starch content (70 DASP) (Fig. 11). During this period, the reduction in luminosity due to high precipitation (Fig. 1) could also increase the carbohydrates competition between latent buds and inflorescences development leading to an insufficient sugar supply to attend bud metabolic activity. Bud anatomy revealed why the number of clusters per shoot (real fruitfulness) during the growing

cycle in the wet season (after dry season pruning) is lower as compared to grape cluster production during the dry season (after wet season pruning), as already observed by FAVERO *et al.* (2011). The reasons of this partial fruitfulness reduction after winter pruning was attributed by those authors to the bad environmental conditions (rainy and cloudy months) occurring during the first stages of latent bud differentiation (from January to April). It was reported in grapevine that light exposure and temperature could affect bud fruitfulness (SÁNCHEZ and DOKOOZLIAN 2005). This study showed that, in our experimental conditions, low bud fruitfulness of SP vines do not result from a deficit of IP but rather from later bud necrosis. Our data suggest that, beyond climatic factors, the changes on carbohydrate levels and pruning management may contribute to primary bud necrosis. In our current knowledge, these observations explain why a double pruning strategy remains essential to ensure a sufficient production cycle during the winter in subtropical climates.

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