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ORIGINAL RESEARCH ARTICLE

Assessment of bud fruitfulness of three grapevine varieties grown in northwest Portugal

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ABSTRACT

Assessing bud fruitfulness (fertility) before budburst can provide useful information for determining the yield potential for the following growing season and allows the number of buds per vine to be adjusted according to the productive potential of each season. The present study aims to assess bud fruitfulness and the incidence of necrotic buds using different analysis techniques in three spur pruned (cordon) grapevine white varieties planted in the Vinhos Verdes Demarcated Region (VVDR), NW Portugal. The first two dormant buds and cane samples were collected before winter pruning in December 2016 and 2017 from three (*Vitis vinifera* L.) varieties (Alvarinho, Fernão-Pires and Loureiro) in two different VVDR sub-regions. The fruitfulness and the incidence of bud necrosis were determined using anatomical bud analysis and by forcing bud growth under controlled environmental conditions. Additional analyses were performed to determine the total soluble sugars and starch in cane samples on node and internode regions. There were significant differences in fruitfulness among varieties over the two growing seasons (2016/2017 and 2017/2018). The Fernão-Pires variety showed the highest bud fruitfulness for the two first buds of the three varieties. Total soluble sugars and starch content were influenced by the site, variety and position on the cane. A year-to-year variation was observed, with a decrease in bud necrosis and an increase in the fruitfulness indices and carbohydrates content from 2016/2017 to 2017/2018. To our knowledge, no previous studies combining anatomical and forced bud growth techniques have been carried out on these three white varieties.

KEYWORDS: bud fertility, bud necrosis, carbohydrate reserves, inflorescence primordia, *Vitis vinifera* L.

INTRODUCTION

In temperate climates, grapevine reproductive development is a long and complex process that extends over two growing seasons, starting with the inflorescence primordia differentiation in the developing latent buds during the previous year (primary bud or R2 meristems R2 and secondary buds or R3 meristems) (Torregrosa *et al*., 2021). The formation of the yield component continues with the growth of the inflorescences, the differentiation of the flowers, flower fecundation, berry set and berry development in the next growing season (Vasconcelos *et al*., 2009; Guilpart *et al*., 2014; Keller, 2015). It comprises a sequence of morphological, biochemical and physiological events influenced by environmental and endogenous factors, which have an important influence on fruitfulness and hence yield (Srinivasan and Mullins, 1981; Vasconcelos *et al*., 2009; Monteiro *et al*., 2021). Temperature, light, water status, carbohydrate reserves, genetic and hormonal balance are all factors which influence the formation of the reproductive structure over the two growing years (Srinivasan and Mullins, 1981; Vasconcelos *et al*., 2009; Li-Mallet *et al*., 2016).

Bud fruitfulness, expressed by the number of inflorescence primordia per winter bud, represents the first measure of the yield components for the following season (Dry, 2000; Ferrer *et al*., 2004; Guilpart *et al*., 2014; Collins *et al*., 2020). However, it is not a constant value, as it depends on numerous factors, namely: variety, bud type (primary or secondary) and bud position in the cane, climate and vine physiology influence on the differentiation of the inflorescence primordia (Magalhães, 2015). An evaluation of bud fruitfulness can thus provide useful information for determining yield potential for the next growing season (May and Antcliff, 1973; Dry, 2000; Meneguzzi *et al*., 2020; Monteiro *et al*., 2021). The number of inflorescences, and therefore clusters per vine, can be responsible for about 60 % of seasonal yield variation, while the number of berries for approximately 30 % (Clingeleffer *et al*., 2001; Guilpart *et al*., 2014). The evolution of berry volume is an important variable to consider for grapevine yield production (Deloire *et al*., 2021). Quantifying bud fruitfulness during grapevine dormancy requires the use of specific laboratory techniques and procedures to observe the internal bud organogenesis and to assess the primordia of identification of inflorescences (Ramos, 1991; Toda, 1991; Rawnsley and Collins, 2005). Bud dissection, histological observations and forcing bud growth under controlled environmental conditions are techniques that can be used to determine bud fruitfulness while the bud is dormant (May and Antcliff, 1963; Toda, 1991). Because these techniques are destructive, they presuppose the use of buds from canes that will be removed in winter pruning (Ramos, 1991). Meanwhile, observations can be helpful for the identification of bud damage; in particular to diagnose any possible tissue necrosis in the bud that can compromise its integrity and development, as well as to evaluate the seriousness of the problem in the vineyard (Vasudevan *et al*., 1998; Rawnsley and Collins, 2005; Collins *et al*., 2006). A high percentage of bud necrosis is a serious problem as it compromises bud development and consequently negatively affects yield due to the loss of fruitful buds (Collins *et al*., 2006).

Budburst and bud development involve the mobilisation of carbohydrate reserves (e.g., soluble sugars and starch) stored in the vine perennial organs (roots, trunk and canes) until the leaves become a source of carbohydrates around flowering (Zapata *et al*., 2004; Vaillant-Gaveau *et al*., 2014). Therefore, the availability of starch and soluble sugars is crucial for the development of the new primary shoots and inflorescences (Scholefield *et al*., 1978; Vaillant-Gaveau *et al*., 2014).

Moreover, Lavee *et al*. (1981) concluded that a low concentration of carbohydrates can affect budburst and cause bud necrosis, resulting in reduced fruitfulness. In line with these views and facts, the aim of this study is to use different techniques to assess bud fruitfulness and the necrosis incidence during ecodormancy in the three white grapes varieties, Alvarinho, Fernão-Pires and Loureiro. These three varieties were cordon spur-pruned and trained in the vertical shoot position, VSP (predominant at the VVDR). The concentration of carbohydrate reserves (total soluble sugars and starch) present in winter canes was analysed and its relationship with percentage budburst and bud fruitfulness was explored.

MATERIALS AND METHODS

1. Site description and plant material

The experiments were carried out at two commercial wine production vineyards located in two VVDR sub-regions: Celorico de Basto (41°25'15"N, 7°58'10"W, 243 m) and Penafiel (41°12'24"N 8°17'56"W, 199 m) during the 2016/2017 and the 2017/2018 growing seasons. The climate of the two sites is classified as Csb by the Köppen-Geiger climate classification (warm temperate climate with dry and warm summers). The meteorological conditions were recorded during the study period using an automatic weather station placed near the vineyards (Figure 1).

In both sites, three white grape varieties (*Vitis vinifera* L.) of identical age were selected: Alvarinho, Fernão-Pires and Loureiro. They were selected for their adaptability to local edaphoclimatic conditions and their high oenological potential to produce Vinho Verde white wines. Alvarinho and Loureiro vines were 14 years old and Fernão-Pires 13 years old. All the varieties used in the experiment were grafted onto 110 Richter rootstocks; The distances between and along the rows were 2.5 m and 1.0 m respectively. The vines were trained using a vertical shoot positioning system and spur-pruned onto a bilateral cordon with 20 to 24 buds/vine (about 2 buds/spur). Both of the vineyards had been managed according to the commercial cultivation practices. The soil at the Celorico de Basto site is classified as umbric leptosols, while at the Penafiel site it is classified as cumulic anthrosol (Agroconsultores and Geometral, 1995).

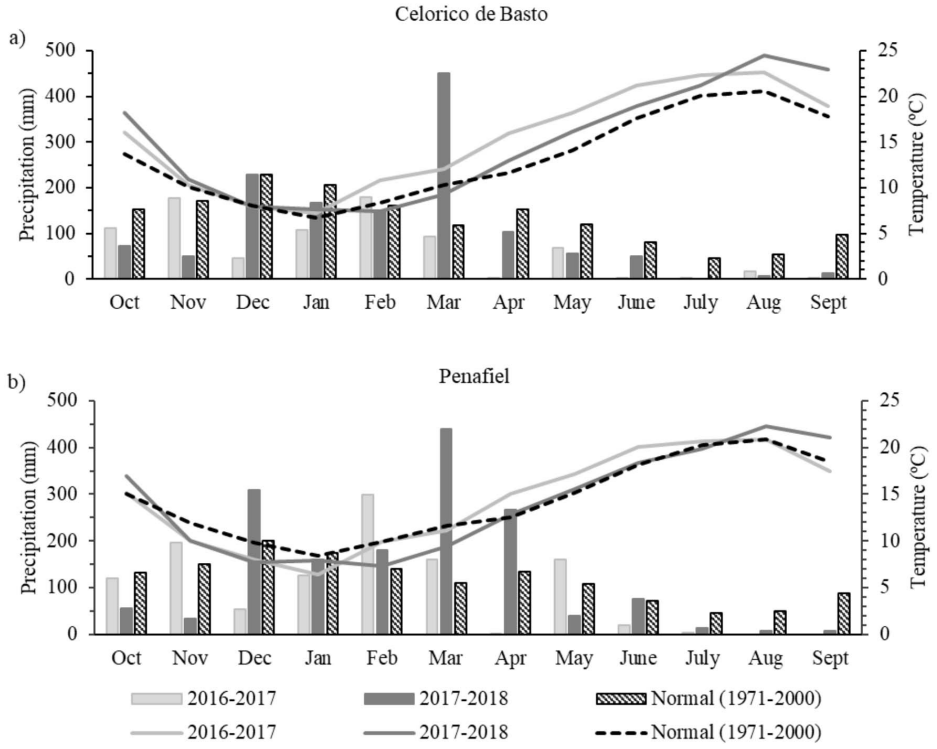


FIGURE 1. Monthly precipitation (vertical bars) and monthly mean temperature (lines) recorded in Celorico de Basto (a) and Penafiel (b) in the 2016/2017 and 2017/2018 growing seasons and 1971-2000 long-term average.

For dissection and histological bud analysis, the first two buds in the cane were collected from 30 randomly selected vines in December 2016 and December 2017 before winter pruning (a total of 60 buds per variety/site). The dormant buds were stored in hermetic bags and then transported on ice to the University of Trás-os-Montes e Alto Douro histology laboratory for analysis. A total of 30 dormant buds were used for each anatomical study technique: bud dissection analysis and histological preparations.

The viability and fruitfulness indices were further determined by forcing bud growth under controlled environmental conditions (Khanduja and Abbas, 1973; Toda, 1991; Rawnsley and Collins, 2005). A total of 45 canes were randomly cut down before winter pruning (October 2016 and 2017) from the middle third of the cordon (n = 45 canes per variety and site), packaged in plastic bags with relative humidity close to saturation and stored in a cold room at 4 ºC for 30 days to ensure enough chilling hours and the artificial breaking of dormancy (Pellegrino *et al*., 2020).

2. Assessment of fruitfulness by bud analysis

The winter buds were used for fruitfulness assessment by counting the number of inflorescence primordia per bud and calculating the percentage of healthy buds (i.e., green coloured and absence of damage, malformations or signs of pest) and the percentage of necrotic buds.

2.1. Bud dissection analysis

Before dissection, the buds were placed in distilled water to prevent them dehydrating and to facilitate the removal of scales and epidermal hairs. The buds were carefully hand-cut in transversal and longitudinal sections with the help of scalpel and tweezers, and then observed under a stereomicroscope (Swift Stereo Eighty lighted, Swift International Instruments, Tokyo, Japan) equipped with a digital camera (Moticam 1080, Motic) to easily identify the inflorescence primordia (Toda, 1991; Rawnsley and Collins, 2005).

2.2. Histological bud observations

The buds from which the scales and epidermal hairs had been removed were fixed in a FAA solution (formaldehyde, acetic acid, and ethanol 70 % 1:1:18) for 48h. Then, they were dehydrated in an alcohol series, cleared and embedded in paraffin wax (Paraplast Scientific). Longitudinal and transverse serial sections 7µm thick were made using a rotary microtome (Leica RM 2135, Germany) (Johansen, 1940; Berlyn *et al*., 1976). The cut sections were stained with 0,1 % toluidine blue and finally mounted using Entellan (Merck, Germany) (O'Brien *et al*., 1964). The bud preparations were observed using an inverted optical microscope Olympus IX51 (Olympus Biosystem, Munich, Germany) equipped with a digital camera (Color View III) and magnifications of x40. Composite photographs were assembled using the Photoshop 5 software.

2.3. Forcing bud growth under controlled environmental conditions

After dormancy had been broken, canes from each of the 3 varieties were cut into short two-node segments. Thereafter, they were placed in distilled water for 24h. The base canes were immersed in 0.8 % indolebutyric acid (IBA) (Seradix®, Bayer, Germany) to enhance rhizogenesis.

Thus, each set of canes (n = 45 per variety and site) was planted in a polypropylene seedling tray (56 holes, 42x7x30 mm) containing a mixture (3:1) of peat (SiroTurfa, Siro, Portugal) and pearlite (Perlite Gramoflor premium 2-6mm, Gramoflor GmbH and Co., Germany). The trays were placed in a walk-in growth chamber FitoClima 10000 HP (Aralab, Albarraque, Portugal) where they were kept for 60 days. During forced growth, the chamber environmental conditions were constant: the temperature was 25 ºC/18 ºC (day/night) and the photoperiod 16h. The relative humidity was maintained at 60 % and the photosynthetic photon flux density at 300 µmol m^{-2}/s. The canes were irrigated every 3 days up to the field capacity using tap water.

2.3.1. Budburst and fruitfulness indices

Phenological stages were monitored weekly according to the Baggiollini phenological scale (Baggiollini, 1952). When 50 % of the buds were at phenological stage C, the budburst percentage was calculated using the following equations (Equations 1 and 2).

After budburst, when inflorescences on the developing shoot were visible, thus corresponding to phenological stage G (Baggiollini, 1952), the potential fruitfulness index (IFpot) and the practical fruitfulness index (IFprat) were calculated as follows (Equations 3, 4 and 5).

3. Non-structural carbohydrate analysis

Total soluble sugar (TSS) and starch quantifications were performed in the node and internode of the remaining wood canes used for the forced bud growth. The nodes and internodes were dried at 60 °C for 72h and were posteriorly ground using a Wiley knife mill to obtain a homogenous fine powder for extraction. TSS was quantified using the anthrone method adapted by Irigoyen *et al*. (1992). Samples of 100 mg of powder wood were suspending in 5mL of ethanol/distilled water (80:20, v/v) in a 80 ºC water bath for 60 min. Two hundred µL of the supernatant was mixed with 3mL of anthrone (Merck, Germany) followed by a new incubation at 100 ºC for 10 min. After cooling, the absorbance was read at 625nm in a spectrophotometer UV/Vis Varian (Cary 100 Bio, Australia). The glucose was used as the standard and the results were expressed as mg/g of dry weight (DW).

Starch content was quantified using a colourimetric and enzymatic method as adopted from Rasmussen and Henry (1990). Five hundred mg of powered wood sample were added to 2.5 mL of sodium acetate buffer 1M (pH 6) and 20µL of α-amylase (EC 3.2.1.1) (Sigma A 4582, Sigma Aldrich, Germany). The suspension was heated in a boiling water bath for 30 min and 10 µL of Amyloglucosidase was added. Posteriorly, after overnight incubation at 60 ºC, the samples were centrifuged at 1000 g for 10 min. Then, 5 mL of distilled water, 200 µL of glucose standard and 5 µl of glucose oxidase (*Aspergillus niger*, Sigma G 2133, Sigma Aldrich, Germany) were added to 200 µL of sample extract, which was then all mixed and incubated at 40 ºC for 15 min. After cooling in a dark room, the absorbance was recorded at 505 nm in a spectrophotometer. Glucose was used as the standard and results were expressed as mg/g of DW.

4. Statistical analysis

Statistical analysis was performed using the statistical software program SPSS for Windows version 25 (IBM SPSS Statistics for Windows, version 25, Orchard Road- Armonk, New York, USA). Statistical differences among sites and varieties were evaluated by two-way factorial ANOVA (site × variety), followed by the post hoc Tukey's test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

1. Morphological and histological observations

The internal anatomical observation confirmed the complex structure comprising the primary bud (R2 meristem) in the centre and two small meristems (R3 meristems or secondary buds) on either side of the primary (Figure 2). As was observed in the longitudinal (Figure 2a-d) and transverse sections (Figure 2e-h), the R2 meristem was the largest and showed a clear advancement in development compared to the others, with well-developed inflorescence and leaf primordia. Inflorescence primordia is a branched structure with small protuberances, that correspond to the meristems that will form a cluster of flowers on a system of branches in the future (Figure 1b and d); it is usually near the vegetative apex and is surrounded by epidermal hairs and leaf primordia.

Healthy buds are easily distinguished from necrotic buds due to their colour: a healthy bud is green with distinguishable brownish areas at the apex of the bud that correspond to the

- **Equation 1:** $Budburst(\%) = \frac{numberofburstbudspervine}{totalnumberofbudspervine} \times 100$
- **Equation 2**: $Budburtsposition \in thecane(\%) = \frac{numberofburstbudsposition \in thecane}{totalnumberofbudsposition \in thecane} \times 100$
- **Equation 3**: $IFpot = \frac{numberofinflorescencesperbud}{totalnumberofburstbud}$
- **Equation 4**: $IFpotorderofthenode \in thecane = \frac{numberofinflorescencesperbud \in order \in thecane}{totalnumberofburstbud \in order \in thecane}$
- **Equation 5**: $IFprat = \frac{numberofinflorescencesperbud}{totalnumberofbudspervine}$

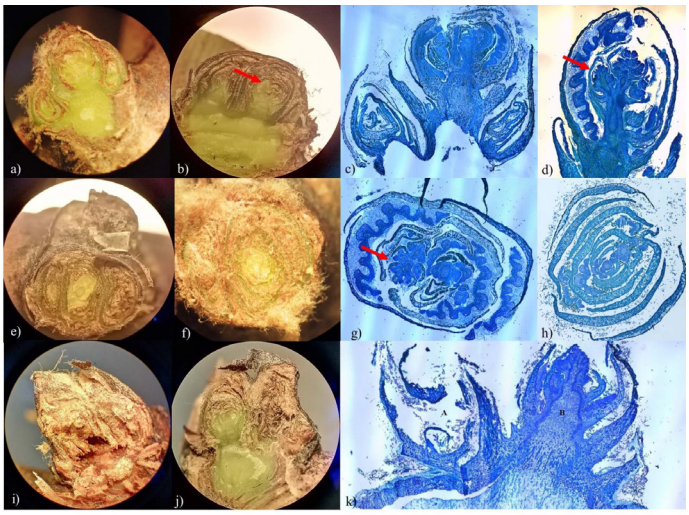


FIGURE 2. Bud analysis to assess fruitfulness and necrosis incidence by bud dissection (a-b, e-f and i-j) and histological observations stained with Toluidine blue (c-d, g-h and k) during dormancy (Stage A, Baggiollini scale). Longitudinal and transversal sections were obtained by dissection (a-b and e-f) and histological sections (c-d and g-h) showing healthy primary and secondary buds (a-b). Scale bars = 500 µm. The red arrow marks an inflorescence primordium in the primary bud. Complete necrotic buds (i) and buds showing a Primary Bud Necrosis (PBN) characterised by the death of primary buds.

epidermal hairs (Figure 2a and b). Meanwhile, necrotic buds are brown in colour and are dry in appearance due to the necrosis of the tissues (cell death), which consequently results in the death of the bud (Figure 2i and j). In Figures 2j and k, the visible necrosis is identical to that described as Primary Bud Necrosis (PBN) when the primary bud is completely necrotic but the secondary ones remain unaffected.

Table 1 shows the results of the bud dissection and histological analysis for the Alvarinho, Fernão-Pires and Loureiro varieties in Celorico de Basto and Penafiel for both growing seasons. There were no statistically significant differences for site, variety and interaction among variables in terms of percentage of healthy and necrotic buds. Both techniques showed that the percentage of healthy buds were significantly higher than the necrotic buds in all the varieties. In 2016/2017 the healthy bud percentage was over 77.3 %, with a slight increase to over 82.6 % in the following season. Bud dissection and histological techniques exhibited a similar percentage of healthy buds (Table 1).

The necrotic bud percentage was lower than 30 % (2016/2017) and 14 % (2017/2018) for each variety. Most of the observed necrosis corresponded to buds with total necrosis when it affected all the dormant buds. Although primary bud necrosis (PBN) was observed, the incidence was low, with percentages below 3 % for both growing seasons (not shown).

Furthermore, it was important to evaluate how many of these healthy buds were fruitful. Significant differences between the varieties ($p < 0,05$) in terms of fruitfulness were only observed in the histological analysis (Table 1). The Fernão-Pires variety showed significantly higher values, compared to the other varieties, ranging from 1.10 to 1.17 (2016/2017), with a slight increase in the following season to 1.20 and 1.30 inflorescences per bud. The Alvarinho variety exhibited the lowest bud fruitfulness for both growing seasons, ranging from 0.78 to 0.86 in 2016/2017, while in the following season there was a slight increase to 0.90 and 1.00 inflorescence per bud. The results of both techniques showed a similar growing season pattern: there was an increase in the number of healthy buds and bud fruitfulness.

2. Forced budburst under controlled environmental conditions

The budburst percentage was significantly affected by site × variety interaction ($p < 0.05$) in both seasons (Table 2). The budburst percentage ranged from 52.2 to 71.1 % in the first season, and from 46.7 to 64.4 % in 2017/2018. Bud position in the cane (first and second node) was found to be significant per variety, but only for the first bud, ($p < 0.005$). In the Fernão-Pires variety, the budburst percentage in the first bud position (BB1) was always significantly higher than all the other varieties (Table 2).

When analysing the fruitfulness indices, we observed statistically significant differences between varieties in terms of potential fruitfulness index (IFpot) ($p < 0.01$) and practical fruitfulness index (IFprat) ($p < 0.01$) and the two-growing seasons. Higher values of IFpot were observed for Fernão-Pires, followed by Loureiro and lastly Alvarinho in both growing seasons. In 2016/2017, IFpot was 0.84 and 1.09 for Fernão-Pires in Penafiel and Celorico de Basto respectively. In the next season, the values were higher than in the previous, with IFpot equal to 1.62 and 1.72. For the Loureiro variety, IFpot ranged from 0.78 to 0.90 in 2016/2017, while in 2017/2018 it varied from 1.49 to 1.69 in Celorico de Basto and Penafiel respectively. The Alvarinho variety showed the lowest IFpot in both sites and seasons, with 0.55 and 0.76 (2016/2017) and 1.40 and 1.42 (2017/2018) inflorescences per bud.

TABLE 1. Percentage (%) of healthy buds, fruitfulness and percentage (%) of necrosis incidence in Alvarinho (AL), Fernão-Pires (FP) and Loureiro (LO) dormant buds from Celorico de Basto (CB) and Penafiel (PE) analysed by bud dissection and histological techniques in two growing seasons (2016/2017 and 2017/2018). Values are the means (n = 30). Different characters in the columns represents statistically significant differences ($p < 0.05$).

Season	Site (S)	Variety (V)	Bud dissection			Histological technique		
			Healthy buds (%)	Fruitfulness index	Necrotic buds (%)	Healthy buds (%)	Fruitfulness index	Necrotic buds (%)
2016/2017	CB	AL	73.3	0.63	26.7	79.0	0.86	21.0
		FP	83.3	0.60	16.7	85.7	1.17	14.3
		LO	83.3	0.72	16.7	77.3	0.94	22.7
	PE	AL	80.0	0.87	20.0	81.8	0.78	18.2
		FP	93.3	0.71	6.7	87.0	1.10	13.0
		LO	86.7	0.80	13.3	81.0	0.94	19.0
	p-value	S	ns	ns	ns	ns	ns	ns
		V	ns	ns	ns	ns	*	ns
		S × V	ns	ns	ns	ns	ns	ns
2017/2018	CB	AL	90.0	0.80	10.0	91.7	1.00	8.3
		FP	93.3	0.78	6.7	96.0	1.30	4.0
		LO	86.7	0.76	13.3	83.3	1.10	16.7
	PE	AL	90.0	0.88	10.0	84.0	0.90	16.0
		FP	96.7	0.72	3.3	88.5	1.20	11.5
		LO	80.3	0.75	19.7	82.6	1.10	17.4
	p-value	S	ns	ns	ns	ns	ns	ns
		V	ns	ns	ns	ns	*	ns
		S × V	ns	ns	ns	ns	ns	ns

* Symbols 'ns' indicate not-significant differences ($p > 0.05$), and * $p < 0.05$, indicates significant differences between sites and varieties.

Regarding the node position in the cane, the potential fruitfulness index in the first node position (IFpot1) ($p < 0.05$) showed significant differences between varieties in 2016/2017 only, with Fernão-Pires displaying the highest values when compared to the other varieties. The practical fruitfulness index (IFprat) was similar in pattern to IFpot. However, the values of this index were slightly lower than IFpot (Table 2). When analysing these indices between seasons, there was a significant increase in 2017/2018 compared to 2016/2017, with fruitfulness indices higher than 1 inflorescence per bud.

3. Carbohydrate reserves in wood canes

Figure 3 shows the total soluble sugars (TSS) and starch content measured on node and internode regions in the two growing seasons. Significant differences between site, variety and position (node and internode) and interactions were observed in both years. The results showed significantly higher TSS and starch concentrations in varieties from Celorico de Basto compared to Penafiel. Meanwhile, a clear trend between varieties in terms of the carbohydrate content was observed (Loureiro > Fernão-Pires > Alvarinho). Alvarinho stood out from the other two varieties as having the lowest TSS and starch content in both growing seasons.

Significant differences between node and internode in TSS and starch content were observed in 2016/2017 and 2017/2018 (Figure 3). In general, TSS content was significantly higher in the node than in the internode. Although in terms of starch content there were also differences between node and internode, a similar pattern to the TSS content was not found for either of the growing seasons. In 2016/2017, higher starch content was found in the node, while in 2017/2018 it was higher in the internode. Furthermore, carbohydrate content was slightly higher in 2017/2018 compared to 2016/2017 in both vineyards.

To understand the role of TSS and starch content in budburst and fruitfulness indices, these variables were combined in Figure 4. Higher percentages of budburst were associated with an increase in fruitfulness indices (IFpot and IFprat) in 2016/2017. TSS and starch content found in nodes and internode of the Alvarinho and Fernão-Pires varieties were related to an increase in budburst percentage and fruitfulness indices. Although Loureiro had the highest TSS and starch content, an increase in budburst percentage and fruitfulness indices was not observed. In 2017/2018, an increase in non-structural carbohydrates and fruitfulness was observed for varieties and sites compared to the values recorded in the previous season.

TABLE 2. Percentage (%) of budburst (BB), budburst in first node position (BB1), budburst in second node position (BB2), potential fruitfulness index (IFpot), potential fruitfulness index in first node position (IFpot 1), potential fruitfulness index in second node position (IFpot 2) and practical fruitfulness index (IFprat) in Alvarinho (AL), Fernão-Pires (FP) and Loureiro (LO) varieties from Celorico de Basto (CB) and Penafiel (PE) by forcing bud growth under controlled environmental conditions in two growing seasons. Values are the means (n = 45). The lower-case letters in the columns represents statistically significant differences ($p < 0.05$).

Season	Site (S)	Variety (V)	BB	BB1	BB2	IFpot	IFpot1	IFpot 2	IFprat
2016/2017	CB	AL	63.3 ab	44.4	82.2	0.55	0.32	0.40	0.30
		FP	71.1 a	75.5	64.4	1.09	1.13	0.68	0.66
		LO	60.0 ab	57.8	62.2	0.90	0.43	0.70	0.47
	PE	AL	52.2 b	50.0	55.0	0.76	0.48	0.37	0.42
		FP	60.0 ab	66.7	55.6	0.84	0.87	0.57	0.54
		LO	68.1 a	60.0	75.6	0.78	0.74	0.55	0.50
	p-value	S	ns	ns	ns	ns	ns	ns	ns
		V	ns	*	ns	**	*	ns	**
		S × V	*	ns	ns	ns	ns	ns	ns
2017/2018	CB	AL	62.2 ab	56.1	66.7	1.42	1.35	1.50	0.87
		FP	63.3 a	63.9	62.8	1.72	1.65	1.45	1.04
		LO	46.7 b	22.8	70.0	1.65	1.22	1.66	0.76
	PE	AL	58.9 ab	40.0	77.8	1.40	1.09	1.47	0.80
		FP	64.4 a	68.3	58.9	1.62	1.31	1.48	0.95
		LO	63.3 a	61.1	65.6	1.49	1.50	1.45	0.88
	p-value	S	ns	ns	ns	ns	ns	ns	ns
		V	ns	*	ns	**	ns	ns	**
		S × V	*	ns	ns	ns	ns	ns	ns

* Symbols 'ns' indicates non-significant differences ($p > 0.05$), and * $p < 0.05$ indicates significant differences between sites and white varieties.

DISCUSSION

The present study confirms that morphological and histological analyses and the bud forcing technique under controlled environmental conditions are important tools for assessing winter bud fertility and diagnosing bud damage. The anatomical observations showed a high number of visually healthy buds (> 73 % in 2016/2017 and > 80 % in 2017/2018), indicating that the normal process of induction and differentiation of inflorescence primordia had taken place. Budburst percentage does not only depend on the variety, but also on the environmental conditions during the year and on vine physiology/functioning (Magalhães, 2015; Monteiro *et al*., 2022). Significant differences between varieties and sites in terms of budburst percentage were found, which can be related to different vine responses to weather conditions (e.g., high temperatures during ecodormancy or low carbohydrate wood reserves). Furthermore, during forced growth under controlled environmental conditions, the formation of adventitious roots in the cane at budburst was observed. These physiological processes require high energy consumption, for which the carbohydrate wood reserves may not be sufficient, thus having a potential detrimental impact on budburst.

Between varieties, there were different responses in terms of position of the bud, which can be explained by the budburst inhibition of basal buds due to apical dominance (Reynier, 1990; Magalhães, 2015).

Bud fruitfulness is a manifestation of the grapevine's productive capacity: the higher the fruitfulness, the higher the productive potential in the following season (Ramos, 1991). A significant effect of variety on fruitfulness variables was found in both seasons in the present study. In the literature, the Alvarinho and Loureiro varieties are referred to as having high fruitfulness, with a mean of 1.8 and 2.0 inflorescences per bud respectively (Böhm, 2010). The Fernão-Pires variety is described as having good fruitfulness in basal buds, with an average rate of 1.6 inflorescences per bud (Böhm, 2010). Compared to the values published in the literature, fruitfulness was lower in 2016/2017 in all varieties and vineyards.

In 2017/2018, an increase in fruitfulness was observed in all varieties, regardless of the technique. Considering the monthly temperatures and precipitation in both seasons, it can be stated that 2017 was warmer and dryer compared to 2016, especially from May to June (IPMA, 2021; Figure 1) when floral induction and differentiation occurred; this may have had a positive impact in the 2017/2018 growing season, with

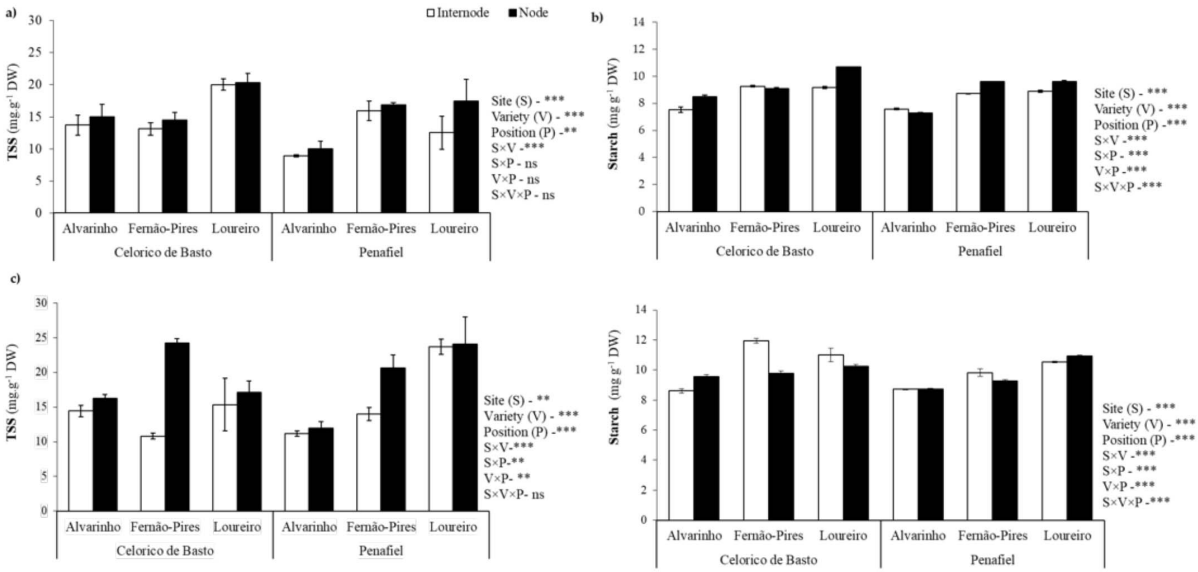


FIGURE 3. Total soluble sugars (TSS, mg g-1 DW) (a, c) and starch content (mg g-1 DW) (c, d) in node and internode for Alvarinho, Fernão-Pires and Loureiro varieties in Celorico de Basto and Penafiel for two growing seasons: 2016/2017 (a,b) and 2017/2018 (c,d). Each column expresses the mean ± SE (n = 4). Statistical significance differences are indicated with an asterisk (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and ns - not significant, according to the Tukey test.

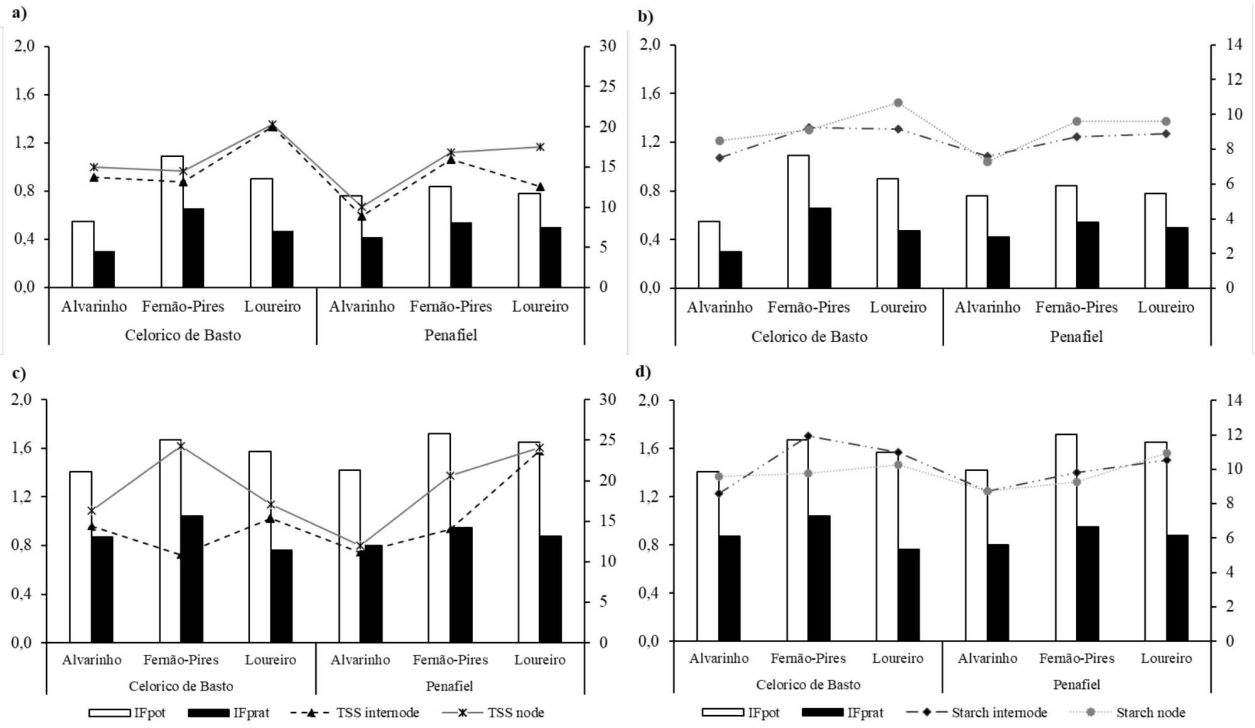


FIGURE 4. Relationship between budburst percentage, fruitfulness indices (potential, IFpot and practical, IFprat fruitfulness indices) and non-structural carbohydrates (total soluble sugar (TSS) and starch) in Alvarinho, Fernão-Pires and Loureiro varieties for two growing seasons: 2016/2017 (a,b) and 2017/2018 (c,d).

a greater number of inflorescence primordia observed per bud (thus enhancing the number of healthy buds and inflorescence formed). Although bud fruitfulness is a varietal characteristic, it can vary between seasons depending on environmental and vine physiology, which directly and indirectly influences the induction and differentiation of inflorescence primordia and, consequently, affects the yield (Monteiro *et al.*, 2021). According to Sanchez and Dokoozlian (2005), depending on the variety, the fruitfulness of the basal buds can be lower in the first and second positions, and it increases throughout the shoot, decreasing again in the distal positions of the node (Huglin, 1978).

Although the number of healthy buds was higher than the necrotic ones, it is necessary to understand how this can affect

future yield, especially in years when a lower fruitfulness is expected. Several studies have identified necrosis as one of the main causes of reduced yield. However, the type of necrosis and the percentage of incidence vary greatly depending on the variety, location and year (Lavee *et al*., 1981; Lavee, 1987; Vasudevan *et al*., 1998; Rawnsley and Collins, 2005; Collins *et al*., 2006). The present study showed that total bud necrosis was the most prevalent type of necrosis for both growing seasons. Furthermore, primary bud necrosis (PBN) was also observed, but with a lower percentage (below 3 %). In both types of observed bud necrosis, there was a decrease in potential yield due to the loss of fruitful buds, but they were less or more significant depending on the variety. For example, in the case of PBN, budburst was guaranteed by the R3 meristem of the winter bud (Torregrosa *et al*., 2021), as in the case of the secondary buds (R3 meristem), which remain healthy and develop to compensate for the loss of the primary bud (R2 meristem). However, depending on the variety, they can be less fruitful and form small clusters (Dry and Coombe, 1994; Rawnsley and Collins, 2005; Collins *et al*., 2006; Cox *et al*., 2012). Little is known about the Alvarinho, Fernão-Pires and Loureiro varietal sensitivity to this problem and how it affects their productivity. Several studies have shown a high sensitivity of the Shiraz/Syrah, Riesling, Chardonnay, Thompson seedless, Askari and Queen of Vineyard varieties to PBN, which has been identified in many wine regions as being one of the main causes of low yield (Lavee *et al*., 1981; Perez and Kliewer, 1990; Dry and Coombe, 1994; Wolf and Warren, 1995; Collins *et al*., 2006; Kavoosi *et al*., 2012; Junior *et al*., 2019; Cox *et al*., 2012). Vasudevan *et al*. (1998) showed that the incidence of bud necrosis in the Syrah variety in American vineyards was 77 %. More recently, Collins *et al*. (2006) found that in some Australian vineyards the incidence was close to 90 %.

At the beginning of vegetative growth, energy needs are fulfilled by the soluble sugars and starch reserves stored in the perennial organs (Zapata *et al*., 2004; Smith and Holzapfel, 2009; Bennett *et al*., 2005). Considering that the mobilisation of reserves occurs until young leaves acquire an export capacity for photoassimilates, that is when they reach 50 % of the final size (Scholefield *et al*., 1978; Vaillant-Gaveau *et al*., 2014), the availability of reserves is presumably of great importance for budburst, especially in situations of forced bursting under environmental conditions, in which the canes are dependent on their reserves. The regulation of photoassimilate translocation from source to sink organs is important for vegetative growth and reproductive development (induction and differentiation of inflorescence primordia). During budburst, low soluble sugars and starch content can cause unequal budburst and the irregular development of fruitful buds, ascarbohydrate reserves stored in woody parts provide energy for bud growth and development until the photoassimilates are synthesised by the leaves (Vasconcelos *et al*., 2009; Vaillant-Gaveau *et al*., 2014).

Our results show that the carbohydrate reserves in canes were influenced by site, variety and position (node and internode). Edaphoclimatic conditions at each site (e.g., air temperature, light exposure, soil, carbon and water status and nutrient viability) can favour the synthesis of photoassimilates while protecting the canes in low temperatures during dormancy (Loescher *et al*., 1990; Jones *et al*., 1998). Since the canes were collected on the same day, all the varieties benefited from similar weather conditions, and therefore we can attribute the differences to the varietal characteristics. In general, there was a greater accumulation of soluble sugar and starch in the node than in the internode. This may be due to the supplementary needs in these structures, especially during budburst, and nodes can function as small deposits closer to the bud. From 2016/2017 to 2017/2018, there was an increase in soluble sugars and starch, which indicates that the accumulation of reserves in the perennial parts of the vine varied over the years. Temperature, light or water status have a high impact on this variation, as well as additional energy needs during the previous season, vine age or cultural practices (e.g., defoliation) (Bennett *et al*., 2005; Vaillant-Gaveau *et al*., 2014; Pellegrino *et al*., 2020). The seasonal dynamics of carbohydrate reserves is linked to the balance between canopy assimilation and carbon demand during the season. Moreover, the early harvest in 2016/2017, which took place about 30 days earlier than expected, may also have contributed to this increase in reserves in 2017/2018. The accumulation of carbohydrate reserves starts before harvest when the plateau of berry sugar accumulation is reached (Rossouw *et al*., 2017; Antalick *et al*., 2021). In this case, the lower need for photoassimilates that results from an increase in photosynthetic activity due to the removal of fruit during harvest may explain the majority of the carbohydrate reserves observed in the 2017/2018 wood (Zapata *et al*., 2004; Vaillant-Gaveau *et al*., 2014). As noted by Smith and Holzapfel (2009) the accumulation of carbohydrates reserves in wood tissues depends on the variety.

CONCLUSION

The three methodologies performed in this study (bud dissection, histological observations and forced bud growth under controlled environmental conditions) allowed us to assess the bud fruitfulness of the Alvarinho, Fernão-Pires and Loureiro varieties in two VVDR vineyards over two growing seasons.

The study showed that the number of inflorescences varied between varieties, vineyards and years. The Fernão-Pires variety showed the highest bud fruitfulness (spur pruned cordon), while Alvarinho the lowest; Fernão-Pires was in between the two. In the 2017/2018 growing season, fruitfulness was higher than in 2016/2017, and consequently the number of inflorescences and clusters was also higher. The increase in the fruitfulness indices and the decrease in the number of necrotic buds may be related to the increase in cane carbohydrate reserves observed in that year, which results from the environmental conditions of the previous

season, especially during the period in which inflorescence primordia differentiation occurs.

Considering the variation in fruitfulness for each situation (variety and season), the present study highlights the importance of bud fruitfulness assessments in indicating the potential number of clusters that will be obtained the following spring. In this way, during the dormant period winegrowers will have a first estimate of yield potential, which from a practical point of view serves as a guide to crop load at winter pruning, i.e., less or more bud load depending on the estimates. Production should thus be optimised, as a balance between the vegetative and productive parts of the grapevine is maintained, and yield irregularities minimised. A minimum of 30 buds per vineyard is necessary to obtain a reliable and meaningful fruitfulness assessment, since the fragility of the inflorescence primordia and the difficulty of removing the epidermal hairs can lead to significant sample losses (buds).

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