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Abstract: Microscopic bud dissection can be used to assess grapevine bud fruitfulness prior to winter pruning and long before actual bud fruitfulness can be measured in the vineyard the following spring. Bud dissections should be performed by qualified and trained personnel because inflorescence primordia are difficult to distinguish in some varieties. In the Puglia region, Southeastern Italy, in 2018 and 2019, potential fruitfulness using bud dissection and actual fruitfulness observed in the vineyard were compared for seventeen table grape varieties. The percentage of fertile buds, the number of inflorescence primordia (IP) per node, and the incidence of primary bud necrosis (PBN) were detected with bud dissection to be used either for managing winter pruning or for predicting yield during the successive season. The data were successively compared with fertile buds and actual bud fruitfulness observed in the vineyard during spring. The table grape varieties examined had similar values of fertile buds and fruitfulness both with bud dissection and in the vineyard. The application of longitudinal sections in bud dissections can be an alternative approach (or can be integrated into traditional cross sections) to distinguish IP in some difficult varieties, but the two techniques can be used together for more repeatable results. The bud dissection technique (with both cross and longitudinal sections) can provide useful insights for viticulturist to help guide winter pruning (intensity of pruning and number of canes) and to predict potential yield.

Keywords: bud fertility; primary bud necrosis; blind buds; winter pruning

1. Introduction

The Puglia region in Southeastern Italy is the most important area in the country in terms of table grape cultivation. In 2020, the region produced 591,480 tons from within an area of 24,355 ha compared to 974,154 tons and 43,502 ha within the whole country [1]. Many varieties have been introduced for cultivation, mainly seedless ones because of consumer demand. However, seedless varieties often have lower bud fruitfulness (0.5–0.9 cluster per bud) compared to seeded varieties (>1), with negative consequences for yield. Different factors could determine the low bud fruitfulness: the varieties, environmental conditions (temperature, light intensity, and shade), and management systems (pruning, irrigation, nutrition, and hormone application) [2–9]. In particular, water stress, light, and temperature are important factors that affect inflorescence induction and differentiation [10–13]. Nitrogen deficiency can also reduce inflorescence formation, and it is a fundamental nutrient for optimal formation of inflorescence primordia and for differentiation of flowers [9,14].

Grapevine yield formation spans over two consecutive years, with floral induction and partial differentiation occurring in the first year, followed by flower initiation and complete differentiation at around the flowering time during the second year [15]. This first year is fundamental for yield formation because it defines the potential number of clusters that the vine could bear the next year. Variations in the number of clusters not only have a direct effect on yield but also may have undesirable effects on the size and quality of berries [16], which are very important aspects for table grape, more so than for wine grape.



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Some authors have reported that the seasonal yield of grapes vary greatly, ranging from 15% up to 35% and even higher [17–19]. Almost 2/3 of grape yield is determined by the number of clusters, whereas the number and weight of berries accounts for the rest [17,20]. Since the yield depends on the number of clusters, knowing this number during the first season (inflorescence primordia) can be very helpful for viticulturists.

During this first year of inflorescence (cluster) formation, there are two main steps in growing buds on grapevine: the formation of uncommitted primordia (also called anlagen) and differentiation of the uncommitted primordium into an inflorescence or a tendril [8,14]. Inflorescence primordia (IP) are formed by extensive and consecutive branching of the anlagen [6,8]. Shade over buds during the vegetative season reduces inflorescence formation, which may be due either to their lower carbon status or reduced assimilate supply to the buds [20–22]. Shaded canes often are present in vineyards with vigorous table grape varieties, resulting in negative consequences on bud differentiation.

In the differentiation process, the distal inflorescences are less differentiated and much smaller than the proximal ones according to an acropetal gradient. At the end of the first season (September–November in the northern hemisphere), the buds enter dormancy. The inflorescences continue to develop at budburst in the successive season (March–April), with resumption of inflorescence branching and differentiation of individual flowers just before anthesis [23,24].

The position of the buds on the cane provides important information about the plant fertility, since bud fruitfulness generally increases from the base to the middle and decreases again toward the distal part [25]. Water stress reduces bud fruitfulness regardless of the bud position on the cane, but the intensity of water stress can reduce inflorescence primordia on more distal nodes due to a shortage of carbohydrates [11]. Irrigation in dry areas during bud formation could be an important factor for potential crop yield [11].

During winter dormancy, the potential grapevine yield for the successive season can be evaluated by assessments of bud fruitfulness [5] using the bud dissection technique. Grapevine compound buds usually consist of a primary bud, which is predominantly responsible for bud fruitfulness, and 1–2 but rarely more secondary smaller lateral buds [26]. If the primary bud is damaged or killed, the secondary buds may partly compensate for potential yield loss associated with the primary bud, depending on the variety. However, secondary and tertiary buds are generally less fruitful (fewer and smaller clusters) than primary buds and, therefore, produce less fruit [20,27]. Primary bud necrosis (PBN) is a physiological disorder that causes the death of primary buds, somehow favored by high shoot vigor and low carbohydrate levels [28–30]. A necrotic bud is a dead bud that externally looks similar to a normal bud but, internally, has necrotized tissues (i.e., has turned black). Necrosis can affect primary bud axis tissue, secondary or tertiary bud axes, or even the whole bud. Furthermore, cane diameter, GA₃ application, and node position affect PBN incidence [28,31], with the first associated with the (high) vigor of the vine and with the latter being a consequence of reduced bud development.

The assessment of bud fruitfulness using the dissection technique is useful in predicting a vineyard's yield potential and in providing the opportunity to modify the same yield by using a management practice such as winter pruning. Furthermore, knowledge of the position of fertile buds on the cane for each cultivar could support more balanced winter pruning in order to achieve the optimal yield, i.e., shorter and more canes or longer and less canes left per vine. Factors such as spur pruning vs. cane pruning or the number of nodes per cane (10, 15, and 20) can have a big impact on bud fruitfulness [32]. Moreover, clusters from basal nodes of 15 node canes on the Thompson Seedless variety were heavier than clusters from basal nodes on 20 node canes, but clusters from apical shoots on the shorter canes were lighter than clusters from apical shoots on longer canes [32].

The bud fruitfulness observed with bud dissections can be defined as potential bud fruitfulness, since the number of inflorescence primordia may not correspond to the number of clusters observed in the field (actual bud fruitfulness) during the successive season in spring–summer and is generally lower that what is observed in the field (incidence of blind buds).

The method of bud dissection was developed by the Commonwealth Scientific and Industrial Research Organization (CSIRO) and the Victorian Department of Agriculture (Australia) in the early 1970s. The analysis of a cane with ten buds needs 20–30 min and takes about 3–5 h for an experienced and qualified technician to estimate the bud fruitfulness of a homogeneous vineyard for 10–15 canes. This bud dissection service can also be provided by the academic spin-off Agridatalog of the University of Bari 'Aldo Moro'.

The objective of this work was (1) to assess the bud fruitfulness of 17 table grape varieties, either potentially in the lab during bud dormancy or actually in the field during the successive growing season; (2) to determine the bud fruitfulness at each node along a 10 node cane; and (3) to determine the incidence of PBN and blind buds and the percentage of fertile buds (in either the lab or vineyard).

2. Materials and Methods

2.1. Experimental Site and Varieties

During the seasons in 2018 and 2019, 17 table grape varieties were analyzed, either seeded or seedless: Sugranineteen, Red Globe, Black Magic, Princess, Sugraone, Victoria, Luisa, Michele Palieri, Pink Muscat, White Seedless, Black Pearl, Fiammetta, Apulia, Crimson Seedless, Summer Royal, Italia, and Regal Seedless. The vineyards were located in an area of a few square kilometers in the countryside of the cities of Noicattaro, Rutigliano, and Adelfia in Bari province of the Puglia region. The vines were 5–7 years old, grafted, cane pruned in winter, and subjected to different summer pruning operations (cluster and berry thinning, lateral removal, shoot thinning and positioning, and leaf removal). The vines were trained with an overhead training system ('tendone system') and subjected to common viticultural practices (winter and summer pruning, irrigation, fertigation, nutrition, and pest control) adopted in this important area for table grape production in Italy.

During both years, bud fruitfulness was assessed for two periods: (1) in winter at pruning time (Figure 1) and (2) in the field during spring around flowering (Figure 2).



Figure 1. Photograph of the vineyard before winter pruning when the canes were collected.



Figure 2. Photograph of the vineyard at flowering to collect data on fertile buds, blind buds, and bud fruitfulness.

A few days before winter pruning, for each variety, 15 vines were selected and 2 canes, with at least 10 nodes, were sampled and collected per vine. The canes were stored in black plastic bags at 4-6 °C until dissection at room temperature. The canes were cut into ten nodes excluding the basal crown buds. Buds at positions 1 to 3 were classified as basal, buds at positions 4 to 6 were considered medium, and buds at positions 7 to 10 were classified as apical.

2.2. Bud Dissection

The buds were dissected with a sharp razor blade; in 2018, cross (transverse) sections were made (Figure 3) while, in 2019, longitudinal sections were also performed (Figure 4) in order to check which methodology is better and to integrate the data for more repeatable results. The compound buds were then assessed for inflorescence primordia number and primary bud necrosis (PBN) using a stereomicroscope Nikon SMZ800 equipped with a camera connected to the computer for image capture.

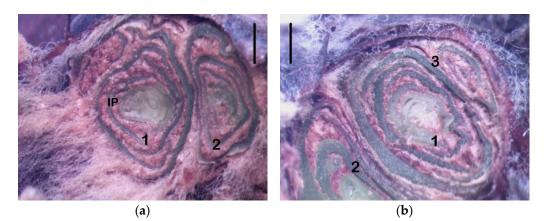


Figure 3. Transverse stereomicrographs of compound buds showing primary (1) and secondary (2) buds with an inflorescence primordia (IP) clearly visible in (**a**) and primary (1), secondary (2), and tertiary buds (3, very small) in (**b**). Scale bars indicate 1 mm.

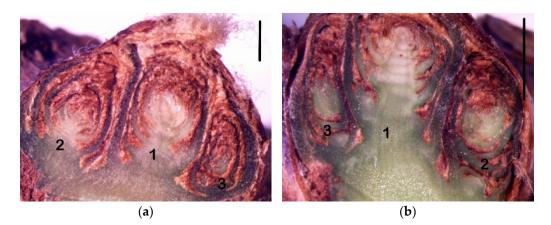


Figure 4. Longitudinal stereomicrographs of compound buds showing primary (1), secondary (2), and poorly developed tertiary buds (3) (**a**) and well-developed primary (1), secondary (2), and tertiary (3) buds (**b**). Scale bars indicate 1 mm.

If PBN was present in the primary buds (Figure 5), the secondary buds were assessed for IP number. In order to distinguish the inflorescence primordia, the magnification was not fixed but selected depending on the variety and how easy was to see the primordia since the differences among the varieties were significant. For all of the varieties, magnifications from 10 to $63 \times$ were used. By using bud dissection in a lab, the following parameters were measured: (1) potential fertile buds (percentage of fertile buds in the 10 nodes), (2) potential bud fruitfulness (as the number of inflorescence primordia per bud), and (3) primary bud necrosis (PBN) (as the percentage of necrotic primary buds) at each node.

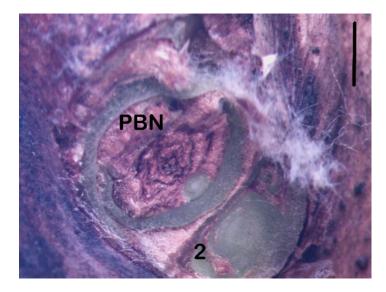


Figure 5. Transverse stereomicrograph showing a Primary Bud Necrosis (PBN) with one developed secondary bud (2). A prompt bud close to PBN is visible. Scale bar indicates 1 mm.

In the vineyards, on the same 15 vines selected for bud dissection in the lab, two canes were tagged at anthesis. The following parameters were measured: (1) actual fertile buds (percentage of fertile buds in the 10 nodes), (2) actual bud fruitfulness (as the number of inflorescences per bud), and (3) blind buds (as the percentage of buds not sprouting) at each node.

The experiment was arranged in a completely randomized design, with 3 replicates of 10 canes with 10 buds each. Data expressed as percentages were transformed by arcsin. The data were assessed by analysis of variance (ANOVA) at 0.05 p, and the REGWQ test

was performed to compare the two methodologies for bud fruitfulness for each variety and to compare the fruitfulness at each bud position within the single variety.

3. Results

3.1. Fertility Parameters

The values for the different fertility parameters varied greatly among the varieties analyzed (Table 1). With regard to potential fertile buds, varieties such as Italia (95.5%), Black Magic (94.0%), Summer Royal, and Sugranineteen (90.0%) showed the highest percentages of fertile buds, whereas Sugraone presented a very low value (46.0%). As expected, the highest values of potential bud fruitfulness were observed for the varieties with more fertile buds, in particular Black Magic (1.76), Victoria (1.53), Italia (1.49), and Pink Muscat (1.49) (Table 1). Many seedless varieties presented values of bud fruitfulness below 1.00 such as Superior Seedless, White Seedless, Black Pearl, and Princess (Table 1). The percentage of PBN was in the range of 0–20%, with the highest value detected in Luisa and with no primary bud necrosis reported only for the buds of Fiammetta. Many varieties presented a physiological necrotic value within 2–8% (Table 1). Besides PBN (Figure 6), necrosis was observed for the secondary buds (Figure 7) and even for the whole bud (Figure 8).

Table 1. Fertility parameters of buds o	f seedless and seeded table	grape varieties (av	verage between two seasons).
		0 1 - (

Potential Variety Fertile Buds (%)		Potential Bud Fruitfulness (n.)	Primary Bud Necrosis (%)	Actual Fertile Buds (%)	Actual Bud Fruitfulness (n.)	Blind Buds (%)	
Sugranineteen	90.0 a	1.26 bd	8.0 ab	84.0 a	1.21 be	6.5	
Red Globe	81.0 a	1.01 ce	12.0 ab	76.4 a	1.08 cg	5.5	
Black Magic	94.0 a	1.76 a	6.0 ab	86.9 a	2.36 a	10.5	
Princess	64.0 ab	0.90 de	4.0 ab	65.0 ab	0.72 eh	18.1	
Sugrone	46.0 b	0.51 e	10.0 ab	43.4 b	0.46 h	22.1	
Victoria	90.0 a	1.53 ab	2.0 ab	79.5 a	1.49 bc	18.0	
Luisa	70.0 ab	0.83 de	2.0 ab	62.5 ab	0.69 fh	12.0	
Michele Palieri	84.0 a	1.18 bd	14.0 ab	83.9 a	1.29 bd	8.0	
Pink Muscat	80.0 a	1.49 ac	18.0 a	75.0 a	1.63 b	12.5	
White Seedless	68.0 ab	0.82 de	8.0 ab	67.5 ab	0.78 dh	20.5	
Black Pearl	70.0 ab	0.84 de	4.0 ab	54.5 ab	0.59 gh	23.5	
Fiammetta	85.0 a	1.23 bd	0.0 b	80.5 a	1.24 bd	12.0	
Apulia	77.5 a	1.21 bd	5.5 ab	75.8 a	1.19 bf	14.5	
Crimson Seedless	72.0 ab	0.87 de	3.5 ab	57.6 ab	0.61 gh	20.6	
Summer Royal	92.0 a	1.33 ad	4.5 ab	81.5 a	1.21 be	11.3	
Italia	95.5 a	1.49 ac	2.0 ab	86.9 a	1.42 bc	10.1	
Regal Seedless	84.0 a	1.37 ad	2.0 ab	73.3 ab	1.27 bd	18.3	

For each parameter, the letters indicate statistical differences between the varieties at p < 0.05 according to the REGWQ test. Potential fertile buds (as a percentage of buds on the cane with at least one cluster according to the bud dissection), potential bud fruitfulness (number of clusters per node according to the bud dissection), primary bud necrosis (as a percentage of necrotic buds on the cane according to the bud dissection), actual fertile buds (as a percentage of buds on the cane with at least one cluster according to the observation in the vineyard), actual bud fruitfulness (number of clusters per node according to the observation in the vineyard), and blind buds (as a percentage of buds that did not sprout in the vineyard).

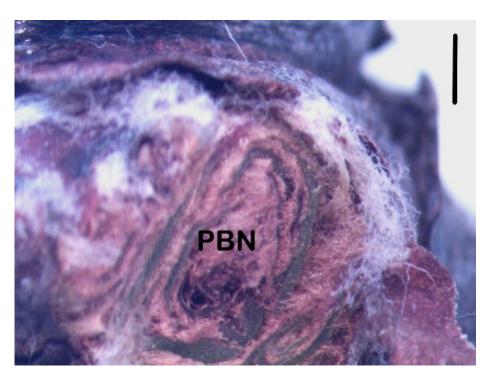


Figure 6. Transverse stereomicrograph showing clear Primary Bud Necrosis (PBN). Scale bar indicates 1 mm.

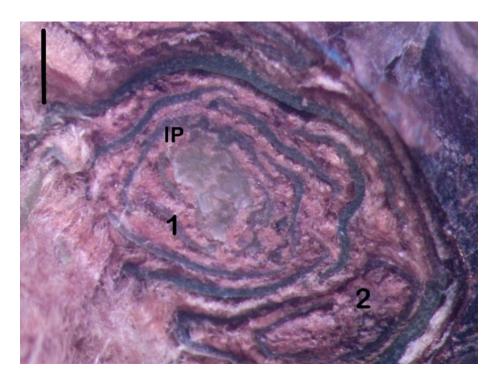


Figure 7. Transverse stereomicrograph showing a primary bud (1) with inflorescence primordia (IP) and a secondary (2) necrotic bud. Scale bar indicates 1 mm.

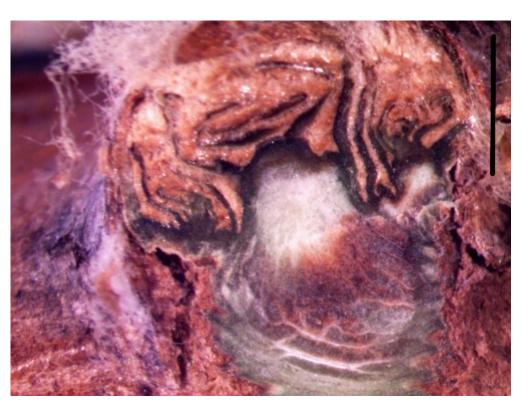


Figure 8. Longitudinal stereomicrograph showing a whole necrotic (dead) bud. Scale bar indicates 1 mm.

The data for actual fertile buds followed the same pattern of bud dissection (Table 1). The percentages of actual fertile buds were highest in Italia and Black Magic (86.9%) followed by Sugranineteen (84.0%) and Michele Palieri (83.9%). Crimson Seedless, Black Pearl, and Sugraone showed the lowest values of actual fertile buds (57.6, 54.5, and 43.4%, respectively).

Black Magic had the highest actual bud fruitfulness (2.36) followed by Pink Muscat (1.63), Victoria (1.49), and Italia (1.42); as for potential values, actual fruitfulness was present in some seedless varieties with less than one cluster per node, with the lowest value registered in Sugraone (0.46) (Table 1). The percentages of blind buds (Table 1) were the highest in varieties such as Black Pearl (23.5%), Sugraone (22.1%), Crimson Seedless (20.6%), and White Seedless (20.5%) and were the lowest in Sugranineteen and Red Globe (6.5 and 5.5%, respectively).

When considering both the potential and actual bud fruitfulness values along the cane, the pattern was very different among the varieties analyzed (Table 2). However, for most of the varieties, potential and actual bud fruitfulness were not different between the two years, with the exception of Crimson Seedless, which showed a higher potential bud fruitfulness compared to the actual one. Italia and Summer Royal also showed higher potential fertile percentages compared to actual values probably because of the incidence of blind buds and inflorescence primordia in the secondary buds. Similar data for the two fruitfulness measures (bud dissection and direct observation during spring in the vineyard) among the two years also indicate that fruitfulness is a stable parameter for each variety, at least in the same environmental and agronomical conditions.

Variety	Buds Node										
		1	2	3	4	5	6	7	8	9	10
	PBF	0.60 d	1.00 cd	1.19 bc	1.20 bc	1.40 ac	1.21 bc	1.62 ab	1.80 a	1.58 ab	1.00 cd
Sugranineteen	PBN (%)	36	9	0	17	0	18	0	0	0	0
	ABF	0.50 b	1.10 a	1.00 ab	1.25 a	1.35 a	1.30 a	1.30 a	1.25 a	1.55 a	1.50 a
	BB (%)	34	11	9	6	5	0	0	0	0	0
	PBF	0.20 b	0.80 a	1.00 a	1.22 a	1.21 a	1.20 a	1.18 a	1.09 a	1.05 a	1.05 a
Red Globe	PBN (%) ABF	44 0.25 c	40 0.60 bc	17 1.35 a	0 1.15 ab	0 1.30 a	0 1.47 a	23 1.38 a	0 1.20 ab	0 1.10 ab	0 1.00 ab
	BB (%)	35	10	1.55 a 0	1.15 ab 5	1.50 a 0	1. 1 7 a	1.50 a 0	1.20 ab	0	1.00 ab
	PBF	1.00 b	1.60 a	1.78 a	1.80 a	2.00 a	1.90 a	1.82 a	1.95 a	1.79 a	1.91 a
Dlash Masia	PBN (%)	38	22	0	0	2.00 u 0	0	0	0	0	0
Black Magic	ABF	1.45 b	1.90 ab	2.00 ab	2.75 a	2.45 ab	2.70 a	2.60 a	2.58 a	2.76 a	2.43 ab
	BB (%)	35	15	15	0	10	5	0	11	0	14
	PBF	0.60	0.78	0.80	0.98	1.18	1.02	0.82	1.22	1.00	0.60
Princess	PBN (%)	20	20	0	0	0	0	0	0	0	0
111111111111	ABF	0.05 d 85	0.25 cd 48	0.50 bc 17	0.75 ab	0.90 ab	0.85 ab 14	0.75 ab	1.10 a 0	1.11 a 6	0.93 ab
	BB (%)				6	0		5			0
	PBF PBN (%)	0.05 b 40	0.20 b 40	0.58 ab 20	0.40 ab 0	0.60 ab 0	0.61 ab 0	0.62 ab 0	0.60 ab 0	0.80 a 0	0.59 ab 0
Sugraone	ABF	40 0 b	0.20 ab	0.45 ab	0.65 a	0.45 ab	0.45 ab	0.60 a	0.53 a	0.67 a	0.60 a
	BB (%)	80	45	15	0	15	20	5	0	28	13
	PBF	0.40 d	0.80 c	1.40 b	1.60 ab	1.80 ab	1.81 ab	1.75 ab	1.95 a	2.00 a	1.79 ab
V ² - t - ··· -	PBN (%)	20	0	0	0	0	0	0	0	0	0
Victoria	ABF	0.55 b	1.15 ab	1.30 a	1.60 a	1.79 a	1.45 a	1.55 a	1.90 a	1.81 a	1.80 a
	BB (%)	60	35	15	15	5	15	20	5	5	5
	PBF	0.40 c	0.40 c	0.40 c	0.60 bc	1.00 ab	1.20 a	1.00 ab	1.00 ab	1.10 a	1.20 a
Luisa	PBN (%) ABF	20 0.20 b	0 0.35 b	0 0.35 b	0 0.65 ab	0 0.65 ab	0 0.90 a	0 0.95 a	0 0.95 a	0 0.85 a	0 1.05 a
	BB (%)	0.20 D 55	0.33 D 40	0.33 D 10	0.65 ab	0.65 ab 5	0.90 a 0	0.95 a 0	0.95 a 5	0.85 a 0	1.05 a 6
	PBF	0.60 b	1.00 ab	1.00 ab	1.20 ab	1.38 a	1.60 a	1.42 a	1.40 a	1.00 ab	1.20 ab
Michele	PBN (%)	60	20	20	0	0	0	20	20	0	0
Palieri	ABF	0.90	1.25	1.35	1.15	1.60	1.55	1.30	1.47	1.18	1.18
	BB (%)	25	10	15	10	5	5	5	5	0	0
	PBF	0.40 c	1.00 b	1.40 ab	1.40 ab	1.80 a	1.60 ab	1.80 a	1.60 ab	2.00 a	1.90 a
Pink Muscat	PBN (%)	40	40	20	20	0	20	20	20	0	0
1 million about	ABF BB (%)	0.60 b 40	1.15 ab 25	1.40 ab 5	1.65 a 10	1.90 a 10	2.00 a 5	1.75 a 0	2.10 a 5	1.90 a 15	1.80 a 10
									-		
White	PBF PBN (%)	0.20 b 20	0.60 ab 20	0.60 ab 20	0.98 a 0	0.80 a 0	1.02 a 0	1.00 a 0	0.80 a 20	1.00 a 0	1.20 a 0
Seedless	ABF	0.25 b	0.75 ab	0.55 ab	1.00 a	1.05 a	0.75 ab	0.90 a	0.86 a	0.85 a	0.85 a
beculess	BB (%)	70	25	25	5	0	30	5	10	25	10
	PBF	0.20 c	0.80 ab	0.40 bc	1.00 a	1.20 a	1.20 a	1.00 a	0.80 ab	0.80 ab	1.00 a
	PBN (%)	20	20	0	0	0	0	0	0	0	0
Black Pearl	ABF	0.05 b	0.20 b	0.25 b	0.50 ab	0.70 a	0.70 a	0.85 a	0.85 a	0.85 a	0.90 a
	BB (%)	80	50	30	0	5	20	15	10	10	15
	PBF	0.35 c	0.55 c	1.20 b	1.30 ab	1.65 a	1.45 ab	1.55 ab	1.35 ab	1.45 ab	1.40 ab
Fiammetta	PBN (%)		0	0	0	0	0	0	0	0	0
1 iaiiiiiletta	ABF BB (%)	0.25 d 45	0.63 c 33	1.18 b 18	1.35 ab 9	1.58 ab 0	1.40 ab 5	1.70 a 0	1.44 ab 5	1.43 ab 4	1.48 ab 0
	. ,										
	PBF PBN (%)	0.20 c 0	0.45 c 10	1.00 b 0	1.30 ab 5	1.65 a 0	1.70 a 10	1.40 ab 10	1.55 a 0	1.45 a 10	1.35 ab 10
Apulia	ABF	0.25 c	0.45 c	1.13 b	1.20 ab	1.68 a	1.50 ab	1.60 ab	1.33 ab	1.43 ab	1.33 ab
	BB (%)	48	35	15	13	5	7	8	5	7	3
	00 (70)	40	55	10	13	5	1	0	5	/	3

Table 2. Potential and actual fruitfulness, primary bud necrosis and blind buds for each node of seedless and seeded table grape varieties (average between two seasons).

Variety	Buds	Node									
		1	2	3	4	5	6	7	8	9	10
Crimson	PBF	0.30 d	0.65 cd	0.75 bc	1.00 ac	1.20 a	1.10 ab	1.00 ac	0.85 ac	0.95 ac	0.90 ac
	PBN (%)	20	0	0	15	0	0	0	0	0	0
Seedless	ABF	0.18 b	0.45 ab	0.50 ab	0.67 a	0.63 a	0.65 a	0.78 a	0.73 a	0.77 a	0.77 a
	BB (%)	66	29	27	15	18	14	5	15	12	5
Summer Royal	PBF	0.90 e	0.95 de	1.20 ce	1.55 ac	1.45 ac	1.60 ab	1.70 a	1.30 bc	1.35 bc	1.25 cd
	PBN (%)	15	0	10	0	10	0	0	0	0	10
	ABF	0.78 b	0.80 b	1.03 ab	1.20 ab	1.50 a	1.40 a	1.50 a	1.25 ab	1.35 a	1.33 a
	BB (%)	25	25	5	15	5	5	5	10	8	10
Italia	PBF	1.15 b	1.40 ab	1.65 a	1.68 a	1.55 ab	1.70 a	1.45 ab	1.65 a	1.45 ab	1.25 ab
	PBN (%)	10	0	0	5	0	0	0	0	0	5
	ABF	1.08	1.43	1.55	1.58	1.45	1.43	1.38	1.53	1.43	1.33
	BB (%)	28	13	5	3	3	15	10	10	5	13
	PBF	0.20 c	0.55 b	1.50 a	1.60 a	1.45 a	1.80 a	1.75 a	1.50 a	1.75 a	1.6 a
Regal	PBN (%)	10	10	0	0	0	0	0	0	0	0
Seedless	ABF	0.10 d	0.13 d	1.23 c	1.43 ac	1.47 ac	1.70 ab	1.70 ab	1.35 bc	1.87 a	1.75 ab
	BB (%)	63	55	5	10	10	10	5	15	0	10

Table 2. Cont.

For each parameter (PBF and ABF), the letters indicate statistical differences within the varieties at p < 0.05 according to the REGWQ test. Potential Bud Fruitfulness (PBF, as the number of clusters/node according to the bud dissection), Primary Bud Necrosis (PBN, as a percentage of necrotic buds according to the bud dissection), Actual Bud Fruitfulness (ABF, as the number of clusters/node according to the visual observations in the vineyard), and blind buds (as a percentage of buds that did not sprout according to visual observations in the vineyard).

The use of bud dissection for a priori determination of bud fruitfulness gave repeatable data with the exception of varieties with >2–3 bud fruitfulness or with small primordia, which made the observation of some inflorescence primordia using the microscope difficult since these inflorescences were much less developed. However, we think a qualified and trained (for such varieties) technician could obtain good results even for 'difficult' varieties to be used for winter pruning. A brief description of the bud fruitfulness (potential and actual) of each variety is given below together with their percentages of PBN and blind buds in 10 node canes (Table 2).

3.2. Notes for Potential and Actual Bud Fruitfulness for Each Variety

3.2.1. Sugranineteen

The fruitfulness of this variety is always higher than 1.00 with the exception of node 1; the highest values (both potential and actual fruitfulness) were measured in the nodes from 4–5 onward. PBN and blind buds were observed only in nodes 1–6.

3.2.2. Red Globe

The fruitfulness of Red Globe was around 1.00, with the highest values in nodes 4–7. Both PBN and blind buds were generally observed in the basal nodes, but necrotic and blind buds were also observed in some other nodes of the cane.

3.2.3. Black Magic

This variety showed a high fruitfulness even from the first node. The actual fruitfulness was higher than the potential one because of the difficulty to detect the third inflorescence primordia in many buds with bud dissection (uncertain identification). PBN was observed in nodes 1–2, whereas blind buds were observed in almost all of the nodes on the cane.

3.2.4. Princess

Both actual and potential bud fruitfulness were below 1.00 in many nodes, with a few exceptions. PBN was observed only for the first nodes on a cane, whereas the incidence of blind buds was very high in the first two nodes (85 and 48%, respectively).

3.2.5. Sugraone

This variety showed low actual fruitfulness (0.40–0.80) in almost all nodes, with very low values in nodes 1–2 (<0.20). PBN was very significant in the basal nodes, with high values (40%) in nodes 1–2. Additionally, blind buds were observed almost in all of the nodes on the cane at variable percentages and up to 80% in node 1.

3.2.6. Victoria

Potential bud fruitfulness was below 1.00 only in the first two nodes, and that in the successive nodes was higher than 1.40. PBN was observed only in node 1, at 20% (Table 2). Blind buds were noticed in all nodes, with higher percentages in the basal part of the cane and around 5% in the distal nodes.

3.2.7. Luisa

Potential bud fruitfulness was lower than 1 in the basal nodes but increased in the middle and distal nodes (1.00–1.20). PBN was only observed in node 1. The actual bud fruitfulness followed the same pattern as that of potential fruitfulness although with lower values since fruitfulness was <1 in almost all buds, with a few exceptions. Blind buds were in the range 55–10% in the basal nodes but were lower, at almost 0%, in the middle-distal nodes.

3.2.8. Michele Palieri

Both actual and potential bud fruitfulness were higher than 1.00 in all nodes except node 1. Greater fruitfulness was observed in the nodes located at the middle portion of the cane. PBN was observed in the basal nodes and some distal nodes, whereas blind buds were observed in all of the nodes except the last two.

3.2.9. Pink Muscat

Potential bud fruitfulness was always higher than 1 with only node 1 as an exception. The same pattern was followed by the actual bud fruitfulness, with values >2. PBN was observed in almost all nodes with only a few exceptions (mainly in distal nodes), whereas blind buds were observed in all nodes but with values generally not exceeding 10–15% except in nodes 1–2.

3.2.10. White Seedless

Potential bud fruitfulness was lower than 1 in many nodes, with only a couple of exceptions, and actual bud fruitfulness was similar but with even smaller values. PBN was observed only in the first three nodes of a cane. The low actual fruitfulness was favored by the significant incidence of blind buds in almost all nodes of a cane.

3.2.11. Black Pearl

Both potential and actual bud fruitfulness were very low in the basal nodes but were higher in the middle cane, in particular for potential fruitfulness. PBN was observed only in nodes 1 and 2, whereas blind buds were significant in almost all nodes, with very high values in the basal ones.

3.2.12. Fiammetta

With the exception of nodes 1 and 2, bud fruitfulness of this variety was always higher than 1, with values around 1.5 in many nodes. PBN was not observed at all for this variety, whereas blind buds were <10% of buds with the exception of the basal nodes.

3.2.13. Apulia

Potential bud fruitfulness was higher than 1 from node 3 onward, and a similar pattern was followed by the actual bud fruitfulness. The values of PBN did not exceed 10% in 6 out

of 10 nodes, but blind buds were seen in all nodes at low values except for nodes 1 and 2 (48 and 35%, respectively).

3.2.14. Crimson Seedless

Potential bud fruitfulness was higher than 1 only in the nodes in the middle part of the cane, whereas actual fruitfulness was below 1 in all nodes, with lower values in the basal nodes. The incidence of PBN was very low because it was observed only in a couple of nodes; blind buds were observed in all nodes, at up to 66% in the first one.

3.2.15. Summer Royal

Both potential and actual bud fruitfulness were higher than 1 from node 3 onward or close to 1 (0.80–0.95) in the first two nodes. Bud fruitfulness was higher in the middle nodes with respect to the distal ones. PBN was only present in a few nodes but at low percentages, whereas blind buds were in all nodes in the range 5–25%.

3.2.16. Italia

For this variety, both potential and actual bud fruitfulness were higher than 1 in all nodes; in particular, the nodes with the highest fruitfulness were in the middle-basal nodes. PBN was observed at a low percentage only in three nodes, and blind buds, although observed in all nodes, were observed at a tolerable percentage taking into account the fruitfulness of the variety.

3.2.17. Regal Seedless

Potential bud fruitfulness of this variety was >1.5 in all nodes except nodes 1 and 2, with values close to 2 in some nodes. Actual bud fruitfulness was similar to the potential one and showed a very low fruitfulness in the first two nodes. PBN (10%) was only observed in nodes 1 and 2, whereas blind buds were observed in all nodes (except node 9), with values around 60% in nodes 1 and 2.

4. Discussion

The percentages of actual fertile buds (in the field) were lower than the potential values (in the lab). This difference can be ascribed to the incidence of blind buds during spring being counted as fertile using bud dissection in winter. Potential bud fruitfulness included inflorescence primordia (when visible) from secondary (or even tertiary) buds not emerging in spring and thus not contributing to the actual bud fruitfulness (although to a small extent) as also reported for Flame Seedless and Thompson Seedless in California [25].

Actual bud fruitfulness did not vary between the two seasons possibly because vineyard management was similar between the two seasons and climatic conditions did not differ much in the different vineyards. The highest values of fertility detected in this trial may indicate, apart from the genetic characteristics, a better adaptation of such varieties to the pedo-climatic conditions of the Puglia region (Italia, Victoria, Michele Palieri, Black Magic, Sugranineteen, etc.). Many international wine grape varieties generally show highly fertile buds in different worldwide environments, confirming their adaptability to various pedo-climatic conditions and vineyard managements, i.e., pruning, irrigation, fertilization, etc. [33,34].

In a recent paper [34], two wine varieties, Merlot and Cabernet Franc, presented 90 and 94%, respectively, of actual fertile buds, higher than our values, but some other varieties such as Prosecco, Fiano, or Sagrantino had 30–40% of fertile buds, even lower than Sugraone in this trial. In a study on table grape varieties (Black Magic, Sugraone, Red Globe, Victoria, Michele Palieri, and Italia) conducted in Sardinia region, Italy [35], the actual fertile bud values were quite similar for Black Magic (86.9 vs. 86.0%), Sugraone (43.4 vs. 46.0%), and Michele Palieri (83.9 vs. 75.0%) but different for Red Globe (76.4 vs. 57.0%), Victoria (79.5 vs. 93.0%), and Italia (86.9 vs. 59.0%). Actual bud fruitfulness was similar for Black Magic (2.36 vs. 2.10), Sugraone (0.46 vs. 0.49), and Michele Palieri (1.29

vs. 1.18) but were different for Red Globe (1.08 vs. 0.77), Victoria (1.49 vs. 1.73), and Italia (1.42 vs. 0.82). Blind buds were very similar for Victoria (18.0 vs. 18.9), slightly different for Black Magic (10.5 vs. 16.1) and Sugraone (22.1 vs. 16.9), and significantly different for Red Globe (5.5 vs. 11.7), Michele Palieri (8.0 vs. 14.3), and Italia (10.1 vs. 25.6). These differences for the examined parameters may be ascribed to the genetic material (rootstock and variety), the pedo-climatic conditions, and the viticultural practices adopted in the area. In particular, our trial was carried out in 2018 to 2019 whereas, for Sardinia, the trials were carried out from 2005 to 2007 and the climatic conditions of the seasons could have affected the results since climatic variations have a strong effect on yield and the quality of table grape varieties [36]. With regards to the viticultural practices, the pruning intensity, from 6 to 14 buds left, can influence the potential fertile buds, as reported for Midnight Beauty[®] in Brazil, where higher fertile buds were observed in the treatment with a bud load of 8–10 buds per cane [37]. A recent bud dissection study [32] with very long canes (30-nodes) of the Fiesta and Selma Pete varieties showed that nodes in the middle of the cane were the most fruitful.

Climatic conditions seem to exert a strong influence on bud fruitfulness on both wine grape varieties [38] and table grape ones [39]. Crimson seedless grown in the São Francisco Valley region (Brazil) showed a bud fruitfulness of 0.17–0.39 in a 9 node cane [39], much lower than our mean value of 0.61, thus confirming the role of pedo-climatic conditions regardless of viticultural practices. Environmental conditions have a great influence on bud fertility, and light intensity, temperatures, and water availability are the most important factors. High light intensity and temperatures promote synthesis of cytokinins (CKs) that favor differentiation of the anlagen into inflorescence primordia instead of tendrils [40]. Cytokinins (mainly transported from the roots) are essential for the transition from undifferentiated cells to differentiated primordia and for successive branching.

Temperature exerts a strong effect on inflorescence differentiation during season 1, with optimal temperatures in the range 20–35 °C, but high temperatures are fundamental for the differentiation of second and third inflorescences in many varieties [41], a more important aspect for the yield of table grape varieties compared to wine grape ones. Among climatic conditions, light is a key factor for inflorescence initiation and development, and exposure of buds to sun exposure increases the number of inflorescence primordia, whereas dense canopies have an opposite effect [41].

The most critical period for bud fruitfulness is around flowering (pre- and postflowering), and even mild water or nitrogen stresses at this time can negatively influence the fruitfulness of the successive season [42]. The rootstocks can also affect the bud fruitfulness and PBN, as reported in a study on Shiraz grafted onto 4 different rootstocks [43]. Bud fruitfulness was often lower at the basal positions and increased along the shoot, with a few exceptions, but it can decline at distal nodes, depending on the variety and trellis system adopted in the vineyard [25]. The highest values of bud fruitfulness have been reported in the medium and apical buds for some new table grape selections in Brazil [39]. The position of the fertile bud influences the pruning system to be adopted, and for varieties that have low fertility of basal buds, it is necessary to adopt a long or mixed pruning system (cane system). By adapting this type of pruning at the position of the most fertile buds, it is possible to increase productivity, as there will be an increase in the number of inflorescences and consequently an increased number of clusters per vine [6,34].

Bud fruitfulness of grapevine varieties can also be increased by foliar N applications, as recently reported for Trebbiano Romagnolo [44], and the best results (increased bud fruitfulness in the successive season) were attained when N was applied at floral initiation and differentiation. Similar positive effects of N fertilization were obtained in a two-year study conducted in France [42], where bud fertility during season 2 was significantly correlated with leaf N concentration during season 1. Apart from N, Pand K also seemed to influence the bud fruitfulness of the vine [41].

Defoliation at bloom and during the two successive weeks can negatively affect the formation of inflorescence primordia in the growing bud close to the leaf, thus affecting

the bud fruitfulness for the successive season [45]. Severe effects of defoliation have been reported when performed early in the season (pre-flowering) with the removal of primary leaves and secondary (lateral) shoots from the first six nodes in Aragonez variety cultivated in the Lisbon winegrowing region [46]. A reduction in bud fruitfulness was observed also during the successive seasons [46], indicating a carryover effect because of the reduced availability of carbohydrate to be used by the buds during the initiation and differentiation of inflorescence primordia at flowering time [8].

Excess shoot vigor can lead to a reduction in bud fertility in grapevines, which is correlated with the occurrence of primary bud necrosis, i.e., PBN [43,47]. Thus, the adoption of crop practices to control shoot vigor and to increase the intensity of light on buds can ameliorate bud fertility (i.e., delayed winter pruning time). The practice of shoot thinning was shown to significantly increase the inflorescence primordia in the wine variety Semillon, probably as a consequence of both the increase in light interception at the bud zone [48] and more sources (carbohydrates) stored in the bud tissues or in the closest tissues (parenchymatic rays, xylem, and phloem) of the cane to be used either for inflorescences formation during the first season or at bud break during the second season for ongoing inflorescence are affected not only by C reserves accumulated in buds and close portions of the cane during season 1 but also by the carbohydrates of the season 2 from leaves to the developing inflorescences [41].

Cultural practices in the vineyard influence the bud fruitfulness during the successive season, but varietal differences have also been reported, i.e., in Shiraz, bud fruitfulness was more sensitive to water deficits, whereas in Aranel, bud fruitfulness was more sensitive to nitrogen deficiencies [42]. Water status of the vine also has a significant effect on inflorescence initiation, since stressed vines reduce bud fruitfulness as a consequence of reduced photo-assimilates (carbohydrates) [41]. Shortage of water during the differentiation period can reduce inflorescence primordia along the cane and more in the distal nodes [11].

Canopy management practices affected the primary buds, and no effects were reported for secondary/tertiary buds [48], since these secondary/tertiary buds are 'emergency buds' for wine in case of adverse conditions.

The application of hormones is a common practice used in table grape vineyards to thin berries and to increase berry size. In particular, gibberellins (GAs) are commonly adopted for seedless grapes during berry growth, a stage occurring at bud differentiation on the shoot. GAs reduce bud fruitfulness, and external applications of this hormone promote the formation of tendrils or intermediate structures more than inflorescences [41]. CKs have an opposite action to that of GAs, favoring the formation of inflorescences instead of tendrils [41].

In this two-year study, we did not find any significant relationship between PBN and actual fruitfulness, as reported for Shiraz in Australia [43]. The PBN incidence was higher at basal node positions, as also reported in previous works [28,47] and more recently for Riesling and Cabernet Sauvignon [33]. Elevated CO₂ because of climate change or the adoption of some viticultural techniques (plastic covered trellis systems) did not affect the number of IPs and the incidence of PBN at individual node positions for two wine varieties, Riesling and Cabernet Sauvignon, grown in Germany [33].

Potential fruitfulness measured at dormancy (prior to pruning) could be a good indicator of cluster number in the following spring. Our results seemed to provide information on many of the examined varieties to predict yield for the successive season. However, canopy management techniques may not only influence production in the current season but also could have a carryover effect on the potential yield components for the next season [48]. We did not detect differences for the varieties between seasons possibly because the management techniques adopted in the different vineyards were similar between the two seasons and within the same vineyard and climatic conditions did not differ much too.

Similar (or lower) correlations have been found between potential and actual fruitfulness for some wine grape varieties such as Riesling, Cabernet Sauvignon [25,33], and Shiraz [43] and were very similar for table grape varieties such as Flame Seedless and Thompson Seedless [25]. These values can suggest to growers the optimal pruning method in order to achieve the most balanced yield for the vineyard, varying either the length of canes and number of nodes (intensity of pruning), the number of canes to be kept (depending on the fruitfulness at each node), or if a spur pruning system can be adopted. Although pruning long canes can increase the overall vine productivity, vines with long canes had reduced budbreak, fewer clusters per node, and less soluble solids per berry (Brix) than vines with short canes [32]. In raisin grapes, shorter canes produced less fruit with higher soluble solids, while long canes produced more fruit with lower soluble solids and the soluble solids decreased with node position, regardless of cane length or variety [32]. This is a factor to also be considered when choosing the number of nodes to be left after pruning.

5. Conclusions

Knowing the (potential) bud fruitfulness or PBN at each node can be a valuable tool for balanced winter pruning of table grape varieties, thus suggesting that the pruning system can be used in the vineyard during winter. Varieties that have low bud fruitfulness at the basal nodes should be pruned with the long (10–12) system, but varieties with high bud fruitfulness in the middle nodes can adopt a short (7–8 node canes) system of pruning with more canes left. A priori knowledge of PBN at each node is useful for pruning in order to reach the desired yield in the vineyard. The use of both cross (transverse) and longitudinal sections of the buds gave more accurate data to better distinguish the IP in difficult varieties.

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