GRAPEVINE BUD FERTILITY UNDER ELEVATED CARBON DIOXIDE

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Abstract:

Aims: Microscopic bud dissection is a common tool used to assess grapevine bud fertility and therefore to predict the yield of the following season. Grapevine yield has been shown to increase under elevated carbon dioxide (eCO_2) concentration and was demonstrated under Free Air Carbon dioxide Enrichment (FACE) conditions. The effect of eCO_2 on bud fertility in regards to this yield gain has not been investigated. However, little is understood about which yield components are affected and at what stage of development this occurs. The aim of this study was to determine the number and cross sectional area of the inflorescence primordia (IP), and the levels of primary bud necrosis (PBN) found in grapevine compound buds grown under two different CO_2 conditions and relate this data to yield parameters at harvest of field grown vines.

Methods and results: Plant material was collected in February 2016 and 2017 from two *Vitis vinifera* cvs., Riesling and Cabernet Sauvignon growing in the VineyardFACE experimental site at Hochschule Geisenheim University (49° 59′ N, 7° 57′ E) in the Rheingau wine region, Germany. Bud dissections were performed at the University of Adelaide's Waite Research Institute, Australia. There canes were stored at 4°C until dissection at room temperature. The first eight nodes of every cane were dissected and the compounds buds were assessed for primary bud necrosis (PBN), IP number and the cross sectional area of IP using image analysis.

No difference in IP number per node and subsequent number of bunches per shoot was observed between treatments in Riesling. However, larger cross sectional areas of IP were found in the compound buds grown under eCO_2. This was not supported by higher bunch weights and yield of Riesling for the eCO_2 treatment over the two years. Cabernet Sauvignon showed a higher IP number per node under eCO_2 but no changes in bunch number per shoot for the two seasons. A larger cross sectional area of IP was observed under eCO_2 treatment. This did translate into significantly higher bunch weights and yields of Cabernet Sauvignonover both seasons. Percentage of PBN was highest in the most basal node position along the fruiting cane. However, average PBN was not affected by eCO_2 for both cultivars along the cane.

Conclusions: Microscopic bud dissection can be used as a predictive tool to capture an increased bunch size at an early stage of vine development. There was evidence of a cultivar dependent response to bud fruitfulness under eCO_2. It will be of future interest to investigate whether higher carbohydrate levels could be responsible for the increase in IP area detectable at a very early stage of development under eCO_2.

Significance and impact of the study: This study contributes to an improvement in ourexisting knowledge about grapevine bud fertility and yield potential particularly under changing climatic conditions.

Keywords:

1.Introduction

Elevated CO_2 (eCO_2) concentration is one of the main drivers of a changing climate; however, the impact of this on bud fertility of field-grown grapevines has not yet been investigated. Studies that focused on the influence of elevated CO_2 concentration on grapevines, particularly in the field showed increased vegetative and fruit biomass under eCO_2 due to higher photosynthetic rates (Bindi *et al*., 2001; Moutinho-Pereira *et al*., 2009; Edwards *et al*., 2017; Wohlfahrt *et al*., 2018). Furthermore, eCO_2 treatment showed no effect on bunch number per vine, but increased bunch and berry weight (Bindi *et al*., 1996; Moutinho-Pereira *et al*., 2009; Wohlfahrt *et al*., 2018).

During winter dormancy grapevine yield potential for the next growing season can be evaluated and measured through an assessment of bud fruitfulness (May and Antcliff, 1973). Bud fruitfulness is dependent on various factors such as cultivar, management system, position of the bud along the cane, nutritional status of the vine and environmental influences such as climatic conditions (Huglin, 1958; Baldwin, 1964; Baldwin, 1966; Buttrose, 1970; May and Antcliff, 1973; Dry *et al*., 2010). For example, light and temperature are important factors of inflorescence induction and differentiation (Buttrose, 1974a; Dunn and Martin, 2000; Petrie and Clingeleffer, 2005).

Grapevine compound buds usually consist of a primary bud, which is predominantly responsible for bud fruitfulness and two or more secondary buds (May, 2000). If the primary bud is damaged or becomes necrotic, the secondary buds may in part compensate for the loss (Rawnsley and Collins, 2005). However, they are known to be less fruitful and therefore produce less yield (Pratt, 1974; Dry, 2000; Rawnsley and Collins, 2005). Primary bud necrosis (PBN) is a physiological disorder that results in the death of the primary bud and has been associated to changes in shoot vigour and carbohydrate levels (Dry and Coombe, 1994; Wolf and Warren, 1995; Vasudevan *et al*., 1998a; Rawnsley and Collins, 2005). Furthermore, Kavoosi *et al*. (2013) demonstrated an effect of the cane diameter and node position on PBN incidence. As such, bud fertility assessments are a valuable tool to predict a vineyard's yield potential and provide the opportunity to modify yield using management practices (Rawnsley and Collins, 2005).

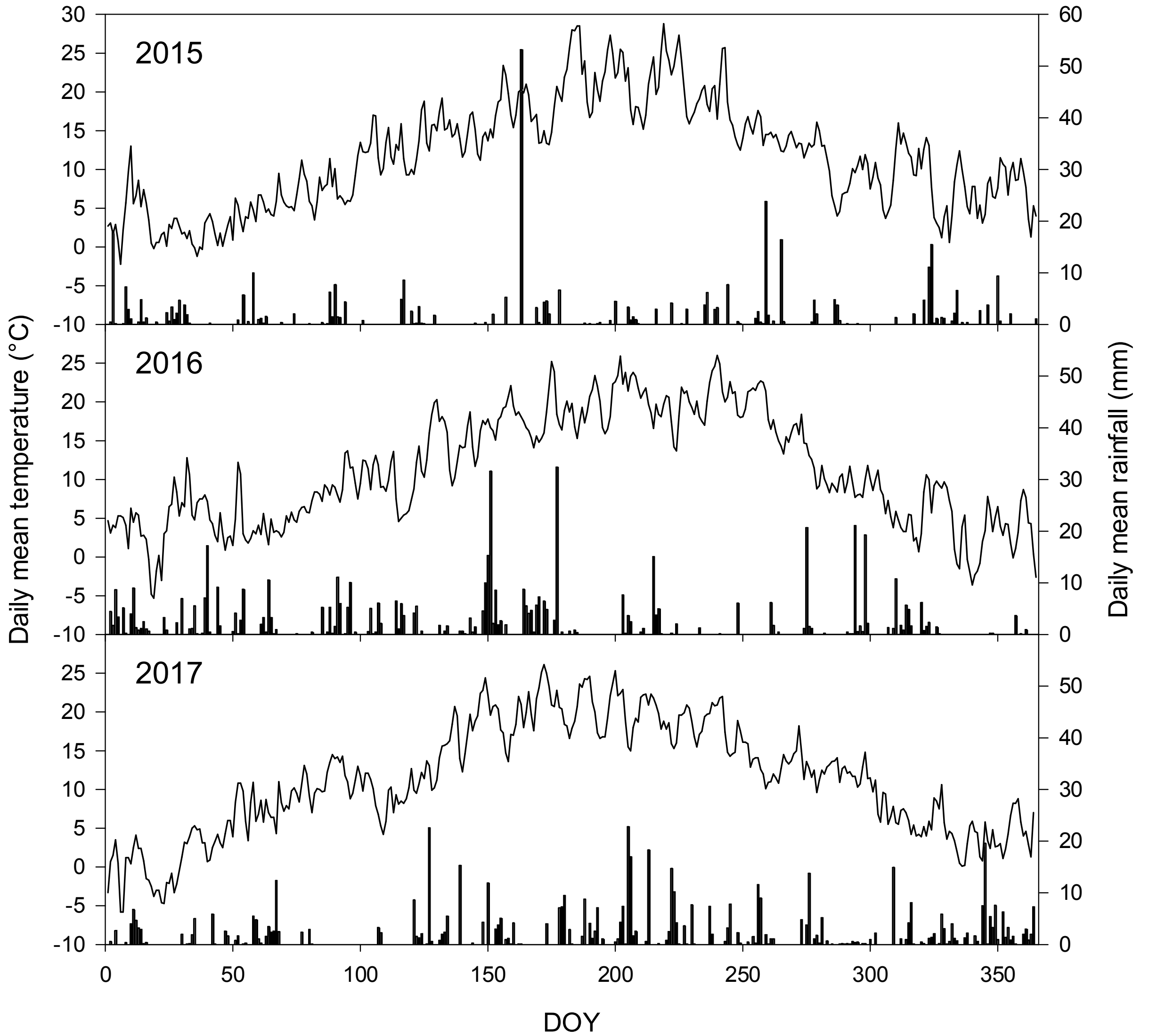
The aim of this study was to determine the number and cross sectional area of IP and the levels of PBN in compound buds of Cabernet Sauvignon and Riesling cvs. grown under two different CO_2 conditions. The relationship between bud fertility and yield at harvest was also investigated.

2.Materials and Methods

2.1. Experimental site and plant material

The experiments were conducted in 2016 and 2017 at the VineyardFACE field site, which is located at the Hochschule Geisenheim University (49° 59' N, 7° 57' E) in the Rheingau Valley, Germany. The vines were planted in 2012 within a six ring FACE-system at a planting density of 6170 vines ha^{-1} (planting distance 1.8 x 0.9 m), with North South oriented rows. The training system used was a vertical shoot positioning system (VSP) with one year old canes pruned to 8 nodes per vine or 5 nodes per m^2. The two *Vitis vinifera* L. cultivars used in the field experiment were Riesling (clone 198-30 Gm) grafted to rootstock SO4 (clone 47 Gm), and Cabernet Sauvignon (clone 170) grafted to rootstock 161-49 Couderc.

The soil at the field site is a sandy loam and climate conditions are characterized by a temperate oceanic climate with mild winters and warm summers. Long term averages (1981 to 2010) for annual temperature and rainfall are 10.5 °C and 543 mm, respectively. Weather data were collected from a weather station within the FACE experimental site. Annual rainfall and air temperature for the seasons 2015, 2016 and 2017 are shown in Supplementary Fig. 1. Average annual temperatures were 11.7 °C in 2015, 11.2 °C in 2016 and 11.3 °C in 2017. Average annual rainfall was 396, 583 and 590 mm respectively.



Supplementary Figure 1. Daily mean air temperature (solid line) and rainfall (black bars) in 2015, 2016 and 2017 at the Geisenheim VineyardFACE. DOY=day of year.

2.2. The VineyardFACE system / Carbon dioxide treatments

The VineyardFACE system was set up as a ring design, where two levels of the main effect CO_2, ambient (aCO_2, 400 ppm) and elevated (eCO_2: aCO_2 + 20%), were replicated by three 12-m diameter rings as shown in Supplementary Fig. 2. Elevated CO_2 rings were specified as E1, E2 and E3 whereas ambient CO_2 rings as A1, A2 and A3. Each ring consisted of seven rows, which were planted alternately with Riesling and Cabernet Sauvignon across a central divide with 67 plants per ring. Only the inner three rows of each ring were used for data collection. Within the VineyardFACE system wind direction and wind velocity were used to determine the release of + 20 % CO_2 from blowers in elevated rings as previously described (Wohlfahrt *et al.*, 2018). Blowers in aCO_2-rings were operated parallel to blowers in eCO_2 rings (E1-A1, E2-A2 and E3-A3) and were therefore defined as blocks. Elevated CO_2 concentrations were maintained from sunrise to sunset 365 days a year since 2014.

Supplementary Figure 2. VineyardFACE experimental site at Hochschule Geisenheim University with corresponding CO_2 tank (bottom right corner). The FACE-rings A1, A2 and A3 were related to ambient CO_2 level (aCO_2), whereas E1, E2 and E3 were related to elevated CO_2 level (eCO_2). Associated numbers to FACE-rings 1, 2 and 3 were defined as blocks.

2.3. Bud dissection assessment and image analysis

One day prior to winter pruning, at the end of February in 2016 and 2017, plant material was collected at the VineyardFACE. Two canes from nine vines per cultivar were chosen within the inner three rows of each FACE-ring. A total number of 216 one-year-old canes were labelled, cut down to approximately 10 nodes, packaged and stored below 4 °C. Subsequently plant material was brought by plane to the University of Adelaide following Australian Government quarantine standards. Bud dissections were then performed at the Waite Research Institute in the Department of Primary Industries and Regions South Australia (PIRSA) facilities in Adelaide. Canes were stored in plastic bags at 4°C until dissection at room temperature. Canes were then cut to eight nodes, excluding basal buds, and weighed before dissection. Internode length between the second and third node was also measured with a manual caliper (Mitutoyo, Japan). Nodes were dissected with a single edged razor blade (Personna, USA) and compounds buds were assessed for the number of IP and occurrence of PBN as illustrated in Fig. 1 by using a Leica MS5 Stereomicroscope (Leica, Germany). If PBN was present in primary buds the secondary buds were assessed for IP number and IP cross sectional area. Images of inflorescence primordia (Fig. 1) were taken from one cane per vine with a TLI Digital Eye-Piece MD500 (TLI, Australia) and the cross sectional area of IP was measured using ImageJ software (NIH, USA).

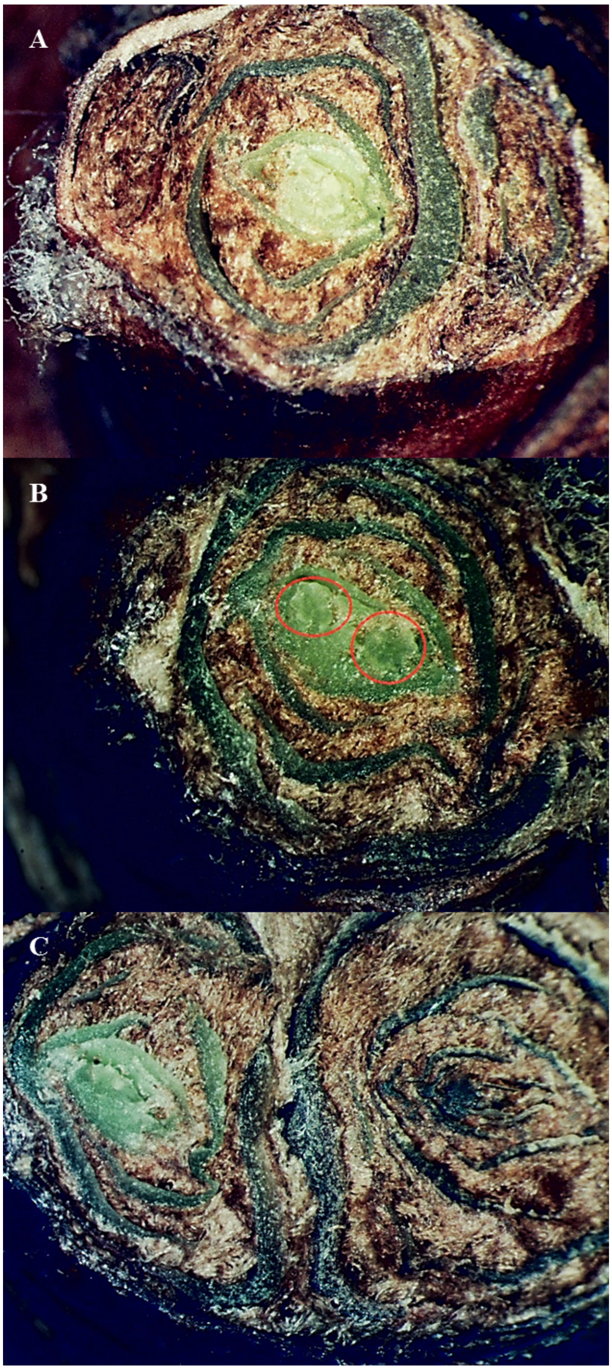


Figure 1. Bud dissection of bud fruitfulness and primary bud necrosis of Cabernet Sauvignon. Compound bud (A) with one healthy primary bud (middle) and two secondary buds. Two inflorescence primordia of first (B, red circle, right) and second order (B, red circle, left) visible in the primary bud (B) used for image analysis. Primary bud necrosis (C, right), secondary bud has enlarged to compensate for the loss of the primary bud (C, left).

2.4. Yield measurements

Bunch number per vine was assessed before veraison (July 2016 and 2017) on the same vines where material was collected for bud dissection analysis. Yield per vine was determined at harvest by weighing the fruit from individual vines. Average bunch weight per vine was calculated from yield and bunch number assessments.

2.5. Statistical analysis

Statistical analyses were performed with the statistical software R, version 3.4.2. Data for all parameters were tested using multi-factor (treatment, block, year and interaction treatment x year) analysis of variance (ANOVA) and Tukey`s honestly significant difference (HSD) test for significant differences at the $p \leq 0.05$ level. For all parameters, measured averages per FACE-ring were calculated and used for statistical analyses.

3.Results

All results of the ANOVA and the Tukey's test are shown in Table 1. Cane parameters of Riesling and Cabernet Sauvignon were not affected by eCO_2 levels. In both years, one year old canes had similar internode length, cane diameter and cane weight for both treatments (Table 2). Cabernet Sauvignon showed higher internode length and cane weights than Riesling.

Table 1. Results of the multi-factor ANOVA and the Tukey's test for the two cultivars and tested parameters.

*, ** and *** indicate statistical significance ($p < 0.05$, $p < 0.01$ and $p < 0.001$) of the main effects, ns = not significant.

Parameter	Treatment	Year	Block	Interaction treatment:year	Treatment	Year	Block	Interaction treatment:year
	Riesling				Cabernet Sauvignon			
Internode length	ns	ns	ns	ns	ns	ns	ns	ns
Cane diameter	ns	ns	ns	ns	ns	ns	ns	ns
Cane weight	ns	ns	ns	ns	ns	ns	ns	ns
IP no node position 1	ns	**	ns	ns	ns	ns	ns	*
IP no node position 2	ns	ns	ns	ns	ns	ns	ns	ns
IP no node position 3	*	**	ns	ns	ns	ns	ns	ns
IP no node position 4	ns	ns	ns	ns	*	ns	ns	ns
IP no node position 5	*	ns	ns	ns	*	ns	ns	ns
IP no node position 6	ns	ns	ns	ns	ns	*	ns	ns
IP no node position 7	ns	ns	ns	ns	ns	ns	ns	ns
IP no node position 8	ns	ns	ns	ns	ns	ns	ns	ns
PBN % node position 1	ns	*	ns	ns	*	**	ns	ns
PBN % node position 2	ns	*	ns	ns	ns	*	ns	ns
PBN % node position 3	ns	ns	ns	ns	ns	*	ns	ns
PBN % node position 4	ns	*	ns	ns	ns	ns	ns	ns
PBN % node position 5	ns	ns	ns	ns	ns	*	ns	ns
PBN % node position 6	ns	ns	ns	ns	ns	*	ns	ns
PBN % node position 7	ns	ns	ns	ns	ns	*	ns	ns
PBN % node position 8	ns	ns	ns	ns	ns	ns	ns	ns
PBN % average	ns	ns	ns	ns	ns	ns	ns	ns
IP no per node	ns	ns	ns	ns	*	ns	ns	ns
Bunch no per shoot	ns	**	ns	ns	ns	*	ns	ns
IP no PB	*	**	ns	ns	*	ns	ns	ns
IP no SB	ns	ns	ns	ns	ns	ns	ns	ns
IP area	*	**	ns	ns	**	***	ns	ns
Bunch weight	ns	*	ns	ns	*	ns	ns	ns
Bunch no per vine	ns	*	ns	ns	ns	*	ns	ns
Yield per vine	ns	**	ns	ns	*	ns	ns	ns

Table 2. Cane parameters for the two cultivars, Riesling and Cabernet Sauvignon, grown under different CO_2 treatments (aCO_2: ambient, eCO_2: elevated) for two seasons 2016 and 2017.

Internode length and cane diameter were measured between second and third node, cane weight was measured of the first eight node positions from the base of the cane. Means ± SD.

Variety	Year	Treatment	Internode length [cm]	Cane diameter [cm]	Cane weight [g]
Riesling	2016	aCO_2	4.77 ± 0.38	0.85 ± 0.07	30.88 ± 6.36
		eCO_2	4.85 ± 0.39	0.85 ± 0.02	31.47 ± 3.39
	2017	aCO_2	4.64 ± 0.40	0.90 ± 0.02	31.94 ± 3.35
		eCO_2	4.95 ± 0.19	0.91 ± 0.05	31.14 ± 5.23
Cabernet Sauvignon	2016	aCO_2	5.83 ± 0.38	0.84 ± 0.07	32.55 ± 5.26
		eCO_2	5.50 ± 0.50	0.90 ± 0.03	35.51 ± 2.82
	2017	aCO_2	4.97 ± 0.22	0.93 ± 0.06	35.28 ± 3.98
		eCO_2	5.57 ± 0.28	0.91 ± 0.01	33.03 ± 1.01

Primary bud fruitfulness of single node positions are shown for Riesling in Figure 2 and Cabernet Sauvignon in Figure 3. Cabernet Sauvignon had a lower average IP number per bud compared to Riesling. IP number was significantly higher at node positions 3 and 5 for Riesling under eCO_2 treatment over both years and influenced by the year at node positions 1 and 3 with higher IP numbers in 2017 (Table 1). Fruitfulness of Riesling increased along the cane in both years (Figure 2). Significant differences in CO_2 treatment were found in IP number of Cabernet Sauvignon with higher numbers at node positions 4 and 5. IP number at node position 6 was influenced by the year (Table 1). An interaction of treatment and year occurred for Cabernet Sauvignon IP number at node position 1.

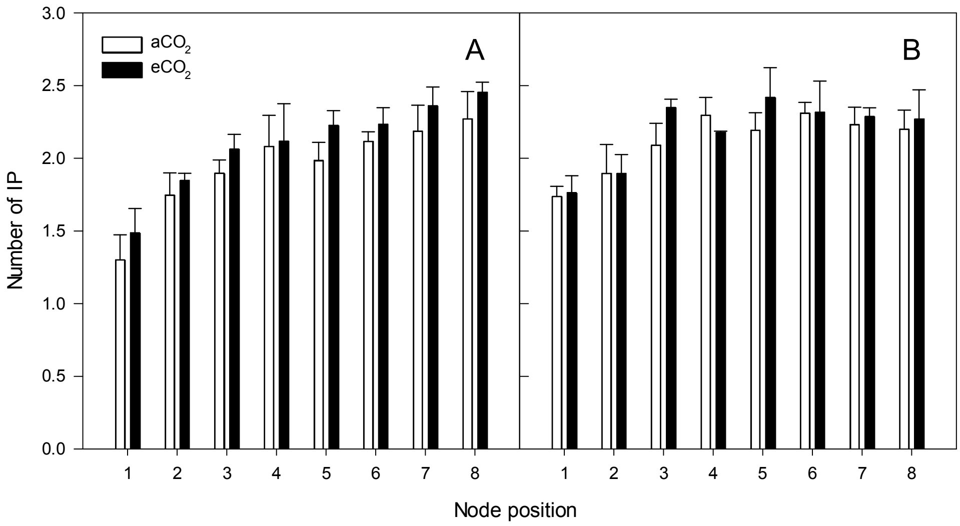


Figure 2. Number of inflorescence primordia (IP) per primary bud along the fruiting cane (node position 1 to 8) of Riesling grown under ambient CO_2 (aCO_2) and elevated CO_2 (eCO_2) in 2016 (A) and 2017 (B). Means ± SD.

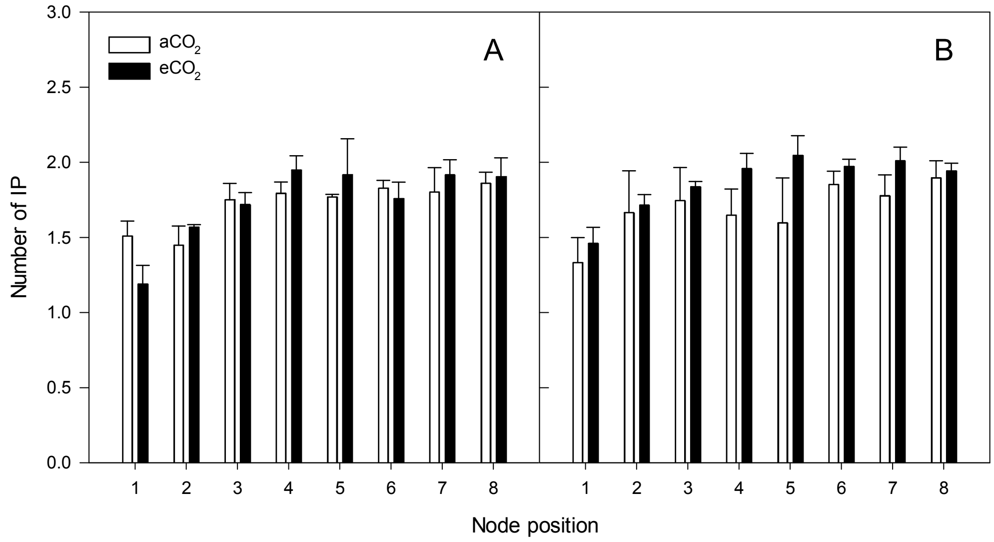


Figure 3. Number of inflorescence primordia (IP) per primary bud along the fruiting cane (node position 1 to 8) of Cabernet Sauvignon grown under aCO_2 (ambient CO_2) and eCO_2 (elevated CO_2) in 2016 (A) and 2017 (B). Means ± SD.

No significant differences in average PBN between treatments and years for both varieties were observed (Tables 1 and 3). Average PBN was lower in Riesling than Cabernet Sauvignon at a mean of 8.6 % less PBN per cane. PBN of single node positions showed only significant differences for the first node position of Cabernet Sauvignon (Table 1) with lower PBN levels for the eCO_2 treatment (Table 3). PBN of Riesling was not affected by eCO_2 (Table 3). The year had a significant effect on PBN of Riesling at node position 1, 2 and 4, whereas Cabernet Sauvignon was affected for almost all node positions, except node positions 4 and 8 (Table 1). Riesling had lower PBN levels at middle node positions compared to the basal and the distal ends of the cane, where occurrence of PBN was higher. Cabernet Sauvignon did show a similar behavior to Riesling in 2016 for PBN but differed in its occurrence with almost increased levels of PBN along the cane in 2017. This is shown by the higher influence of the year on Cabernet Sauvignons PBN incidence but not by effects of the treatment.

Table 3. Percentage of primary bud necrosis (PBN) of the compound buds along the fruiting cane (node position 1 to 8) and average per cane (AV) for two cultivars, CO_2 treatments (aCO_2: ambient, eCO_2: elevated) and years. Means ± SD.

Position along fruiting cane			1	2	3	4	5	6	7	8	AV
Variety	**Year**	**Treatment**	**PBN [%]**								
Riesling	2016	aCO_2	37.0 ± 3.2	18.5 ± 11.6	5.6 ± 0.0	16.7 ± 5.6	7.4 ± 6.4	7.4 ± 6.4	9.3 ± 6.4	14.8 ± 6.4	14.6 ± 5.7
		eCO_2	25.9 ± 3.2	14.8 ± 3.2	9.3 ± 11.6	9.3 ± 8.5	7.4 ± 8.5	7.4 ± 3.2	5.6 ± 5.6	1.9 ± 3.2	10.2 ± 5.9
	2017	aCO_2	24.1 ± 14.0	24.1 ± 6.4	13.0 ± 3.2	3.7 ± 3.2	13.0 ± 8.5	9.3 ± 3.2	11.1 ± 5.6	13.0 ± 12.8	13.9 ± 7.1
		eCO_2	13.0 ± 6.4	31.5 ± 6.4	27.8 ± 14.7	3.7 ± 6.4	13.0 ± 6.4	13.0 ± 3.2	14.8 ± 6.4	13.0 ± 3.2	16.2 ± 6.6
Cabernet Sauvignon	2016	aCO_2	46.3 ± 3.2	24.1 ± 6.4	20.4 ± 3.2	5.6 ± 5.6	13.0 ± 8.5	13.0 ± 12.8	20.4 ± 3.2	18.5 ± 8.5	20.1 ± 6.4
		eCO_2	37.0 ± 8.5	27.8 ± 9.6	22.2 ± 5.6	14.8 ± 8.5	9.3 ± 8.5	9.3 ± 11.6	13.0 ± 6.4	16.7 ± 5.6	18.8 ± 8.0
	2017	aCO_2	20.4 ± 3.2	11.1 ± 5.6	16.7 ± 5.6	9.3 ± 8.5	20.4 ± 11.6	29.6 ± 11.6	25.9 ± 12.8	31.5 ± 27.4	20.6 ± 10.8
		eCO_2	9.3 ± 6.4	13.0 ± 6.4	13.0 ± 3.2	18.5 ± 6.4	33.3 ± 5.6	38.9 ± 9.6	40.7 ± 11.6	31.5 ± 17.9	24.8 ± 8.4

Figure 4 shows the average IP number per node counted at dormancy and the average bunch number per shoot counted before veraison (July). On average, Riesling bunch number was predicted through IP number with a 96% accuracy for aCO_2 treatment whereas eCO_2 was predicted with an accuracy of 98% over the two years. Assessment of Cabernet Sauvignon IP number was higher with 25% for aCO_2 and 35% for eCO_2 treatment compared to the bunch numbers counted before veraison (Fig. 4). Average IP number of Cabernet Sauvignon was significantly higher under eCO_2 (Table 1). Bunch number per shoot of Riesling and Cabernet Sauvignon was affected by year as shown in Fig. 4 with lower levels in 2016.

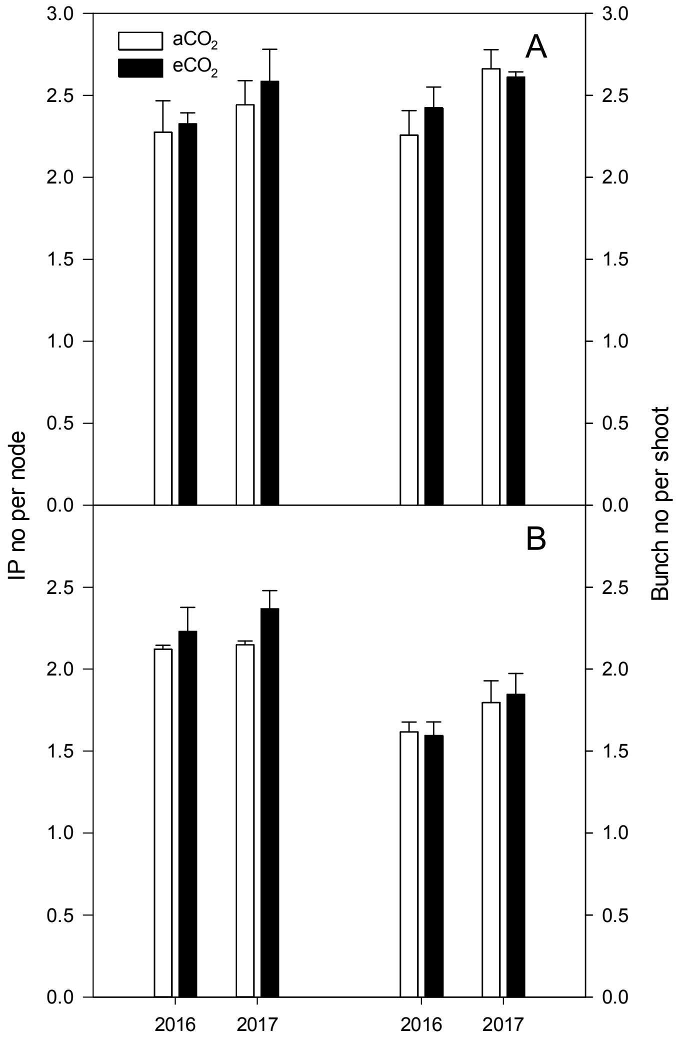


Figure 4. Average inflorescence primordia (IP) number per node (A and B, left) and average bunch number per shoot (A and B, right) for Riesling (A) and Cabernet Sauvignon (B) grown under aCO_2 (ambient CO_2) and eCO_2 (elevated CO_2) in 2016 and 2017. Means ± SD.

As shown in Table 4 the average number of IP in primary buds were higher than in the secondary buds. Secondary buds were less in number but they were only assessed when primary buds were damaged due to PBN. The number of IP in primary buds was significantly influenced by the treatment for both varieties and was also affected for Riesling by the year (Table 1). Both varieties showed a higher number of IP in primary buds under eCO_2 treatment (Table 4). Number of IP in secondary buds were not influenced by eCO_2 concentration or year. Between varieties, Riesling had higher IP numbers in the primary buds than Cabernet Sauvignon.

Table 4. Average number of inflorescence primordia (IP no) of primary (PB) and secondary buds (SB) of the compound buds for the two cultivars grown under different CO_2 treatments (aCO_2: ambient, eCO_2: elevated) in the two seasons.

IP of secondary buds were assessed when primary buds were damaged due to PBN. Means ± SD.

Variety	Year	Treatment	IP no	
			PB	SB
Riesling	2016	aCO_2	1.95 ± 0.07	0.33 ± 0.12
		eCO_2	2.10 ± 0.03	0.23 ± 0.07
	2017	aCO_2	2.12 ± 0.07	0.32 ± 0.16
		eCO_2	2.18 ± 0.03	0.40 ± 0.17
Cabernet Sauvignon	2016	aCO_2	1.72 ± 0.03	0.40 ± 0.02
		eCO_2	1.74 ± 0.03	0.49 ± 0.12
	2017	aCO_2	1.69 ± 0.11	0.46 ± 0.13
		eCO_2	1.87 ± 0.05	0.50 ± 0.10

Average IP area significantly increased under eCO_2 for Cabernet Sauvignon and Riesling over the two years of examination (Fig. 5). The year did also affect IP area of both varieties (Table 1) and showed lower values of IP area in 2017. Bunch weights significantly differed between treatments for Cabernet Sauvignon showing higher bunch weights under eCO_2 treatment. Riesling differed in bunch weights between years (Table 1), showing higher bunch weights in 2017 compared to 2016, whereas IP area was less in 2017 and higher in 2016 (Fig. 5). Cabernet Sauvignon showed higher IP area and bunch weights compared to Riesling (Fig. 5). The average area size of IP was 24.1 % higher in 2016 and 19.8 % higher in 2017 for Cabernet Sauvignon compared to Riesling.

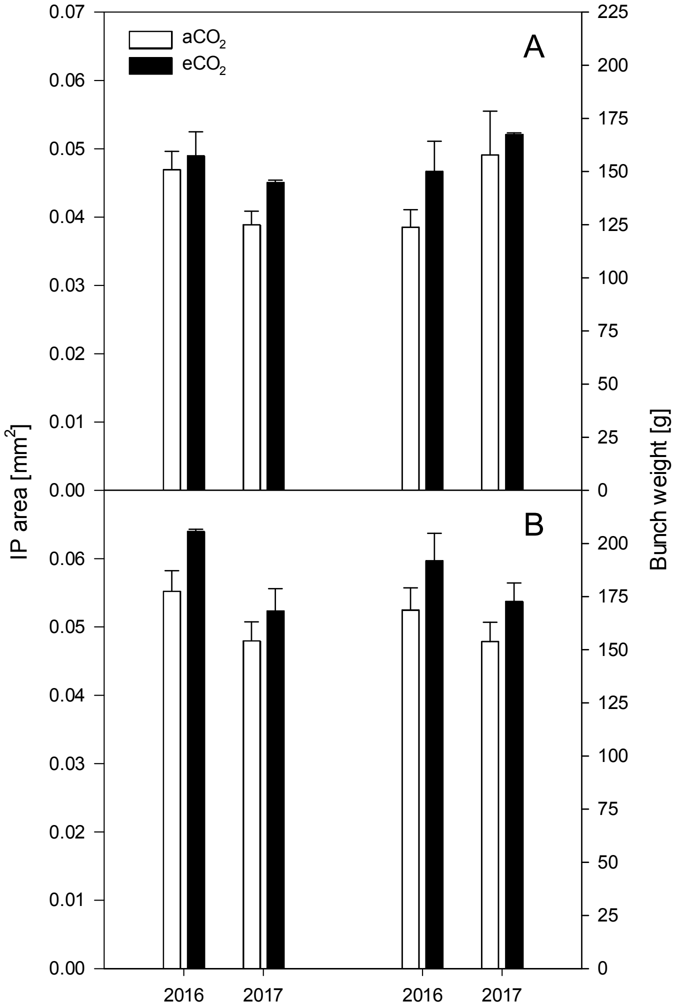


Figure 5. Average inflorescence primordia (IP) area (A and B, left) and average bunch weight (A and B, right) for Riesling (A) and Cabernet Sauvignon (B) grown under aCO_2 (ambient) and eCO_2 (elevated) in 2016 and 2017. Means ± SD.

As shown in Table 5, CO_2 concentration had no impact on bunch number of both varieties but was affected by the year (Table 1). Riesling bunch number differed between seasons with a higher number of bunches in 2017 with an average of 2.4 to 3.2 more bunches. Seasonal differences of Cabernet Sauvignon ranged from 0.9 to 1.4 bunches more per vine in 2017 compared to 2016 (Table 5). Yield did significantly increase under eCO_2 for Cabernet Sauvignon but not for Riesling (Table 1). Riesling differed in yield among years with higher values in 2017.

Table 5. Bunch number and yield per vine for the two cultivars, Riesling and Cabernet Sauvignon, grown under different CO_2 treatments (aCO_2: ambient, eCO_2: elevated) for two seasons 2016 and 2017.

Means ± SD. Bunch number was recorded before veraison (July) and yield at harvest in 2016 and 2017.

Variety	Year	Treatment	Bunch number	Yield [kg vine^{-1}]
Riesling	2016	aCO_2	17.1 ± 2.1	2.11 ± 0.39
		eCO_2	17.7 ± 0.8	2.65 ± 0.26
	2017	aCO_2	20.3 ± 1.7	3.16 ± 0.47
		eCO_2	20.1 ± 1.0	3.30 ± 0.10
Cabernet Sauvignon	2016	aCO_2	12.6 ± 0.6	2.12 ± 0.20
		eCO_2	12.3 ± 0.5	2.33 ± 0.21
	2017	aCO_2	13.4 ± 0.7	1.97 ± 0.16
		eCO_2	13.7 ± 0.5	2.38 ± 0.19

4.Discussion

In the present study, the effect of elevated carbon dioxide levels on bud fruitfulness was investigated for two *V. vinifera* cultivars. The number of IP and the incidence of PBN detected at the single node positions was not affected by eCO_2 while the area of IP did change. The effects of eCO_2 also differed between Riesling and Cabernet Sauvignon and the two seasons of investigation, 2016 and 2017.

The examined cane parameters are indicators of shoot vigour and can be related to environmental conditions in the previous season and to cultivar dependent responses (Smart, 1985) as illustrated by higher Cabernet Sauvignon cane weights compared to Riesling. A high shoot vigour expressed as an increased cane diameter and internode length has been associated with a high incidence of PBN (Lavee *et al*., 1981; Dry and Coombe, 1994; Wolf and Warren, 1995). Nevertheless, this was not confirmed for the two varieties under eCO_2 treatment. Even though an increased growth in terms of lateral leaf area and vegetative biomass was reported earlier for the two cultivars grown under eCO_2 (Wohlfahrt *et al.*, 2018), primary shoot growth was unaffected by eCO_2 in the present study.

Comparing the two cultivars, for Cabernet Sauvignon it was confirmed that higher internode lengths and cane weights were accompanied with higher average PBN (Lavee *et al*., 1981; Dry and Coombe, 1994; Wolf and Warren, 1995). Both cultivars used within this study have been reported to be susceptible to PBN (Wolf and Warren,1995; Vasudevan *et al.*, 1998b; Rawnsley and Collins, 2005; Sanchez and Dokoozlian, 2005), but also a rootstock influence could be considered, as the two cultivars were grafted to different rootstocks (Cox *et al*., 2012; Kidman *et al*., 2013).

Bud fruitfulness is often lower in the first and second node position, and increases along the shoot but can decline at distal node positions depending on cultivar and trellis system (Sommer *et al.*, 2000; Sanchez and Dokoozlian, 2005). This was confirmed for basal buds of Riesling and Cabernet Sauvignon in both years with less IP at lower node positions. PBN incidence was opposite to this and tended to be higher at basal node positions (Lavee *et al*., 1981; Dry and Coombe 1994; Rawnsley and Collins, 2005; Kavoosi *et al*., 2013). In this study, results for Riesling agree with previous research where the highest

PBN levels occurred mostly in basal node positions, which corresponded to lower fruitfulness at the same node positions. PBN at single node positions of Cabernet Sauvignon was influenced by the year, represented by higher levels at the distal node positions in 2017. Hence, these finding supports the influence of cultivar driven responses regarding occurrence of PBN related to the node position (Lavee, 1987; Vasudevan *et al.*, 1998a).

Primary and secondary bud fruitfulness was examined and the primary buds were more fruitful than secondary buds for both cultivars. Secondary buds exhibited less growth and were reported to be less fruitful than the primary buds with smaller IP (Pratt, 1974; Srinivasan and Mullins, 1981; May, 2000; Rawnsley and Collins, 2005; Sanchez and Dokoozlian, 2005). Interestingly, the number of IP detected in primary buds was significantly increased under eCO_2 for both cultivars but not for IP number observed in secondary buds. It could be of importance that eCO_2 might induce higher primary bud fruitfulness in grapevines, but through the variation of seasonal climatic or environmental conditions as described by Sommer *et al.* (2000), these effects may be reduced depending on the sensitivity of the cultivar. Similarly, the average IP number per node was higher under eCO_2 for Cabernet Sauvignon, but was not validated by differences in bunch numbers per shoot examined before veraison. A seasonal impact was observed for both cultivars on bunch number per shoot as well as on bunch number per vine, which was described earlier in a three-year study conducted within the VineyardFACE (Wohlfahrt *et al*., 2018). The lower number of bunches in 2016 could have been induced due to the drier growing period in 2015 with only 227 mm of rainfall, whereas higher numbers in 2017 were possibly induced by the wet spring conditions in 2016 with 185 mm of rainfall that occurred in March, April and May as shown in supplementary Fig. 1. Buttrose (1974b) described in previous studies that fruitfulness as the number of bunch primordia is depressed with increasing water stress, and this could explain the fewer bunches in 2016 and was confirmed by lower levels of pre-dawn leaf water potential in 2015 compared to 2016 (Wohlfahrt *et al*., 2018).Nonetheless, Riesling was found to have higher variability in bunch number between seasons 2016 and 2017 than Cabernet Sauvignon. This varietal response to climatic conditions shows the sensitivity of Riesling when the number and size of inflorescence primordia are determined (Dry, 2000; Clingeleffer, 2010; Guilpart *et al*., 2014).

Elevated CO_2 did influence grapevine fruitfulness by increasing the IP area, which was confirmed for Riesling and Cabernet Sauvignon over the two years. More growth and vigour were caused by higher photosynthesis rates under eCO_2 (Wohlfahrt *et al*., 2018), therefore the development of compound buds and their IP may have been promoted by a higher accumulation of photosynthesis assimilates (Shaulis and May, 1971). Cabernet Sauvignon had higher IP area and mostly higher bunch weights compared to Riesling, but considering yield parameters, Riesling did compensate by higher number of bunches and therefore by higher yields than Cabernet Sauvignon. In addition, this compensation was also detected in higher number of berries per bunch under eCO_2 for Riesling compared to Cabernet Sauvignon (Wohlfahrt *et al*., 2018).

Cross sectional area of IP was less in 2017 than in 2016 for both cultivars, and Cabernet Sauvignon followed this pattern for bunch weight and yield. Cabernet Sauvignon had lower bunch weights in 2017, compared to 2016. Riesling differed in bunch weights and yields for the 2016 season with lower values for both treatments, whereas IP area was lower in 2017 compared to 2016. This could be due to a spring frost event in late April (28.04.2016) when phenological development of Riesling was more advanced compared to Cabernet Sauvignon. A monitoring of the frost damage revealed 14.4 % affected buds within Riesling whereas Cabernet Sauvignon had less damage at 8.1 % (data not shown). Due to this spring frost damage in 2016 within Riesling, secondary buds possibly compensated for the loss of primary shoots, which have been shown to be less fruitful with smaller bunches (Pratt, 1974; Dry, 2000; Rawnsley and Collins, 2005) and may explain the lower bunch weights observed for Riesling in 2016. Cabernet Sauvignon showed no spring frost damage in 2016 and remained less affected by climatic conditions than Riesling (Schultz and Jones, 2010).

When relating IP number per node, which was assessed during winter dormancy to the bunch number per shoot determined before veraison, the prediction of Riesling was surprisingly close to the actual number counted in the field. For Cabernet Sauvignon an overestimation of IP number compared to bunch number was observed and has been reported earlier (Cox *et al*., 2012).

In the present study, where near future climate conditions of rising CO_2 levels were simulated, a higher risk of PBN, which is negatively affecting bud fruitfulness, was not detected. Nevertheless, carbohydrate deficiency has been found to contribute to PBN incidence (Vasudevan *et al.*, 1998a) and needs to be investigated further under different CO_2 regimes conducted in the VineyardFACE. Therefore, it will be of future interest to prove if higher carbohydrate levels in buds could be responsible for the increase in IP area, which were detectable at a very early stage of development under eCO_2.

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