Received 11 March 2019 | Accepted: 17 April 2019 | Published: 24 May 2019
DOI: 10.20870/oeno-one.2019.53.2.2428

Grapevine bud fertility under conditions of elevated carbon dioxide

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This article is published in cooperation with the 21th GIESCO International Meeting, June 23-28 2019, Thessaloniki, Greece. Guests editors: Stefanos Koundouras and Laurent Torregrosa

ABSTRACT

Aims: Microscopic bud dissection is commonly used to assess grapevine bud fertility and thereby predict yield for the following season. Grapevine yield has been shown to increase in response to elevated carbon dioxide concentration (eCO_2), and this yield gain has been demonstrated under Free Air Carbon dioxide Enrichment (FACE). However, the effects of eCO_2 on bud fertility and their relation to yield gain have not been investigated. Little is known about which stages of development and which yield components are affected. The aim of this study was to determine the number of inflorescence primordia (IP) per node, the cross-sectional area of the IP, and the incidence of primary bud necrosis (PBN) in grapevine compound buds grown under conditions of ambient CO_2 concentration (aCO_2) and eCO_2, and to relate the data to yield parameters of field-grown vines at harvest.

Methods and results: Plant material was collected in February 2016 and February 2017 from two *Vitis vinifera* L. cultivars, Riesling and Cabernet-Sauvignon, growing at the VineyardFACE experimental site of 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. Canes were stored at 4 °C until dissection at room temperature. The first eight nodes of each cane were dissected and the compound buds assessed; IP number, IP cross-sectional area (evaluated by image analysis) and PBN incidence were recorded.

In Riesling, no differences were found between plants grown under the two CO_2 treatments in terms of IP number per node or subsequent number of bunches per shoot. Compound buds of Riesling plants grown under eCO_2 had larger IP cross-sectional area; however, this did not result in higher bunch weight or yield in response to eCO_2 over the 2 years. In Cabernet-Sauvignon, IP number per node was higher in plants grown under eCO_2 but no changes in bunch number per shoot were found in either season. In contrast, the larger IP cross-sectional area in Cabernet-Sauvignon in response to eCO_2 translated into higher bunch weight and yield in both seasons. PBN incidence of both cultivars was highest at basal node positions along the fruiting cane, except for Cabernet-Sauvignon in 2017, where both treatments showed higher incidence at the distal ends. In both cultivars, average PBN incidence along the cane was unaffected by eCO_2.

Conclusions: Microscopic bud dissection can be used at an early stage of vine development to predict increased bunch weight. There was evidence of a cultivar-dependent response to eCO_2 in terms of bud fruitfulness. In future, it would be interesting to investigate whether higher carbohydrate levels are responsible for the increase in IP area detectable at a very early stage of development in plants grown under eCO_2.

Significance and impact of the study: The findings of this study contribute to our understanding of grapevine bud fertility and yield potential, particularly under changing climatic conditions.

KEYWORDS

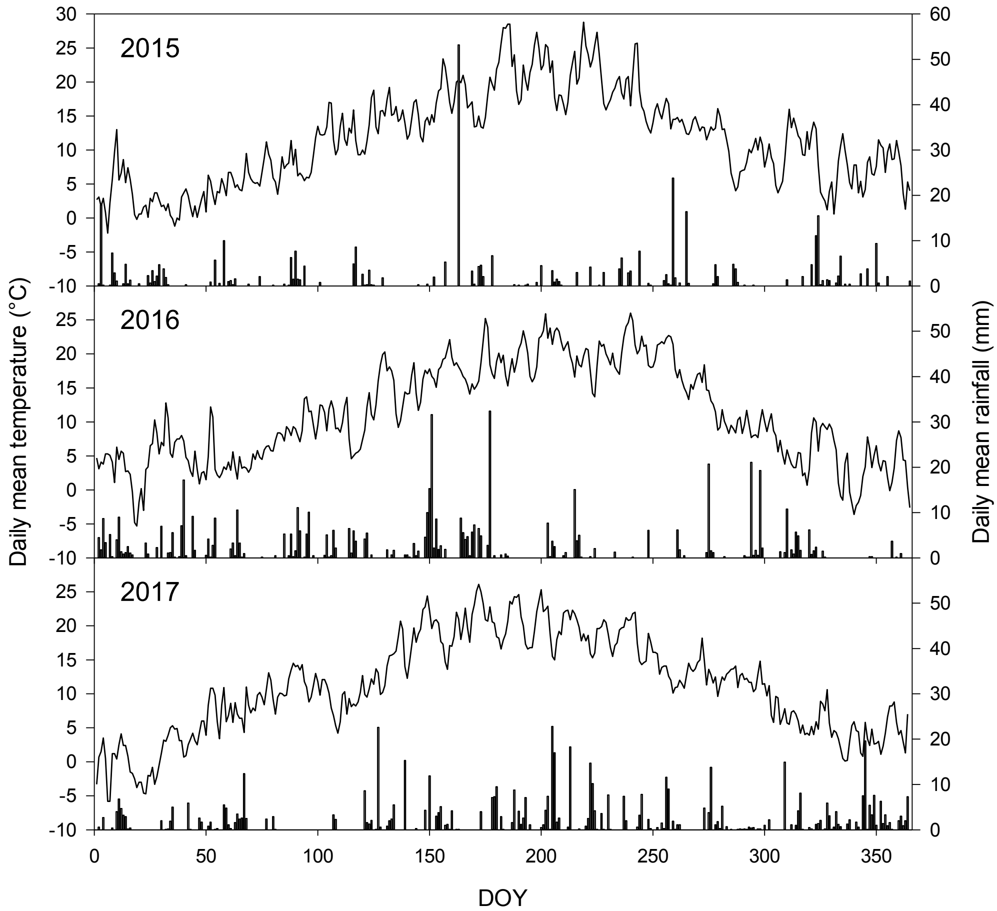
Vitis vinifera, carbon dioxide, bud fruitfulness, inflorescence primordia, primary bud necrosis, bunch weight

INTRODUCTION

Elevated CO_2 concentration (eCO_2) is one of the main drivers of climate change; however, its impact on the bud fertility of field-grown grapevines has not yet been investigated. Studies that focused on the influence of eCO_2 on grapevines, particularly in the field, have shown increased vegetative and fruit biomass under eCO_2, due to higher rates of photosynthesis (Bindi *et al.*, 2001 ; Moutinho-Pereira *et al.*, 2009; Edwards *et al.*, 2017; Wohlfahrt *et al.*, 2018). Although eCO_2 had no effect on the number of bunches per vine, bunch and berry weight were increased (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 by assessment of bud fruitfulness (May and Antcliff, 1973). Bud fruitfulness depends on various factors, including 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 affecting 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 partly compensate for the loss (Rawnsley and Collins, 2005). However, they are less fruitful and therefore produce a lower yield (Pratt, 1974; Dry, 2000 ; Rawnsley and Collins, 2005). Primary bud necrosis (PBN), a physiological disorder that results in death of the primary bud, has been found to be associated with reduced shoot vigour and carbohydrate levels (Dry and Coombe, 1994 ; Wolf and Warren, 1995 ;



SUPPLEMENTARY FIGURE 1. Daily mean air temperature (solid line) and rainfall (black bars) at the VineyardFACE experimental site of Hochschule Geisenheim University in 2015, 2016 and 2017. DOY, day of year.

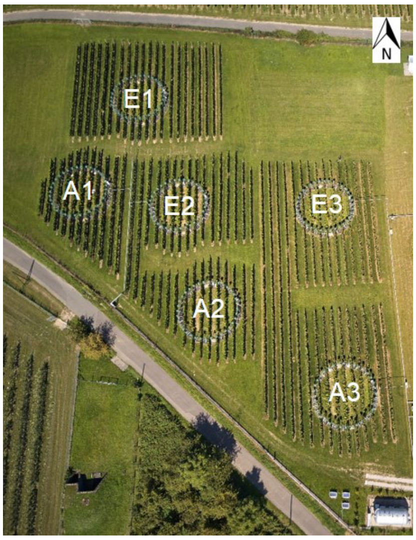
Vasudevan *et al.*, 1998a; Rawnsley and Collins, 2005). Furthermore, cane diameter and node position have been shown to affect PBN incidence (Kavoosi *et al.*, 2013). As such, bud fertility assessments are a valuable tool to predict a vineyard's yield potential and provide the opportunity to modify yield using management practices (Rawnsley and Collins, 2005).

The aim of this study was to determine the number of inflorescence primordia (IP) per node, the cross-sectional area of the IP, and the incidence of PBN in compound buds of the *V. vinifera* cultivars Riesling and Cabernet-Sauvignon grown under conditions of ambient CO_2 concentration (aCO_2) and eCO_2. The relation between bud fertility and yield at harvest was also investigated.

MATERIALS AND METHODS

1. Experimental site and plant material

The experiments were conducted in 2016 and 2017 at the VineyardFACE experimental site, which is located at 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 per ha (planting distance 1.8 m x 0.9 m), and North South oriented rows.



Supplementary Figure 2. The VineyardFACE experimental site of Hochschule Geisenheim University. The corresponding CO_2 tank is shown at the bottom right corner. Vines in rings A1, A2 and A3 were grown under ambient CO_2 concentration (aCO_2), and those in rings E1, E2 and E3 were grown under elevated CO_2 concentration (eCO_2). Rings A1–E1, A2–E2 and A3–E3 were defined as blocks.

The training system was a vertical shoot positioning system (VSP) with 1-year-old canes pruned to eight nodes per vine or five nodes per m^2. The two 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 experimental site is a sandy loam, and the climate is temperate oceanic, characterized by mild winters and warm summers. Long-term averages (1981–2010) for annual temperature and rainfall are 10.5°C and 543 mm, respectively. Weather data were collected from a weather station within the site. Annual rainfall and air temperature for the seasons 2015, 2016 and 2017 are shown in Supplementary Figure 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.

2. The VineyardFACE system and carbon dioxide treatments

The VineyardFACE system was set up as a ring design, where two levels of 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 Figure 2. The aCO_2 rings were specified as A1, A2 and A3, and the eCO_2 rings as E1, E2 and E3. Each ring consisted of seven rows, which were planted alternately with Riesling and Cabernet-Sauvignon with 67 plants per ring. Only material from plants growing in 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 the aCO_2 rings were operated in parallel with blowers in the eCO_2 rings, therefore A1–E1, A2–E2 and A3–E3 were defined as blocks. From 2014 onwards, eCO_2 had been maintained from sunrise to sunset, 365 days a year.

3. Bud dissection, bud assessment and image analysis

Plant material was collected from the experimental site one day before winter pruning, at the end of February in 2016 and 2017. Two canes from nine vines per cultivar were chosen out of the three inner rows of each FACE-ring. A total of 216 1-year-old canes were labelled, cut down to approximately 10 nodes, packaged and stored below 4 °C. The plant material was then transported by air to the University of Adelaide, following Australian Government quarantine standards.

Bud dissections were performed at the Waite Research Institute at the Department of Primary Industries and Regions South Australia (PIRSA)

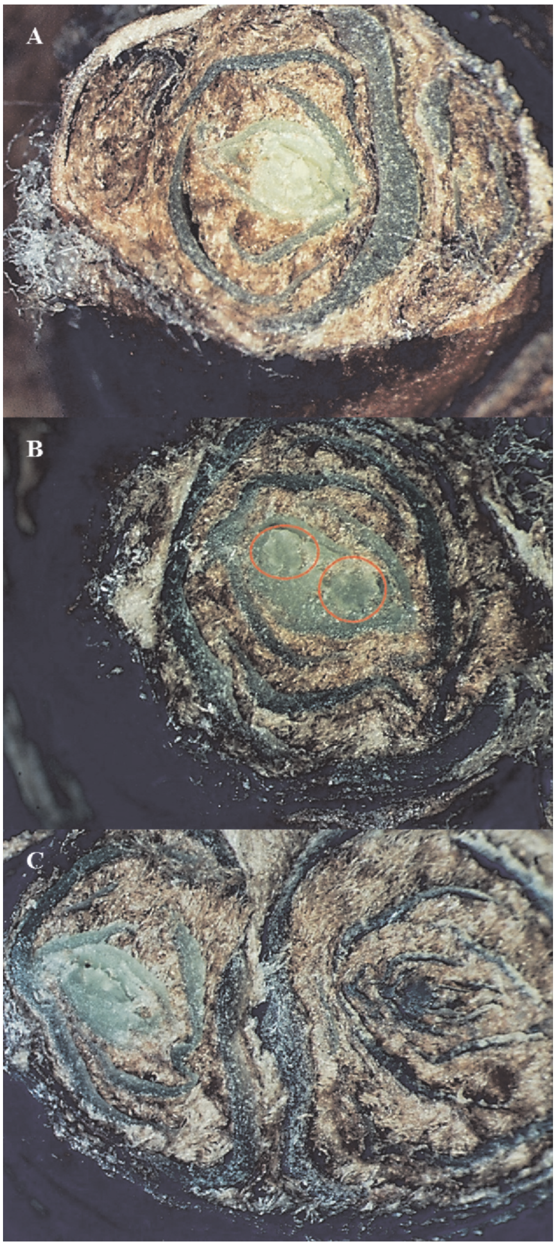


FIGURE 1. Bud dissection to assess bud fruitfulness and primary bud necrosis (example from Cabernet-Sauvignon). A, Compound bud with one healthy primary bud (middle) and two secondary buds. B, Primary bud with two inflorescence primordia (red circles) used for image analysis. C, Enlargement of the secondary bud (left) to compensate for loss of the primary bud (right) through primary bud necrosis (PBN).

facilities in Adelaide. Canes were stored in plastic bags at 4 °C until dissection at room temperature. The canes were cut to eight nodes, excluding the basal buds, and weighed before dissection. Internode length from the second to the third node was measured with a manual caliper (Mitutoyo, Kawasaki, Japan). Nodes were dissected with a single edged razor blade (Personna, Verona, VA, USA). The compound buds were then assessed for IP number and PBN, as illustrated in Figure 1, by using a Leica MS5 Stereomicroscope (Leica, Wetzlar, Germany). If PBN was present in the primary buds, the secondary buds were assessed for IP number. Then images of the IP, from one cane per vine, were obtained by using a TLI Digital Eye-Piece MD500 (TLI, Illawong, NSW, Australia) and used for the measurement of IP cross-sectional area by image analysis with ImageJ software (National Institutes of Health, Bethesda, MD, USA) (see Figure 1).

4. Yield measurements

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

5. Statistical analysis

Statistical analyses were performed with the statistical software R, version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria). Data for all parameters were tested using multi-factor (treatment, block, year and interaction treatment ´ year) analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test for significant differences (at the level P£0.05). For all parameters, measured averages per ring were calculated and used for statistical analyses.

RESULTS

Results of the ANOVA and Tukey's test are shown in Table 1. Cane parameters for Riesling and Cabernet-Sauvignon were unaffected by eCO_2. In 2016 and 2017, 1-year-old canes had similar internode length, cane diameter and cane weight, regardless of CO_2 treatment, and both internode length and cane weight were higher in Cabernet-Sauvignon than in Riesling (Table 2).

The results for primary bud fruitfulness at individual node positions are shown for Riesling in Figure 2 and for Cabernet-Sauvignon in Figure 3. Average IP number per bud was lower

TABLE 1. Results of the multifactor ANOVA and Tukey's test for the two *V. vinifera* cultivars and the parameters tested.

Parameter	Riesling				Cabernet-Sauvignon			
	Treatment	Year	Block	Interaction: treatment ↔ year	Treatment	Year	Block	Interaction: treatment ↔ year
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., primary buds	*	**	NS	NS	*	NS	NS	NS
IP no., secondary buds	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

IP, inflorescence primordia; PBN, primary bud necrosis. *, ** and *** indicate statistical significance at the levels of $P<0.05$, $P<0.01$ and $P<0.001$, respectively. NS, not significant.

for Cabernet-Sauvignon than for Riesling. For Riesling, IP number was significantly higher at node positions 3 and 5 under eCO_2 over both years and was influenced by 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). For Cabernet-Sauvignon, IP number was significantly higher at node positions 4 and 5 under eCO_2 over both years, and IP number at node position 6 was influenced by year (Table 1). An interaction of treatment and year occurred for Cabernet-Sauvignon IP number at node position 1.

No significant differences were found in average PBN incidence between the CO_2 treatments or between years for either cultivar (Tables 1 and 3). Average PBN incidence was lower in Riesling than in Cabernet-Sauvignon, with a mean of 8.6 % lower PBN incidence per cane. PBN incidence at individual node positions showed a significant difference for the first node position in Cabernet-Sauvignon (Table 1), with lower PBN incidence under eCO_2 (Table 3). In Riesling, PBN incidence was unaffected by eCO_2 (Table 3).

The year had a significant effect on PBN incidence in Riesling at node positions 1, 2 and 4, and in Cabernet-Sauvignon at all node positions except 4 and 8 (Table 1). In Riesling, PBN incidence was lower at the middle node positions than at the basal and distal ends of the cane (Table 3). In 2016, Cabernet-Sauvignon showed a similar pattern of PBN incidence to that of Riesling, but in 2017, PBN incidence tended to increase along the cane (Table 3). This

TABLE 2. Cane parameters for the two *V. vinifera* cultivars grown under ambient CO_2 concentration (aCO_2) and elevated CO_2 concentration (eCO_2) in 2016 and 2017.

Cultivar and 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

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

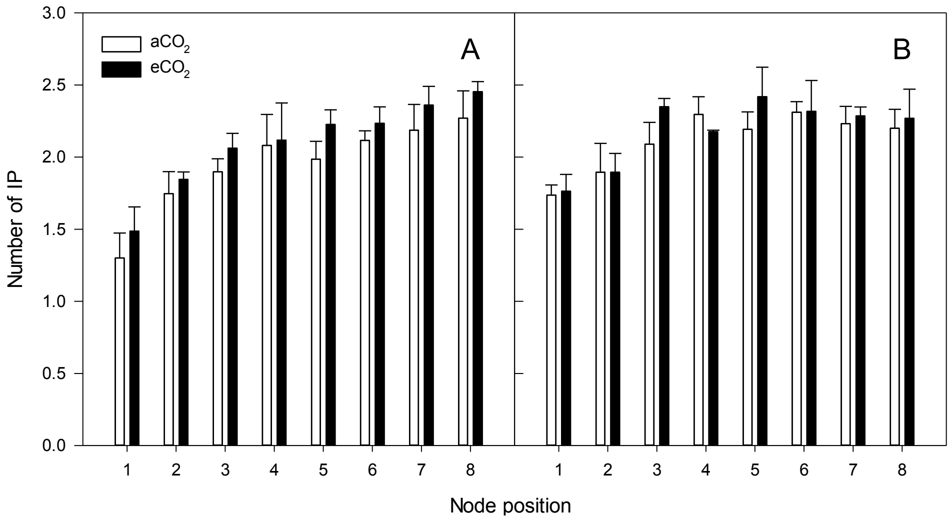


FIGURE 2. Number of inflorescence primordia (IP) per primary bud along the fruiting cane (node positions 1–8) for Riesling grown under ambient CO_2 concentration (aCO_2) and elevated CO_2 concentration (eCO_2) in 2016 (A) and 2017 (B). Means ± SD.

effect is reflected by the greater influence of the year on PBN incidence in Cabernet-Sauvignon, compared with the effect of the treatment (Table 1).

Figure 4 shows the average number of IP per node at dormancy and the average number of bunches per shoot before veraison (July). Over the 2 years, in Riesling, IP number predicted bunch number with 96 % and 98 % accuracy under aCO_2 and eCO_2, respectively. Assessment of Cabernet-Sauvignon IP number was higher, with 25 % and 35 % for aCO_2 and eCO_2 treatment, respectively, compared with respective bunch numbers before veraison.

In Cabernet-Sauvignon, average IP number was significantly higher under eCO_2 (Table 1). In Riesling and Cabernet-Sauvignon, bunch number per shoot was affected by year (Table 1), being lower in both cultivars in 2016 (Figure 4).

As shown in Table 4, the average number of IP in primary buds was higher than in secondary buds for both cultivars, Riesling and Cabernet-Sauvignon. Compared with primary buds, fewer secondary buds were assessed; secondary buds were assessed only when primary buds were damaged due to PBN.

For both cultivars, the number of IP in primary buds was significantly influenced by treatment, and for Riesling, it was also affected by year (Table 1). Both cultivars had a higher number of IP in primary buds under eCO_2 (Table 4). The number of IP in secondary buds was not influenced by treatment or year (Table 1). The number of IP in primary buds was higher in Riesling than in Cabernet-Sauvignon (Table 4).

Average IP cross-sectional area increased significantly under eCO_2 for Riesling and Cabernet-Sauvignon over the 2 years (Figure 5). Year also affected the IP area of both cultivars (Table 1), with lower values in 2017 (Figure 5). In Cabernet-Sauvignon, bunch weight was significantly higher under eCO_2 than under aCO_2 (Table 1). In Riesling, bunch weight differed between years (Table 1), being higher in 2017 than in 2016, and conversely, IP area was lower in 2017 and higher in 2016 (Figure 5). Both IP area and bunch weight were higher in Cabernet-Sauvignon than in Riesling (Figure 5). Average IP area was 24.1 % higher in 2016 and 19.8 % higher in 2017 for Cabernet-Sauvignon compared with Riesling.

As shown in Table 5, CO_2 concentration had no impact on bunch number in either cultivar, but it was affected by year (Table 1). In Riesling, bunch number differed between seasons, with a higher number of bunches in 2017 (average, 2.4–3.2 more bunches) (Table 5). In Cabernet-Sauvignon, seasonal differences ranged from 0.9 to 1.4 more bunches per vine in 2017 compared with 2016 (Table 5). Yield increased significantly under eCO_2 for Cabernet-Sauvignon but not for Riesling (Table 1). Riesling differed in yield between years, with higher values in 2017 (Table 5).

DISCUSSION

In the present study, the effect of eCO_2 on bud fruitfulness was investigated for two *V. vinifera* cultivars. The number of IP and the incidence of PBN at individual node positions were unaffected by eCO_2; however, the cross-sectional area of the IP did change. Additionally, the effects of eCO_2 differed between Riesling and Cabernet-Sauvignon and between the two seasons investigated, 2016 and 2017.

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

The results confirmed that the higher internode lengths and cane weights of Cabernet-Sauvignon are accompanied by higher average incidence of PBN (Lavee *et al.*, 1981; Dry and Coombe,

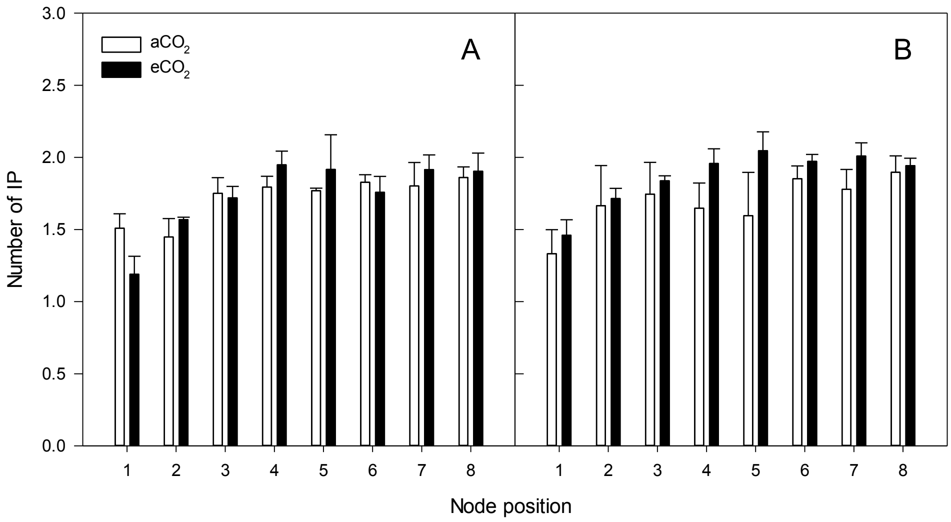


FIGURE 3. Number of inflorescence primordia (IP) per primary bud along the fruiting cane (node positions 1–8) for Cabernet-Sauvignon grown under ambient CO_2 concentration (aCO_2) and elevated CO_2 concentration (eCO_2) in 2016 (A) and 2017 (B). Means ± SD.

TABLE 3. Incidence of primary bud necrosis (PBN) in compound buds along the fruiting cane (node positions 1–8) and average incidence per cane (Av.) for the two *V. vinifera* cultivars under ambient CO_2 concentration (aCO_2) and elevated CO_2 concentration (eCO_2) in 2016 and 2017.

Cultivar and year	Treatment	PBN [%] at node position 1	2	3	4	5	6	7	8	Av. 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

1994; Wolf and Warren, 1995). Both cultivars used in the present 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 the influence of rootstock could also be considered, because the two cultivars were grafted to different rootstocks (Cox *et al.*, 2012; Kidman *et al.*, 2013).

Bud fruitfulness is often lower at the first and second node positions and increases along the shoot, but it can decline at distal node positions, depending on the cultivar and trellis system (Sommer *et al.*, 2000; Sanchez and Dokoozlian, 2005). This was confirmed for the basal buds of Riesling and Cabernet-Sauvignon in both years,

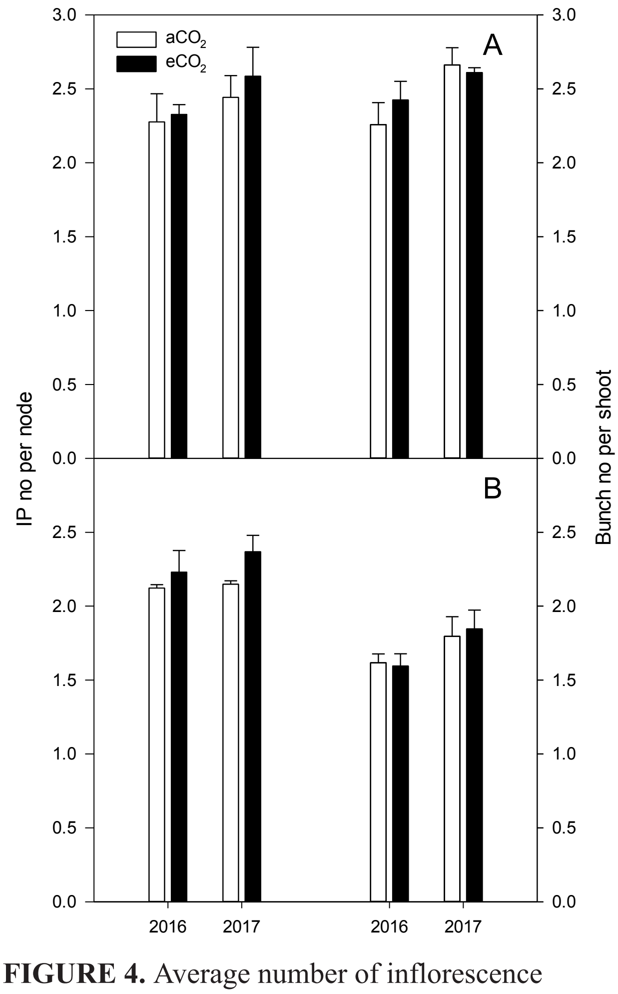


FIGURE 4. Average number of inflorescence primordia (IP) per node (four bars on left of each panel) and average number of bunches per shoot (four bars on right of each panel) for Riesling (A) and Cabernet-Sauvignon (B) grown under ambient CO_2 concentration (aCO_2) and elevated CO_2 concentration (eCO_2) in 2016 and 2017. Means ± SD.

TABLE 4. Average number of inflorescence primordia (IP no) in primary and secondary buds of the compound buds of the two *V. vinifera* cultivars grown under ambient CO_2 concentration (aCO_2) and elevated CO_2 concentration (eCO_2) in 2016 and 2017.

Cultivar and year	Treatment	IP no	
		Primary buds	Secondary buds
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

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

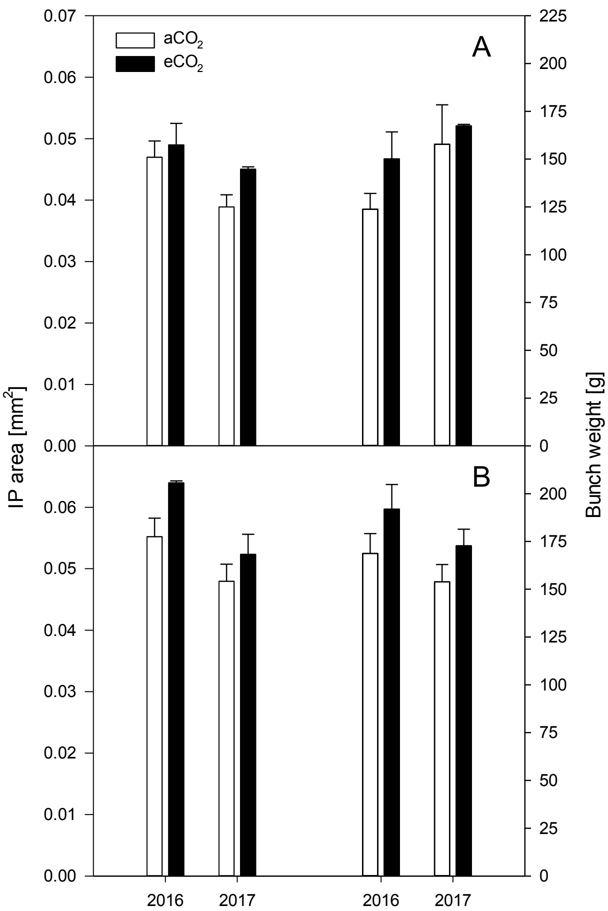


FIGURE 5. Average cross-sectional area of inflorescence primordia (IP) (four bars on left of each panel) and average bunch weight (four bars on right of each panel) for Riesling (A) and Cabernet-Sauvignon (B) grown under ambient CO_2 concentration (aCO_2) and elevated CO_2 concentration (eCO_2) in 2016 and 2017. Means ± SD.

with fewer IP at the lower node positions. The opposite was found for PBN incidence, which 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 the present study, the results for Riesling agree with the findings of previous research, in that PBN occurred mostly in the basal nodes, corresponding with lower fruitfulness at these positions. In Cabernet-Sauvignon, PBN at individual node positions was influenced by year, with a higher incidence at the distal node positions in 2017. Hence, these findings support the influence of cultivar-driven responses regarding occurrence of PBN related to the node position (Lavee, 1987 ; Vasudevan *et al.*, 1998a).

For both cultivars, primary buds were more fruitful than secondary buds. Secondary buds exhibited less growth and have been reported to be less fruitful than primary buds with smaller IP (Pratt, 1974 ; Srinivasan and Mullins, 1981 ; May, 2000 ; Rawnsley and Collins, 2005 ; Sanchez and Dokoozlian, 2005). Interestingly, although the number of IP detected in primary buds was significantly increased in both cultivars under eCO_2, there was no such effect in secondary buds. It is possible that eCO_2 increased primary bud fruitfulness in Riesling and Cabernet-Sauvignon grapevines, but through variation in seasonal climatic or environmental conditions, as described by Sommer *et al.* (2000), these effects may be limited by the sensitivity of the cultivar.

The average number of IP per node was higher under eCO_2 for Cabernet-Sauvignon. However, this observation was not reflected in differences

TABLE 5. Bunch number and yield per vine for the two V. vinifera cultivars grown under ambient CO_2 concentration (aCO_2) and elevated CO_2 concentration (eCO_2) in 2016 and 2017.

Cultivar and 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

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

in the number of bunches per shoot, recorded before veraison. A seasonal impact was found for both cultivars in terms of bunch number per shoot as well as bunch number per vine, and has previously been shown by the results of a 3-year study conducted within the VineyardFACE (Wohlfahrt *et al.*, 2018). The lower number of bunches in 2016 could have been a consequence of the drier growing period in 2015, with only 227 mm of rainfall, whereas the higher number in 2017 may have been due to the wet spring conditions in 2016, with 185 mm of rainfall in March, April and May of that year. Buttrose (1974b) has described the results of previous studies showing that fruitfulness (in terms of number of bunch primordia) is depressed by increasing water stress ; this could explain the fewer bunches in 2016 and has been confirmed by lower levels of pre-dawn leaf water potential in 2015 compared with 2016 (Wohlfahrt *et al.*, 2018).

Between seasons 2016 and 2017, bunch number was found to be more variable in Riesling than in Cabernet-Sauvignon. This varietal response to climatic conditions shows the sensitivity of Riesling, when the number and size of IP are determined (Dry, 2000 ; Clingeleffer, 2010 ; Guilpart *et al.*, 2014).

Elevated CO_2 concentration influenced grapevine fruitfulness by increasing IP area, as confirmed by the results for Riesling and Cabernet-Sauvignon over the 2 years. The increased growth and vigour caused by higher rates of photosynthesis under eCO_2 (Wohlfahrt *et al.*, 2018) ; the development of compound buds and their IP may have been promoted by greater accumulation of photosynthetic assimilates (Shaulis and May, 1971). Compared with Riesling, Cabernet-Sauvignon had higher IP area and mostly higher bunch weights, but when yield parameters are considered, Riesling compensated with a higher number of bunches and therefore higher yields than Cabernet-Sauvignon. In addition, this compensation was also detected in higher numbers of berries per bunch under eCO_2 for Riesling compared with Cabernet-Sauvignon (Wohlfahrt *et al.*, 2018).

In both cultivars, the cross-sectional area of IP was smaller in 2017 than in 2016. In Cabernet-Sauvignon, bunch weight and yield followed the same pattern. Riesling differed in bunch weight and yield for the 2016 season, with lower values for both CO_2 treatments ; in contrast, IP area was lower in 2017 than in 2016. This finding could have been due to a spring frost event in late April (28 April 2016), when the phenological development of Riesling was more advanced than that of Cabernet-Sauvignon. Monitoring of the frost damage revealed that 14.4 % of Riesling buds were affected, whereas Cabernet-Sauvignon had less damage, with 8.1 % of buds affected (data not shown). Because of the spring frost damage in 2016, the secondary buds of Riesling 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). This may explain the lower bunch weights for Riesling in 2016. Cabernet-Sauvignon showed almost no spring frost damage in 2016 and, compared with Riesling, remained less affected by climatic conditions (Schultz and Jones, 2010).

When number of IP per node, assessed during winter dormancy, was related to number of bunches per shoot, determined before veraison, the predicted Riesling yield was surprisingly close to the actual bunch number recorded in the field. For Cabernet-Sauvignon, bunch number was lower than would have been expected from the IP number, and this finding is consistent with those of previous studies (Cox *et al*., 2012).

In the present study, in which near-future climatic conditions of rising CO_2 levels were simulated, a higher risk of PBN, which reduces bud fruitfulness, was not detected. However, carbohydrate deficiency has been found to contribute to the incidence of PBN (Vasudevan *et al.*, 1998a) and needs to be investigated further under different CO_2 regimes in experiments conducted within the VineyardFACE. Therefore, in future it would be interesting to determine whether higher carbohydrate levels in buds could be responsible for the increase in IP area detectable at a very early stage of development under eCO_2.

Acknowledgements: The authors would like to thank the LOEWE excellence cluster FACE2FACE of the Hessian State Ministry of Higher Education, Research and the Arts for funding this work. For funding the research exchange with the University of Adelaide, the authors are grateful to the association Gesellschaft zur Förderung der Hochschule Geisenheim e.V. (GFHG) and the University platform Bordeaux–Adelaide–Geisenheim (BAG). For the opportunity to conduct bud dissections at the quarantine laboratory, we thank Pauline Glocke and Deborah Blackman of Primary Industries and Regions SA – PIRSA, Adelaide. For her assistance with bud dissection, we thank Annette James. Furthermore, we would like to thank J. A. Dittrich, F. Franke and W. Hölzer for their help in cutting and packaging canes at Hochschule Geisenheim University (HGU).

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