Double cropping in *Vitis vinifera* L. cv. Pinot Noir: agronomical and physiological validation

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Abstract

Background and Aims: Environmental effects of global warming are leading to extended ripening seasons, which may either require or allow new vineyard management techniques. An innovative double-cropping technique is proposed for temperate climate areas.

Methods and Results: The principle technique was to maintain the primary crop as well as to obtain a second, late ripening crop through release of dormancy of the auxiliary buds during the current season. Potted Pinot Noir vines were subjected to two forcing treatments over 2 years: trimming all the primary shoots at six nodes and removing any developing laterals at the end of the flowering and pea size stages. Vine growth, yield components and grape composition were monitored on both primary and forced shoots. In the second season, seasonal whole-vine gas exchange was evaluated in detail. Forced shoots carried 40–50% of the vine crop compared to primary shoots and fruit quality was greatly enhanced and higher TA was observed. Forcing treatments reached a similar net carbon dioxide exchange rate per vine compared to unforced Control about 2 weeks following auxiliary budburst. For the remainder of the season, forcing treatments maintained much higher net carbon dioxide exchange rate and water-use efficiency than unforced Control vines.

Conclusions: Detailed agronomical and physiological evaluation for 2 years confirmed the reliability of the double-cropping technique without compromising the pruning point selection for the next cropping year.

Significance of the Study: Future field application may disclose that this forcing technique is able to warrant two crops potentially suited to different wine styles within a single season.

Keywords: *bud dormancy, gas exchange, grape composition, summer pruning, yield*

Introduction

The impact of global warming coupled with a higher frequency of extreme weather events is a growing concern in several viticulture areas of the world. Research is currently active in finding either short-term adaptation solutions or long-term strategies, such as selection of new genotypes or alternative geographical distribution of grape cultivars (Schultz 2000, Jones et al. 2005, Hannah et al. 2013, Palliotti et al. 2014).

Within its complexity, climate change has also brought some advantages, paving the way towards some new and unexpected possibilities. The steady and still unhalted rise in atmospheric CO_2 concentration in C3 species such as grapevine is a driver to increased photosynthetic rates. Flore and Lakso (2011) in a review devoted to factors affecting leaf physiology in different fruit crops reported for grapes maximum assimilation rates (A) of about 12.5 μmol/(m^2· s) at about 330 μL/L of [CO_2]. Bindi et al. (2001) have reported that elevated [CO_2] had a significant effect on current season total dry mass with increases that ranged from 40 to 45% in the 550 μL/L treatment and from 45 to 50% in the 700 μL/L treatment when compared to the ambient [CO_2] recorded at around 370 μL/L. Adapting to a rapidly warming earth may include expansion of viticulture to cooler or elevated areas (van Leeuwen and Darriet 2016, Alessandrini et al. 2017, Mercenaro et al. 2019) or reduction of the impact of specific pests or diseases (Reineke and Thiéry 2016, Bois et al. 2017).

Another common trait of global warming impacting viticulture worldwide is that a specific heat requirement that would match ideal ripening for a given cultivar and resulting wine quality is achieved earlier in the season, causing progressive advancement of grape harvesting (Webb et al. 2012, Cook and Wolkovich 2016) with likely loss of grape quality (Petrie and Sadras 2008) and logistic issues in terms of fruit delivery to the winery. A longer 'growing' season is also expected as budburst starts earlier and potentially increases the risk of spring frost (Poling 2008, Schultze et al. 2014); in contrast, the postharvest season can be prolonged considerably (Schultze et al. 2014).

An extended growing season and therefore a higher potential for ripening is a scenario that, especially for early ripening cultivars, can attract the challenge of a feasible double cropping within a single season. An annual double cropping system in subtropical are as is a reasonably consolidated approach in tablegrape production (Bo et al. 2016) based on the integrated use of pruning, defoliation and chemical treatments. A case for such an approach has been reported for the winegrape cultivar Shiraz grown in Minas Geiras, Brazil, where a summer and a winter crop were obtained through pruning after the first harvest (Favero et al. 2011). Indeed, the technique, transferred to winegrapes grown in a temperate climate where vines

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typically undergo a long dormant season, sounds contemporary, innovative and challenging.

A double pruning/cropping approach, however, was originally tested in Australia by Dry in 1987 on Shiraz grapevines. The technique consisted of trimming growing shoots in the summer to six nodes and concurrently removing all laterals and bunches to force the dormant N+2 compound bud to push before entering *endo*-dormancy. This delay in the crop cycle positioned fruit ripening into a cooler period of the season, and the grape composition was significantly enhanced. The same technique was more recently applied by Gu et al. (2012) in the hot climate of Fresno, California. The study compared four dates of dormant bud forcing on Cabernet Sauvignon. Fruit maturation was delayed by up to 2 months when compared to the unforced control (hedging was performed between 21 and 42 days after anthesis). Interestingly, berry flavonoids increased linearly until harvest in forced vines while flavonoids in the control peaked and began to decrease. Martinez De Toda et al. (2019) tested different dates of forcing in Tempranillo and Maturana Tinta (Trousseau) cultivars, trimming the primary shoots between the second and the third internode. A consistent delay in ripening was observed (up to 2 months vs the unforced control) among all the forced treatments; this result, however, came at the expense of a large decrease in yield per vine and bud fruitfulness. Similar results were obtained by Lavado et al. (2019) and Martínez-Moreno et al. (2019) with Tempranillo.

Despite the observed effectiveness of bud forcing at maintaining fruit quality in some studies, the practice has a low likelihood of being adopted by growers due to three main limitations: (i) the drastic trimming of shoots and removal of all leaves on the primary shoot cause a strong source limitation that can severely impair the current and next season's yield; (ii) the removal of all primary bunches as well as main and lateral leaves (needed to unlock the dormant compound buds) can be extremely time consuming; and (iii) most importantly, convincing a grapegrower to drop the entire primary crop based on the trust that a second crop will originate from the unlocked dormant compound buds is a difficult task. In a recent proof of concept paper (Poni et al. 2020), it was successfully hypothesised that severe early spring trimming of main shoots coupled with removal of developing laterals can promote the growth of the compound bud in Pinot Noir grapevines. The newly originated forced shoots warranted a second delayed crop, enhancing yield and shifting maturity into a much cooler period without compromising the primary crop.

The objective of this work was to provide a more comprehensive agronomical and physiological understanding of the technique applied for two consecutive seasons on Pinot Noir grapevines. Besides evaluating the overall vine performance, this study focused on: (i) assessing any carryover effect on bud induction for cropping potential in the next year; and (ii) providing a whole-season assessment of gas exchange (namely net photosynthesis and transpiration) of the entire canopy using a whole-plant enclosure system to better evaluate the ripening potential for the second crop.

Materials and methods

Plant material and experimental layout

The experiment was carried out in 2019 and 2020 at the Department of Sustainable Crop Production (DIPROVES) of the Università Cattolica del Sacro Cuore in Piacenza (45°02′ N, 9°43′ E, 54 masl), Italy, using 12, 4-year-old grapevines cv. Pinot Noir (clone VCR18 grafted on Kober 5BB) grown outside in 35 L pots filled with a mixture of sand, peat and loamy soil (30, 20 and 50% by volume, respectively). Each year, the vines were spur-pruned to leave 4–5 two count-node spurs per vine on a 1 m cordon length. In case of two shoots per primary node, the secondary was removed as were any shoots coming from the base buds.

From the initial pool, each vine was randomly assigned to one of the three following treatments (four vines per treatment): unforced Control (UC), meaning that primary shoots were left to grow and were trimmed to retain 13–15 main leaves only once they outgrew the top foliage wire; forcing one (F1), where all the main shoots were trimmed above node 6 at BBCH 69 (Lorenz et al. 1995) (end of flowering); and forcing two (F2) was performed in the same way but at BBCH 73 (pea-sized berries). Concurrently, already developed lateral shoots were removed from the retained nodes of F1 and F2 vines. The lateral shoots were removed bi-weekly until they no longer developed. In 2019, the resulting forcing dates were day of the year (DOY) 154 for F1 and DOY 164 for F2; in 2020, forcing was executed on DOY 153 in F1 and on DOY 162 in F2. To better assess carryover effects of the technique, treatments were applied on the same vines in both the seasons. Details of the crop forcing techniques are given in Figure S1. Another batch of nine extra vines was prepared and treated accordingly (i.e. three vines per treatment) to provide destructive sampling material for determination of leaf area along the season.

In the F treatments, normal crop from trimmed primary shoots was maintained. The shoots growing from the forced compound buds were lightly trimmed when they outgrew the top wire (2019 season) or when they started to hit the roof of the plastic chambers (2020 season) hampering gas circulation through the chambers to the top outlets. Bunches developed on the forced shoots were not thinned and/or standardised. In all the treatments, the date of budburst was assessed visually as the swollen stage (BBCH 09), whereas veraison was set at 5% berry colour change.

Pots were painted white to minimise overheating of the root system. The number and intensity of daily irrigation events were calibrated to assure pot water capacity did not decrease below 90%. Disease management was primarily focused against downy and powdery mildews using sustainable local practices.

Vine measurements

Three primary shoots per vine originating from the distal node of three different spurs were tagged to make specific measurements. The day before F1 was applied (DOY 151) total vine leaf area (LA) in each treatment was estimated based on leaf counts and mean single blade area measured with a LI-3000A desk leaf area meter (LI-COR Biosciences, Lincoln, NE, USA) on 27 leaves per treatment sampled from the extra vines. To be representative of the leaf size gradient along the stem, one basal, one median and one apical leaf were removed. The same procedure was applied to estimate pending LA/vine in F2 on DOY 162 (1 day before forcing), in UC on DOY 192 and in all treatments on DOY 222.

At the time of forcing, on each tagged shoot, the main and lateral leaves were removed and separated, their fresh mass recorded and then processed through the leaf area meter. Vine LA removed with light trimming on DOY

193 in UC and on DOY 223 in all treatments was measured with the same method. Retained LA in the F treatments was calculated from node counts and leaf area of basal leaves sampled from extra vines of the same initial batch.

The number of total primary and forced shoots per vine was recorded along with their fertility given as the number of bunches per main or forced shoot. On each trimmed shoot, the node position originating a forced shoot from the compound dormant bud was registered. After harvesting the forced crop, in all the treatments, the primary tagged shoots and forced shoots were individually defoliated, recording the number of leaves, and their leaf area was recorded and separately measured. In UC, the contribution of laterals developed prior to or after trimming was also added. The main single leaf area for primary, forced and lateral shoots was calculated. Immediately after leaf fall, the number of nodes on the primary and forced shoots as well as on the UC lateral shoots was counted. The final leaf area for each type of shoot was estimated based on node counts and leaf blade areas. Total vine leaf area was then calculated as a sum of the three components.

In each season, berry samples from the primary crop of each treatment were collected bi-weekly to measure progression of TSS, pH and TA. The primary crop was harvested when fruit of each treatment (UC, F1, F2) achieved a TSS of ~18°Brix and a TA of ~8 g/L, identified as the optimal thresholds for Pinot Noir premium sparkling wines. In the first season the primary crop of UC was harvested August 7 (DOY 219) whereas the F1 and F2 primary crop was picked 11 and 7 days later, respectively. In 2020, due to a more compressed ripening, primary crop of all treatments was harvested on August 11 (DOY 223).

The mass of the primary crop was measured with a field portable scale and the number of bunches recorded. Mean bunch mass was then calculated accordingly. The bunches from tagged primary shoots (three per vine) were used to determine rachis length, number of berries per bunch, berry mass and bunch compactness, calculated as unit (g) of fruit mass per unit (cm) of rachis.

To explore the maximum ripening delay potential of the forcing technique, the harvest date was about the same in both years (DOY 280, 7 October in 2019 and DOY 281, 8 October in 2020) for all the forced shoots. This was the latest date available before the berries started to dehydrate and/or rot. Yield components were analysed as previously described for the primary crop. Vine balance indexed as the total leaf area-to-yield ratio was calculated for primary and forced shoots, as well as on a whole vine basis.

Fruit composition

Every season, at harvest, for both primary and secondary crop, a sample of 100 and 50 berries per vine and crop type, respectively, was crushed to obtain a must. Three berries per bunch were sampled taking care to cover top, median and bottom bunch zones. The TSS was measured using a temperature-compensating RX 5000 refractometer (Atago, Bellevue, WA, USA) and pH analysed with a digital PHM82 pH meter (Radiometer Analytical, Villeurbanne, France). For determination of TA, 10 mL of juice solution was titrated against a standardised 0.1 N NaOH solution to a pH 8.2 end-point and expressed as g/L of tartaric acid equivalents using a Crison Compact Titrator (Crison, Barcelona, Spain). Tartaric acid and malic acid were quantified via HPLC (Agilent Technologies, Santa Clara, CA, USA) after filtering the juice through a 0.22 μm polypropylene syringe into auto-sampler vials. For this analysis, an Allure organic acid column, 300 × 4.6 mm, 5 μm (Restek, Bellefonte, PA, USA) was used. Acids were separated under isocratic conditions using water, pH adjusted at 2.5 with ortho-phosphoric acid. The column temperature was maintained at 30 ± 0.1°C, and 15 μL of the sample was injected. The elution was monitored at 200–700 nm with detection by UV–Vis absorption with a diode array detector (DAD) at 210 nm. Organic acids were identified using authentic standards, and quantification was based on peak areas and performed by external calibration with standards.

Total anthocyanins and phenolic substances were determined on 50 and 25 berries per vine, either primary or forced crop, respectively. The berries were homogenised at 10 000 rpm with the Ultra-Turrax T25 (Rose Scientific, Edmonton, AB, Canada) homogeniser for 1 min; then 2 g of the homogenate was transferred to a pre-tared centrifuge tube, enriched with 10 mL aqueous ethanol (50%, pH 5.0), capped and mixed periodically for 1 h before centrifugation at 959 *g* for 5 min. A portion of the extract (0.5 mL) was added to 10 mL 1 mol/L HCl, mixed and allowed to stand for 3 h; absorbance was then measured at 520 and 280 nm on a Jasco V-530 UV spectrophotometer (Jasco Analytical Instruments, Easton, MD, USA). The concentration of total anthocyanins and phenolic substances was expressed as mg per g of berry fresh mass.

Whole-canopy and single leaf gas exchange

Whole-canopy net CO_2 exchange rate (NCER) was measured using the multi-chamber system (Poni et al. 2014). It features alternating current, centrifugal blowers (Vorticent C25/2 M Vortice, Milan, Italy) delivering a maximum air flow of 950 m^3/h, flexible plastic polyethylene chambers allowing 88% light transmission, 6% diffused light enrichment and no alteration of the light spectrum, a CIRAS-3 DC CO_2/H_2O differential gas analyser (PP-Systems, Amesbury, MA, USA), and a CR1000 data logger wired to an AM16/32B Multiplexer (Campbell Scientific, Shepshed, England). Switching of air sampling between chambers was programmed at a 60 s time interval using a set of solenoid valves; the air-flow rate to each chamber was controlled by a butterfly valve and measured with the Testo 510 digital manometer (Farnell, Lainate, Italy) following the flow-restriction method described by Osborne (1979).

In 2020, each test vine was chambered on DOY 140 (19 May), 12 days before applying the first forcing treatment, and operated 24 h a day until DOY 280 (6 October), when they were finally dismantled. Within this long period of 140 measuring days, short dismantling periods occurred as follows: from DOY 171 to 173 to allow disease assessment, light shoot-tipping of Control vines and system repair and maintenance; from DOY 190 to 193 due an energy black out caused by a heavy storm, which required laborious fixing of several electrical components; and on DOY 231 (18 August) when the single leaf readings were performed.

The air flow rate fed to the chambers was progressively adjusted according to the increasing leaf area in the chambers. It varied from 8.5 L/s between DOY 140–207, after which it was then raised to 15.0 L/s on DOY 208 until DOY 226 and then lowered again to 10 L/s on DOY 229 until the end measurement (DOY 280). Ambient (inlet) air temperature and the air temperature at each chamber outlet were measured by shielded 1/0.2 mm diameter perfluoroalkoxy(PFA) – teflon insulated type-T thermocouples (Omega Engineering, Stamford,

CT, USA); direct and diffuse radiation were measured with a BF2 sunshine sensor (Delta-T Devices, Cambridge, England) placed horizontally on top of a support stake next to the chambers enclosing the canopies. Vine net CO_2 exchange rate ($NCER_{vine}$ as μmol CO_2/s) and transpiration (T_{vine} as mmol H_2O/s) were calculated from flow rates and CO_2 and H_2O differentials. Daily *NCER* and T_c rates were then calculated by averaging instantaneous records taken from dawn until dusk. Whole vine water-use efficiency (WUE_{vine}) was then calculated as *NCER/T* ratio and given as μmol CO_2/mmol H_2O. The *NCER* and *T* data were also normalised versus total leaf area enclosed into each chamber and reported on a per leaf area unit basis.

In 2020, upon temporary dismantling of the chambers, single leaf gas-exchange measurements were taken on DOY 230 (18 August) on three primary and forced shoots per vine. Specifically, the readings regarded the third (3P) and the ninth (9P) leaf of the UC treatment shoots, whereas in the F treatments, the third leaf (3P) on the primary trimmed cane and a leaf inserted at node 3 (3F) of the apical forced shoot were measured. The readings were taken between 1030 and 1230, under saturating light conditions [*PAR*- > 1400 mmol/(m^2· s)], using a portable, gas-exchange open system, namely the LCi infrared gas analyser (ADC Bio Scientific, Hoddesdon, England). The system was equipped with a broad leaf chamber with a 6.25 cm^2 window, and all the readings were taken at ambient RH with an airflow adjusted to 350 mL/min. The assimilation rate (leaf *A*) and the transpiration rate (leaf *E*) were derived from inlet and outlet CO_2 and H_2O concentration and instantaneous water use efficiency (*WUE*) calculated as the *A/E* ratio.

Assessment of bud fruitfulness

Bud fruitfulness as affected by 2019 treatments was determined in 2020 as shoot fruitfulness on each node of retained spurs. To gain an estimate of the effects of the second forcing season on next year (i.e. 2021) bud fertility, three canes per vine (12 per treatment) were collected in early November 2020 and their six basal dormant buds dissected under a stereo microscope (40× magnification), as described by Sánchez and Dokoozlian (2005). The number of inflorescence primordia in each primary bud was recorded, and potential bud fruitfulness according to their cane position was expressed as the number of inflorescence primordia per primary bud. In F1 and F2, if bud dormancy was broken during the season in a specific node position as a consequence of the application of the F treatment, a note was recorded and the position was excluded by the count.

Data analysis

Vine performance data were subjected to a two-way ANOVA using the SigmaStat software package (Systat Software, San Jose, CA, USA). Homogeneity of error variances for the data taken on the same individuals over different years was assessed with Bartlett's test. The year was considered as a random variable, and the error term for the treatment factor was the year × treatment interaction mean square. Since variances were in all cases homogeneous, the year × treatment effects were tested using the pooled error mean square as an error term. Treatment comparison was performed using the Student–Neuman–Keuls test at $P \leq 0.05$. Year × treatment interaction was partitioned only when the *F* test was significant.

Results

Weather course and vine performance

In 2019, growing degree days (GDD) calculated according to Winkler Index (1 April–31 October) were 2274°C (Figure S2a). The season experienced a wet and cool spring, whereas June was dry and hot having a GDD of 468°C and a mean daily temperature of 25.6°C. Thermal trends of July and August were similar to June, whereas September was cool with a mean daily temperature of 20.8°C.

In 2020, GDD summed up to 2247°C; spring was rather dry and warm, whereas the time window including the two forcing dates was characterised by several cloudy days with a temperature below average (Figure S2b). The first week of August had the highest air temperature, up to 36.2°C.

The time elapsed between the application of the forcing treatments and the release from dormancy of the primary apical buds (Figure S1) varied significantly between the two seasons; in 2019 it took 14 to 29 days for F1 and F2 treatments to exit dormancy, whereas in 2020, bud dormancy breaking was quite delayed (33 to 42 days after shoot trimming) and was concentrated around DOY 195.

Leaf area (LA) per vine removed with shoot trimming was, on a 2-year basis, 11.5 and 45.2% of the final total LA in F1 and F2, respectively (Table 1). Forcing 2 achieved higher LA compensation than F1 compared to the unforced Control, although this effect was also contributed by a lower number of forced shoots in F1 versus F2. Laterals accounted for 28.1% of the total LA per vine in UC, whereas lateral development in the forced shoots was negligible. The year 2020 was more conducive to vegetative growth than 2019, primarily due to a higher shoot number per vine depending

Table 1. Effects of dormant bud forcing on canopy components, leaf area removed with treatments application and final vine leaf area in Pinot Noir grapevines in 2019 and 2020.

	Main shoots (No./vine)	Forced shoots (No./vine)	LA removed (m^2/vine)	Final main LA (m^2/vine)	Final lateral LA (m^2/vine)	Final forced LA (m^2/vine)	Final vine LA (m^2/vine)
Treatments (T)							
UC	12 ± 2	–	–	1.07 ± 0.19 a	0.42 ± 0.18 a	-	1.49 ± 0.16 a
F1	13 ± 2	9 ± 1 b	0.23 ± 0.06 b	0.53 ± 0.26 b	0.07 ± 0.02 b	0.53 ± 0.07 b	1.13 ± 0.11 b
F2	12 ± 2	11 ± 1 a	0.62 ± 0.18 a	0.54 ± 0.11 b	0.06 ± 0.03 b	0.77 ± 0.03 a	1.37 ± 0.07 a
P	*0.268*	*0.036*	*0.000*	*0.000*	*0.000*	*0.000*	*0.006*
Year (Y)							
2019	10 ± 1 b	7 ± 2 b	0.36 ± 0.07 b	0.54 ± 0.13 b	0.17 ± 0.08	0.31 ± 0.04 b	1.02 ± 0.08 b
2020	15 ± 1 a	12 ± 2 a	0.47 ± 0.04 a	0.85 ± 0.10 a	0.20 ± 0.05	0.52 ± 0.06 a	1.57 ± 0.11 a
P	*0.000*	*0.000*	*0.001*	*0.000*	*0.562*	*0.001*	*0.000*
T × Y	*0.202*	*0.026*	*0.265*	*0.562*	*0.216*	*0.581*	*0.423*

Within column and factor levels, mean separation was performed by Student–Newman–Keuls (SNK) test for treatment levels and by *t*-test for year level and indicated by lower case letters. LA, leaf area; F1, first forcing time; F2, second forcing time; UC, unforced Control.

upon completion of spur selection on the permanent cordons. No significant T × Y interactions were found for vegetative parameters.

Yield per vine and its main components highlighted few significant effects among treatments when the comparison was made within shoot (cane) categories, i.e. primary and forced (Table 2). A minor deviation from the above pattern was reported for bunch mass, which, on a 2-year basis, was reduced in the F2 treatment. Conversely, features of crop developed on forced shoots largely differed from those on primary shoots. Regarding timing of forcing, bunches were smaller, with fewer berries and less compact, whereas the total yield reached through forcing was 52 and 39% of the primary yield in F1 and F2, respectively (Table 2). Together the primary and forced crop gave a significantly higher total yield/vine in F1 and F2 versus UC, corresponding to +31 and +20%, respectively (Table 2).

As per carryover effects on the cropping potential of the next year, fertility recorded in 2020 on shoots originated on basal primary nodes averaged 1.0, 0.9 and 1.1 bunches/shoot for UC, F1 and F2, respectively; the predicted fertility for the 2021 season through bud dissection analysis (Table S1) was 1.6, 1.7 and 1.6 bunches/shoot, respectively, for UC, F1 and F2.

Whole vine balance indexed as LA/Y ratio was reduced in F1 compared to UC. When this ratio, however, was calculated for each crop type (primary vs forced), it was relevant that within each forcing treatment, LA available to ripe fruit carried by the forced shoot was much higher than the LA available for ripening the primary crop.

Grape composition at harvest was markedly affected by treatments (Table 3). Overall, the effects of the treatments within the primary crop type were moderate. All the treatments reached a suitable composition for sparkling wine production; significant differences concerned tartaric acid concentration (i.e. higher in F1 compared to UC), the total anthocyanins which were reduced in the F1 treatment and the total phenolics which in contrast increased in F2 versus UC.

Grape composition of the forced crop showed, on a 2-year basis, the higher TSS in F1 and F2 (about 2°Brix more than C), lower pH, and a distinctly higher TA attributed to a significant increase in malic acid and a major enhancement in the concentration of total anthocyanins which at harvest was 47.2 and 43.8% higher in F1 and F2 versus the unforced Control.

Gas exchange

Daily direct *PAR* values, averaged from dawn to dusk, showed a prevalence of mostly clear days [i.e. mean *PAR* higher than 600 μmol/(m^2·s)], although a series of cloudy days occurred immediately after imposition of the F1 treatment. For the remainder of the season cloudy days occurred occasionally. Daily mean air VPD peaked at about 2.5 kPa in early August and then progressively declined during the season (Figure 1).

In 2020, $NCER_{vine}$ averaged over the whole measuring period (DOY 140–281) was 6.54 μmol/s in UC vines and hence significantly lower ($P < 0.01$) than the rate of 8.91 and 8.41 μmol/s measured in F1 and F2, respectively (Figure 1b). Main shoot trimming performed in F1 on DOY 152 curtailed $NCER_{vine}$ by 54% based on the mean $NCER_{vine}$ rate measured over 2 days before and after trimming. Although the release of bud dormancy started only around DOY 188, and active new leaf formation was apparent from

Table 2. Effects of dormant bud forcing on yield and bunch components assessed in Pinot Noir grapevines in 2019 and 2020.

	Rachis length (cm)		Berries/bunch (No.)		Bunch compactness (g/cm)		Berry mass (g)		Bunch mass (g)		Bunches/vine (No.)		Yield/vine (kg)			Forced/primary yield ratio (%)	LA/Y ratio (m^2/kg)		
	P	F	P	F	P	F	P	F	P	F	P	F	P	F	Total		P	F	Total
Treatments (T)																			
UC	7.3 ± 0.8	–	104 ± 8	–	12.8 ± 1.7	–	0.90±0.04	–	94 ± 5A	–	12 ± 2	–	1.09 ± 0.07	–	1.09 ± 0.07B	–	1.37 ± 0.22A	–	1.37 ± 0.22A
F1	6.81 ± 0.9	8.2 ± 0.6	118 ± 28^{a}	67 ± 20^{b}	13.4 ± 2.8^{a}	8.5 ± 1.7^{b}	0.79 ± 0.12	0.95 ± 0.07	90 ± 12^{a}AB	70 ± 7^{b}	12 ± 2^{a}	8 ± 1^{b}	1.03 ± 0.12^{a}	0.54 ± 0.12^{b}	A1.57 ± 0.17	52 ± 15	0.58 ± 0.06^{b}B	0.98 ± 0.22^{a}A	0.72 ± 0.25B
F2	6.56 ± 1.0	8.0 ± 0.4	96 ± 18^{a}	68 ± 11^{b}	12.5 ± 1.2^{a}	7.9 ± 1.0^{b}	0.85 ± 0.10	0.87 ± 0.10	82 ± 6^{a}B	63 ± 2^{b}	12 ± 2^{a}	7 ± 1^{b}	0.97 ± 0.12	0.38 ± 0.14^{b}	1.35 ± 0.08A	39 ± 18	0.62 ± 0.09^{b}B	2.02 ± 0.32^{a}B	1.0 ± 0.40AB
P	*0.383*	*0.327*	*0.790*	*0.192*	*0.811*	*0.507*	*0.691*	*0.498*	*0.035*	*0.687*	*0.414*	*0.453*	*0.452*	*0.500*	*0.000*	*0.269*	*0.000*	*0.000*	*0.043*
Year (Y)																			
2019	7.6 ± 0.1	8.0 ± 1.1	133 ± 18^{a}A	94 ± 13^{b}A	14.7 ± 2.32^{a}A	9.7 ± 1.31^{b}A	0.84 ± 0.07	0.83 ± 0.07	112 ± 12^{a}A	78 ± 13^{b}	10 ± 1^{a}B	7 ± 1^{b}	1.06 ± 0.08^{a}	0.54 ± 0.15^{b}	1.42 ± 0.07	51 ± 19	0.67 ± 0.12B	0.57 ± 0.13B	0.64 ± 0.16B
2020	6.3 ± 0.8	9.8 ± 1.6	76 ± 9^{a}B	55 ± 5^{b}B	10.5 ± 0.79^{aB}	6.2 ± 0.64^{bB}	0.86 ± 0.07^{a}	1.00 ± 0.09^{b}	66 ± 7B	61 ± 10	15 ± 1^{a}A	8 ± 1^{b}	0.98 ± 0.13^{a}	0.46 ± 0.09^{b}	1.29 ± 0.11	47 ± 10	1.07 ± 0.16A	1.13 ± 0.36A	1.09 ± 0.19A
P	*0.893*	*0.234*	*0.001*	*0.002*	*0.019*	*0.021*	*0.122*	*0.106*	*0.000*	*0.666*	*0.000*	*0.877*	*0.612*	*0.720*	*0.762*	*0.384*	*0.000*	*0.000*	*0.000*
T × Y	*0.115*	*0.473*	*0.622*	*0.122*	*0.735*	*0.213*	*0.023*	*0.700*	*0.233*	*0.507*	*0.487*	*0.177*	*0.245*	*0.992*	*0.168*	*0.256*	*0.369*	*0.596*	*0.274*

Within column and factor levels, mean separation was performed by Student–Newman–Keuls test for treatment levels and by *t*-test for year level and indicated by upper-case letters. Within row, pair comparison between P and F were made using *t*-test and mean separation shown as lower-case letters. F, forced; F1, first forcing time; F2, second forcing time; LA, leaf area; P, primary; UC, unforced Control; Y, yield.

Table 3. Effects of dormant bud forcing on fruit composition of Pinot Noir grapevines in 2019 and 2020.

	TSS (°Brix)		pH		TA (g/L)		TSS/TA		Malate (g/L)		Tartrate (g/L)		Total anthocyanins (mg/g)		Total phenolic s (mg/g)	
	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F
Treatments (T)																
UC	18.6 ± 0.6	–	3.37 ± 0.03	–	7.3 ± 0.45	–	2.6 ± 0.45	–	2.6 ± 0.26	–	6.7 ± 0.69B	–	0.500 ± 0.021A	–	3.280 ± 0.076B	–
F1	18.9 ± 0.7	20.8 ± 0.4B	3.28 ± 0.03[a]	2.94 ± 0.046[b]	7.4 ± 0.35[b]	13.6 ± 2.46[a]	2.6 ± 0.35[a]	1.60 ± 0.20[b]	2.8 ± 0.33[b]	6.2 ± 0.88[a]	9.4 ± 1.12A	9.2 ± 0.51	0.344 ± 0.024[b]B	0.651 ± 0.067[a]B	B3.359 ± 0.121A	3.709 ± 0.088B
F2	b19.7 ± 0.6	21.9 ± 0.6[a]A	3.31 ± 0.03[a]	2.95 ± 0.043[b]	6.9 ± 0.55[b]	14.5 ± 2.13[a]	2.9 ± 0.28[a]	1.54 ± 0.15[b]	2.2 ± 0.53[b]	5.2 ± 0.46[a]	B8.4 ± 1.09A	8.3 ± 0.48	B0.481 ± 0.020[b]A	0.855 ± 0.045[a]A	3.492 ± 0.103A	3.942 ± 0.124A
P	*0.223*	*0.020*	*0.145*	*0.779*	*0.699*	*0.589*	*0.323*	*0.598*	*0.064*	*0.857*	*0.002*	*0.206*	*0.016*	*0.000*	*0.004*	*0.044*
Year (Y)																
2019	18.0 ± 0.6[b]B	22.8 ± 0.7[a]A	3.15 ± 0.02AB	2.92 ± 0.043[b]	8.0 ± 0.38[b]	13.6 ± 1.13[a]	2.2 ± 0.35[a]B	1.64 ± 0.06[b]	2.2 ± 0.24[b]B	5.7 ± 0.47[a]	8.2 ± 0.25	7.2 ± 0.43B	0.429 ± 0.019[b]	0.870 ± 0.054[a]A	3.294 ± 0.154	3.523 ± 0.212B
2020	19.9 ± 0.5A	19.8 ± 0.7B	3.49 ± 0.02[a]A	2.97 ± 0.046[b]	6.3 ± 0.26[b]	13.9 ± 1.21[a]	3.1 ± 0.15[a]A	1.49 ± 0.08[b]	2.9 ± 0.13[b]A	5.5 ± 0.38[a]	8.0 ± 0.33[b]	11.4 ± 0.56A	0.450 ± 0.017[b]	0.636 ± 0.041[a]B	3.477 ± 0.141	4.172 ± 0.186A
P	*0.009*	*0.001*	*0.000*	*0.466*	*0.144*	*0.796*	*0.005*	*0.438*	*0.011*	*0.252*	*0.457*	*0.000*	*0.573*	*0.000*	*0.834*	*0.001*
T × Y	*0.056*	*0.363*	*0.182*	*0.528*	*0.849*	*0.662*	*0.387*	*0.234*	*0.098*	*0.218*	*0.059*	*0.044*	*0.095*	*0.280*	*0.222*	*0.048*

Within column and factor levels, mean separation was performed by Student–Newman–Keuls test for treatment levels and by *t*-test for year level and indicated by upper-case letters. Within row, pair comparison between *P* and *F* were made using *t*-test and mean separation shown as lower-case letters. F, forced; F1, first forcing time; F2, second forcing time; LA, leaf area; P, primary; UC, unforced Control; Y, yield.

DOY 194 onward, $NCER_{vine}$ increased by about 48% from DOY 153 (1 day after trimming) until DOY 194 (resumption of active vegetative growth). Over the same period, $NCER_{vine}$ reduction in F1 compared to UC was 58.5%. Upon F1 leaf area resumption, a fast $NCER_{vine}$ recovery occurred, with F1 reaching similar $NCER_{vine}$ to UC at around DOY 205 and then gaining a progressive and an increasing advantage. At the onset of veraison of the forced bunches (DOY 255), $NCER_{vine}$ was 9.33 μmol/s in F1 versus 5.33 in UC (+43%). During the time interval between veraison and the harvest (DOY 255–DOY 281) of the second crop, this gap widened and was set at +58% in favour of F1.

Shoot trimming in F2 on DOY 163 (11 days after F1 trimming) reduced $NCER_{vine}$ by 74.8% compared to 2-day-averaged pre-trimming rate. As seen in F1, over time (DOY 164–194), when new leaf formation was still prevented by unleashed bud dormancy, a marked $NCER_{vine}$ compensation was reported, as the $NCER_{vine}$ rate of F2 rose by 68.6% from DOY 164 (1 day after trimming) to DOY 194. Over the same period, the $NCER_{vine}$ reduction in F2 compared to UC was 57.3%. Net CO_2 exchange rate /vine of F2 likewise recovered abruptly overlaying new leaf area development, and a similar rate to that of UC vines was achieved around the same time as F1. At the appearance of berry colour (DOY 255), the daily $NCER_{vine}$ rate of F2 was 46.8% higher than that of the UC, and over the DOY 255–281 period, such difference increased by 59.2%.

Transpiration (T) per vine averaged over the whole 141-day-long measuring period did not differ among treatments at 2.05 ± 0.08, 2.14 ± 0.08 and 2.08 ± 0.10 mmol/s in UC, F1 and F2, respectively (Figure 1c). Upon trimming of F1, T_{vine} decreased by 23.6% versus the 2-day averaged pre-trimming rate. In parallel with $NCER_{vine}$ parameter, a consistent compensation occurred for the F1 T_{vine} rate over the DOY 153–194-time period when T/vine increased in F1 by 39.2%. Over the same period, T_{vine} reduction in F1 compared to UC was 34.2%. New leaf area formation caused by bud dormancy release coupled with several clear days conducive to high transpiration allowed F1 to reach UC T_{vine} level already at the end of July (DOY 212). Thereafter and until the end of measurements, T_{vine} in UC and the F1 treatment was similar (1.94 ± 0.11 mmol/s in UC and 2.04 ± 0.11 mmol/s in F1).

Shoot trimming on DOY 163 in F2 vines caused a 44% reduction of T_{vine} compared to the pre-trimming rate. Due to several cloudy days preceding trimming, however, a comparison was made with T_{vine} recorded on DOY156–157 under high direct PAR and non-limiting VPD. Post trimming, T_{vine} compensation calculated from DOY 164 to DOY 194 in F2 was 36.1%. Throughout the same time interval, the mean T_{vine} reduction in F2 was 35.3%. Analogous to F1 and compared to UC vines, F2 reached full compensation in the T_{vine} rate, around DOY 210, and then until the end of measurements.

Whole vine water-use efficiency (WUE_{vine}) indexed as the $NCER/T$ ratio was homogeneous among treatments before application of shoot trimming (Figure 1d). Upon trimming, in both F treatments, WUE_{vine} registered a sharp drop (−39.4 and −65.9% compared to pre-trimming values). Both the F treatments showed some recovery over the post-trimming period until the day of bud dormancy release. Over the DOY 153–194 interval, however, WUE_{vine} of F1 was 35.7% lower than that calculated in UC plants; whereas in F2, the reduction referring to the interval DOY

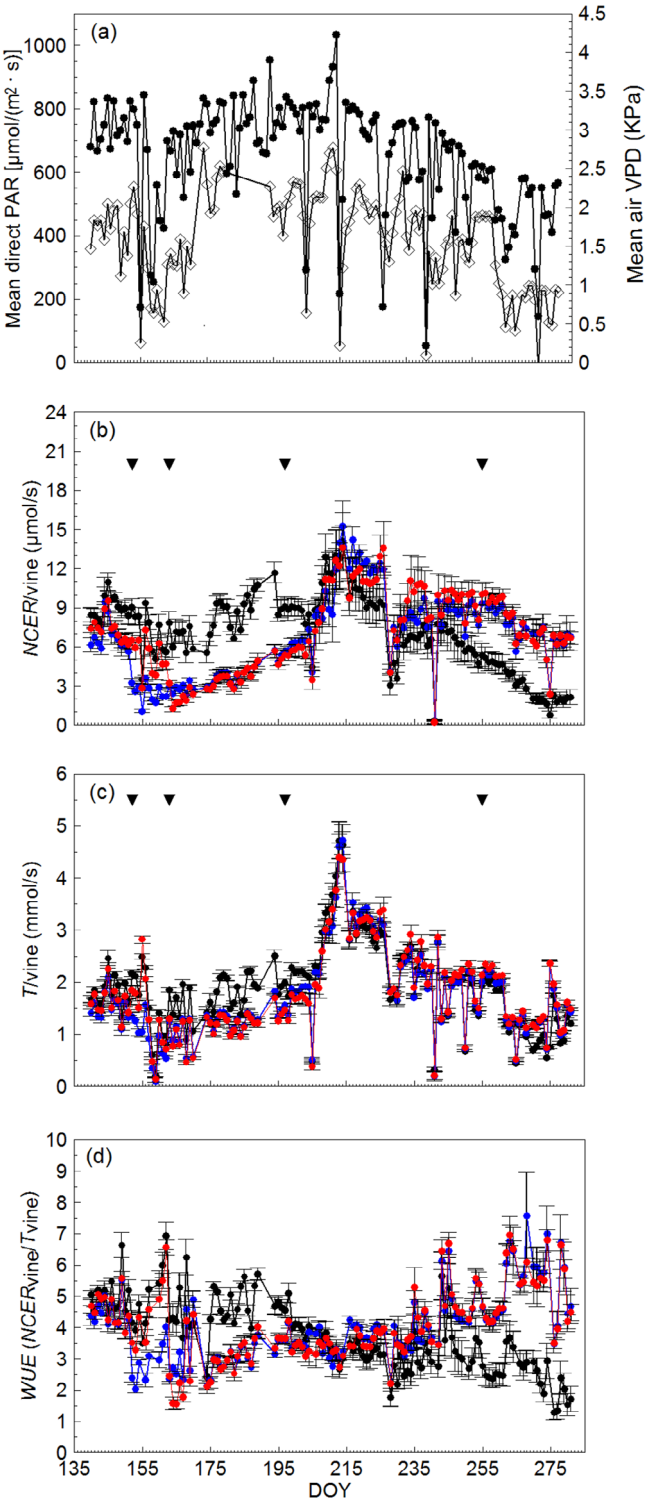

Figure 1. (a) Seasonal direct photosynthetic active radiation [PAR (●)] and air vapour pressure deficit [VPD (◊)]; (b) net CO_2 carbon exchange rate (*NCER*) per vine on forcing treatments F1 (●) and F2 (●) compared to that of the unforced Control (●); (c) total transpiration (*T*) per vine recorded daily in 2020 from day of year (DOY) 140 until DOY 281; and (d) the calculated vine *WUE*. In panels (b) and (c), from left to right, arrows indicate dates of F1, F2, onset of veraison in primary crop and onset of veraison in forced crop. Daily means ± SE are calculated on the four vine replicates per treatment.

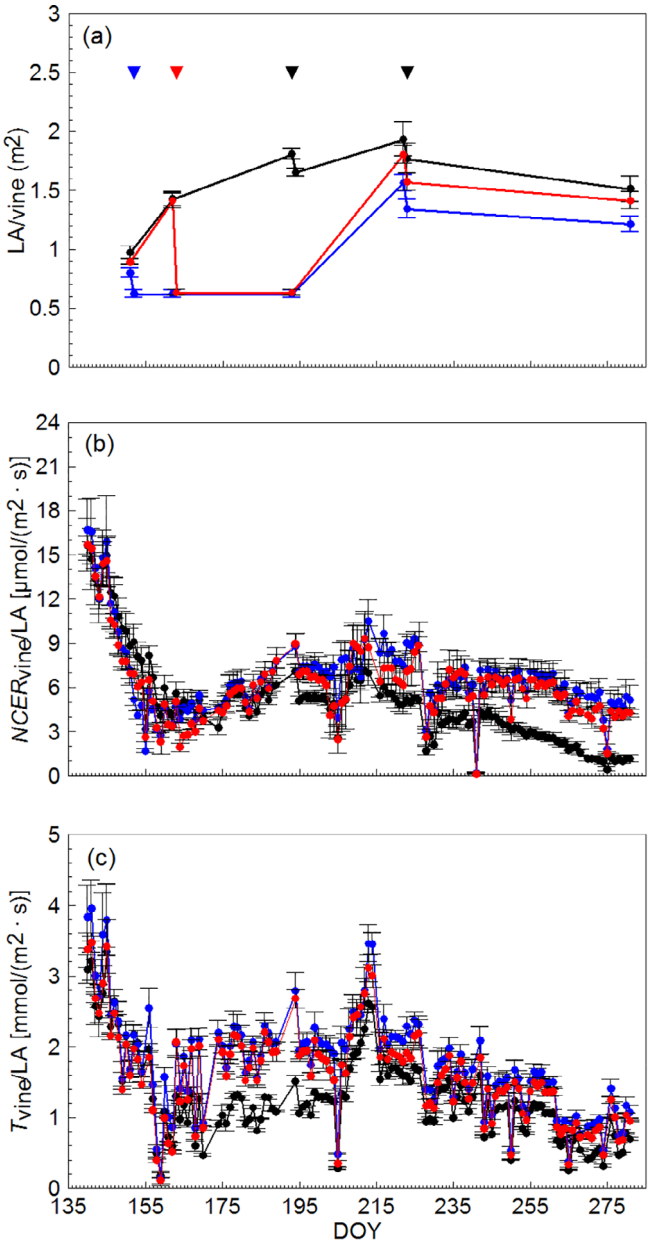

Figure 2. (a) Seasonal total vine leaf area, (b) net CO_2 exchange rate (*NCER*) and (c) transpiration (*T*) rate per unit leaf area calculated from daily whole-canopy gas exchange assessment in 2020 from day of year (DOY) 140 until DOY 281 in forcing treatments F1 (●) and F2 (●) compared to that of the unforced Control (●). In panel (a) arrows from left to right indicate the DOY of trimming for F1 and F2 treatments, shoot trimming in UC vines and shoot trimming in all treatments. Daily means ± SE are calculated on the four vine replicates per treatment. LA/vine data back to the start of gas exchange measurements (DOY 140) were derived by linear interpolation between budburst date (estimated at DOY 91 for all treatments) and the LA estimated on DOY 151 (i.e. the day before F1 treatment setup).

164–194 was 37.6%. With the progressive development of leaf area on forced shoots, WUE_{vine} of F1 and F2 treatments notably increased and, starting from DOY 247 until the end of measurement, it was always significantly higher than the value calculated for UC. At veraison (DOY 255), F1 and F2 had 40.4 and 40.9% higher WUE_{vine} than UC, respectively, and this fraction largely increased over the last part of season when WUE_{vine} in the F treatment was often twofold greater than the value found in the UC vine.

Due to the quite diverse pattern of leaf area development (Figure 2a), seasonal trends of $NCER_{vine}$ per unit leaf area [µmol/(m^2· s)] showed a different behaviour when compared to the whole-vine data (Figure 2b). For instance, in F1, mean post-trimming $NCER_{vine}$/LA calculated over

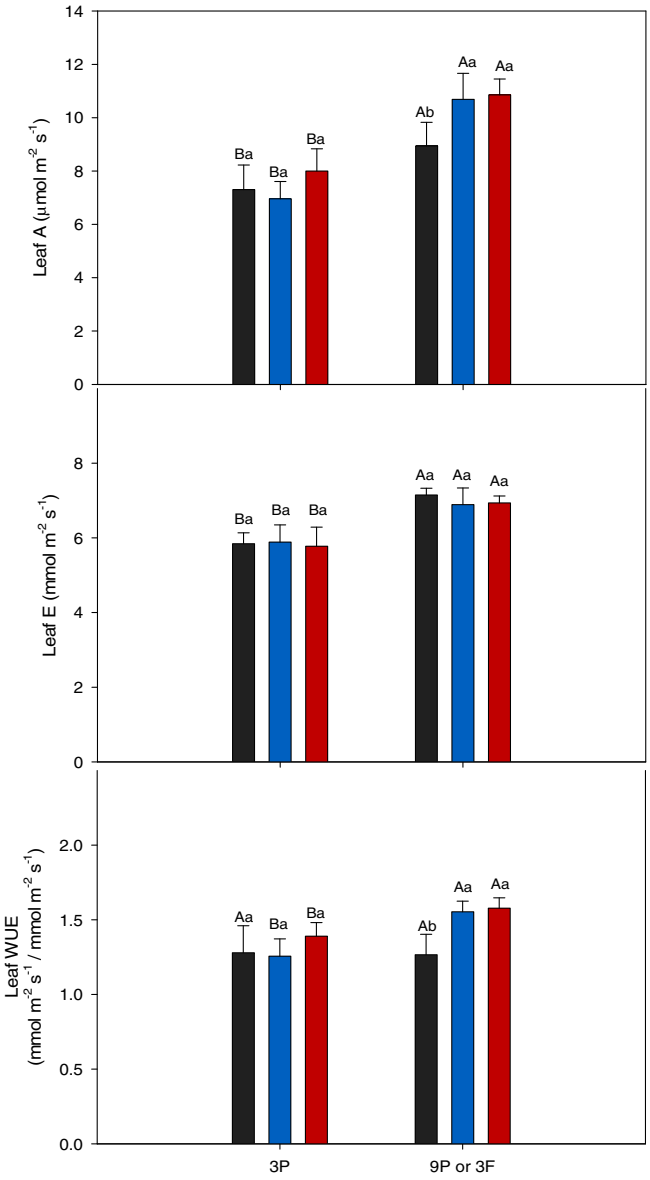

Figure 3. (a) Leaf net assimilation (*A*) rate, (b) transpiration (*E*) rate and (c) water use efficiency (*WUE* calculated as *A/E* ratio) measured on DOY 230 (18 August 2020) on vines subjected to forcing treatments on DOY 152 [F1 (■)] and 163 [F2 (■)] and on unforced Control vines [UC (■)]. For UC vines, gas exchange was measured on leaves at nodes 3 (3P)and 9 (9P) of primary shoots. For F1 and F2 vines, data were taken on leaves at node 3 of primary shoots (3P) and at node 3 of forced shoots (3F). Lower case letters indicate a significant difference at $P \leq 0.05$ (Student–Neuman–Keuls test) among treatments within node position; upper case letters indicate a significant difference at $P < 0.05$ among different node positions for each single treatment.

DOY 153–194 was 5.08 ± 0.25 versus 5.23 ± 0.22 μmol/(m^2· s) in UC vines; therefore, offsetting differences showed when the data were calculated on a whole-vine basis. Notably, starting about 2 weeks after bud dormancy release, both the F treatments maintained a distinctly higher $NCER_{vine}$/LA rate than the UC vines. Moreover, at veraison (DOY 255), $NCER_{vine}$/LA of F1 and F2 was 57.9 and 54.1% higher than the rate measured the same day on the UC vines. Averaging *NCER*/LA over the whole post-veraison period (DOY 256–281) confirmed that the $NCER_{vine}$/LA rate retained by F1 and F2 was, in the order of, 5.44 ± 0.23 and 4.75 ± 0.23 μmol/(m^2· s) compared to 1.69 ± 0.14 μmol/(m^2· s) registered in the UC vines.

Vine transpiration (*T*) rate measured on a per unit leaf area basis (T_{vine}/LA) showed a quite different response compared to the $NCER_{vine}$/LA data (Figure 2c). Regardless of the timing, shoot trimming did not significantly modify the T_{vine}/LA rate as compared to the pre-trimming values; throughout the time period until the beginning of forced leaf area development, T_{vine}/LA was consistently higher in F1 and F2 [respectively, 1.71 ± 0.12 and 1.78 ± 0.11 mmol/(m^2· s), versus 1.07 ± 0.070 mmol/(m^2· s) in the UC vines]. Conversely, resumption of vegetative growth in F1 and F2 tended to smooth the differences among treatments. At veraison (DOY 255), F1 and F2 still retained a higher T_{vine}/LA than the UC vines; T_{vine}/LA averaged over the post-veraison–harvest period was 33.1 and 25.3% higher in F1 and F2 treatments, respectively, compared to the UC vines.

Single leaf gas exchange assessed on a single date (DOY 230, 18 August) upon temporary opening of the chambers showed limited variation in leaf *A*, *E* and *WUE* for readings measured on leaves located on the third node of the primary shoots of each treatment (Figure 3). Conversely, comparing the *A* rate measured on leaf 9 on the stem of UC vines and on leaf 3 of a forced shoot confirmed a higher value for the latter. Since the *E* rate was not affected, leaf *WUE* was also significantly improved.

Discussion

A double cropping approach applied in a *V. vinifera* cultivar grown in a temperate–semi-continental climate—where plants typically undergo a dormant season—must overcome some potential drawbacks: (i) unlocking dormant buds during the first year of induction needs to result in an acceptable yield and grape composition; (ii) yield and ripening of bunches on primary shoots need to be assured; and (iii) useful pruning points for next year cropping have to be preserved to warrant long-term sustainability of the technique.

Removal of leaf area with trimming and the subsequent leaf area replenishment by the forced shoots caused a complex variation in the vine leaf area development both in terms of amount and function, significantly impacting the whole vine gas exchange. First, the fractional reduction of $NCER_{vine}$ occurring in F1 and F2 compared to the pre-trimming rates was more than proportional to the decrease, which could have been expected simply based on the amount of the removed leaf area (25 and 53%, respectively). Such an effect was well confirmed by the *NCER* data when normalised for leaf area, reporting, in F1 and F2, respectively, a 43.3 and 74.5% reduction over 48 h after treatment. Mechanisms involved in this limiting response may suggest several factors related to the vine source–sink balance. Considering that the age span of the six retained basal leaves (roughly 40 to 60 days) is considered to be optimal for maximum photosynthesis (Kriedemann et al. 1970, Poni et al. 1994, Patakas et al. 1997), the main limitation is likely due to an abrupt change of the source–sink relationship where the sudden removal of all main vegetative sinks (main shoot tip and any developing laterals) may have caused a feedback inhibition of photosynthesis (Quereix et al. 2001). On the one hand, in vitro experiments have demonstrated that mesophyll carbohydrate concentration, which depends on the local balance between assimilation and export, can modify the expression of photosynthetic gene promoters (Jang and Sheen 1994, Cook and Wolkovich 2016). In contrast, several contributions have reported that photosynthetic rates decline in direct response to a build-up of carbohydrate in the plant other than in the

mesophyll, including in the sinks themselves, via a currently unidentified biochemical signal (Quereix et al. 2001, Flore and Lakso 2011).

Continuous long-term gas exchange monitoring in the vine enclosure system also clarified that a few days after trimming and with no new vegetative sinks developing, a consistent photosynthetic compensation occurred in the forced treatments, as reported by Koblet et al. (1997). An interesting term of comparison are the data reported by Poni and Giachino (2000) who applied a severe shoot trimming (six main leaves retained) with concurrent removal of any developing lateral at flowering on Cabernet Sauvignon potted vines. Fifty-five days after trimming, the treated vines had a mean leaf *A* rate of 10.4 versus 7.1 μmol/(m^2· s) reported in the un-trimmed control vines, thereby suggesting the hypothesis that a consistent photosynthetic compensation is apparent only after some time from the source–sink manipulation.

In our study, both the forcing treatments reached a $NCER_{vine}$ rate similar to that of the UC vines due to either new leaf area production and higher $NCER_{vine}$ per unit leaf area by the end of July. This response fully validates one of the main assumptions made in this study, that is, that the forced crop can benefit greatly from a large source potential assured by a relatively young hence more efficient canopy established in August and September. Due to this effect and an extremely favourable leaf-to-fruit ratio (Table 2), the ripening potential of the forced crop is high, confirmed by the data related to the concentration of sugar and total anthocyanins. Notably, in none of our treatments, removal of primary crop affected the postharvest $NCER_{vine}$/LA rate which was quite similar to that recorded over the 2 days prior to harvest, thus confirming results reported by Greer (2020) in cv. Chardonnay grown in New South Wales, Australia.

Interestingly, the impact of the forcing treatments on whole-vine water use did not closely mirror the $NCER_{vine}$ behaviour. The first significant difference was the *T*/vine decrease, caused by trimming, was rather proportional to the amount of removed leaf area. Hereafter, during the period preceding bud unlocking, both the F treatments always had T_{vine}/LA higher than UC suggesting higher compensation capacity compared to what was reported for *NCER*. Indeed, under strong sink limitation, mechanisms by which retained leaf area can compensate for balancing water use are diverse. One hypothesis is that root hydraulic conductance is adjusted and stomatal conductance (g_s) consequently increased (Lovisolo et al. 2002). Poni and Giachino (2000) reported that trimmed shoots deprived of laterals had, a few weeks after trimming, a leaf g_s of 166 mmol/(m^2· s) that more than doubled the value recorded on the untrimmed Control shoots [74 mmol/(m^2· s)]. Petrie et al. (2000) demonstrated that at a high level of leaf removal in Pinot Noir, vine stomatal conductance was increased, which is also supported by previous work of Candolfi-Vasconcelos and Koblet (1991). Moreover, it cannot be ruled out that the removal of all nodes above node 6 improved light interception, thereby enhancing the transpiration capacity of the retained basal leaves. An objection can be made that a similar effect should have also been seen in NCER, but this is common knowledge (Keller and Pharr 2017) that while the leaf assimilation rate shows a saturating light response curve, transpiration is linearly related to the amount of intercepted light.

Another remarkable difference between the $NCER_{vine}$ and *T*/vine patterns was that within the time period that included vegetative growth of the forced shoots (DOY 195–281), whole-vine water use never differed among treatments. An explanation for this should be sought within the interaction between a relatively 'young' foliage that develops late in the season and a season that is rapidly cooling. It is well known that *T*/vine is a function of intercepted radiation, canopy conductance and vapour pressure deficit (VPD) at the leaf–air interface (Williams and Ayars 2005). Under well-watered and not light-limited conditions, it is also abundantly demonstrated that VPD represents the prevailing factor and that higher VPD leads to an increase in *T*/vine despite stomatal conductance might start to decline. In fact, under well-watered conditions, Edwards et al. (2011) have shown a threefold increase in vine transpiration, despite a slight reduction in stomatal conductance, while air temperature was increased by 10°C. Likewise, Bonada et al. (2018) reported that increased VPD modulated through an apparatus of row heating in the field led to higher vine transpiration. In our study, environmental conditions during the DOY 195–281 registered an air T_{mean} of 23.4°C, a mean VPD of 1.55 kPa, a quite moderate value, and GDD summation of only 1075°C. Taken together, these factors may justify a limitation of the transpiration potential of the second flush of vegetative growth.

Seasonal variation in whole vine *WUE* is an obvious reflection of the relative changes in $NCER_{vine}$ and *T*/vine. Two main items, however, must be noted: when the main vegetative sink is removed and only mature leaves are left on the vine, *WUE* decreases as photosynthesis is more inhibited than transpiration. Conversely, a 'young' canopy developing reasonably late in the season achieves remarkably higher *WUE* than an 'old' canopy. Both the effects can be justified when the seasonal interaction between *WUE* and leaf/canopy age is considered. A pioneer study by Poni et al. (1994) on field-grown Sangiovese vines showed that at any sampling date after veraison, leaf extrinsic *WUE* (assimilation to transpiration ratio) of apical leaves was higher than the values calculated for the median and basal leaves. This outcome matches the findings in this study (Figure 3) comparing leaf *WUE* of 9P and 3F positions. Upscaling to the whole canopy level does not appear to alter the *WUE*; several authors have shown, under well-watered conditions, that canopy aging is also conducive to lower *WUE* (Poni and Intrieri 2001, Flexas et al. 2010, Douthe et al. 2018).

From an agronomic standpoint, this research demonstrated, on a 2-year basis, that the forced to primary shoot sprouting ratio was 70% in F1 and 92% in F2. These ratios are close to the optimal value sought of 100%, meaning that one forced shoot develops from the apical or subapical node of each trimmed cane and then the subtending nodes stay dormant to allow standard winter pruning. In a preliminary proof of concept paper on the technique, Poni et al. (2020) have shown that the response of each single trimmed shoot to the forcing treatment depends upon its size, that is basal diameter. It is likely that the pot environment has constrained vine size, therefore limiting, although not to great extent, the number of developed forced shoots. Additional concerns that forcing would have either reduced fertility of the forced shoot due to incomplete bud induction and/or negatively affected the next season bud induction of the basal primary nodes are negated by our data. In the forced shoots, fertility as bunch per shoot was 0.88 and 0.64 for F1 and F2, respectively, and the basal primary node fruitfulness was practically unchanged oscillating around 1.0 bunches/

shoot in 2020 and predicted to be high for the 2021 season (1.7 and 1.6 bunches/shoot in F1 and F2, respectively, from the bud dissection date referred to node positions 1–5 compared to the estimated 1.6 bunches/shoot in UC. While it is unlikely that a source limitation imposed at BBCH 73 (pea-size berries) will impair bud induction in the next year, leaf area reduced at the end of flowering (BBCH 69) might still interfere with the process (Srinivasan and Mullins 1978); however, our bud fertility data suggest that this was not the case.

The yield potential of the forced shoots, ranging between 39 and 52% of the respective primary crop load, should not be disregarded for two good reasons: it is a consistent and rewarding yield improvement and, most importantly, grape composition at harvest is of special interest and should be interpreted as a mutual interaction between an especially functional late season canopy and a rapidly changing weather pattern, where the final ripening events take place under much cooler conditions than a standard season. This can easily explain why the forced crop has largely improved TSS and colour while retaining much higher acidity, especially malic acid. The scenario is especially challenging for future field applications where an even longer ripening season—a feature fed by worldwide global warming—associated to the absence of soil volume constrictions can allow TA to be smoothed further to reach a balance that will be suited to an high value aged wine.

Conclusions

A bud-forcing technique that aimed to obtain two crops per season in Pinot Noir vines grown in pots has proven to be feasible and envisages the chance that the second, late season crop, due to distinctive grape composition features, might be intended for higher value wines. Feasibility is based on a series of positive responses observed on 2 years of research, confirming that several required responses, such as good quality grapes at both harvests and no compromise of cropping in the next year, can be fulfilled. The technique followed two different strategies: (i) longer ripening seasons allowed by global warming create conditions favourable to this management practice which, starting with early ripening cultivars, can extend to others; and (ii) economic sustainability of the wine business may indeed benefit from such an intensive approach, since yield is increased, and the two harvests may originate from different grape composition. This creates a gateway to a new and competitive market strategy. Field applications of the technique, which may benefit from the preliminary assessment reported in this study, are in progress to focus on agronomic response under different environments and genotypes and to assess the degree of mechanisation of the practice.

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Figure S1. Schematic representation of the different phases of the crop forcing technique: (a) red cuts indicate main shoot trimming above node 6 and removal of any developing lateral; (b) ripening of primary crop has been reached whereas forced crop are at about fruitset; (c) forced crop is now ripen, while some of the basal main leaves have already dropped; (d) a detail of the trimming cut on the primary shoot and, underneath, initial growing of the forced dormant bud; and (e) a detail of two bunches each born on two adjacent forced shoots.

Figure S2. Seasonal daily trends for (a) 2019 and (b) 2020 of minimum air temperature (■), mean air temperature (■), and maximum air temperature (■) recorded at the experimental site.

Table S1. Potential fruitfulness (inflorescence/primary bud) observed with a stereo-microscope in dormant buds sampled from counts nodes 1–6 in each treatment (0) at the end of the 2020 season. Position 0 is referred to the base bud.