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Grapevine Response to Stress Generated by Excessive Temperatures during the Budburst

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Abstract: At springtime, the formation of stem somatic traits (stem elongation and leaf growth) and reproductive activity (flowering and fruit set) occur simultaneously. They are all competing carbon sinks, with an extremely high demand for carbohydrates. The shoot growth rate is strongly related to environmental temperature, which, according to climate change scenarios, is expected to increase also in extremes. Our hypothesis was that the increase in temperature during budburst could increase the vegetative carbon sink strength reducing the carbon stock available for the reproductive activity jeopardizing flowering of the next year. In our experiment, we artificially conditioned grapevine budburst by exposing the growing shoots to different temperature regimes. Higher temperatures during the spring vegetative growth favored shoot stem extension at the detriment of shoot leaf area. This caused a reduction in vine CO_2 assimilation, which, combined with the competition of the growing vegetative organs, affected the concurrent reproductive activity, with carry-over effects on the following year, resulting in a limited flower initiation in dormant buds. These results suggest that the increase in springtime temperature can alter canopy development and vine physiology, resulting in the reduction in reproductive activity with an economical negative impact on grapevine productivity.

Keywords: grapevine; temperature; carbon partitioning; climate change; bud fruitfulness; yield

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1. Introduction

Shoot growth is related to endogenous and environmental factors; endogenous factors are mainly related to carbohydrate availability, plant water relations, and hormonal signals. Environmental factors are primarily linked to temperature. In fact, temperature, often indexed as growing degree days (GDD), is the main driver of shoot growth, plastochron, and phenological stages development, when there are no other limiting factors [1–4].

In grapevine, shoot growth occurs after budburst in spring and continues until mid-summer. During this period, vegetative growth is the most important carbohydrate sink for the formation and development of plant structures, such as leaves, buds, and shoot apex. Leaves become a source of carbohydrate once they undergo the transition from heterotrophy to autotrophy [5,6]. For instance, in the grapevine, young and rapidly expanding leaves are sinks for photosynthates, and they become active carbon exporting organs only when they reach about 30% to 50% of their final size [7]. Shoots become active exporters of carbohydrates before flowering when they reach about six to eight mature leaves [8].

During early vegetative plant growth, other fundamental reproductive processes occur. Flowering, fruit and seed set, fruit growth, and induction of next year flowers are

concomitant to shoot growth, and all these processes compete for carbon; in grapevine, the allocation of carbon to clusters is negatively correlated with the allocation to leaves [9]. In early summer, excessive vegetative growth competes with the reproductive activity, resulting in a reduction in flower initiation [10,11], relative fruit set, and, in general, limiting the amount of carbohydrates allocated to reproductive structures [12]. The balance among different co-occurring sinks strength is influenced by the combination of the various factors affecting the expression of somatic and reproductive traits in horticultural species.

Water potential regulates plant vigor [13–15], mineral nutrition enhances somatic traits through the stimulation of photosynthetic activity [16], whereas nitrogen excess causes excessive vigor, which in turn negatively affects flower initiation and fruit growth [17]. However, temperature plays a pivotal role in determining the developmental cycle of plants and the growth rate of vegetative and reproductive organs [18]. Indeed, temperature influences both carbon assimilation in source organs as well as the activity of carbon sinks. During budburst, T is between 10 and 15 °C; optimum temperature for photosynthesis is between 25 and 35 °C, while when the temperature is below 10 °C and above 40 °C physiological processes decline [19,20]. The growth rate of flowers, fruits, and shoots can be promoted or inhibited by temperature, according to different sensitivity thresholds, and this may result in the alteration of the balance between vegetative growth and reproductive activity [21]. In grapevine, Pouget [22] and Keller et al. [23] reported an increase in fruit yield caused by the artificial increase in temperature on single shoots between budburst and initial stages of flower appearance. This suggests that temperature plays an important role in regulating the partitioning of carbohydrates not just between different plant organs (i.e., roots and shoots) but also between somatic and reproductive traits [24].

The relevance of temperature in regulating these processes may increase due to climate change. In fact, the expected changes in temperature over the next 30–50 years will be in the range of 2–3 °C [25], and Chmielewski and Rötzer [26] reported that in the period 1969–1998 across Europe, temperatures increased in the early spring (February–April) by 1 °C inducing a 7-day earlier start of the growing season in four forest tree species. Many studies have focused on the effects of climate change and climate extremes (i.e., drought, hot spells, spring frost, etc.) on plant phenology and physiology [18,27] and on plant reproduction [28]. However, poor attention has been devoted to the effect of temperature in regulating the balance between the expression of somatic traits and reproductive activity, which may have a pivotal role in agricultural production. Furthermore, there is no information regarding any potential carry-over effect of spring temperature on the reproductive activity of perennials related to modified carbon partitioning on next year's flower induction. Our hypothesis was that different temperature regimes after budburst affects the partitioning of carbohydrates in grapevines growing shoots with a detrimental impact on the reproductive activity in the current year and a carry-over effect on next year's cropping.

The aim of the present work was to investigate the effect of simulated environmental conditions (increasingly warmer springs) during the initial stage of vine development induced with a delayed budburst. Specifically, we aimed at determining the effect of different spring temperature scenarios on vegetative growth, carbon assimilation, whole vine physiology, and the allocation of carbohydrates to reproductive organs.

2. Materials and Methods

2.1. 2017 Experiment

The experiment was conducted in 2017 in Piacenza (45°02′ N, 9°43′ E), Italy, on twelve five-year-old spur-pruned cv. 'Sangiovese' (clone R10) vines grafted on SO_4 and grown outdoors in 40 L pots. On February 15th, all the vines were placed in a walk-in (3 × 2 m growth chamber (Monelletta srl, Perugia, Italy) located in a greenhouse and equipped with a glass roof allowing a maximum natural solar radiation ~1000 µmol m^{-2} s^{-1}. Temperature was kept at 4 ± 1 °C during the storage of the vines in the growth chamber. On March 16th (T0), May 2nd (T1), and June 5th (T2), 4 vines per each date/treatment, were transferred outside and were arranged along a single, vertically shoot-positioned cordon 35° NE-SW

oriented and hedgerow-trained row (Table 1). Each vine had a ~1 m long cordon on which 10–12 winter buds were retained on six spurs. The cordon was trained 90 cm from the ground with three pairs of top catch wires for a canopy wall extending about 1.3 m above the main wire. The pots were filled with a mixture of sand, loam, and clay (65%, 20%, and 15% by volume, respectively) and kept well-watered throughout the season. Pots were pale-green colored to limit radiation-induced overheating.

Table 1. Timing (DOY) of experiment phases and key phenological stages for the three treatments and mean temperature (T_{mean}) during the days between each phase.

Experiment Phases and Phenological Stages	DOY			T_{mean} (°C)		
	T0	T1	T2	T0	T1	T2
Vines removed from refrigerated chamber	75	122	156			
Budburst (BBCH 05)	90	133	160	14.45	15.79	22.94
Full bloom (BBCH 65)	142	158	181	16.02	22.29	25.9
Shoot topping	160	205	205	23.56	26.02	26.2
Veraison (BBCH 83)	186	212	236	26.17	26.65	26.27
Harvest	241	268	289	26.2	22.45	18.17

Whole-canopy net CO_2 exchange rate (NCER) measurements were taken using the multi-chamber system after Poni et al. [29]. The system features alternating current, centrifugal blowers (Vorticent C25/2 M Vortice, Milan, Italy) delivering a maximum air flow of 950 m^3 h^{-1}, flexible plastic polyethylene chambers allowing 88% light transmission, 6% diffuse light enrichment, and no alteration of the light spectrum, a CIRAS-EGM4 single-channel absolute CO_2 infrared gas analyzer (PP-Systems, Amesbury, MA) and a CR1000 data logger wired to an AM16/32B Multiplexer (Campbell Sci., Shepshed, U.K.). Switching of air sampling from one chamber to another was at programmed time intervals using a set of solenoid valves; the air-flow rate to each chamber was controlled by a butterfly valve and measured with a Testo 510 digital manometer (Farnell, Lainate, Italy) after the flow restriction method described in Osborne [30]. Twelve chambers, enclosing all the canopy with the exception of the apical spur on the cordon of each experimental vine, were mounted, in each treatment, after budburst when shoots were ~5 cm long: more specifically, it was on DOY 108, DOY 140 and DOY 167 in the T0, T1, and T2 treatment, respectively. Chambers operated continuously, 24 h a day, until October 24th (DOY 297). The air-flow rate fed to the chambers was progressively adjusted according to the increasing vine leaf area. They were 6.3 s^{-1} between DOY 108–142, DOY 140–205, and DOY 167–205 in T0, T1, and T2 treatments, respectively, and they were raised to 10.1 L s^{-1} between DOY 143–205, DOY 206–297, and DOY 206–297 in T0, T1, and T2, respectively. In T0, the air-flow rate was further adjusted at 10.2 L s^{-1} on DOY 235 and maintained constant for the remainder of the season. Ambient (inlet) air temperature and the air temperature at each chamber outlet were measured by shielded 1/0.2 mm diameter PFA-Teflon insulated type-T thermocouples (Omega Eng. INC, Stamford, Connecticut); direct and diffuse radiation were measured with a BF2 sunshine sensor (Delta-T Devices Ltd., Cambridge, U.K.) placed horizontally on top of a support stake next to the chambers enclosing the canopies. Canopy NCER (µmol CO_2/s) was calculated from flow rates and CO_2 differentials after Long and Hallgren [31].

Starting when shoots were about 5 cm long, shoot length, leaf length of green leaves (senescent leaves were excluded), and the number of nodes was recorded every 3–4 days up to mid-August when shoot growth slowed down in all treatments; thereafter, measurements were taken every 2 weeks. Shoots used for these measurements were those not enclosed in the growth chamber (two per vine). The whole vine leaf area was calculated from the measurements on these shoots by multiplying the shoot leaf area (obtained by regression between leaf length and leaf area measured by photographs that were then processed by Sigmascan Pro 5.0 software (Systat Software Inc., San Jose, CA, USA) with the total number of shoots per vine. Shoot topping was carried out on DOY 160 in the T0 treatment and on DOY 205 in the other two treatments.

Vines were harvested on DOY 241, 268, and 289 for T0, T1, and T2 treatments, respectively. The number of clusters per vine was counted, and yield per vine was recorded. Cluster weight was calculated. On all clusters, berries were separated from the rachis and counted, berry weight was then measured, and mean berry weight was calculated by dividing the cluster weight by the number of berries per cluster. Berries were then crushed, and the concentration of total soluble solids (TSS, °Brix) was determined by a temperature-compensating refractometer (RX-5000 Atago USA, Bellevue, WA, USA). Must pH was assessed with a digital PHM82 pH-meter (Radiometer Analytical s.a.s., Villeurbanne Cedex, France), and TA was measured titrating with 0.1 N NaOH (pH 8.2 end-point) and given as g/L of tartaric acid equivalents. On January 10th 2018, vines were pruned: main canes were separated from laterals, and pruning wood was weighted after being oven-dried.

At the end of leaf fall, all the canes used for shoot growth measurements were collected and brought to the lab. Subsequently, all dormant buds underwent bud dissections under a stereo microscope (40× magnification) by slicing thin sections perpendicular to the buds axes with a razor blade [32]. The number of inflorescence primordia in primary buds was recorded. Then, the potential fruitfulness of buds according to their position on the cane was expressed as the number of inflorescence primordia per primary bud.

Integrated leaf area per season was obtained as

$$\int_{BB}^{LF} \boldsymbol{LA}\ (\boldsymbol{DABB})\ \boldsymbol{dDABB} \tag{1}$$

where ***BB*** was budburst, ***LF*** was leaf fall, ***LA*** was leaf area as an empirical function of ***DABB*** (Figure 1) and represented the area subtended by the three curves reported in Figure 1.

Weighted mean vine leaf area (***WMLA***) was calculated as

$$\boldsymbol{WMLA} = \frac{\sum(\boldsymbol{LA} \times \boldsymbol{D})}{\boldsymbol{D_s}} \tag{2}$$

where ***LA*** was the vine leaf area calculated per each record, ***D*** were the days to the next record, and $\boldsymbol{D_s}$ were the days from ***BB*** to ***LF***.

Shoot CO_2 assimilation to shoot ***RGR*** ratio (***NCER/RGR***) was calculated as

$$\boldsymbol{NCER/RGR} = \frac{(\int_{t_1}^{t_2} \boldsymbol{NCER}(\boldsymbol{t})\ \boldsymbol{dt}) \times \boldsymbol{SL_1} \times (\boldsymbol{t_2} - \boldsymbol{t_1})}{\boldsymbol{SL_2} - \boldsymbol{SL_1}} \tag{3}$$

where ***NCER*** was the vine net CO_2 exchange rate divided by the number of shoots, $\boldsymbol{SL_1}$ and $\boldsymbol{SL_2}$ were the mean shoot length of the two shoots per vine at the time ($\boldsymbol{t_2}$ and $\boldsymbol{t_1}$) of two subsequent records of shoot growth.

2.2. 2018 Experiment

In spring 2018, 16 vines of cv Montepulciano grafted on SO_4 rootstock planted on the same potting medium as the ones used in the previous experiment were placed on March 1th in the same growth chamber of the previous year. A total of 4 vines were then removed from the cold storage on March 16th (DOY 75), 4 vines on April 13th (DOY 103), 4 vines on May 3rd (DOY 123) and 4 vines on May 25th (DOY 145). All vines were then placed in the external area where the 2017 experiment was carried out. Vines were trained as described in the previous experiment. From March to the end of June, the shoot length and node number of all the shoots formed were recorded, and in May 2019, the number of inflorescences per cane was counted.

2.3. Statistical Analysis

Statistical data treatment was performed by linear and non-linear regression analyses using Sigmaplot 8.0 (SystatSoftware Inc., San Jose, CA, USA), and R^2 significance was assessed by ANOVA. Treatments were analyzed by one-way ANOVA with a significance level set at 0.05, and means were separated by Tukey's w-procedure at $p = 0.05$.

3. Results

3.1. Shoot Growth

The seasonal dynamic of vine leaf area development differed among treatments (Figure 1). In particular, leaf area reached a maximum of 2.57 ± 0.02, 1.82 ± 0.35, and 1.88 ± 0.31 m^2 on 116, 65, and 42 days after budburst (DABB) in T0, T1, and T2 treatments, respectively (Figure 1). The maximum leaf area in T1 and T2 treatments overlapped with the measurements carried out before shoot topping. In T0 treatment, shoot topping was carried out 70 DABB but did not correspond with the maximum leaf area since shoot topping stimulated lateral shoot growth, which led to the formation of new leaf area. Integrating vine leaf area over the season, T0, T1, and T2, respectively, had 283.71 ± 12.54, 144.75 ± 25.19, and 140.54 ± 29.67 m^2 days of photosynthetically functional leaf area over the season, while they respectively had a weighted mean vine leaf area (WMLA) of 1.26 ± 0.07, 0.91 ± 0.16, and 1.03 ± 0.22 m^2.

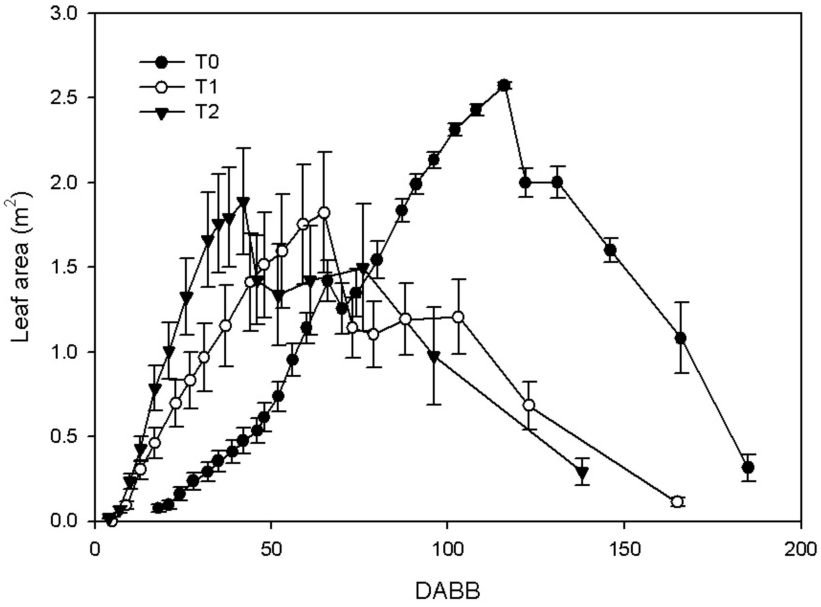

Figure 1. Leaf area (mean ±SE) of T0, T1, and T2 vines (n = 4) vs. day after budburst (DABB).

Vines were removed from the refrigerated growth chamber on DOY 76, 123, and 157, respectively, 47 and 81 days later from the first timing (T0), and transferred to outside growing conditions. Between budburst and shoot topping, on average, T_{min}, $T_{mean,}$ and T_{max} were, respectively, 11.5, 17.9, and 24.1 °C for T0 treatment, 17.2, 24.6, and 31.9 °C for T1 treatment and 18.9, 26.3 and 33.4 °C for T2 treatment (Figure 2A). Budburst occurred on DOY 90, 133, and 160 for the T0, T1, and T2 treatments, respectively (Figure 2B). The time elapsed between transferring the vines from the refrigerated growth chamber to the outside conditions and budburst was reduced in the T1 and T2 treatments when compared to the T1 or T0 treatment. In T0, the mean daily temperature at budburst was 15.5 °C, while in T1 and T2, it was 20.1 and 25.5 °C, respectively. In T0 mean daily temperature at full bloom was 21.1 °C, while in T1 and T2, it was 21.8 and 22.7, respectively

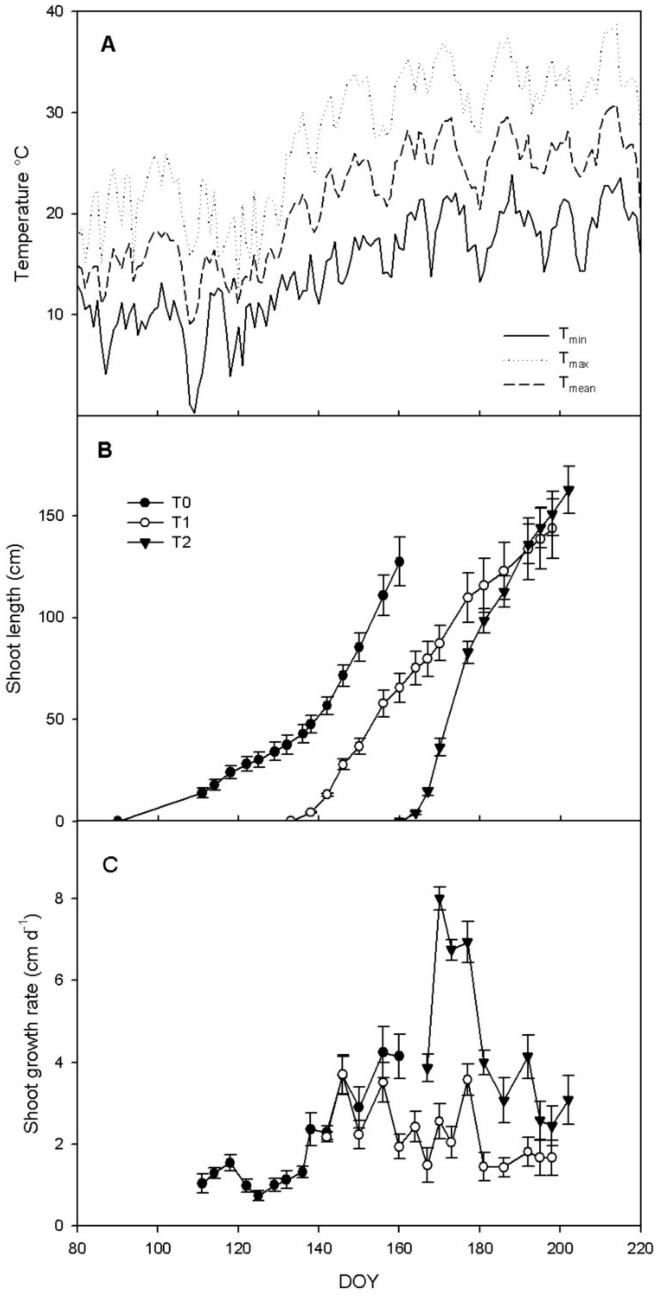

Figure 2. Air temperature between the experiment start and DOY 220 (**A**). Shoot length (**B**) and shoot growth rate (**C**) in vines (n = 4) of T0, T1, and T2 treatments. Mean ± SE (n = 4).

In T0, shoot growth was almost linear up to DOY 140 and, thereafter, shoot growth rate increased, tracking the rise of daily temperature (Figure 2B). In T1 and T2 treatments, shoot length linearly increased until DOY 177 to slow down right after. Maximum shoot length before topping was. Respectively, higher in T1 (+16 cm) and T2 (+35 cm) treatments when compared to the T0 treatment. The shoot growth rate increased over the season from 1.04 ± 0.23 to 4.24 ± 0.62 cm day^{-1} in T0 (Figure 2C). In T1, the shoot growth rate decreased over the season ranging between 1.44 ± 0.23 and 3.69 ± 0.47 cm day^{-1}. In T2, the shoot growth rate decreased over the season ranging between 2.44 ± 0.47 and 8 ± 0.27 cm day^{-1}.

Shoot growth rate was linearly correlated with daily growing degree (GD) (R^2 = 0.75 $p < 0.001$) (Figure 3) in T0. Instead, in T1 and T2 treatment, there was no significant correlation between shoot growth rate and daily GD.

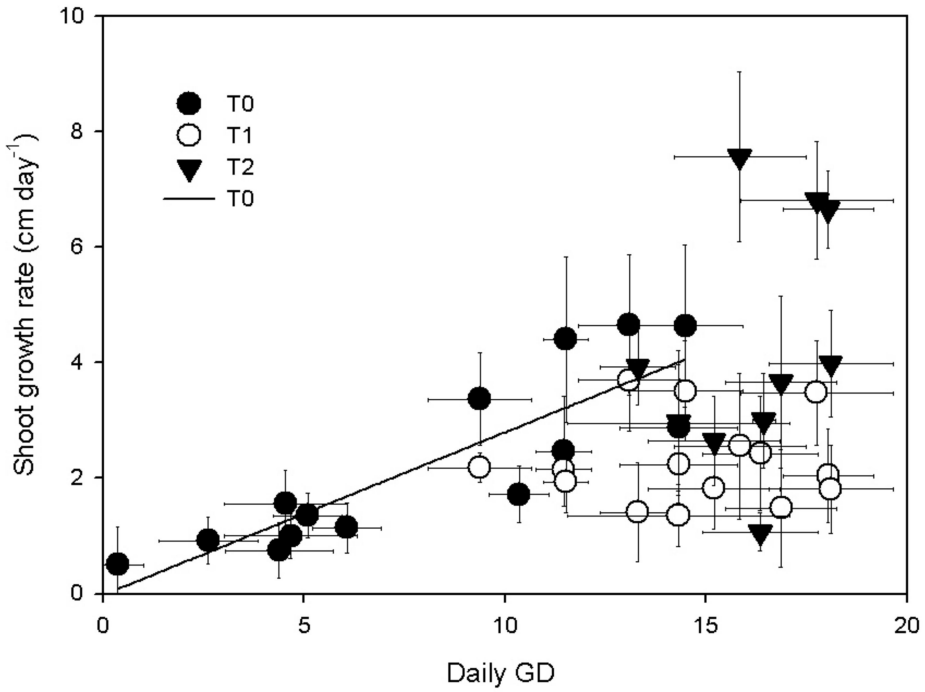

Figure 3. Shoot growth rate vs. mean daily growing degree (GD) in T0 (*Shoot growth rate* = 0.28 × Daily GD − 0.02), $R^2 = 0.75$, $p < 0.0001$) T1 and T2. Mean ± SE ($n = 4$).

Shoot leaf area per shoot length increased in T0 and T1 treatments until shoot length was above 37.5 ± 4.5 and 79.7 ± 8.2 cm, respectively, and reached a plateau when the shoots were 110.9 ± 9.9 and 133.7 ± 14.2 cm long before decreasing after in both treatments due to senescence of basal leaves (Figure 4). In T2 shoot, leaf area per unit shoot length (cm^2 cm^{-1}) gradually increased, reaching a maximum value of 11.6 ± 0.7 cm^2 cm^{-1} at 151 ± 10.9; in T2 shoot, leaf area per unit shoot length was reduced by about 13% in comparison with the other two treatments.

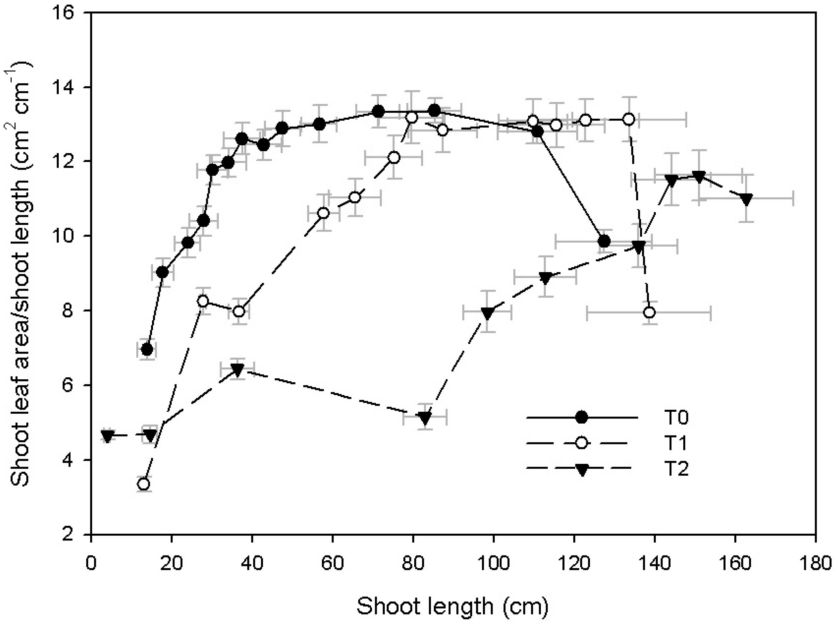

Figure 4. Shoot leaf area/shoot length vs. shoot length in T0, T1, and T2 vines ($n = 4$). Mean ± SE.

3.2. Vine Gas Exchange

Vine NCER increased later after budburst in T0 treatment, in comparison with T1 and T2, as the budburst process started later and advanced slower (Figure 5). In T0, maximum vine NCER was reached on DABB 60 with a maximum daily NCER of 0.96 ± 0.05 mol of CO_2. In T1 treatment, vine NCER increased up to DABB 26 when the maximum daily NCER was 0.40 ± 0.05 mol of CO_2. In T2 treatment, vine NCER increased up to DABB 28 when maximum daily NCER was 0.47 ± 0.04 mol of CO_2 day^{-1}. After reaching the peak, vine NCER decreased over the rest of the season in all the treatments. Overall, from budburst to leaf fall, T0, T1, and T2 treatments assimilated a total of 59.12 ± 4.95, 25.23 ± 2.90, and 23.56 ± 1.92 mol CO_2 $vine^{-1}$, respectively.

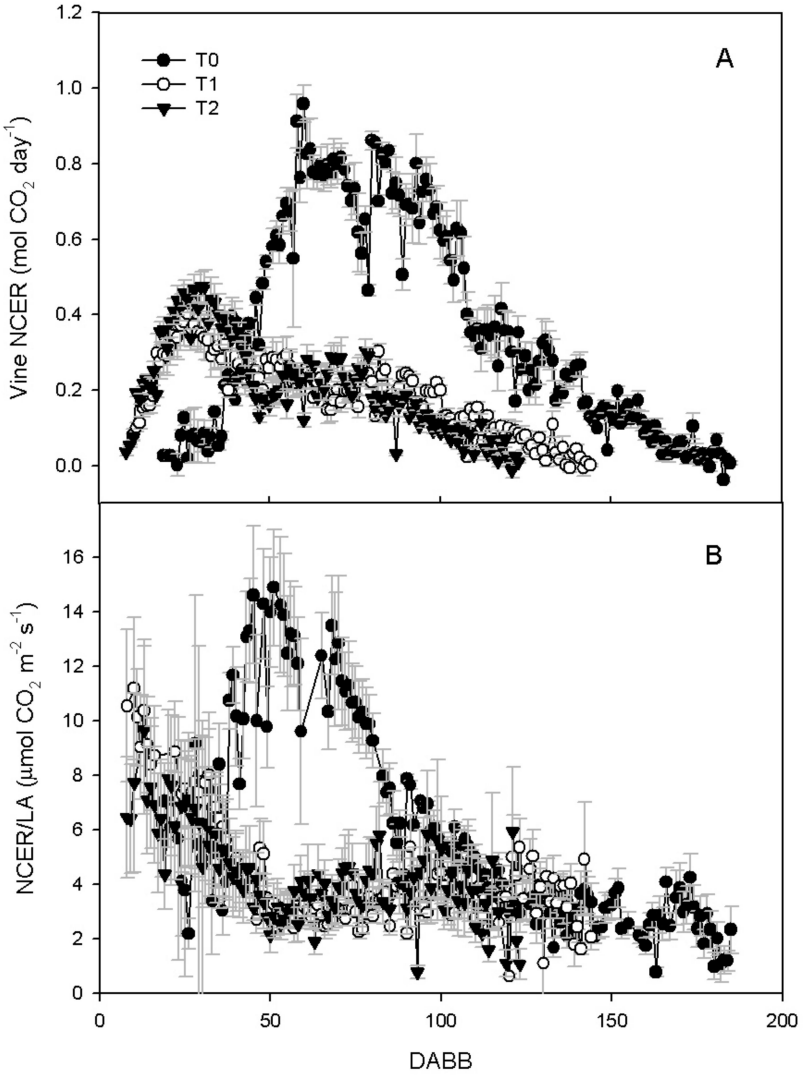

Figure 5. Vine net CO_2 exchange rate (NCER) (**A**) and daily mean leaf-specific NCER (**B**) from budburst to leaf fall vs. day after budburst (DABB) in T0, T1, and T2 vines (n = 4). Mean ± SE.

Mean daily LA-specific NCER increased in T0 until DABB 45, reaching a maximum NCER/LA of 14.61±2.52 and gradually decreased over the rest of the season (Figure 5B). In T1 and T2, maximum NCER/LA was 10.36 ± 2.61 and 9.66 ± 3.07 µmol m^{-2} s^{-1}, 10 and 13 DABB, respectively, and decreased sharply until 46 and 49 DABB, respectively, when it reached the minimum (2.70 ± 0.48 and 2.79 ± 0.67 µmol m^{-2} s^{-1}). After that, NCER/LA in T1 and T2 over the remaining part of the season until the moment before leaf fall. Vine NCER between budburst and max leaf area development was closely correlated to vine

leaf area in T0, while in T1 and T2 treatments, vine NCER increased more gradually than in the other treatments, up to 0.93 and 1.32 m^2 $vine^{-1}$ (Figure 6).

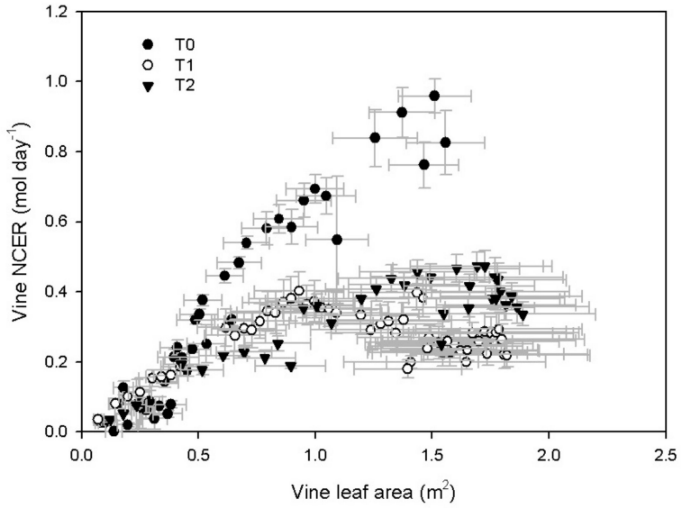

Figure 6. Vine net CO_2 exchange rate (NCER) from budburst to shoot topping vs. vine leaf area in T0, T1, and T2 vines (n = 4). Mean ± SE.

In T1 and T2 treatments, further leaf area development did not increase vine NCER. Shoot CO_2 assimilation to shoot RGR ratio increased during the season faster in T0 treatment when compared to T1 and T2 treatment (Figure 7).

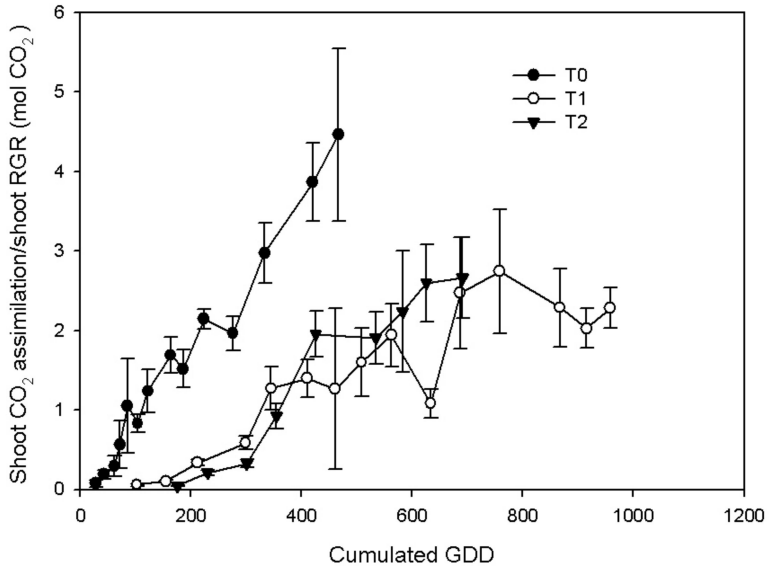

Figure 7. Shoot CO_2 assimilation/shoot RGR vs. cumulated GDD from budburst to shoot topping in T0, T1, and T2 vines (n = 4). Mean ± SE.

3.3. Reproductive Activity

Vine yield and cluster weight were significantly smaller in T1 and T2 treatments than in T0 vines, though the number of clusters per vine was similar (Table 2). Sugar concentration was higher in the T0 treatment in comparison with that of others, while pH was not affected by any of the treatments. Titratable acidity was higher in the fruit of T2 treatment, as compared to the other two treatments

Table 2. Vine yield, number of clusters, mean cluster weight, total soluble solids (°Brix), pH, and titratable acidity (g/L tartaric acid) of fruit in the tree treatment. Each number is the mean of 4 vines (n = 4) ± SE. Different letters indicate statistically significant means per $p < 0.05$ (Tukey test).

	T0				T1				T2			
Yield (kg vine^{-1})	1.56	±	0.37	a	0.29	±	0.08	b	0.17	±	0.06	b
Clusters (n vine^{-1})	10.00	±	0.41	a	7.50	±	1.71	a	9.75	±	0.95	a
Cluster weight (g)	154.63	±	36.63	a	36.28	±	6.09	b	16.61	±	5.22	b
Berry weight (g)	2.3	±	0.25	a	0.99	±	0.08	b	0.84	±	0.09	b
Berries per cluster (n)	66.4		1.4	a	36.2		0.5	b	19.9		1.2	b
Brix	16.55	±	0.68	a	14.58	±	1.35	ab	14.76	±	0.32	b
pH	3.26	±	0.09	a	3.34	±	0.06	a	3.40	±	0.03	a
Titratable acidity	6.04	±	1.59	b	6.80	±	0.16	b	8.89	±	0.47	a
Pruning weight (g dw vine^{-1})												
Primary shoots	155	±	10.08	a	82.5	±	8.98	b	96.25	±	12.5	b
Lateral shoots	26.75	±	5.02	a	3.62	±	0.61	c	11.25	±	3.64	b
Total	181.75	±	6.41	a	86.12	±	9.28	c	107.5	±	13.4	b

Vine yield was negatively correlated with shoot growth rates from budburst to full bloom (R^2 = 0.90, $p < 0.0001$) (Figure 8).

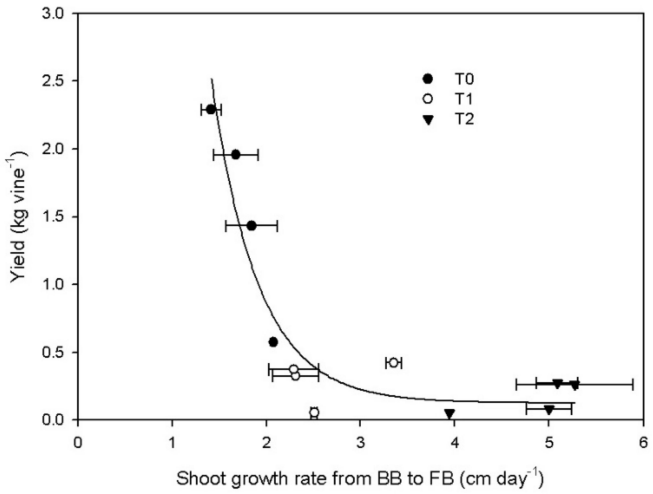

Figure 8. Vine yield vs. shoot growth rate from budburst (BB) to full bloom (FB) in T0, T1, and T2 vines (n = 4). Mean ± SE ($Yield = 0.13 + 41.26 \times \exp(-2.01 \times$ shoot growth rate), $R^2 = 0.90$, $p < 0.0001$).

The correlation was linear up to a shoot growth rate of about 2.5 cm day^{-1}; at higher shoot growth rates, there was no further significant reduction in yield. Mean number of inflorescences primordia in winter buds carried on the five basal nodes of the canes was 1.58 ± 0.14 in T0, 0.91 ± 0.15 in T1, and 0.58 ± 0.23 in T2. It was negatively correlated with mean shoot growth rate during the formation of the first five nodes (R^2 = 0.76, $p < 0.0001$) (Figure 9).

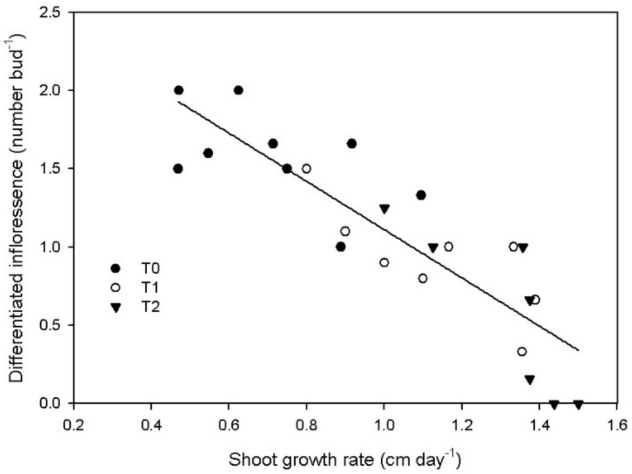

Figure 9. Mean bud fruitfulness on the 5 basal nodes vs. shoot growth rate from budburst to the appearance of the 5th nodes (*Differentiate inflorescence* = −1.54 × shoot growth rate + 2.65, R^2 = 0.76, $p < 0.0001$). Each point represents one shoot.

Pruning weight was higher in the T0 treatment than in T2 and T1 treatment, respectively (Table 2). Such difference was due to a lower primary cane dry weight, which was similar between T1 and T2 treatments, and laterals dry weight that was lower in T2 and T1 treatment, respectively.

In the 2018 experiment, shoot growth was relatively more intense in vines removed from the cold storage on DOY 145, 123, and 103 than those removed on DOY 75 (Figure 10). However, in these three treatments, shoot elongation was more limited than in vines of DOY 75 treatment.

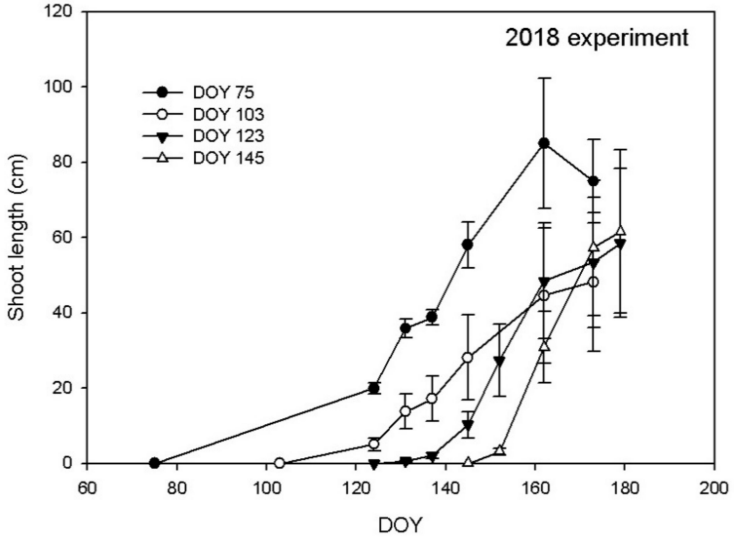

Figure 10. Shoot length over the experiment carried out in 2018 in Montepulciano vines removed from the cold storage on DOY 75, 103, 123, and 145. Mean ± SE (n = 4). Different letters indicate statistically significant means per $p < 0.05$ (Tukey test).

Shoot fruitfulness on the next year (2019) was significantly reduced in DOY 123 and DOY 145 treatments in comparison with the other two treatments (Figure 11).

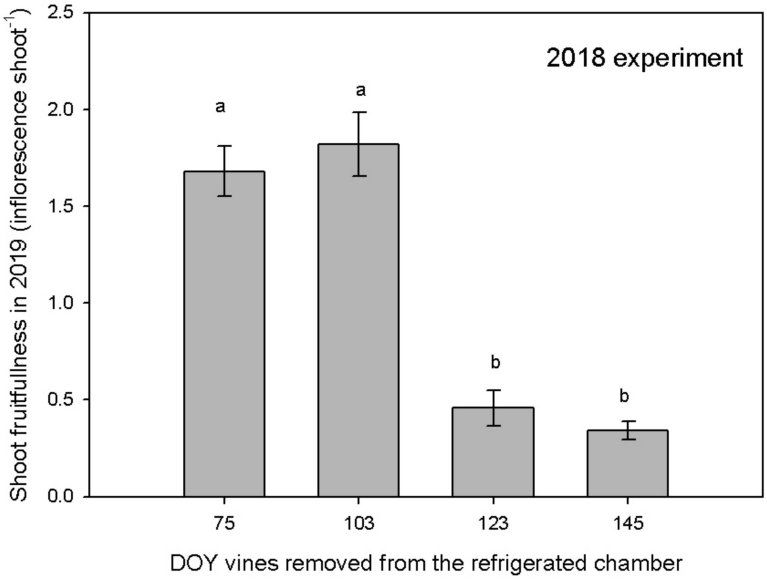

Figure 11. Shoot fruitfulness in 2019 in Montepulciano vines removed from the cold storage on DOY 75, 103, 123, and 145 in 2018. Mean ± SE (n = 4). Different letters indicate statistically significant means per $p < 0.05$ (Tukey test).

4. Discussion

Temperature has a pivotal role in regulating shoot growth rate, especially during the early plant phenological stages, which influences shoot growth rate over the rest of the season [3]. In our experiment, we simulated different temperature conditions during and after budburst by inducing grapevine budburst at different times during the season. The range of temperature increases is higher than the mean temperature increase projected by IPCC scenarios but could be considered representative of the different growing conditions in the wide area where viticulture is practiced. Vines were exposed to consistently higher daily minimum, maximum, and mean temperatures, which caused different shoot growth dynamics by increasing shoot growth rate during the first phenological stages (Figure 2C). In T1 and T2, shoot extension was not limited by temperature as in T0 (Figure 3). In these two treatments, faster shoot growth (measured on two shoots per vine left outside the gas exchange measurement chamber) caused a faster increase in leaf area due to (1) higher shoot growth during the first period before budburst (Figure 2C) (2) the advancement of the phenological stages (Figure 1, Table 1). Based on these data, it could be reasonable to expect higher seasonal vine CO_2 assimilation in T1 and T2 treatments due to faster development of active vine leaf areas. In fact, during the first period of leaf area development, T1 and T2 had an earlier increase in vine NCER than T0 vines (Figure 5), but after 26 and 28 DABB vine, NCER started to decline in T1 and T2, respectively. This was partly due to the decrease in leaf-specific NCER rate (Figure 5B) and to the reduced leaf area per shoot length in these two treatments in comparison with T0 (Figure 4). Indeed, in T1 and T2, temperature forced earlier shoot growth, investing primarily in node formation and internodes elongation while leaf size was greatly reduced (Figure 4). In particular, T1 vines reached a leaf area-to-shoot length ratio (cm^2 cm^{-1}) similar to that measured in T0 when shoots were 80 cm long vs. the 37 cm scored in T0; T2 vines never reached similar values. Due to the need to continuously monitor shoot growth, internal shoots may have been exposed to micro-environmental conditions slightly different to that of shoots inside the gas exchange chamber; for instance, at full bloom in the three thesis (DOY 142, 158, 181), the mean temperature inside the chambers was 0.20, 0.66, and 0.41 °C higher than in the outside environment. However, no morphological difference was noted between the inside and outside shoots.

After budburst, the largest amount of shoot dry mass is allocated to leaves (82%) and the remaining to stem [33]. In our experiment, temperature forced shoot stem growth at the detriment of leaves, as indicated by the different correlation between vine leaf area and NCER. The relationship between vine leaf area and NCER was almost linear in T0 vines (Figure 6); in the other two treatments, vine NCER increased at different rates until reaching a plateau when vine leaf area was larger than 0.93 m^2 $vine^{-1}$ and 1.32 m^2 $vine^{-1}$(Figure 6). T1 and T2 had a lower photosynthetic efficiency than T0 when the vine leaf area was larger than 0.5 m^2 (Figure 6): this occurred 46, 23, and 17 DABB in T0, T1, and T2, respectively (Figure 1). At this stage (46, 23 and 17 DABB), shoots were 42.83 ± 4.50, 57.81 ± 6.5, and 82.94 ± 5.39 cm long (Figure 2B) and leaf area per shoot length was 12.45 ± 0.40, 10.61 ± 0.48, and 5.39 ± 0.35 cm^2 cm^{-1} in T0, T1, and T2 (Figure 4). This indicates a relatively lower investment in leaf structures, with respect to stem elongation, in T1 and T2 in comparison with T0. Moreover, in T1 and T2, the lack of vine NCER increased as the leaf area increased over 0.5 m^2 (Figure 6), and the fast decline of leaf area after 65 and 42 DABB indicate a faster loss of leaf functionality of the oldest leaves in the latter two treatments when compared to the T0 treatment. Reduced leaf functionality combined with a reduced allocation to leaf area caused a reduction in the photosynthetic efficiency that resulted in consistent lower CO_2 assimilation right before full bloom (that occurred 52, 25, and 21 DAFB in T0, T1, and T2, respectively) and from that stage onwards. Noteworthy, it has been demonstrated that, at full bloom, use of reserve carbohydrate diminishes, and the vine becomes dependent upon recently assimilated photosynthate [34,35].

For plants, the allocation of carbon in somatic traits, such as shoots and leaves, is an investment to intercept more light to assimilate more carbon [36]. This can either decrease the amount of carbon allocated to reproduction (because of competition with vegetative growth) or increase the seasonal plant CO_2 assimilation (because of larger leaf area) with benefit, at least in the long term, for the reproduction activity too. In our experiment, Shoot CO_2 assimilation per shoot relative growth rate (RGR), which represents an index of the carbon return after the investment in somatic traits (shoot growth), shows as the carbon return of the carbon investment in somatic traits in T1 and T2 came later and it was overall reduced than in T0 (Figure 7). Although the latter two treatments developed their leaf area in a shorter time due to the fast stem growth rate, during this period, the carbon investment (which was mainly based on reserve remobilization) resulted in a slower return in terms of photosynthesis (i.e., more thermal time was needed to have an increase in carbon assimilation with the contribution of the new growth). The lower carbon assimilation return was due to the lower photosynthetic efficiency, which, in turn, was related to increased stem growth instead of leaf tissue. This caused a carbon shortage that was evident at full bloom when carbon reserves presumably decreased [34,35], stem elongation was intense, and leaf area and leaf photosynthetic efficiency were not adequate to support all the sinks. Under these conditions, after full bloom, even vegetative growth was limited (Figure 2C). This can account for the lack of correlation between daily GD and shoot growth rate, indicating that the limiting factor was not the daily temperature, such as in T0 treatments, rather the carbon pool available for vegetative growth. In our experiment, the exposure of the whole vine to different temperatures during budburst produced different effects on shoot growth and yield (see below) in comparison with those reported by Keller and Tarara [37] and Keller et al. [23], which conditioned the temperature of single shoots. In their experiment, heating the single shoot resulted in a larger leaf area, which, according to our data, could be explained by compensation of carbon shortage coming from neighboring shoots. Such a phenomenon is quite common when an imbalance between source and sinks occurs on single shoots [38]. Furthermore, they did not evaluate the leaf area per shoot length ratio, which played a pivotal role in our experiment.

Carbon shortage caused a consistent slowdown of vegetative activity (coincided with the period after shoot topping) which partially re-equilibrated the ratio between stem growth and leaf area. However, the imbalance between stem growth and leaf area expansion caused a lower canopy efficiency that resulted in a reduction in the seasonal CO_2

assimilation by 57% and 60% in the T1 and T2 treatments. Perennial species, such as trees, are considered K-strategist: they invest in long terms traits, such as somatic traits, because the individual ecological fitness depends on the size of the progeny generated over the whole plant life [39]. Thus, if the pool of resources is limiting, somatic traits have priority over reproductive traits.

In our study, higher temperature ultimately caused a limitation of the carbohydrate pool. The first consequence of such limitation was the strong reduction in yield, which in T1 and T2 treatment was, in terms of fresh weight, 18% and 10% of that in T0. Such decrease was due to the reduction in berry per cluster (−46% and −71% in T1 and T2, respectively) and berry weight (−56% and −63% in T1 and T2, respectively). Indeed, grape yield was negatively correlated with shoot growth rate from budburst to full bloom (Figure 8). The competition during this phase drastically limited the number of berries, their weight, and finally, yield (Table 2). These data confirm that climate anomalies in this specific vine growth stage (budburst-bloom), combined with site (i.e., soil fertility) and plant management strategies (i.e., canopy management, floor management, fertilization) that induce excessive vegetative growth, can have a dramatic effect on carbon allocation to reproductive organs [40]. On the other hand, these results contrast with the positive effect of artificially increased spring temperature on single shoot yield reported by Pouget [22] and Keller et al. [23], which explored the effect of limited increase in temperature. This indicates that the forcing effect of temperature on vegetative growth can be positive only if carbon sources are not limiting; presumably, in the Keller et al. experiment, supplementary carbon for supporting stronger shoot growth, derived from other shoots, on the same vine, that were not heated during the experiment.

Previous studies reported that different yield levels reduced the plant investment in leaf and shoot biomass, and this effect becomes more evident as the grape develops [41]. In our experiment, yield was not affected when shoot growth was linearly correlated to temperature (Figure 3) as in T0. This indicates that temperature was the limiting factor. When shoot growth is not correlated with temperature, it means that other factors are limiting, and in our experiment, carbohydrate availability was the most likely candidate. Under strong carbon limitation, such as in our experiment, vine carbon allocation prioritizes stem structure over leaves and fruits. Such conclusion is supported by the Buttrose [42] experiment in which a higher percentage of carbon allocation in leaf biomass was obtained at the lowest temperature regime he tested (15 °C). In our experiment, T1 and T2 were exposed to a longer photoperiod and larger light availability. This should have contributed to increasing vine photosynthesis, as recorded in the period immediately after budburst. However, according to this experimental design, it is not possible to assess if the different light regimes may have had any effect on the vine physiology in this experiment.

Noteworthy, the yield variability among T0 vines was largely explained by the shoot growth rate, emphasizing the importance of a parameter usually underestimated in commercial vineyards. According to our data, the carbon pool limitation induced by the environmental condition also influenced the flower induction process. In fact, differentiated inflorescences in the basal five nodes were significantly less in T1 and T2 in comparison with T0. Furthermore, differentiated inflorescences in the basal five nodes were negatively correlated to the shoot growth rate during the formation of the basal five nodes (Figure 9), indicating that high-temperature regimes, such as those tested in our experiment, during the first part of the season can have consistent negative carry-over effects on the next year production. The results of the 2018 experiment confirmed a negative effect of similar treatments on shoot fruitfulness in the next year in another cv, Montepulciano. In this experiment, too, the shoot growth in the same year was negatively affected by the warmer temperatures (data not shown) at which vines were exposed during budburst as a consequence of DOY 123 and 145. DOY 103 had no effect on shoot fruitfulness, presumably because it was carried out 19 days before the timing of T1 in the 2017 experiment.

To our knowledge, this is the first report of the negative effect of high temperatures during and after budburst on the next season reproductive activity (related to the limitation of

flowers differentiated in the basal buds) due to an unbalance of carbon partitioning between stem and leaves. According to climate change scenarios, springtime temperatures are expected to increase, particularly in temperate climate areas [26]. However, it should be noted that in our experiment, the increase in T experienced between budburst and bloom was quite consistent (+6 and +10 °C between T0 and T1 and between T0 and T2, respectively). The outcomes of this study suggest that further to the widely investigated effects on the vine phenology, such climatic trend could progressively jeopardize reproductive activity and fruit production, particularly in warmest viticultural areas, by inducing plants to allocate more carbon on stems, and, later in the season, on leaves with primary consequences on the whole vine carbon assimilation and on crop yield efficiency. In our experiment, the impact of the different temperature regimes after budburst decreased vine carbon diurnal assimilation by about 57.3% (T1) and 60.1% (T2) and reduced the carbon allocated in berries as sugar (parameter sensible to carbon budget alteration) from 15.4% (T0) to 6.0% (T1) and 3.7% (T2) of seasonal diurnal assimilation. This accounted for a reduction in carbon allocated to fruit of 83.4% (T1) and 90.4% (T2) in comparison to T0. On the other hand, pruning dry weight was reduced by 52% and 40% in T1 and T2 treatments, respectively, and maximum leaf dry weight was reduced by 28% in T1 and 24% in T2. These data may appear in contrast with the postulated decrease in carbon allocation in the leaf. Indeed, after the imbalance produced at the beginning of the season, the leaf area re-equilibrated the larger stem growth that occurred at the beginning of the season (Figure 4). This was performed at the detriment of reproductive activity: leaf area-to-shoot length ratio recovery occurred after full bloom, and the amount of carbon synthesized was not used for the flower initiation in basal buds. Our data indicate that temperature increase during the early phase of shoot growth induces a sink prioritization that, at the end of the season, favors leaf area and stem growth on reproductive activity, which was almost zeroed on the same year and strongly reduced in the next one. This experiment did not evaluate the carbon storage in reserves. Literature suggests that a limitation of carbon assimilation induces depletion of carbon storage [43]. We can then hypothesize that reserves were affected by the treatments, but further experiments are needed to address this point. Finally, according to our experimental design, we cannot exclude that other environmental factors such as day length, light intensity, and humidity may have concurred with the results reported here. Further experiments are needed to clarify the role played by these variables in the post-budburst phase.

5. Conclusions

The simulated increase in temperature during and after budburst resulted in an advantage for the elongation of shoot stem, which competes with the formation of leaf area. The delayed establishment of vine leaf area resulted in carbon starvation, which had as a primary effect the reduction vine yield and flower initiation and the slowdown of vegetative growth, which resulted in reduced seasonal assimilation of CO_2. Carbon starvation combined with the lack of fruits as active sinks favored the allocation of newly assimilated carbon in leaf structures. These results suggest that the increase in temperature during and after budburst could negatively affect canopy development and ultimately reduce carbon fixation efficiency and yield in the same year, with a potential carry-over effect on the next year's yield. These phenomena could cause severe economic consequences on perennial tree crops as well as alter the reproductive fitness of perennial species.

Author Contributions: S.T. and S.P. conceived and planned the study. F.B., P.Z. and S.T. carried out the experiment and analyzed the data. S.T. wrote the first draft of the manuscript. F.B. and P.Z. managed the plants that were used in the study. S.P., P.S., F.G., T.F. and A.P. revised and edited the manuscript. A.P. helped in the analysis of the data and revised and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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