

Article

Effect of Time of Girdling on Leaf Photosynthetic Performance and Kiwifruit Quality Characteristics at Harvest and Post-Storage

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Abstract: The present study investigated the impact of cane girdling on the ‘Hayward’ kiwifruit cultivar, both in terms of leaf physiological functions and fruit quality attributes, at harvest and post-storage. Four treatments were conducted: the control cane girdling conducted separately in August (GA), in September (GS), and both in August and September (double girdling) (GAS), using different canes. The results indicated that the carbon assimilation rate was reduced in girdled canes. Nevertheless, girdling resulted in increased fruit dry matter (by 1.7%), weight (by 6.4%), and dimensions without altering fruit shape. Additionally, fruits produced on girdled canes exhibited higher total soluble solids content (by almost 13%) and TSS-to-TA ratio post-storage. There were no significant differences in chlorophyll and carotenoid concentrations, organic acids, and most sugars assessed, both at harvest and post-storage. There were no significant differences among the treatments at harvest regarding total phenolic compounds, except for total flavonoids, which were lowest in the GA treatment. Post-storage, girdling (especially GAS and GS) was found to enhance the fruits’ total phenols and total flavanols, as well as its antioxidant capacity (1.88 $\mu\text{mol equiv. Trolox g}^{-1}$ FW based on DPPH assay under GS versus 0.53 $\mu\text{mol equiv. Trolox g}^{-1}$ FW under control). Overall, cane girdling can improve the quality of kiwifruit in terms of both fruit size and functional fruit properties.

Keywords: antioxidant capacity; organic acids; phenolic compounds; photosynthesis; sugars



Citation: Roussos, P.A.; Denaxa, N.-K.; Tsafouros, A.; Ntanos, E. Effect of Time of Girdling on Leaf Photosynthetic Performance and Kiwifruit Quality Characteristics at Harvest and Post-Storage. *Appl. Sci.* **2023**, *13*, 11087. <https://doi.org/10.3390/app131911087>

Academic Editor: Monica Gallo

Received: 12 September 2023

Revised: 27 September 2023

Accepted: 7 October 2023

Published: 9 October 2023



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1. Introduction

Kiwifruit, known for its high nutritional value and delightful taste [1], is a significant fruit crop in many countries around the globe and for many consumers. The widely cultivated ‘Hayward’ cultivar, with its green flesh, dominates the global market, while yellow and red flesh cultivars have been recently introduced to the global market. Growers, the packing and storage industry, as well as consumer acceptance are influenced by quality characteristics such as fruit size, dry matter content, and sugar levels [2,3], which all play a crucial role in determining the success of a kiwifruit cultivar and cultivation management. Additionally, these quality indexes have a great impact on the post-harvest behavior of the fruit [4]. Kiwifruit quality can be influenced by various factors, including extreme environmental conditions (e.g., high temperatures, radiation) and cultivation practices such as fertilization, irrigation, pruning, netting [5], or girdling [6].

Girdling, a traditional horticultural practice involving the partial removal of a ring of bark around the trunk or branch of a plant, is widely recognized for its impact on various agronomical traits, both quantitative and qualitative, and has been applied in several crops, including grapes [7], citrus [8], apples [9], peaches [10], and even in kiwifruit orchard management [6,11].

At the physiological level, by selectively interrupting the basipetal flow, shoot girdling alters the source–sink relationships within the plant. This disruption redistributes the

flow of assimilates, such as carbohydrates, and acropetally-produced compounds, such as auxins, towards the roots, leading to carbohydrate accumulation above the girdle zone [12] and provoking changes in mineral partitioning [12]. Furthermore, it can affect stomatal conductance [13], decrease leaf photosynthesis [14], and alter primary or secondary metabolism [13].

The impact of girdling on growth, fruit quality, and yield depends on the species, cultivar [9,15], and timing of application [6,11,16]. However, despite its significance, the changes in fruit at the metabolic level caused by girdling are not extensively studied [16]. In avocados [17], kiwifruit [18], and mandarins [8], girdling increased fruit set or the number of fruits per tree and enhanced fruit maturity [18,19]. Additionally, girdling has been found to reduce fruit drop in apple and Japanese persimmon [20,21], increase fruit size and coloring [19], and raise the sugar content in grapes and citrus [4,15,22]. In plums, amino acids, organic acids, anthocyanins, and other phenols were accumulated in the fruit after girdling [16]. In apples, the soluble solids concentration and acidity increased, but the growth of the fruit was not affected, while firmness was improved [9]. Nonetheless, some species do not exhibit any significant changes in the studied fruit quality parameters [23].

Girdling of canes is a widely applicable practice in 'Hayward' kiwifruit vines. Wounded areas typically heal within 3–6 weeks after application, allowing the root system to retain its assimilate supply. Girdling of the main trunk produces even more fascinating results, increasing both the total soluble solids concentration and dry matter content of the fruits [6], which are two major quality parameters associated with fruit sweetness [24] and storage behavior [25]. In all cases, it has been reported that the timing of girdle application plays a crucial role in the extent of the plant's response [11]. The application of girdling after fruit set has been shown to increase fruit size and, consequently, yield without negatively affecting the plant's vigor [11]. When girdles were applied during the starch accumulation phase, they enhanced the dry-matter content of the fruit, consequently improving its quality [26]. Girdling in autumn, approximately 16–20 weeks after the midpoint of flowering, resulted in increased bud break and flower number in the following season [6,11].

On the other hand, few studies have investigated the effect of girdling on fruit quality attributes both at harvest and post-storage, as well as on fruit phytochemical content [27].

Therefore, the present study aimed to investigate the impact of girdling on the 'Hayward' kiwifruit cultivar, focusing on key physiological changes and their subsequent effects on fruit quality and nutraceutical attributes. Specifically, the primary objectives of the present study were as follows: (a) to assess the effect of girdling (time and frequency) on kiwifruit plant photosynthetic activity during the cultivation period and (b) to evaluate the impact of girdling on fruit development, as well as on fruit physiological and quality attributes at harvest and post-storage.

2. Materials and Methods

2.1. Test Site Location—Plant Material—Treatments—Experimental Design

A field experiment took place in a 5-hectare kiwifruit orchard located in Agrinio County, Western Greece, for two successive years (2018 and 2019). The orchard consisted of 'Hayward' cultivar vines, which were 15 years old and trained as a T-shape with planting distances of 2.0 m × 4 m and a trunk height of 1.8 m. All the vines in the orchard received uniform cultural practices and inputs, including water, fertilizers, and phytosanitary products. The soil in the orchard was identified as loam, with a pH value of 7.25, 3.05% *w/w* CaCO₃ concentration, 1.76% *w/w* organic matter, and an electrical conductivity of 0.310 mS cm⁻¹ (based on soil analysis results provided by the farmer).

Girdling treatments were conducted using a girdling cutter during the following periods: (a) early August (on 9 August 2018 and on 8 August 2019) (referred to as GA, August girdling), (b) early September (on 12 September 2018 and on 10 September 2019) (referred to as GS, September girdling), and (c) both in August and September (referred to as GAS). Girdling consisted of removing a stripe of approximately 5 mm ring of bark at the base of a producing cane (Figure 1). Special attention was given to completely removing

the phloem tissue while minimizing any damage to the xylem tissue. Wounds were completely healed approximately one month after the treatments, as shown in Figure 1D. Each treatment was replicated four times, with four vines per replicate (i.e., 16 girdling applications per treatment). All treatments were applied on the same vines (on different canes) to reduce variability induced by the vine itself. Care was taken to choose canes with similar fruit numbers.



Figure 1. Cane appearance under control (A), single (B), and double girdling (C) and healing of girdling wound after approximately one month (D).

2.2. Photosynthesis and Photosynthetic Parameters

The net photosynthetic rate of the vines was measured with a portable photosynthesis system (Li-COR 6400) (Li-Cor, Lincoln, NE, USA) at the time of each girdling event (early August and early September) and at harvest (which took place on the 11 October 2018 and 18 October 2019), from 08.00 to 11.30 a.m. approximately. The portable photosynthesis system was adjusted to operate at 400 ppm CO₂, PAR was adjusted at 1500 μmol m⁻² s⁻¹ provided by LED arrays, chamber temperature was adjusted to 25 °C, and the flow rate was adjusted to 450 mL min⁻¹. Measurements were taken on four mature, sunlit leaves per vine, and three consecutive measurements were taken per leaf. At the same time, the chlorophyll content of the leaves was estimated using a Minolta SPAD 502 m (Konica Minolta, Inc., Tokyo, Japan) on at least 20 fully expanded sun-oriented (at the time of measurement) leaves per plot. In all cases, fully mature, healthy leaves from the upper canopy level were selected for the measurements.

2.3. Sampling and Physiological Properties Determination

In October (11 October 2018 and 18 October 2019), all fruits per treatment per vine were harvested at commercial maturity index. Then, at least 25 fruits per plot were randomly sampled from the harvested fruits, placed into labeled plastic bags, and immediately transferred via a portable freezer to the Laboratory of Pomology (Agricultural University of Athens) for further analysis.

The weight, diameter, and length of each fruit were measured using an electronic balance (Kern 470, Kern and Sohn, Ziegelei 1, 72336 Balingen, GmbH, Germany) and a digital caliper (Starrett, 727 Series, Athol, New England, MA, USA), respectively. Firmness was measured at two opposite sides of each fruit using a penetrometer with a conical tip (Turoni 53205 fruit pressure tester) (T.R. Turoni srl, via Copernico, 26, 47122 Forlì (FC), Italy). Before the measurement, a small part of the fruit skin was peeled off using a sharp knife. The dry matter percentage of eight fruits per plot was determined by drying an approximately 5 mm wide portion of the fruit (sampled at the equatorial sector) in an oven at 70 °C until a constant weight was achieved. The remaining fruits were peeled and homogenized using a household homogenizer, and the resulting pulp was stored in 50 mL tubes in a freezer at −25 °C until further biochemical analyses were conducted.

The rest of the harvested fruits were stored in the cold rooms of the Agricultural Co-operative of Agrinio 'AC Neapolis' under 0.5 °C and 95% humidity for approximately 128 days. A sample of these fruits was then sent to the Laboratory of Pomology at the Agricultural University of Athens, where the physiological properties post-storage were measured on at least 100 randomly collected fruits per treatment. Finally, the fruits were homogenized as previously described to study the post-storage fruit quality characteristics affected by trunk girdling application.

2.4. Determination of Organoleptic Characteristics

The determination of total soluble solids (TSS), total titratable acidity (TA), and pH in kiwifruit juice was conducted following the methodology outlined by Denaxa et al. [28]. TSS was expressed as °Brix, while TA was expressed as a percentage (% w/v) of citric acid present in the juice.

2.5. Chlorophyll and Carotenoids Determination

To determine the concentration of chlorophylls and carotenoids, 2 g of frozen pulp was used. The pulp was twice extracted using 5 mL of 80% v/v ethanol, and the resulting solution's absorbance was measured at 663 nm and 645 nm for chlorophylls and 470 nm for carotenoids. The concentration of chlorophylls and carotenoids was calculated using the equations described by Lichtenthaler [29] and expressed as mg per 100 g fresh weight.

2.6. Soluble Sugars Determination

Soluble sugar concentration was determined using 2 g of frozen pulp extracted twice with 4 mL of HPLC-grade water in a microwave oven following the method developed by Roussos et al. [30]. Sucrose, glucose, fructose, and inositol were separated using a HPLC system (Shimadzu Nexera X2) equipped with a refractive index detector (RID) (Shimadzu, Kyoto, Japan). The separation was performed using an Adamas Amino 5 µm column (250 × 4.6 mm) from Sepachrom (Via Trento, 33, 20017 Rho MI, Italy). The column was equilibrated at 35 °C, and the mobile phase comprised 80% v/v acetonitrile and 20% v/v water, flowing at a rate of 1.0 mL min⁻¹. The total sugar concentration was determined by summing up the concentrations of the individual sugars detected by HPLC. Each sample was analyzed twice, and the final concentrations were expressed as mg per g of fresh weight.

2.7. Organic Acids Determination

Approximately 0.5 g of frozen pulp was extracted twice with 5 mL of a 3% v/v metaphosphoric acid solution in water, following the procedure outlined by Roussos et al. [5]. The analysis of organic acids was performed using a HPLC system (Shimadzu Nexera X2) equipped with a diode array detector (DAD) (SPDM20A, Shimadzu, Kyoto, Japan). Citric acid, malic acid, and ascorbic acid were identified through isocratic detection by the DAD at a wavelength of 200 nm. The mobile phase consisted of 0.02% v/v formic acid in water, flowing at a rate of 1.0 mL min⁻¹ through a Kinetex C18 EVO column (250 mm × 4.6 mm) (Phenomenex, Torrance, CA, USA). The total concentration of organic acids was calculated by combining the individual acid concentrations, and these were expressed as mg per g of fresh weight.

2.8. Phenolic Compounds Concentration and Antioxidant Capacity Determination

To assess the concentration of phenolic compounds and antioxidant capacity, 2 g of frozen pulp was weighed and extracted using 5 mL of 100% v/v HPLC grade methanol at 38 °C for 15 min with periodic agitation. Following the extraction, the resulting extract was subjected to centrifugation at 4000 × g for 6 min. Subsequently, the supernatant was carefully transferred into a new tube, while the pellet obtained from the centrifugation was subjected to another round of extraction under identical conditions. The concentrations of total phenols, total o-diphenols, total flavonols, and total flavonoids were analyzed and determined in the supernatants following the methodology outlined by Denaxa et al. [28].

The antioxidant capacity was assessed using the DPPH (2,2-diphenyl-1-picryl hydrazyl) and FRAP (ferric reducing antioxidant power), as described by Denaxa et al. [28]. The results were expressed in terms of micromoles of Trolox equivalents (TE) per g of fresh weight.

2.9. Statistical Analysis

The trial was organized as a completely randomized design with four replicates, each consisting of four vines (totaling 16 vines per treatment). Raw data from the two successive growing periods were analyzed together ($n = 8$) as a one-way ANOVA experiment. Significant differences were determined based on Tukey's HSD test at a significance level of $\alpha = 0.05$, separately per sampling event, harvest, and post-storage period. Raw data of total sugars, total phenols, and ascorbic acid content per fruit post-storage, along with the antioxidant capacity per fruit (based on DPPH and FRAP assays), were analyzed based on Dunnett's test to determine significant differences from the control. Principal component analysis of raw data produced separately at harvest and post-storage was used to assess possible differences among treatments with a reduced number of variables. The statistical software Statgraphics Centurion XV (Statgraphics Technologies, Inc., located in The Plains, VA, USA) and JMP 13 (100 SAS Campus Drive Cary, NC 27513, USA) were utilized for the analyses.

3. Results

As shown in Figure 2, girdling exhibited a significant effect on carbon assimilation efficiency. During September, photosynthetic efficiency was higher in the control vines compared to the GA ones (Figure 2a). At harvest, once again, the control vines exhibited the highest photosynthesis, with significant differences from GS and GAS treatments. The intercellular CO_2 did not differ significantly among the treatments during the entire experimental period (Figure 2b). On the contrary, stomatal conductance and transpiration rates were higher under the control treatment in September compared to GA (Figure 2c,e), while no significant differences were observed among treatments at harvest. The photosynthesis versus stomatal conductance ratio was found to be higher in control vines compared to other treatments both in September (against GA) and at harvest (against all other treatments) (Figure 2d). Additionally, no significant differences were determined concerning the SPAD index in September, while at harvest, the GS treatment exhibited the highest SPAD index value (with a significant difference from the GAS treatment, which exhibited the lowest value) (Figure 2f).

The dry matter percentage of the fruit increased from August (13%) until harvest (approximately 17%) and decreased slightly post-storage (16% on average) (Figure 3a). Additionally, it was found that girdling in August led to a significant increase of fruit dry matter (17.2%) within the next 30 days (in September's assessment) compared to control, a value that remained almost the same until harvest. At harvest, no significant differences were detected among the treatments, while post-storage, the control fruits exhibited the lowest dry matter (15.3%) compared to the girdling treatments (16.4% on average). It is also interesting that in August, the dry matter per fruit accounted for 10 g of the total weight of the whole fruit (70 g) (Figure 3b). In September, the fruits from the vines girdled in August had a significantly higher dry matter per fruit (15.9 g) compared to the control (14.9 g). At harvest and post-storage, the control fruits exhibited a decrease in dry matter compared to the girdled ones. Furthermore, the dry matter of control fruits increased by approximately 4.7% during the period from September until post-storage, while in the girdled vines, this increase was calculated to be around 9%.

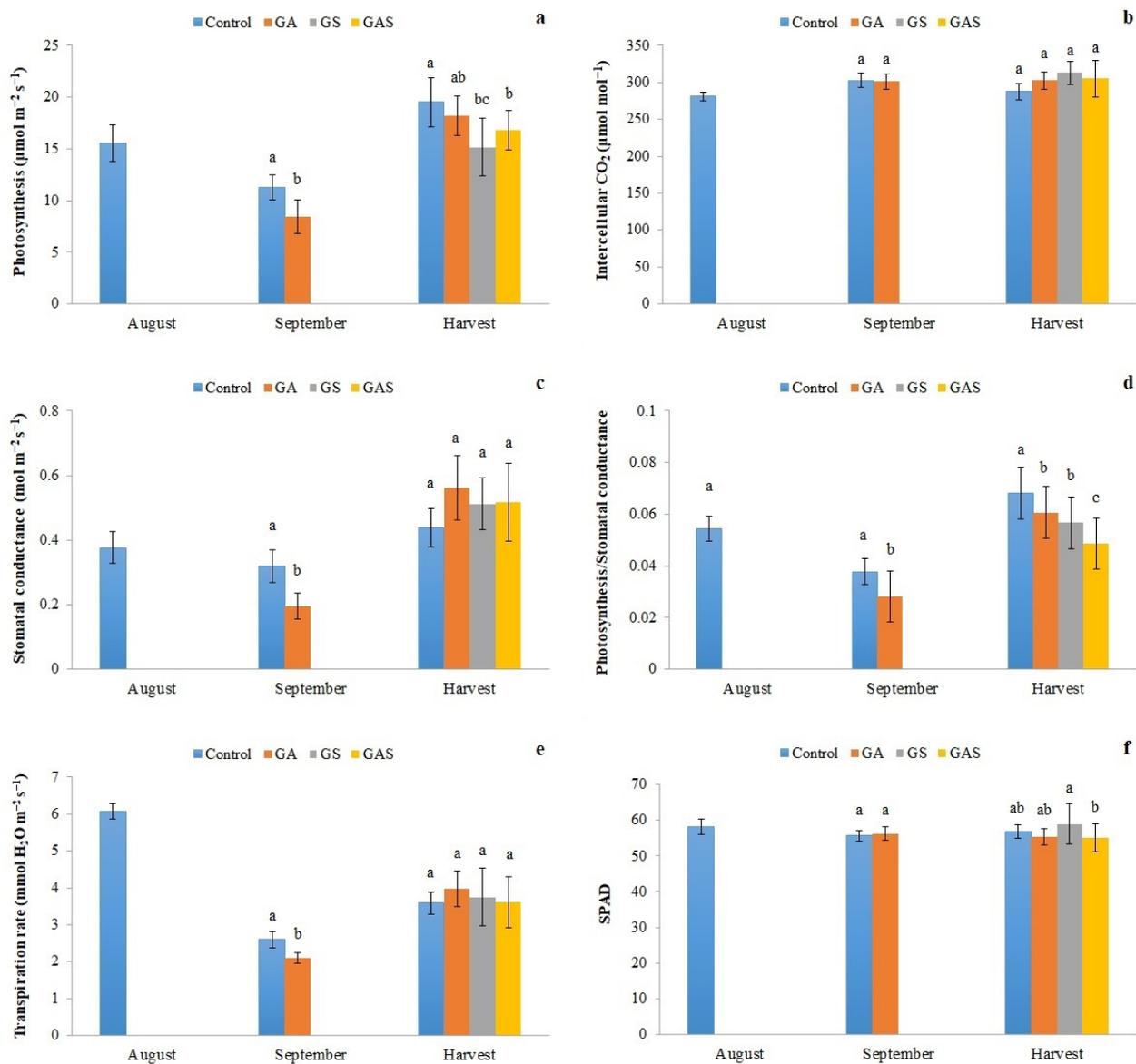


Figure 2. Cane girdling impact on kiwifruits' (a) photosynthesis, (b) intercellular CO_2 , (c) stomatal conductance, (d) ratio of photosynthesis to stomatal conductance, (e) transpiration rate and (f) SPAD index during August, September, and at harvest (October). Abbreviations: GA, kiwifruit cane girdling conducted in August; GS, kiwifruit cane girdling conducted in August; GAS, kiwifruit cane girdling conducted both in August and September. Different letters above columns, separately in September and at harvest, indicate statistically significant differences based on Tukey's HSD test at $\alpha = 0.05$. Bars on the columns indicate the standard deviation.

There were no significant differences concerning the mean fruit weight between the control and GA treatment in September (Figure 3c). However, at harvest, the mean fruit weight produced under the control treatment was 102.60 g. In contrast, the other treatments resulted in slightly higher mean fruit weights, ranging from 104.20 g to 109.14 g, achieved under GS treatment, with a significant difference from the control. It can also be observed that over the 3-month experimental period (August until harvest), the mean fruit weight of GA-treated vines increased from 74.73 g to 108.0 g and from 74.73 g to 109.0 g after GS treatment. This represents an approximately 45% and 46% increase, respectively, compared to only a 37% increase observed under control conditions.

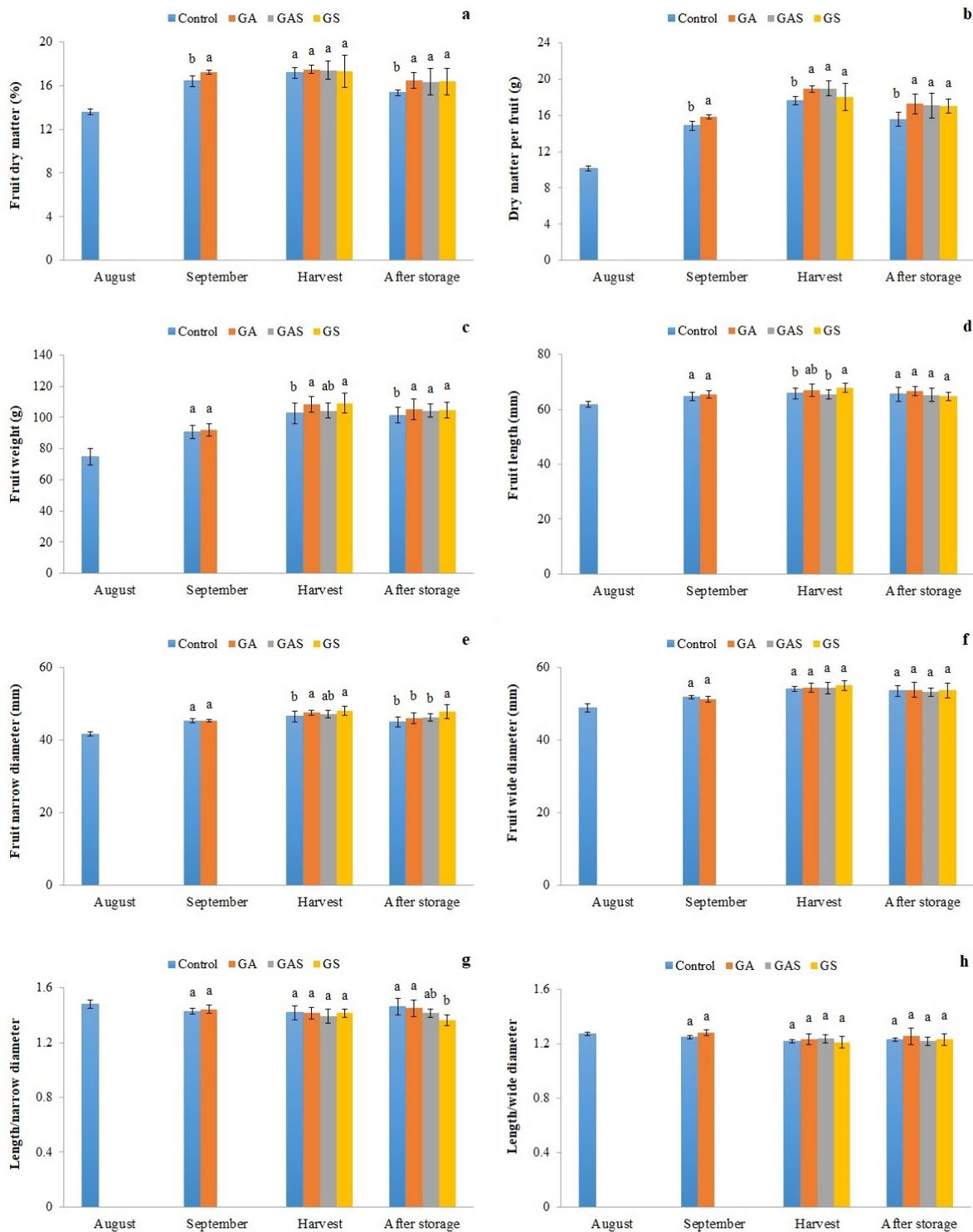


Figure 3. Cane girdling impact on kiwifruits' (a) fruit dry matter percentage (%), (b) dry matter per fruit (g), (c) fruit weight (g), (d) fruit length (mm), (e) fruit narrow diameter (mm), (f) fruit wide diameter (mm), (g) ratio of length to narrow diameter and (h) ratio of length to wide diameter during August, September, at harvest, and post-storage. Abbreviations: GA, kiwifruit cane girdling conducted in August; GS, kiwifruit cane girdling conducted in August; GAS, kiwifruit cane girdling conducted both in August and September. Different letters above columns, separately in September, at harvest, and post-storage, indicate statistically significant differences based on Tukey's HSD test at $\alpha = 0.05$. Bars on the columns indicate the standard deviation.

The length of kiwifruits remained consistent across all treatments during September and post-storage. However, at harvest, the fruits from GA-treated vines were characterized by a greater length compared to the control and GAS treatment (Figure 3d). Furthermore, the fruits of GS-treated vines exhibited the highest narrow diameter both at harvest and post-storage (Figure 3e), while all treatments showed similar wide diameters, with no significant differences observed (Figure 3f).

The effect of girdling treatments on fruit pulp pH, TA, TSS, and the ratio of TSS to TA during harvest was not found to be significant (Table 1). Additionally, control fruits exhibited the highest firmness (38.03 N) without any significant difference from the other treatments. Post-storage, similar pH and TA levels were observed among all treatments. However, the girdling treatments (GA, GS, and GAS) resulted in higher TSS content and higher ratios of TSS to TA compared to the control (Table 1). It was also noted that control fruits displayed the highest firmness (7.66 N), whereas the other treatments showed significantly lower firmness values ranging from 3.84 to 5.00 N.

Table 1. Cane girdling impact on kiwifruits' organoleptic characteristics (pH, total soluble solids (TSS, °Brix), titratable acidity (TA, % *w/v* citric acid), and the ratio of total soluble solids:titratable acidity (TSS:TA)) at harvest and post-storage.

	Control	GA	GS	GAS
Harvest				
pH	2.87 ± 0.09 a	2.86 ± 0.05 a	2.89 ± 0.05 a	2.89 ± 0.05 a
TA	2.59 ± 0.28 a	2.57 ± 0.12 a	2.58 ± 0.29 a	2.58 ± 0.33 a
TSS	6.33 ± 0.49 a	6.15 ± 0.42 a	5.73 ± 0.57 a	6.12 ± 0.27 a
TSS:TA	2.66 ± 0.82 a	2.43 ± 0.57 a	2.26 ± 0.40 a	2.36 ± 0.68 a
Firmness (N)	38.03 ± 1.28 a	35.75 ± 4.76 a	36.41 ± 2.30 a	37.73 ± 3.12 a
Storage				
pH	3.09 ± 0.14 a	3.01 ± 0.04 a	3.01 ± 0.07 a	3.03 ± 0.03 a
TA	2.23 ± 0.24 a	2.25 ± 0.14 a	2.26 ± 0.21 a	2.21 ± 0.14 a
TSS	12.24 ± 0.55 b	13.74 ± 0.39 a	13.68 ± 0.89 a	13.85 ± 0.72 a
TSS:TA	5.54 ± 0.65 b	6.27 ± 0.42 a	6.29 ± 0.57 a	6.09 ± 0.49 a
Firmness (N)	7.66 ± 1.26 a	4.16 ± 0.79 b	5.00 ± 1.66 b	3.84 ± 0.78 b

Mean ± standard deviation within the same row followed by the same letter, do not differ significantly according to Tukey's HSD test, at $\alpha = 0.05$. Abbreviations: GA, kiwifruit cane girdling conducted in August; GS, kiwifruit cane girdling conducted in August; GAS, kiwifruit cane girdling conducted both in August and September.

In Table 2, it can be observed that girdling treatments did not significantly affect the concentration of chlorophylls or carotenoids in the fruits, both at harvest and post-storage.

Table 2. Cane girdling impact on chlorophylls (Chl) and carotenoids concentration (mg 100 g⁻¹ FW) at harvest and post-storage.

	Control	GA	GS	GAS
Harvest				
Chla	0.51 ± 0.07 a	0.54 ± 0.09 a	0.56 ± 0.04 a	0.59 ± 0.16 a
Chlb	0.38 ± 0.02 a	0.43 ± 0.08 a	0.46 ± 0.04 a	0.47 ± 0.19 a
Total Chls	0.89 ± 0.09 a	0.97 ± 0.016 a	1.02 ± 0.07 a	1.07 ± 0.35 a
Carotenoids	0.16 ± 0.02 a	0.19 ± 0.03 a	0.16 ± 0.05 a	0.20 ± 0.05 a
Storage				
Chla	0.35 ± 0.02 a	0.35 ± 0.05 a	0.38 ± 0.09 a	0.37 ± 0.06 a
Chlb	0.19 ± 0.03 a	0.18 ± 0.06 a	0.25 ± 0.12 a	0.20 ± 0.06 a
Total Chls	0.54 ± 0.04 a	0.53 ± 0.10 a	0.63 ± 0.18 a	0.56 ± 0.12 a
Carotenoids	0.15 ± 0.02 a	0.13 ± 0.03 a	0.16 ± 0.05 a	0.16 ± 0.02 a

Mean ± standard deviation within the same row followed by the same letter, do not differ significantly according to Tukey's HSD test, at $\alpha = 0.05$. Abbreviations: GA, kiwifruit cane girdling conducted in August; GS, kiwifruit cane girdling conducted in August; GAS, kiwifruit cane girdling conducted both in August and September.

Table 3 presents the results of the effect of girdling on the fruits' soluble sugars at harvest and post-storage. The data showed that there were no significant differences in the concentration of the sugars detected at harvest. The same was also noticed after the storage period, as there were no significant differences in the concentration of all measured sugars among the treatments (Table 3).

Table 3. Cane girdling impact on kiwifruits' soluble sugars (expressed as mg g⁻¹ FW) at harvest and post-storage.

	Control	GA	GS	GAS
Harvest				
Fructose	11.22 ± 3.19 a	13.54 ± 4.76 a	16.06 ± 5.10 a	14.60 ± 4.62 a
Glucose	6.13 ± 1.59 a	7.16 ± 1.65 a	7.74 ± 1.82 a	7.51 ± 1.66 a
Sucrose	0.74 ± 0.72 a	1.18 ± 0.93 a	0.70 ± 0.75 a	1.40 ± 0.54 a
Inositol	2.31 ± 0.65 a	1.94 ± 0.63 a	2.44 ± 0.75 a	2.23 ± 0.54 a
Total sugars	20.40 ± 5.16 a	23.82 ± 5.18 a	26.94 ± 6.64 a	25.75 ± 7.54 a
Storage				
Fructose	53.08 ± 9.87 a	57.52 ± 3.04 a	56.35 ± 5.80 a	55.97 ± 6.94 a
Glucose	27.19 ± 4.62 a	28.93 ± 1.61 a	28.19 ± 2.80 a	28.04 ± 3.58 a
Sucrose	8.49 ± 1.25 a	9.13 ± 0.59 a	9.07 ± 2.01 a	9.57 ± 1.53 a
Inositol	0.93 ± 1.03 a	1.15 ± 0.22 a	1.16 ± 0.22 a	1.19 ± 0.22 a
Total sugars	89.69 ± 13.25 a	96.73 ± 5.13 a	94.77 ± 9.91 a	94.77 ± 11.89 a

Mean ± standard deviation within the same row followed by the same lowercase letter, do not differ significantly according to Tukey's HSD multiple range test, at $\alpha = 0.05$. Abbreviations: GA, kiwifruit cane girdling conducted in August; GS, kiwifruit cane girdling conducted in August; GAS, kiwifruit cane girdling conducted both in August and September.

The results presented in Table 4 showed that cane girdling did not significantly affect the concentration of the organic acids both at harvest and post-storage. The ascorbic acid was the predominant organic acid, followed by citric acid.

Table 4. Cane girdling impact on kiwifruits' organic acids concentration (expressed as mg g⁻¹ FW) at harvest and post-storage.

	Control	GA	GS	GAS
Harvest				
Malic acid	0.77 ± 0.26 a	0.97 ± 0.64 a	0.82 ± 0.19 a	0.93 ± 0.29 a
Ascorbic acid	6.14 ± 2.20 a	5.79 ± 1.78 a	5.94 ± 1.74 a	5.53 ± 2.15 a
Citric acid	3.85 ± 0.30 a	3.91 ± 0.30 a	3.69 ± 0.28 a	3.99 ± 0.27 a
Total organic acids	10.18 ± 3.0 a	10.31 ± 3.33 a	10.07 ± 2.26 a	10.03 ± 3.01 a
Storage				
Malic acid	0.75 ± 0.39 a	1.06 ± 0.53 a	0.86 ± 0.19 a	1.02 ± 0.44 a
Ascorbic acid	5.35 ± 0.50 a	5.44 ± 0.97 a	4.93 ± 0.41 a	5.24 ± 0.26 a
Citric acid	3.28 ± 0.99 a	3.55 ± 1.47 a	3.30 ± 0.65 a	3.54 ± 1.24 a
Total organic acids	9.38 ± 1.80 a	10.05 ± 1.87 a	9.09 ± 0.77 a	9.80 ± 0.98 a

Mean ± standard deviation within the same row followed by the same letter, do not differ significantly according to Tukey's HSD test, at $\alpha = 0.05$. Abbreviations: GA, kiwifruit cane girdling conducted in August; GS, kiwifruit cane girdling conducted in August; GAS, kiwifruit cane girdling conducted both in August and September.

The total phenolic content did not exhibit any significant difference among the treatments at harvest, ranging from 0.39 to 0.44 mg gallic acid equivalents (GAE) g⁻¹ FW (Table 5). This was also observed regarding the concentration of total *o*-diphenols and total flavanols. Fruits produced under the GA treatment presented the lowest concentration of total flavonoids, with a significant difference from the fruits produced under the other

treatments. Regarding the antioxidant capacity at harvest, it ranged from 1.35 to 1.45 $\mu\text{mol equiv. Trolox g}^{-1}$ FW, as measured by the FRAP assay, and from 0.88 to 1.17 $\mu\text{mol equiv. Trolox g}^{-1}$ FW, as measured by the DPPH assay (Table 5). The lowest antioxidant capacity of the pulp was measured in fruits produced under control conditions (based on both assays), while fruits produced under GS treatment exhibited the highest values, with a significant difference from the control one.

Table 5. Cane girdling impact on kiwifruit total phenolic compounds concentration (total phenols, mg equiv. gallic acid g^{-1} FW; total *o*-diphenols, $\mu\text{g equiv. caffeic acid g}^{-1}$ FW; total flavanols, $\mu\text{g equiv. catechin g}^{-1}$ FW; total flavonoids, $\mu\text{g equiv. catechin g}^{-1}$ FW) and antioxidant capacity ($\mu\text{mol equiv. Trolox g}^{-1}$ FW) at harvest and post-storage.

	Control	GA	GS	GAS
Harvest				
Total phenols	0.42 ± 0.05 a	0.39 ± 0.07 a	0.44 ± 0.05 a	0.43 ± 0.04 a
Total <i>o</i> -diphenols	10.0 ± 0.01 a	8.9 ± 0.01 a	9.6 ± 0.03 a	9.8 ± 0.01 a
Total flavanols	3.3 ± 0.01 a	2.8 ± 0.01 a	2.7 ± 0.01 a	2.6 ± 0.01 a
Total flavonoids	17.0 ± 0.01 a	12.4 ± 0.01 b	15.3 ± 0.01 a	16.0 ± 0.01 a
FRAP	1.35 ± 0.11 b	1.41 ± 0.10 ab	1.45 ± 0.09 a	1.40 ± 0.10 ab
DPPH	0.88 ± 0.17 b	0.97 ± 0.23 ab	1.17 ± 0.36 a	1.08 ± 0.14 ab
Storage				
Total phenols	0.35 ± 0.06 b	0.39 ± 0.05 ab	0.45 ± 0.10 a	0.45 ± 0.06 a
Total <i>o</i> -diphenols	15.6 ± 1.9 a	13.3 ± 1.6 ab	13.1 ± 1.6 b	15.1 ± 2.5 ab
Total flavanols	1.3 ± 0.09 b	2.2 ± 0.1 ab	2.6 ± 0.1 a	2.5 ± 0.2 a
Total flavonoids	15.3 ± 0.10 ab	6.5 ± 0.21 b	16.7 ± 0.17 a	6.6 ± 0.25 b
FRAP	1.43 ± 0.10 a	1.41 ± 0.07 a	1.44 ± 0.12 a	1.46 ± 0.06 a
DPPH	0.53 ± 0.09 b	0.95 ± 0.12 a	1.00 ± 0.20 a	0.88 ± 0.18 a

Mean ± standard deviation within the same row followed by the same letter, do not differ significantly according to Tukey's HSD test, at $\alpha = 0.05$. Abbreviations: GA, kiwifruit cane girdling conducted in August; GS, kiwifruit cane girdling conducted in August; GAS, kiwifruit cane girdling conducted both in August and September; DPPH, antioxidant capacity based on 2,2-diphenyl-1-picrylhydrazyl assay; FRAP, antioxidant capacity based on ferric reducing antioxidant power assay.

Post-storage, the control fruits exhibited the lowest concentration of total phenols and total flavanols, with significant differences from fruits produced after the GA and GAS treatments (Table 5). In contrast, the concentration of total *o*-diphenols was highest under control conditions (15.6 $\mu\text{g g}^{-1}$ FW), while the other treatments ranged from 13.1 to 15.6 $\mu\text{g g}^{-1}$ FW. Furthermore, the GS treatment resulted in the highest concentration of total flavonoids in the fruits (16.7 $\mu\text{g g}^{-1}$ FW), with significant differences from the other two girdling treatments. Based on the FRAP assay, there were not any significant differences among treatments concerning the antioxidant capacity of the fruit, ranging from 1.41 to 1.46 $\mu\text{mol equiv. Trolox g}^{-1}$ FW. However, according to the DPPH assay, the fruits produced under control conditions presented the lowest value of antioxidant activity (0.53 $\mu\text{mol equiv. Trolox g}^{-1}$ FW), while the girdling treatments ranged from 0.88 to 1.00 $\mu\text{mol equiv. Trolox g}^{-1}$ FW, with a significant difference from control (Table 5).

According to Figure 4, consuming a single kiwifruit produced under any of the three girdling treatments provides health benefits compared to consuming a fruit produced under control conditions. This is because the total phenolic content consumed, along with the antioxidant capacity measured by the DPPH assay, is greater.

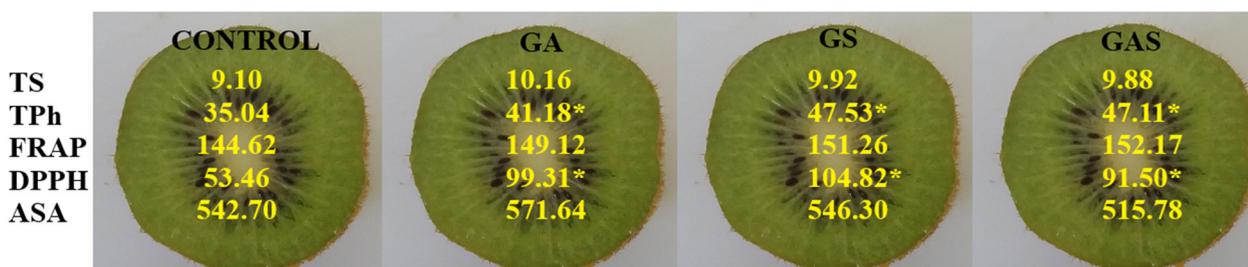


Figure 4. Major nutraceutical and health-promoting compounds per fruit under the various girdling treatments. Abbreviations: TS, total sugars (g); TPh, total phenols (mg); FRAP (μmol equiv. Trolox); DPPH (μmol equiv. Trolox); ASA, ascorbic acid (mg); GA, August girdling; GS, September girdling; GAS, double girdling in August and again in September. The asterisks denote significant differences from the control treatment based on Dunnett's test.

The PCA analysis using all the measured parameters at harvest and after the storage period is presented in Figures S1 and S2, respectively. Both PCAs did not produce significant cumulative percentages, indicating possibly that more data were required to distinguish the treatments' impacts. Nonetheless, kiwifruits at harvest (taking into account both physiological parameters of the leaves and quality characteristics of the fruits) presented similar characteristics under all treatments, as there was no single area describing solely one treatment. On the other hand, though, after the storage period, it was obvious that fruits produced under the GS treatment presented unique properties, making them distinguishable from fruits produced under the control treatment. Based on the components' weight (data not presented), the TSS content, the dry weight percentage, and the dry weight per fruit was mainly used to distinguish between the two treatments (control against GS) post-storage.

4. Discussion

The girdling of shoots or the trunk to prevent the basipetal movement of assimilates and plant growth regulators is an old technique that has been applied with variable success in many fruit tree species [12,31]. In kiwifruit, girdling of canes or the trunk during the early fruit developmental stages aims mainly at improving fruit size, while girdling applied during the later stages of fruit development (stages of slower fruit development) focuses mainly on increasing fruit dry matter [32–35]. In the present experiment, the girdling was applied at the end of summer and early autumn, when fruit growth is slower, aiming at improving fruit quality characteristics and storability.

The most immediate effect of girdling seen in kiwifruit vines was a decrease in the carbon assimilation rate, which occurred within one month after girdling. Both in September as well as at harvest, the leaves in shoots sprouting from girdled canes presented lower photosynthetic rates without any significant changes in intercellular CO_2 or stomatal conductance. Similar results concerning the reduction of photosynthetic rates have been reported by many other researchers, both in kiwifruit [36] as well as in other fruit species [24,37–42], while non-significant impacts have also been observed [31,43]. The reduction of the photosynthetic capacity of the leaf has been attributed to a feedback inhibition mechanism (or feedback sink regulation) [41,42] due to the accumulation of excessive amounts of carbohydrates in the leaves above the girdle zone [24,42,44]. Others attribute this reduction to the reduced stomatal conductance (possibly due to the elevated concentration of ABA in the leaves above the girdle zone) [24], which greatly influences the diffusion of CO_2 into the sub-stomatal area and reduces at the same time transpiration rates [24]. In the present experiment, though, such reductions in stomatal conductance and transpiration rates were recorded only in September's measurement (under the GA treatment) but not at harvest, indicating that the reduction of photosynthesis should not be solely attributed to stomatal limitations. The reduced efficiency of the PS II reaction center has also been proposed as the main factor limiting photosynthesis after girdling [41,45]. In

the present experiment, the slight increase in the intercellular CO₂, although not significant, could mean that non-stomatal limitations may also exist, as has been reported in peach [46], orange tree [45], mango [14], and pistachio [47]. The reduction of the photosynthetic rate due to girdling has also been attributed to the reduction of leaf nitrogen concentration [42]. Although nitrogen concentration was not measured in the present experiment, it would be safe to assume that its reduction would lead to leaf yellowing, as indicated here by the reduced SPAD index at harvest, especially under GAS treatment. Furthermore, it has been reported that girdling accelerates leaf senescence, leading to decreased chlorophyll content [48–50] and thus photosynthetic rates due to reduced cytokinin levels together with increased ABA ones [50]. This reduction of chlorophyll levels was supported in the present experiment by the low SPAD index under the double girdling treatment, which may have accelerated leaf senescence compared to other treatments.

All girdling treatments had a significant effect on increasing fruit dry matter at harvest, with this effect remaining even post-storage. At the same time, fruit fresh weight was increased at harvest under GA and GS treatments compared to the control. To some extent, this was also obvious regarding fruit dimensions, as girdling treatments (especially GS and secondly GA) increased the fruits' narrow diameter without significantly changing the fruits' shape, based on the length-to-diameter ratios. Similar increases in fruit fresh weight as well as in fruit dry matter at harvest have already been reported by other researchers in kiwifruit [6,24,26] as well as in other fruit species [31,51–57], while there are also reports where girdling did not have a significant effect on grape berry weight [58]. The overall increase in the fruit weight and fruit dry matter is attributed to the increased supply of photosynthates to the fruits above the girdle zone [32,34]. According to Le Lievre et al. [32], the modification of carbohydrate supply to the fruits, especially during the second stage of fruit sink development, leads to a significant increase of dry weight through the increased dry matter accumulation, as was shown in the present trial.

This increased accumulation of carbohydrates in the growing fruit leads to the advancement of maturation [12,18,31,32,43,59]. This was not clear in the present experiment, as TSS, chlorophylls, TA, as well as sugar concentration of the pulp and fruit firmness did not exhibit significant differences among treatments. Nonetheless, if one takes into account the absolute content of the aforementioned constituents of the fruit, then it would be clear that girdling increased their content per fruit (approximately 16.4% increase of the total carbohydrates per fruit under GA treatment compared to control). After the storage period, fruits produced under all girdling treatments presented a reduced firmness compared to the control, which indicated faster maturation under storage conditions, also supported by the highest TSS content and TSS versus TA ratio determined. The higher dry matter per fruit at harvest under girdling could have been the source for the higher TSS determined after the storage period. Significant post-storage effects of girdling on the fruits have been described [18], although the later research focuses on physiological disorders appearing post-storage. Similar significant increases during storage in the TSS content of fruits produced after girdling have also been reported in 'Jonagold' apples [60]. Improved storage efficiency of the fruits produced under girdling treatments has also been reported in various other species [57,61,62].

Phenolic compounds, as well as ascorbic acid, contribute greatly to the antioxidant profile of fruit, and this characteristic is becoming more and more desirable by consumers, aiming at bioactive enriched fruits, i.e., 'nutrafruits' [16]. Girdling did not have a significant effect on the phenol content and ascorbic acid concentration of the fruit at harvest, but it significantly increased its antioxidant capacity, especially when girdling was performed in September (GS). Girdling has been found to affect fruit polyphenols in table grapes [54] as well as in cherries [63], mandarins [4], and plums [16], where this action has been attributed to the increases in L-phenylalanine, the substrate of phenylalanine ammonia-lyase, which is the key enzyme of phenolic metabolism. Once again, if one takes into account the overall fruit mass stimulation by girdling and calculates the overall phenol content of the fruit, it becomes obvious that girdling did increase the phenol content per fruit, as

was also clearly shown in Figure 3. Roussos and Tassis [4] attributed such accumulation of secondary metabolites based on the growth differentiation balance hypothesis. Based on this hypothesis, the over-accumulation of carbohydrates to levels exceeding the demands for growth leads to the use of this excess carbon for the biosynthesis of carbon-based secondary metabolites, such as phenolic compounds. As there were no significant differences among treatments concerning the carbohydrate concentration of the fruits, one should not expect a boost in the concentration of secondary metabolites, as was indeed found in the present experiment.

The antioxidant capacity of the fruits was consistently greater under GS treatment (based on FRAP and DPPH assays at harvest and DPPH post-storage), while the consumption of a single fruit produced under any girdling treatment was shown to confer greater antioxidant protection compared to control. Higher antioxidant capacity of fruits produced after girdling has been reported in mandarin [4] and plum [16]. Non-significant differences after girdling treatment have been reported, too [43,64], as well as reduced antioxidant capacity [64], revealing the complexity of the effects of this horticultural technique.

While considering the efficiency of a single agronomic practice like girdling, it is essential not to overlook several other factors. These include the timing of girdling in relation to the plant's physiological stage, the specific plant species, the pedoclimatic conditions, and other cultural management techniques. These factors collectively exert a significant influence on the effectiveness of girdling. Interestingly, when PCA was applied to the data collected at harvest, it did not yield a clear separation of the various treatments. However, post-storage, when the fruit characteristics were assessed, PCA successfully distinguished the control group from the GS treatment. This observation suggests that girdling has a notable impact on the physiological, anatomical, and biochemical properties of the fruit at harvest, which in turn may affect the fruit's behavior during post-harvest cold storage.

5. Conclusions

Kiwifruit is highly appreciated by consumers worldwide due to its pleasant taste and high concentration of vitamin C and antioxidants. Girdling is an ancient practice aimed at improving the production both quantitatively and qualitatively. In the present experiment, girdling was performed during the last stages of fruit development to enhance fruit quality indexes, including total soluble solids, dry matter, and firmness. These traits are highly desirable in the industry, as they extend fruit storability and improve post-harvest quality. Girdling improved kiwifruit quality at harvest by increasing the dry matter accumulated in the fruit as well as its size and weight, without any negative effect on fruit firmness. After the storage period, fruits produced under the influence of girdling exhibited higher TSS content, total phenols, and antioxidant capacity, indicating that these fruits offer greater health benefits than fruits produced under control conditions. Therefore, girdling appears to be a promising practice, not only for inducing larger fruit sizes but also for improving their nutraceutical value. However, further research is needed to elucidate more aspects of the effects of girdling on fruit anatomical, physiological, and biochemical properties, which may influence the behavior and quality of the fruit during the storage period. Due to the variability regarding plant physiology as well as fruit properties that exist among kiwifruit species (i.e., the two major kiwifruit species *Actinidia deliciosa* and *Actinidia chinensis*), the present trial possesses some limitations as it applies only to the 'Hayward' grown under the conditions described above. Any attempt to extrapolate these results to other cultivars may produce erroneous conclusions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app131911087/s1>, Figure S1: Scatterplot of the principal components' weight analysis at harvest. Abbreviations: GA, August girdling, GS, September girdling, GAS, girdling both in August and September. The dark eclipse indicates the control treatment, the red the GA treatment, the green the GS treatment, and the blue the GAS treatment; Figure S2: Scatterplot of the principal components' weight analysis after storage. Abbreviations: GA, August

girdling, GS, September girdling, GAS, girdling both in August and September. The dark eclipse indicates the control treatment, the red the GA treatment, the green the GS treatment, and the blue the GAS treatment.

Author Contributions: Conceptualization, P.A.R.; methodology, P.A.R., N.-K.D., A.T. and E.N.; software, P.A.R. and N.-K.D.; validation, P.A.R. and N.-K.D.; formal analysis, P.A.R., N.-K.D., A.T. and E.N.; investigation, P.A.R., N.-K.D., A.T. and E.N.; resources, P.A.R.; data curation, P.A.R., N.-K.D., A.T. and E.N.; writing—original draft preparation, P.A.R. and N.-K.D.; writing—review and editing, P.A.R. and N.-K.D.; visualization, P.A.R. and N.-K.D.; supervision, P.A.R.; project administration, P.A.R. and N.-K.D.; funding acquisition, P.A.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by ΕΥΔ ΕΠΙΛΕΚ, grant number “Τ1ΕΔΚ-03343”.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We would like to thank the Agricultural Co-operative of Neapolis for their valuable help and equipment and Zarkadoula T. for his excellent cooperation during the two-year experimentation period.

Conflicts of Interest: The authors declare no conflict of interest.

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