

Article



Successive Harvests Modulate the Productive and Physiological Behavior of Three Genovese Pesto Basil Cultivars

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Abstract: In the Italian culinary tradition, young and tender leaves of Genovese basil (Ocimum basilicum L.) are used to prepare pesto sauce, a tasty condiment that attracts the interest of the food processing industry. Like other leafy or aromatic vegetables, basil is harvested more than once during the crop cycle to maximize yield. However, the mechanical stress induced by successive cuts can affect crucial parameters associated with pesto processing (leaf/stem ratio, stem diameter, and dry matter). Our research accordingly aimed to evaluate the impact of successive harvests on three field-grown Genovese basil cultivars ("Aroma 2", "Eleonora" and "Italiano Classico") in terms of production, physiological behavior, and technological parameters. Between the first and second harvest, marketable fresh yield and shoot dry biomass increased by 148.4% and 172.9%, respectively; by contrast, the leaf-to-stem ratio decreased by 22.5%, while the dry matter content was unchanged. The increased fresh yield and shoot dry biomass at the second harvest derived from improved photosynthetic efficiency, which enabled higher net CO_2 assimilation, F_V/F_m and transpiration as well as reduced stomatal resistance. Our findings suggest that, under the Mediterranean environment, "Italiano Classico" carries superior productive performance and optimal technological characteristics in line with industrial requirements. These promising results warrant further investigation of the impact successive harvests may have on the qualitative components of high-yielding basil genotypes with respect to consumer expectations of the final product.

Keywords: *Ocimum basilicum* L.; italiano classico; marketable yield; mineral composition; organic acids; F_v/F_m ; photosynthetic activity

1. Introduction

When attempting to classify the most popular aromatic herbs, sweet basil (*Ocimum basilicum* L.) easily outranks the rest; its versatile use as an ornamental plant for sauce production, for flavoring and garnishing dishes, and as a cosmetic fragrance, justifiably bestowed it the title "king of herbs" [1]. Universally recognized for its aroma, basil is an annual herbaceous plant belonging to the Lamiaceae family [2]. Due to its intrinsic genetic variability, the myriad species of basil identified by researchers and botanists, differ in morphological traits (e.g., color and shape) and in chemical and aromatic composition [1,3]. Hence, making it a multifaceted vegetable highly demanded by the pharmaceutical, cosmetic, and food processing industries [4]. Native to the Asian continent (India, Pakistan, Iran, and Thailand), nowadays basil is widespread and cultivated worldwide [5,6]. Its dispersal to the European continent is mainly ascribed to its gastronomic value as fresh and dried herb in typical regional recipes [7]. Nutritionally, basil has remarkable health-promoting properties owing to its good content of minerals, vitamins, antioxidants, and low caloric value, which attract the growing consumers' interest [8–10]. In Italy, basil cultivation was in the 1990s mainly located in the Liguria region with an overall production



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). of 170 tons per year, mostly in protected environment [11]. However, the ongoing high demand for fresh products all year round and the growing interest of the food industry for this aromatic plant has led to a 60% increase in the area dedicated to its cultivation in the last two decades [12]. In Italy, basil is mainly processed into "pesto", a typical green sauce of the Italian gastronomic tradition, appreciated worldwide [13]. The sensory attributes of Genovese basil used to produce this tasty sauce and the impact of pedoclimatic conditions in the Mediterranean area, unequivocally characterize pesto's organoleptic properties, mainly characterized by phenylpropanoids and monoterpenes produced by specialized glandular trichomes [14,15].

Relevant in basil cultivation is temperature, representing a limiting factor for its development [16]. Walters [17] confirmed that an increase in air temperature up to 29 °C led to an increment in fresh and dry weight, number of nodes, internode length, percentage of flower buds, and plant height. Basil is a long-day plant that blooms in summer and grows well in the full sun [1]. Beaman et al. [18] achieved an increase in edible biomass in basil Genovese cultivars exposed to a photosynthetic photon flux density (PPFD) of 500 μ mol m⁻² s⁻¹. Further studies revealed that increased daily light raised the number of branches per plant and plant height [19]. Like other leafy vegetables, basil for agroindustry is harvested several times during its crop cycle, in the pre-flowering phase (2–3 times depending on the latitude) [20].

In line with the needs of the industrial supply chain, the agri-food practice of successive harvests, other than ensuring earlier production, leads to reduced labor costs by extending the crop cycle, thus circumventing multiple sowings during the growing season [7], and avoiding tanks and greenhouse disinfection. The mechanical stress induced by successive cuts can trigger productive and qualitative responses in the basil crop [7,20,21], warranting more substantial understanding of the impact this agronomic practice may have on parameters (e.g., leaf number, leaf-to-stem ratio, stem diameter, and dry matter) that are critical for industrial processing. In line with the above, our investigation aimed to assess the productive and eco-physiological responses of Genovese basil grown in the Campania region to successive harvests, using three commercial cultivars ordinarily employed for intensive open-field cultivation, which are characterized by an aromatic profile rich in linalool and poor in estragole that is responsible for the undesirable hint of mint [20].

2. Materials and Methods

2.1. Plant Material Tested, Experimental Design, and Growth Conditions

A field experiment was conducted in spring-summer 2019 at the Department of Agriculture of the University of Naples "Federico II", at the experimental farm "Torre Lama", located in Bellizzi (Salerno, Italy; 43°31' N, 14°58' E; 60 m a.s.l). The clay loam soil (46% sand, 24% silt, and 30% clay) had the following chemical and physical characteristics: pH 7.7, electrical conductivity 0.16 dS m⁻¹, organic matter (w/w) 1.21%, total N 0.11 %, extractable phosphorus 88 mg kg⁻¹, and exchangeable potassium 980 mg kg⁻¹ in the first 0-30 cm soil layer. Before transplanting, the soil was plowed and simultaneously manured with 2 kg m^{-2} of mature manure, while during the experiment seedlings were fertigated with soluble 8-12-24 (10) NPK (SO₃) complex fertilizer. The experiment was set up as a factorial combination of three basil cultivars ("Aroma 2", Fenix, Belpasso, Italy; "Eleonora", Enza Zaden, Enkhuizen, The Netherlands; "Italiano Classico", La Semiorto, Sarno, Salerno, Italy) and two successive harvests (first and second corresponding to CT1 and CT2, respectively). The three cultivars were arranged in a randomized complete block design (RCB), with three replicates accounting for nine experimental units (2 square meters each). Basil plants were sown in a mixture of peat and vermiculite on 15 May 2019 and were transplanted on 6 June with a density of 250 plants m^{-2} . During the experiment, the mean air temperature was 28 °C (min: 17 °C; max: 33 °C), while the mean relative humidity was 57.0% (min: 36%; max: 78%). At 1, 25, and 50 days after transplant (DAT), fifteen measurements of PPFD were recorded between 11:00 and 13:00 h with a MSC15 spectral radiometer (Gigahertz Optik, Turkenfeld, Germany). The mean

PPFD was 2012 µmol m⁻² s⁻¹. Precipitation was insufficient during the 2019 growing season and irrigation was provided by a drip irrigation system consisting of a main 32 mm polyethylene pipeline equipped with a series of semi-compensating dripping laterals (t-tape, 16 mm diameter, 200 µm thickness, with 1.5 L h⁻¹ drippers and 10 cm spacing). The ion concentration of the irrigation water (mg L⁻¹) was: HCO₃⁻ (285); Ca²⁺ (86); Cl⁻ (9); Mg²⁺ (20); Na²⁺ (7); NO₃⁻ (4.5); K⁺ (und); SO₄²⁻ (9). The irrigation water electrical conductivity and pH were 0.43 dS m⁻¹ and 7.5, respectively.

2.2. Plant Collection, Yield, and Growth Analysis

At the preflowering stage, basil plants were harvested leaving two internodes to promote regrowth for the second harvest. The experimental trial lasted two months, in which two successive harvests (33 and 54 DAT) were made. For each experimental unit, 100 plants were sampled. At each harvest, plants were separated into leaves and stems to determine the number of leaves per plant, stem diameter (cm), fresh marketable yield (kg of fresh leaves m⁻²), and leaf-to-stem ratio. The sampled material was dried in a ventilated oven at 70 °C to constant weight (72 h) in order to determine total dry shoot biomass (g m⁻²), dry weight (dw) of leaves and stem (g plant⁻¹), and dry matter percentage (%). Samples of dry plants were stored for mineral and organic acids analyses.

2.3. Leaf Gas Exchange, Chlorophyll Fluorescence, and SPAD Index Determination

At both harvests: 33 and 54 DAT, an LCA-4 leaf Chamber Analyser (ADC Bio Scientific Ltd., Hoddesdon, UK) was used to measure net photosynthesis (μ mol CO₂ m⁻² s⁻¹), transpiration (mol H₂O m⁻² s⁻¹), stomatal resistance (m² s mol⁻¹ H₂O). As for intrinsic water use efficiency (WUEi), it was calculated as net photosynthesis/transpiration. Leaf gas exchange parameters were measured between 11:00 and 13:00 at a temperature range of 28–30 °C, on the uppermost fully expanded terminal leaflets. Environmental parameters such as PPFD, relative humidity (RH), and CO₂ concentration were set according to the ambient values (700 ± 50 µmol m⁻² s⁻¹, RH 55 ± 5%, and 390 ± 10 ppm, respectively) while the airflow rate was 400 mL s⁻¹. Eight measurements were made for each replicate.

On the same date, chlorophyll fluorescence was measured using a portable chlorophyll fluorometer (Plant Stress Kit, Opti-Sciences, Hudson, NH, USA) on the same leaf used for the gas exchange determination, after a dark adaptation time for 10 min [22], the chlorophyll fluorescence ratio (F_v/F_m) was recorded.

At 54 DAT, measurements of the leaf chlorophyll index (SPAD) were made on adaxial side of fully expanded leaves from 8 randomly selected plants per each experimental unit with a chlorophyll meter SPAD 502 (Minolta Camera Co., Osaka, Japan). Measurements were taken by avoiding the leaf midrib, and a single average SPAD value for each replicate was calculated.

2.4. Minerals and Organic Acids Determination

Dried tissues of sampled basil plants were finely ground (MF10.1 Wiley laboratory mill, IKA[®], Staufen im Breisgau, Baden-Württemberg, Germany) and analyzed with a gas chromatograph coupled with a conductivity detector (ICS3000, Dionex, Sunnyvale, CA, USA) for determination of mineral composition and organic acids according to the protocol described by Rouphael et al. [23]. Briefly, 250 mg of the dried material were suspended in 50 mL of ultrapure water (Milli-Q, Merck Millipore, Darmstadt, Germany), subjected to three freeze–thaw cycles in liquid nitrogen, centrifuged for 10 min at 6000 rpm (R-10 M, Remi Elektrotechnik Limited, India) and filtered through a 0.20 μ m filter Whatman paper (Whatman International Ltd., Maidstone, UK). The clear supernatant was assayed by ion exchange chromatography. Concentrations of anions, cations, and organic acids were expressed as g kg⁻¹ dry matter.

2.5. Statistical Analysis

All data are presented as the mean \pm Standard Error (SE). A two-way analysis of variance (ANOVA) was conducted with cultivar (CV) and cut (CT) as the main effects. In the absence of significant CV \times CT interaction, mean comparisons for the main effects were performed by Duncan's Multiple Range Test for CV and by *t*-Test for CT. For variables that were subject to significant CV \times CT interaction, one-way ANOVA was performed separately for each cultivar and where CT effect was significant, means were compared by *t*-Test. All experimental data were analyzed using the software IBM SPSS Statistics ver. 10 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Production Response

The production parameters of Genovese basil cultivars in response to successive harvests are shown in Table 1. For all the measured production parameters, no significant interaction between the two tested factors (Cultivar-CV and Cut-CT) was observed. Irrespective of cut order, "Italiano Classico" revealed a significant higher dry shoot biomass of 39.4% compared to "Eleonora". However, "Eleonora" exhibited a 6.5% higher dry matter when compared to "Aroma 2". Neither the number of leaves per plant nor the fresh marketable yield differed among cultivars. When averaged over basil cultivars, the cut resulted in significant differences in all investigated parameters, except for dry matter %. Specifically, the number of leaves per plant, fresh marketable yield (kg m⁻² of fresh leaves), and dry shoot biomass increased at the second cut by 100.0%, 148.4%, and 172.9%, respectively.

Table 1. Effect of cultivar and cut on leaf number, fresh marketable yield, dry shoot biomass, and dry matter of basil.

Source of Variance	Leaf Number	Fresh Marketable Yield	Dry Shoot Biomass	Dry Matter
	(No. plant ⁻¹)	(kg m ⁻²)	(g m ⁻²)	(%)
Cultivar (CV)				
"Aroma 2"	69.58 ± 10.88	3.56 ± 0.75	$889.38\pm219.59~\mathrm{ab}$	11.92 ± 0.15 a
"Eleonora"	65.92 ± 10.23	.23 3.23 ± 0.58 727.17 ± 144.22 b		$11.14\pm0.27~\mathrm{b}$
"Italiano Classico"	69.22 ± 9.85	4.86 ± 1.13	1013.75 ± 194.35 a	$11.78\pm0.16~\mathrm{ab}$
Cut (CT)				
1	$45.50\pm1.24\mathrm{b}$	$2.23\pm0.14\mathrm{b}$	$470.22 \pm 37.13 \text{ b}$	11.57 ± 0.19
2	$90.98\pm1.80~\mathrm{a}$	$5.54\pm0.57~\mathrm{a}$	$\pm 0.57 \text{ a}$ 1283.31 \pm 72.38 a	
ANOVA				
Cultivar	n.s.	n.s.	**	*
Cut	***	***	***	n.s.
Cultivar \times Cut	n.s.	n.s.	n.s.	n.s.

Data are mean values \pm standard error, *n* = 3. Mean comparisons were performed by Duncan's Multiple Range Test for CV and by *t*-Test for C. Different letters within columns indicate significant mean differences. n.s., *, ** and *** denote non-significant or significant effects at $p \le 0.05, 0.01$, and 0.001, respectively.

3.2. Biometric Parameters

Similarly, to the crop production parameters, no significant interaction was observed among the two factors examined (Cultivar-CV and Cut-CT) for the tested morphometric parameters (i.e., leaf Dw, stem Dw, leaf-to-stem ratio, and stem diameter) (Table 2). Irrespective of the cut order, the cultivar effect was evident only for leaf Dw and stem diameter. Interestingly, "Italiano Classico" resulted 60.8% greater in leaf Dw and 9.4% greater in stem diameter than "Eleonora" and "Aroma 2", respectively (Table 2). Successive cuts performed on basil plants led to significant changes in all morphometric parameters mentioned below in Table 2. Specifically, when averaged over cultivars the second cut recorded a decrease in leaf-to-stem ratio (-22.6%). In contrast, leaf Dw, stem Dw, and stem diameter increased by 134.5%, 261.1%, and 31.9%, respectively.

	Leaf Dw	Stem Dw	Leaf-to-Stem	Stem Diameter	
Source of Variance	(g plant ^{-1}) (g plant ^{-1})		Ratio	(cm)	
Cultivar (CV)					
"Aroma 2"	$1.82\pm0.39~\mathrm{ab}$	1.57 ± 0.46	1.16 ± 0.09	$0.51\pm0.04~b$	
"Eleonora"	$1.53\pm0.24\mathrm{b}$	1.38 ± 0.34	1.05 ± 0.07	$0.55\pm0.03~\mathrm{ab}$	
"Italiano Classico"	$2.46\pm0.54~\mathrm{a}$	2.03 ± 0.55	1.10 ± 0.06	$0.58\pm0.04~\mathrm{a}$	
Cut (CT)					
1	$1.16\pm0.10\mathrm{b}$	$0.72\pm0.05~\mathrm{b}$	$1.24\pm0.04~\mathrm{a}$	$0.47\pm0.01~{ m b}$	
2	$2.72\pm0.29~\mathrm{a}$	$2.60\pm0.24~\mathrm{a}$	$0.96\pm0.02b$	$0.62\pm0.01~\mathrm{a}$	
ANOVA					
Cultivar	*	n.s.	n.s.	**	
Cut	***	***	***	***	
Cultivar × Cut	n.s.	n.s.	n.s.	n.s.	

Table 2. Effect of cultivar and cut on leaf dry weight, stem dry weight, leaf-to-stem ratio, and stem diameter.

Data are mean values \pm standard error, n = 3. Mean comparisons were performed by Duncan's Multiple Range Test for CV and by *t*-Test for CT. Different letters within columns indicate significant mean differences. n.s., *, ** and *** denote non-significant or significant effects at $p \le 0.05$, 0.01, and 0.001, respectively.

3.3. Leaf Chlorophyll Index (SPAD), Physiological, and Biochemical Parameters

Significant interaction was observed between cultivar and cut for net photosynthesis, intrinsic water efficiency (WUEi), and maximum quantum yield of photosystem II (F_v/F_m) (Table 3). For net photosynthesis, "Aroma 2" was the source of the interaction since its behavior was different than the other two cultivars during the two cuts, while for WUEi the interaction was observed because the three cultivars behaved differently during the two cuts. For "Aroma 2", at the second cut (CT2), net photosynthesis and WUEi increased by 61.8% and 41.0%, respectively. In contrast, WUEi decreased in "Italiano Classico" by 25.5% while no significant difference was observed for net photosynthesis (Table 3). The F_v/F_m increased significantly from the first to the second cut by 10.0%, 5.5%, and 3.9% in "Aroma 2", "Eleonora", and "Italiano Classico", respectively.

The leaf chlorophyll index (SPAD), stomatal resistance, and transpiration were influenced by cultivar and/or cut factors and no interaction was observed among the two factors. When averaged over basil cultivars, the cut order (from CT1 to CT2), elicited an increase in transpiration (+ 22.1%) and a consequent decrease in stomatal resistance (by 44.8%). Finally, irrespective of cut order the highest leaf chlorophyl index (SPAD) (42.7) and stomatal resistance (9.3 m² s mol⁻¹) were both obtained in "Aroma 2".

3.4. Minerals and Organic Acids

Regardless of the cultivar and cut effect, potassium was found in higher concentrations than the other minerals, with values ranging from 22.4 to 46.4 g kg⁻¹ Dw, obtained in "Italiano Classico" and "Eleonora" at CT1, respectively (Table 4). As shown in Table 4, the concentrations of potassium, phosphorus, and magnesium were significantly influenced by CV × CT interaction (Table 4). For P "Aroma" was the source of interaction since it behaved differently from the other two cultivars during the two cuts, whereas for K and Mg the three cultivars behaved differently during the 2 cuts which caused the interaction. Moreover, at the second cut, K and Mg content in "Italiano Classico" showed an increase by 40.2% and 11.6%, respectively. The opposite trend was observed in "Eleonora", in which K and Mg decreased by 38.5% and 26.0%, respectively. Furthermore, no significant differences occurred in "Aroma 2" for potassium and magnesium after the cuts, while a significant increase in phosphorus (19.4 %) from CT1 to CT2 was observed.

Source of	SPAD Index	Pn	r _s E		WUEi	F /F
Variance		μ mol CO ₂ m ⁻² s ⁻¹	$m^2 s mol^{-1} H_2O$	mol $H_2O m^{-2} s^{-1}$	µmol CO ₂ mol ⁻¹ H ₂ O	Γ_V/Γ_m
Cultivar (CV)						
"Aroma 2"	$42.74\pm0.21~\mathrm{a}$	$17.18\pm1.86~\mathrm{b}$	$9.27\pm1.17~\mathrm{a}$	4.34 ± 0.25	3.96 ± 0.37	$0.74\pm0.02~{\rm c}$
"Eleonora"	$41.08\pm0.19b$	$18.01\pm0.78~\mathrm{b}$	$6.82\pm1.15\mathrm{b}$	4.54 ± 0.36	4.04 ± 0.19	$0.75\pm0.01\mathrm{b}$
"Italiano Classico"	$39.96\pm0.19~\mathrm{c}$	$19.87\pm0.59~\mathrm{a}$	$5.54\pm0.75~b$	4.55 ± 0.23	4.44 ± 0.31	$0.77\pm0.01~\mathrm{a}$
Cut (CT)						
1	41.24 ± 0.39	$16.91\pm1.18\mathrm{b}$	$9.29\pm0.79~\mathrm{a}$	$4.03\pm0.18\mathrm{b}$	4.23 ± 0.28	$0.73\pm0.01\mathrm{b}$
2	41.28 ± 0.48	$19.79\pm0.49~\mathrm{a}$	$5.13\pm0.51b$	$4.92\pm0.15~\mathrm{a}$	4.07 ± 0.21	$0.78\pm0.01~\mathrm{a}$
CV × CT						
interaction						
"Aroma 2"						
CT1	42.42 ± 0.32	$13.13\pm0.36\mathrm{b}$	11.74 ± 0.66	4.04 ± 0.39	$3.29\pm0.21\mathrm{b}$	$0.70\pm0.01\mathrm{b}$
CT2	43.06 ± 0.15	$21.24\pm0.84~\text{a}$	6.81 ± 0.62	4.63 ± 0.26	4.64 ± 0.44 a	$0.77\pm0.01~\mathrm{a}$
"Eleonora"						
CT1	41.33 ± 0.22	16.71 ± 0.93 a	9.09 ± 1.07	3.93 ± 0.44	4.31 ± 0.24 a	$0.73\pm0.01\mathrm{b}$
CT2	40.84 ± 0.26	$19.30\pm0.69~\mathrm{a}$	4.56 ± 0.58	5.15 ± 0.30	$3.77\pm0.20~\mathrm{a}$	$0.77\pm0.01~\mathrm{a}$
"Italiano Classico"						
CT1	39.96 ± 0.35	$20.90\pm0.77~\mathrm{a}$	7.04 ± 0.53	4.12 ± 0.20	$5.09\pm0.18~\mathrm{a}$	$0.76\pm0.01\mathrm{b}$
CT2	39.95 ± 0.23	$18.83\pm0.29~\mathrm{a}$	4.04 ± 0.51	4.99 ± 0.22	$3.79\pm0.18~\mathrm{b}$	$0.79\pm0.01~\mathrm{a}$
ANOVA						
Cultivar	***	**	***	n.s.	n.s.	***
Cut	n.s.	***	***	**	n.s.	***
Cultivar \times Cut	n.s.	***	n.s.	n.s.	***	**

Table 3. Effect of cultivar and cut on leaf chlorophyll index (SPAD), net photosynthesis (Pn), stomatal resistance (r_s), transpiration (E), intrinsic water use efficiency (WUEi), and maximum chlorophyll fluorescence ratio (F_v/F_m).

Data are mean values \pm standard error, n = 3. Mean comparisons were performed by Duncan's Multiple Range Test for CV and by *t*-Test for CT. For variables subject to significant CV \times CT interaction, CT means within cultivars were compared by *t*-Test. Different letters within columns indicate significant mean differences. n.s., ** and *** denote non-significant or significant effects at $p \leq 0.01$ and 0.001, respectively.

-	Table 4. Effect of cultivar and cut on minerals and organic acids accumulation of basil.

Source of Variance	Р	К	Mg	Malic Acid	Tartaric Acid	Citric Acid
Source of variance	(g kg $^{-1}$ Dw)	(g kg $^{-1}$ Dw)	(g kg $^{-1}$ Dw)	(g kg $^{-1}$ Dw)	(g kg $^{-1}$ Dw)	(g kg ⁻¹ Dw)
Cultivar (CV)						
"Aroma 2"	$2.77\pm0.12~\mathrm{a}$	$33.31\pm0.97\mathrm{b}$	$2.88\pm0.04~\mathrm{a}$	$14.96\pm0.39~\mathrm{a}$	14.20 ± 0.57	3.99 ± 0.36 a
"Eleonora"	$2.58\pm0.02b$	$37.49\pm4.25~\mathrm{a}$	$2.71\pm0.18\mathrm{b}$	$13.63\pm0.22\mathrm{b}$	14.30 ± 0.88	$3.26\pm0.22b$
"Italiano Classico"	$2.67\pm0.04~ab$	$26.92\pm2.06~c$	$2.82\pm0.08~ab$	$15.19\pm0.38~\mathrm{a}$	13.74 ± 0.47	$3.85\pm0.55~a$
Cut (CT)						
1	$2.62\pm0.05b$	33.56 ± 3.64	$2.84\pm0.07~\mathrm{a}$	14.64 ± 0.33	15.20 ± 0.40 a	$2.90\pm0.11~\mathrm{b}$
2	$2.73\pm0.07~\mathrm{a}$	31.58 ± 0.97	$2.73\pm0.11~b$	14.55 ± 0.39	$12.95\pm0.29~b$	$4.50\pm0.23~\mathrm{a}$
CV × CT interaction "Aroma 2"						
CT1	$2.53\pm0.09\mathrm{b}$	31.84 ± 1.34 a	$2.73\pm0.05~\mathrm{a}$	14.38 ± 0.08	15.16 ± 0.76	3.20 ± 0.12 b
CT2	$3.02\pm0.01~\mathrm{a}$	$34.78\pm0.86~\mathrm{a}$	$2.92\pm0.04~\mathrm{a}$	15.55 ± 0.64	13.23 ± 0.34	$4.78\pm0.12~\mathrm{a}$
"Eleonora"						
CT1	$2.57\pm0.03~\mathrm{a}$	$46.43\pm3.15~\mathrm{a}$	$3.11\pm0.03~\mathrm{a}$	13.89 ± 0.27	16.09 ± 0.20	$2.85\pm0.16b$
CT2	$2.59\pm0.01~\mathrm{a}$	$28.55\pm0.60b$	$2.30\pm0.05~b$	13.37 ± 0.31	12.51 ± 0.78	$3.67\pm0.22~\mathrm{a}$
"Italiano Classico"						
CT1	$2.75\pm0.04~\mathrm{a}$	$22.41\pm0.73\mathrm{b}$	$2.66\pm0.06b$	15.65 ± 0.61	14.36 ± 0.78	$2.66\pm0.15b$
CT2	$2.59 \pm 0.04 \text{ a}$	$31.43\pm0.68~a$	$2.97\pm0.05~\mathrm{a}$	14.73 ± 0.34	13.11 ± 0.32	$5.04\pm0.22~\mathrm{a}$
ANOVA						
Cultivar	**	***	*	**	n.s.	**
Cut	**	n.s.	**	n.s.	***	***
Cultivar \times Cut	***	***	***	n.s.	n.s.	**

Data are mean values \pm standard error, n = 3. Mean comparisons were performed by Duncan's Multiple Range Test for CV and by *t*-Test for CT. For variables subject to significant CV × CT interaction, CT means within cultivars were compared by *t*-Test. Different letters within columns indicate significant mean differences. n.s., *, ** and *** denote non-significant or significant effects at $p \le 0.05, 0.01$, and 0.001, respectively.

As for the citric acid the interaction observed was an interaction in scale. "Italiano Classico" citric acid was almost doubled during the second cut when compared to the other two cultivars. When averaged over basil cultivars, the cut order (from CT1 to CT2), elicited a decrease in tartaric acid (-14.8%). Malic acid showed significant cultivar-dependent variation, with the lowest value obtained in "Eleonora" (13.63 g kg^{-1}).

4. Discussion

Over the years, basil has gained a prestigious role in the national horticultural markets, mainly for producing the pesto sauce, a condiment with a strong territorial connotation and linked to the Italian gastronomic tradition. On the other hand, the need to supply the industry with fresh leaves all year round [13] has motivated producers to broaden their horizons about traditional cultivation practices, encouraging them to research and develop alternative agronomic strategies. However, careful selection of the most suitable genotype for a specific environment and possible abiotic interferences to which plants are unavoidably exposed is mandatory for maximizing marketable fresh production.

Our research's goal was to assess three Genovese basil cultivars' productive performance for pesto production after two successive cuts in a spring-summer open field cycle. Successive harvests usually performed on basil can elicit different morpho-physiological and biochemical responses, affecting technological properties mandatory for the food processing industry (e.g., leaf-to-stem ratio and dry matter percentage) [20]. Our results confirm a positive correlation between the tested cultivars and the successive cuts for physiological and growth responses.

The present experiment's total production was lower than previously obtained by De Masi et al. [24] on Genovese basil cultivars, although an additional harvest was performed. Regardless of the cultivars, our findings showed increased marketable fresh production (kg of fresh leaves m^{-2}) due to successive harvests, in agreement with the results of a comparable study [21]. The increased leaf number at the second harvest could be attributable to cut-induced mechanical distress or due to the increased number of stems after the cut. Probably, as outlined by Wang et al. [25], the cut would induce higher cytokinin production, which stimulates cell division, regulates leaf primordia number, and reduces stomatal resistance [7,26,27], as supported by our results (Tables 1 and 3).

The increased dry shoot biomass at the second harvest resulted from improved photosynthetic efficiency, which led to higher net photosynthesis and transpiration, reduced stomatal resistance. Although the cut decreased leaf-to-stem ratio and increased the number of leaves per plant, there was no change in the leaf chlorophyll index. Among the tested cultivars, the highest leaf chlorophyll index was obtained in "Aroma 2" (42.7). This index other than giving an indirect measure of the chlorophyll content, is a useful nondestructive tool for leaf greenness measurement, a quality attribute that can influence consumer and industry choices [28,29]. The increased dry shoot biomass could probably result from more root growth in response to the cut, which would have improved the allocation of photosynthates to the plants' epigeal portion, thus fostering the emergence of more leaves and stems [21]. Furthermore, the F_v/F_m increase in all cultivars confirmed that the cut did not pose a damage to the photosynthetic apparatus, specifically to Photosystem II [30–32]. On the contrary, F_v/F_m values at the second harvest were typical of healthy plants with an efficient photosynthetic system [31,32].

As observed by Nicoletto et al. [20] the leaf-to-stem ratio, a key parameter for pesto's industrial processing, did not show significant differences among the tested cultivars due to their genetic suitability for industrial needs. On the other hand, even though the plants at the second harvest had more leaves, the cut resulted in a reduced leaf-to-stem ratio, due to the increased stem percentage and its diameter, results in accordance with Nicoletto et al. [20]. It is necessary for industrial pesto processing to ensure low fiber levels because excessive lignification of tissues, therefore higher dry matter, would extend the processing time and promote oxidation, leading to pesto blackening. Our results showed significant differences in dry matter percentage among the cultivars whose values (11.1–11.9 %) were

well below those obtained by Khalid [33] in a previous study. In contrast, the cut effect did not result in any differences in dry matter accumulation, compared to a recent investigation on basil [34]. However, the dissimilar result achieved by Corrado et al. [34]. could be related to the different genetic material and the different growth conditions and crop management techniques.

The availability of essential minerals and light helps plants to synthesize all compounds they need in order to grow. Phosphorus is a limiting factor for plant development, playing a crucial role in the photosynthetic process [35]. The lack of phosphorus induces a decrease in the ATP/ADP ratio leading to impaired phosphorylation and subsequent photosynthesis carbon depletion [36]. However, the average phosphorus content in the tested cultivars, independent of cut, was in line with the standard values for basil [8]. In light of the above, our experimental results indicate a significant correlation between phosphorus and CO_2 fixation increases after cut, independently of the cultivar. By contrast, all cultivars exhibited no unique behavior for potassium and magnesium buildup after the cut. Nicoletto et al. [20] suggested that this result could be due to the different environmental conditions characterizing the first and second harvests.

Additionally, organic acids are the primary metabolites involved in different biochemical pathways and play a key role in taste definition of horticultural products [37]. Our results showed a lower citric acid level, in accordance with a previous study performed on different basil cultivars [38]. However, organic acids are transported by roots through xylem tissues [39], hence, the increase in citric acid could be justified by the increased transpiration at the second cut. In contrast, the reduction in tartaric acid level at the second harvest could be derived from its role as a precursor in chicoric acid's biosynthesis [40]. Indeed, chicoric acid and most phenolic compounds increase in response to cut-induced stress [41] would lead to a reduction in tartaric acid.

5. Conclusions

The high demand for premium quality basil products poses a challenge for growers and the food industry alike, which must evaluate a plethora of agronomic and technological traits among genotypes that differ in habit, color, and productivity. However, potential environmental interaction with cultivation techniques necessitates careful assessment of suitable cultivars to ensure high yield outputs. Our findings suggest that successive harvests are a useful tool for enhancing productivity. The second harvest resulted in an improved fresh marketable yield (+148.4%) and dry shoot biomass (+172.9%) while not altering the key technological attributes desired by the food industry (i.e., dry matter percentage and color). Among the tested cultivars, "Italiano Classico" performed better under Mediterranean pedoclimatic conditions and successive cuts, resulting in increased productivity (+43.1%). The promising results achieved in this study can pave the way for future investigations to evaluate the qualitative responses of "Italiano Classico" to successive cuts under Mediterranean environmental conditions both in the open field and greenhouse modules.

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