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Production, Leaf Quality and Antioxidants of Perennial Wall Rocket as Affected by Crop Cycle and Mulching Type

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Abstract: The plastic mulch has raised a disposal issue, which has been diverting the research focus on biodegradable film as an alternative. Research was carried out in southern Italy in 2016–2017 and 2017–2018 in order to assess the effects of three crop cycles (autumn-winter, winter, spring) in factorial combination with three soil mulching types (a MaterBi biodegradable black film; a brown photoselective low density polyethylene (LDPE) film; a black-standard LDPE film) and a non-mulched control, on leaves yield, quality and antioxidants of greenhouse grown *Diplotaxis tenuifolia* (L.) D.C. The spring cycle was the shortest and best enhanced plant growth and yield. The non-mulched control caused an 11% yield reduction compared to the mulching treatments average (12.4 t ha^{-1}). The soil temperature was highest under photoselective and standard LDPE films. The Soil Plant Analysis Development (SPAD) index was 17.4% higher in the leaves grown in mulched soil. Winter season and biodegradable mulch led to higher leaf dry residue and organic acids. Leaf nitrate content was highest in winter and under mulching. The spring cycle, the biodegradable and photoselective LDPE film resulted in the highest antioxidant compound content and activity. The biodegradable polymer improved leaf quality, showing suitable features for sustainable production.

Keywords: *Diplotaxis tenuifolia* (L.) D.C.; arugula; biodegradable film; production; polyphenols; ascorbic acid; antioxidant activity

1. Introduction

Perennial wall rocket (*Diplotaxis tenuifolia* (L.) D.C.) belongs to the *Brassicaceae* family, is currently cultivated in several agricultural areas worldwide and, in particular, on about 4800 ha in Italy based on the latest estimation in 2018 [1]. The increasing diffusion of perennial wall rocket in the last two decades, both as a fresh-market and ready-to-use salad oriented crop, is due to its smooth and succulent leaves which meet the expectations of consumers. The leaves of this species are also rich in mineral elements and antioxidants [2], the latter protecting against ultraviolet radiations [3], though their excessive content may be averse to lipid, protein and nucleic acid stability [4].

When the produce is addressed to fresh market, the crop cycles of perennial wall rocket are carried out in sequence from autumn to spring or from spring to summer, the cultivation season showing

significant effects on yield and quality performances of *D. tenuifolia* [5] as well as of other vegetable species [6–8]. The transplant is usually practiced on mulched ridges, as the soil cover allows for a reduction of crop duration, a better weed management and an improved produce quality.

Up to date, plastic material (usually polyethylene) is mostly used for soil mulching worldwide, though biodegradable films have slowly been increasing and currently account for almost 5% out of the total mulched surface area [9]. However, the use of plastic represents a spotlighted issue due to the adverse environmental impact connected to its disposal. In this respect, the agricultural plastic waste on the land is being estimated and mapped by using satellite images, with the aim to monitor and optimize its collection from the farms and their transport to the recycling companies [10,11]; notably, in Barletta-Andria-Trani Province of Apulia Region the agricultural plastic waste amounts to 1.2 tons per hectare [12]. Indeed, plastic degradation is a complex process which is remarkably affected by the plastic composition as well as the biotic and abiotic conditions they are exposed to [13]. In this respect, the biodegradable polymers offer the chance to make a sustainable choice from the environmental and crop management point of view [14,15]. Indeed, biodegradable films are incorporated directly into the soil and decomposed by microorganisms, eventually into CO_2 and H_2O [16]. However, the rate of biodegradation depends on different factors such as polymer characteristics (even thickness), soil and environmental conditions [13]. In this respect, the films can break down too slowly, resulting in problems with persistent small fragments of residual mulch, or too quickly, leaving the crop unprotected [17]. The economic benefit connected to the use of biodegradable mulch may be slightly lower than the plastic one due to the higher current cost of biodegradable films [18], which however do not need to be removed from the soil. Mater-Bi is a starch-based biopolymer which is similar to traditional plastic in terms of agronomic characteristics and to cellulose referring to degradation rate [19]. Mater-Bi black mulch also showed good performances in organic system under the Mediterranean continental climate [20].

A scientific investigation aimed to assess the effects of biodegradable mulch on yield, quality and antioxidant properties of perennial wall rocket leaves was carried out in southern Italy, in order to provide useful details for the cost evaluation of these materials at farm scale, taking into account they are incorporated into soil after their use, and therefore are costless for removal and disposal. In this respect, a biodegradable mulch was compared with a standard and a photoselective LDPE, in interaction with three crop cycles.

2. Material and Methods

2.1. General Analytical Methods

Research on rocket (*Diplotaxis tenuifolia* (L.) D.C.) cultivar Nature was carried out in Portici (Naples, southern Italy) in 2016–2017 and 2017–2018 on a clay-sandy soil; in a three-span polytunnel with each span being 5.0 m wide, 2.0 and 3.5 m tall at wall and roof respectively. The trend of temperature is shown in Figure 1 as mean values of the two research years, because no variable regarding the plant determinations was affected by the year of investigation. The experimental protocol was based on the comparison between three crop cycles in factorial combination with three soil mulching types and a non-mulched control. The three crop cycles corresponded to the following seasons: autumn-winter; winter; spring. The three mulching types were: a MaterBi biodegradable black film (Biodegradable), 15 µm thick EF04P made of corn starch by Novamont S.p.A., Novara, Italy; a brown photoselective light low density polyethylene film (Photoselective LDPE), 25 µm thick Al-Or C-889 by PolyEur S.p.A., which has an 80% transmissivity in the near infrared range (from 780 nm to 2500 nm) and absorbs 96% of the photosynthetically active radiations (PAR); a standard black low light density polyethylene film (Standard LDPE), 45 µm thick. The thicknesses of the three mulch films are different because they are referred to diverse materials, and each of them entails a specific goal: the 45 µm standard LDPE is the commonly used type in Italian vegetable systems, because smaller thickness may result in the material break down; the biodegradable film cannot be as thick as the standard LDPE, because it would show

difficulties to be degraded in that case; the photoselective LDPE is aimed to transmit near IR radiations and, therefore, it is thinner than the standard LDPE. A randomized complete block design was used with three replicates and the elementary plot had a 3.2 m^2 surface area.

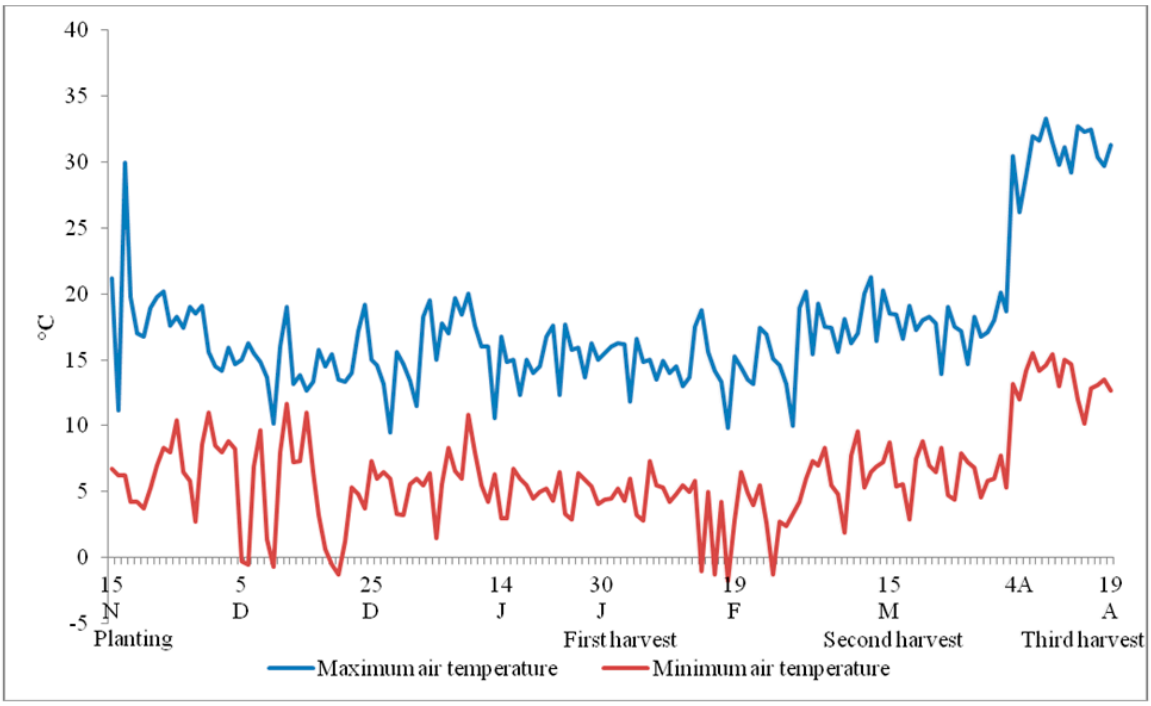

Figure 1. Trend of air temperature inside the greenhouse in Portici (Naples, southern Italy) as an average of 2016–2017 and 2017–2018.

The crops were managed by performing environmentally sustainable farming practices. Prior to transplant, the soil was ploughed and hoed, organically fertilized with 38 kg·ha^{-1} N, 10 P_2O_5, 30 K_2O, arranged in 100 cm wide raised beds which were mulched according to the experimental protocol. The transplant was performed on 16 and 20 November in 2016 and 2017, respectively with plants spaced 20 cm along and between the rows within each bed, with 80 cm between the outer rows of adjacent beds (14.3 alveoli per m^2). The following practices were done during the growing season: 112 kg·ha^{-1} N 30 P_2O_5, and 90 K_2O supply through fertigation; hoeing; drip irrigation when the soil available water capacity in the first 20 cm of soil profile decreased to 80%; six foliar spraying applications (two in the first crop cycle, three in the second and one in the third) for plant protection against fungal diseases and insects using copper (0.7 kg·ha^{-1} copper oxichloride) and azadirachtin (25 mL·ha^{-1} active ingredient).

Harvests of commercially ripe leaves (12 to 15 cm length) were performed, practicing the cut at 3 to 5 cm above the cotyledons in order to allow for efficient vegetative apex regrowth [21], in the following dates: 28 and 31 January in 2017 and 2018, respectively, for the first crop cycle; on 14 and 16 March in 2017 and 2018, respectively, for the second crop cycle; on 19 April both in 2017 and 2018, for the third crop cycle.

The soil temperature (Figure 2) at 10 cm depth, which is the average depth of perennial wall rocket plant roots in this research crop system, was measured every hour in each plot during the three crop cycles, using PT100 sensors connected to the Console Wireless Vantage Pro2, equipped with a data logger (Davis Instruments, Illinois, USA).

At the end of each crop cycle, random plant samples were collected in each plot in order to assess: The total leaf area, using a bench top electronic leaf area meter (Li-Cor3000, Li-Cor, Lincoln, NE, USA); the aboveground dry biomass in an oven at 70 °C until constant weight.

At each harvest, the weight and number of marketable leaves, as well as of leaf mean weight on 100 unit samples were determined in each plot. At the end of both the winter and spring crop cycle,

leaf samples per each experimental treatment were taken and transferred to laboratory in order to perform the following determinations.

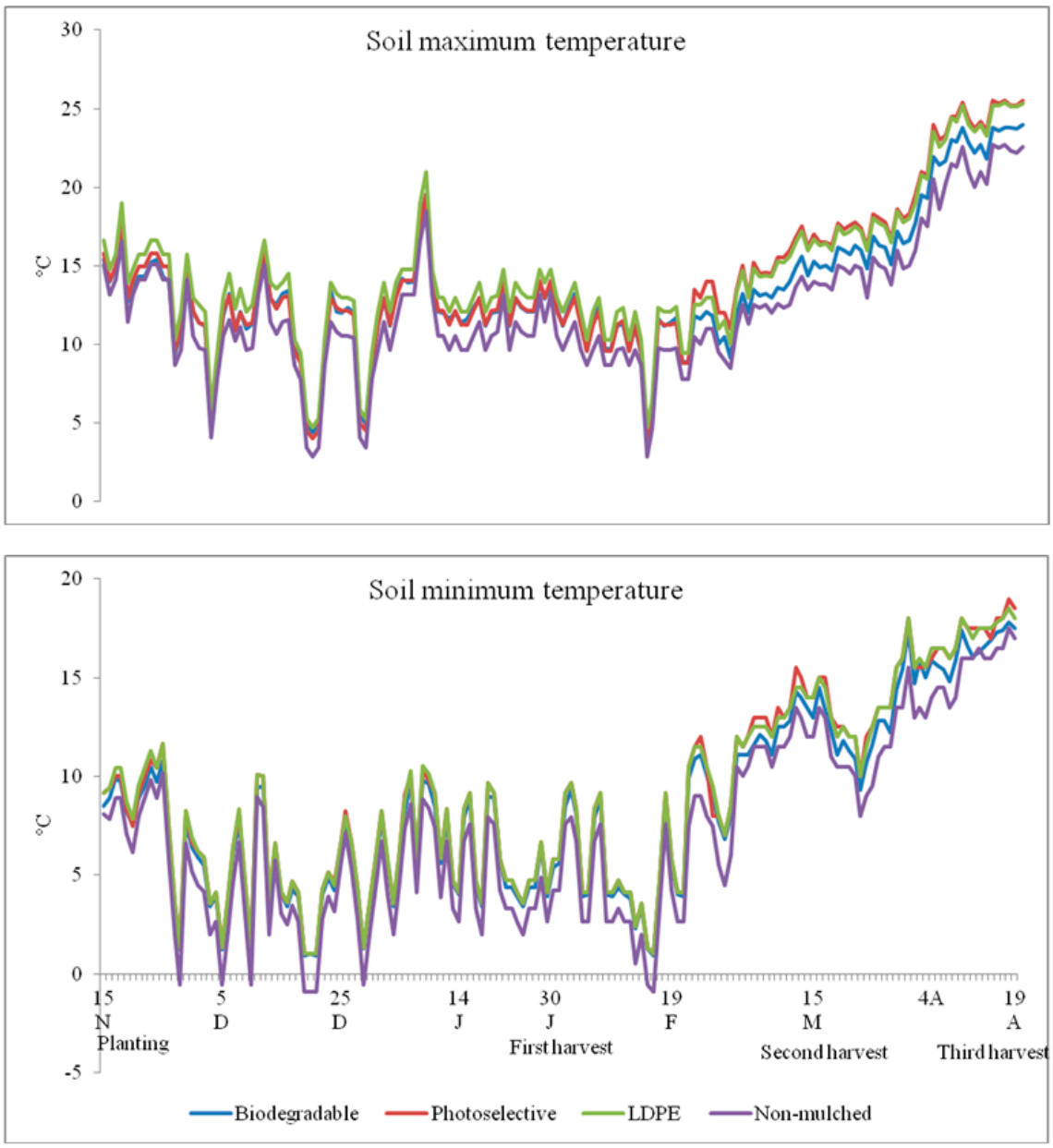

Figure 2. Trend of soil temperature of greenhouse perennial wall rocket grown under different mulching types, as an average of 2016–2017 and 2017–2018.

2.2. SPAD and Leaf Colour Parameters

Just before harvesting, the following measurements were performed as previously described [22]: The Soil Plant Analysis Development (SPAD) index, on twenty undamaged rocket leaves per experimental treatment, by means of a portable chlorophyll meter SPAD-502 (Konica Minolta, Tokyo, Japan); the color parameters L* (lightness, from 0 to 100, i.e., black to white), a* and b* (chroma components from −60 to +60, i.e., from green to red and from blue to yellow for 'a' and 'b' respectively).

2.3. Dry Matter

The leaf dry matter was assessed after dehydration of the fresh samples in a forced-air oven at 70 °C until constant weight.

2.4. Mineral Elements

The content of NO_3-N, P, K, Ca, Mg, Na, S and Cl was assessed in leaf dry tissues ground in a Wiley Mill and then sieved through an 841-microns riddle. For this purpose, 250 mg of leaf tissue powder suspended in 50 mL of ultrapure water (Milli-Q, Merck Millipore, Darmstadt, Germany) underwent three freeze-thaw cycles in liquid nitrogen followed by shaking water bath (ShakeTemp SW22, Julabo, Seelbach, Germany) at 80 °C for 10 min. The mixture obtained was managed according to the procedure by Rouphael et al. [23] and the determinations of the mentioned mineral elements were performed as described by Rouphael et al. [22].

2.5. Antioxidants

2.5.1. Phenols

The total phenols content in methanolic extracts was assessed using the Folin–Ciocalteu method [24] with gallic acid as a standard. For this purpose, 100 mL of the supernatant were combined with 500 mL of Folin–Ciocalteau's reagent (Sigma-Aldrich Inc., Milano, Italy) and 400 mL of 7.5% sodium carbonate/water (*w*/*v*). The solution absorbance was measured after 30 min at 765 nm by an ultraviolet-visible spectrophotometer, expressing the results as mg gallic acid (Sigma-Aldrich Inc.) per 100 g of dry weight.

2.5.2. Ascorbic Acid

The total ascorbic acid was assessed by spectrophotometric detection as described by Kampfenkel et al. [25], by reducing the dehydroascorbate to ascorbic acid upon the sample pre-incubation with dithiothreitol. The solution absorbance was measured at 525 nm, expressing the results as mg ascorbic acid per 100 g fresh weight.

2.6. Antioxidant Activity

The total antioxidant activity was determined by the 1,1-diphenyl-2-picryl-hydrazil (DPPH) test, following the method of Brand-Williams et al. [26], measuring the absorbance decrease at 515 nm wavelength of a 63.4 μM DPPH solution, using 10 μl of extract. The values obtained were interpolated with those from a calibration line built up using Trolox as a reference antioxidant and the results were expressed as mmol Trolox equivalents (TE) per g of fresh weight.

2.7. Statistical Processing

The two-way analysis of variance and the Duncan multiple range test were used for processing the data and performing the mean separations at the 0.05 probability level respectively, by using SPSS software version 21. The angular transformation was applied to percentage data before processing. The variables regarding the plant determinations examined in our research were not significantly affected by the research year and, therefore, only mean data of the two years are reported. Moreover, no significant interactions arose between the two experimental factors "crop cycle" and "mulching type" and for this reason only the data relevant to their main effects are showed.

3. Results and Discussion

3.1. Precocity, Plant Growth and Yield

Rocket earliness, growth and yield parameters resulting from the comparison between the three crop cycles and the four mulching treatments tested are reported in Table 1. The autumn-winter crop cycle was the longest (73 days), showing more than double duration compared to the shortest spring one (35 days). The spring cycle was associated to the most enhanced plant growth, in terms of leaf expansion and dry matter, as well as yield; the latter was the result of the highest mean weight, though the number of leaves was lowest. Conversely, the winter crop cycle produced the highest number of leaves with the lowest mean weight and the yield was not significant different from that corresponding to the autumn-winter crops. The highest yield performance of the spring crops is associated to the most favorable trend of air temperature inside the greenhouse: The temperature in April was always over 20 °C, whereas it dropped just four times below 5 °C in the second half of March. Consistently with other results, Bonasia et al. [27] recorded higher leaf yield of perennial wall-rocket in winter-spring cycle than in autumn-winter (2.25 vs. 1.50 kg m^{-2}). Schiattone et al. [21] reported a marketable yield range between 5 and 18 t ha^{-1} per growing cycle.

As for the comparison between the four mulching treatments (Table 1), no significant differences of average harvest precocity were recorded between the biodegradable, photoselective and LDPE films (49 days on average), whereas the non-mulched control caused the longest cycle (55 days). Consistently with our results, Ibarra et al. [28] found a positive correlation between the crop earliness and the soil heat accumulation under mulching treatments and no significant differences between the plastic and biodegradable mulch.

The biodegradable and photoselective films did not significantly differ from LPDE in terms of growth and yield (12.4 t ha^{-1} as an average); indeed, the traditional plastic mulch resulted in lower dry biomass than that produced by the other two materials. The non-mulched control showed smaller plants and an 11% yield reduction due to the lower number of leaves, whereas their mean weight was not significantly affected by mulching treatment.

In our research, the effects of the biodegradable and photoselective films on yield were not significantly different from LDPE, though the thickness of the latter material is more than triple (45 vs. 15 μm). This mainly suggests that the biodegradable mulch is a good environmentally friendly alternative to the plastic one for managing the perennial wall rocket crop in greenhouse; however, all the mulching treatments encouraged the production compared to bare soil, which highlights the importance of both increasing soil temperature and controlling weeds with no costly manual intervention [29]. Indeed, in our investigation both the maximum and minimum soil temperatures recorded over the whole crop cycles (Figure 2) were lowest in the non-mulched control and highest under photoselective LDPE and standard LDPE film, maybe also due to the higher thickness compared to the biodegradable mulch, in agreement with previous studies [30]. In contrast with our findings relevant to rocket whose leaves do not cover all the soil surface until the end of crop cycle, in previous investigations carried out on open-air grown lettuce [31] and potato [32] the effect of mulch on soil temperature decreased with the full establishment of the plant canopy in the later growth stage. Ngouajio et al. [33] found that the biodegradable mulch led to higher soil temperatures than the polyethylene film in early crop development in open-air conditions. By contrast, Rangarajan and Ingall [34] recorded slightly lower soil temperatures under biodegradable green or black mulch compared to the same colors of polyethylene material.

In all treatments of our research the mean values of soil root-zone temperature fell in the 2 °C to 25 °C range, fitting rocket requirements [2] and thus enhancing physiological processes, such as uptake of water and mineral nutrients, and accordingly growth and yield. However, the maximum soil temperature under standard and photoselective LDPE exceeded 25 °C for an average of seven days in April and this overheating may have damaged the crop.

The differences in the soil temperature among the different mulch types could mainly be attributable to their composition [35]. Notably, the highest soil temperatures reached under LDPE may result from the optical properties of this material, which reflects or transmits less than 10% of solar radiation and absorbs the remaining over 90% fraction [36]. Indeed, if there is a good contact between soil and mulch, significant heat conduction can occur, thus increasing soil temperature during the daytime [37]. The increase of mulch thickness may result in soil temperature rise [38], but it may even not affect the soil heating [33]. The biodegradable mulch resulted on average in lower soil temperatures than LDPE and photoselective film: 0.7 °C compared to both plastic mulches referring to the minimum temperatures; 0.6 and 1.0 °C lower than photoselective LDPE and standard LDPE film respectively, regarding maximum temperatures. This is due to the higher permeability of the biodegradable film tested, which encourages gas exchange with the open air [35,39]. The latter feature, in combination with the soil overheating caused by LDPE mulch for seven days of the spring crop cycle, referring to perennial wall rocket optimal requirements, may explain the not different yield between the two plastic mulches and the biodegradable one. The lowest bare soil temperature is the result of the highest heat loss upon reflectance and evaporation [37]. The highest minimum soil temperatures recorded under LDPE may be explained by the more effective heat accumulation of this material during the day, though the energy loss overnight was higher than the biodegradable mulch as witnessed by the more amplified day-night temperature difference [36,37].

All mulching types reduced the soil temperature fluctuation as compared to bare soil, due to minimum soil temperature increase, as also described by Moreno et al. [30]. Consistently with our plant growth and yield results, other authors [18] reported the positive effect of increasing soil temperature under mulching on peanut leaf area index and pod production compared with the bare soil. The 15% starch-based biodegradable film warmed the soil up with similar effectiveness as PE film in the first two months of peanut cultivation, thereafter the soil temperature under the biodegradable mulch was 0.8–2.8 °C lower compared to PE at 10 cm soil depth. However, no significant difference in terms of leaf area expansion, SPAD index and yield was recorded between the two mulching types, though the number of pods per plant was higher under the plastic mulch. In research carried out on winter oilseed rape [40], the starch-based biodegradable much did not degrade in winter but only in the late spring crop stages. Accordingly, soil temperature differences between plastic and biodegradable mulch were recorded only in the advanced plant development, when the more moderate temperature under the biodegradable mulch resulted in higher tap root length and lateral root mass density in the 20–30 cm layer. Nevertheless, the seed production was not significantly affected by mulching type. In a study carried out on lettuce [41], biodegradable mulching based on hydrolyzed proteins resulted in lower soil temperatures but higher electrical conductivity than those determined by LDPE film, the difference magnitude depending on biodegradable material tested. However, mulching type showed no significant effect on SPAD index, leaf expansion and dry matter. As reported by Waterer [42], no significant differences in soil temperature were recorded between standard and biodegradable mulch and, accordingly, yields of pepper, zucchini, sweet corn, eggplant and melon were not affected by the type of film.

Table 1. Mean values of perennial wall rocket precocity, growth indices and yield components as affected by crop cycle and mulch treatment.

Experimental Treatment	Crop Cycle Duration	Leaf Area Index (LAI)	Plant Dry Matter	Marketable Leaves		
	days	$m^2 \cdot m^{-2}$	$g \cdot m^{-2}$	Yield $t \cdot ha^{-1}$	Number Per Alveolus	Mean Weight g
Crop cycle						
Autumn-winter	73 a	1.36 b	105.8 b	11.9 b	140.7 b	0.61 b
Winter	44 b	1.30 b	101.0 b	11.0 b	155.3 a	0.51 c
Spring	35 c	1.48 a	114.2 a	13.2 a	119.0 c	0.80 a
Mulch treatment						
Biodegradable	50 b	1.41 a	118.3 a	12.5 a	140.9 a	0.67
Photoselective LDPE	49 b	1.45 a	112.4 ab	12.2 a	144.0 a	0.63
Standard LDPE	48 b	1.41 a	105.8 b	12.5 a	143.2 a	0.64
Non-mulched control	55 a	1.24 c	91.6 c	11.0 b	126.5 b	0.63
						n.s.

Within each column, n.s. no statistically significant difference; means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

3.2. Leaf Colour, SPAD Index, Quality and Chemical Composition

The SPAD index was not affected by the crop cycle, whereas the color components a* and b* attained higher values in winter and in spring, respectively. The SPAD index was higher in the leaves grown under mulching (by 17.4% as an average) compared to bare soil (Table 2). All the leaf color components were highest under the biodegradable mulch.

The higher SPAD index observed in rocket plants grown in mulched soil can be associated to the effective enhancement of N uptake efficiency achieved with this farming practice. In fact, the SPAD index, used as a non-invasive and non-destructive estimate of chlorophyll content, is considered a key indicator of the efficiency of chlorophyll biosynthesis and photosynthetic apparatus, which is connected with the crop performance [43].

In previous research [18], the SPAD index under mulching was higher than that recorded in bare soil grown plants; no significant differences were recorded between the 15% starch biodegradable film and the PE film from the pod-setting to pod maturity stage. The SPAD index is generally correlated to chlorophyll content and leaf nitrogen status [44]; in this respect, nitrogen uptake is stimulated by the increase of daylight soil temperature, contrary to the trend of phosphorus and calcium in leaf tissues [45]. However, Sartore et al. [41] reported that at harvest leaves grown in soil mulched with protein hydrolysate combined with polyethylene glycol diglycidyl ether (PH-PEG-ESO) showed a tendency to higher SPAD index compared to epoxidized soybean oil (PH-ESO) and LDPE, though the latter mulch generally showed a higher soil temperature than PH-PEG-ESO.

As reported in Table 3, the leaves harvested at mid-March showed higher values of dry residue, oxalate and isocitrate, whereas malate and citrate concentration did not significantly differ between the winter and spring cycle. As for the comparison between the mulch types, the biodegradable mulch resulted in the highest values of dry residue, though not different from the photoselective LDPE film, and of the organic acids concentration which did not differ from the non-mulched control.

As for the mineral composition (Table 4), potassium and chlorine attained higher concentration in the leaves produced in spring, whereas the nitrate content was higher in winter; no significant differences in the other elements analyzed were recorded between the two crop cycles. Consistently with our results, Caruso et al. [5] reported a higher nitrate concentration in perennial wall rocket leaves grown in autumn-winter cycle compared to winter-spring season.

With regard to the comparison between the mulching treatments, Mg, Na and P were most concentrated in the leaves produced with the biodegradable film, whereas the nitrate accumulation was enhanced by mulching independently on the material type. The bare soil caused the lowest leaf content of the aforementioned elements, not differing from the photoselective LDPE film in Na concentration and even from standard LDPE mulch in Mg and P.

Table 2. Mean values of SPAD index and color components of perennial wall rocket as affected by crop cycle and mulch treatment.

	SPAD	**L***	**a***	**b***
Crop cycle				
Winter	35.9	38.4	−13.5	18.1
Spring	38.1	40.1	−21.1	20.4
	n.s.	n.s.	*	*
Mulch treatment				
Biodegradable	39.1 a	40.5 a	−30.8 a	20.6 a
Photoselective LDPE	38.7 a	33.5 b	−11.2 b	16.7 b
Standard LDPE	37.5 a	32.0 b	−11.1 b	15.3 b
Non-mulched	32.7 b	31.6 b	−10.7 b	14.8 b

Within each column, n.s. no statistically significant difference, * significant difference at $p \leq 0.05$; means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

Table 3. Mean values of leaf quality indicators of perennial wall rocket as affected by crop cycle and mulch treatment.

		Organic Acids			
	Dry Residue	**Malate**	**Oxalate**	**Citrate**	**Isocitrate**
	%	$g \cdot kg^{-1}$ d.w.			
Crop cycle					
Winter	9.13	26.4	0.88	20.7	0.64
Spring	8.57	25.9	0.80	21.1	0.58
	*	n.s.	*	n.s.	*
Mulch treatment					
Biodegradable	9.42 a	28.8 a	0.91 a	22.1 a	0.67 a
Photoselective LDPE	9.19 a	24.4 b	0.81 b	19.7 b	0.58 b
Standard LDPE	8.47 b	24.3 b	0.81 b	20.0 b	0.57 b
Non-mulched	8.32 b	26.9 a	0.84 ab	21.7 a	0.63 ab

DR, dry residue. Within each column, n.s. no statistically significant difference, * significant difference at $p \leq 0.05$; means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

Table 4. Mean values of mineral composition of perennial wall rocket leaves as affected by crop cycle and mulch treatment.

	K	**Ca**	**Mg**	**Na**	**P**	**S**	**NO_3**	**Cl**
	$g \cdot kg^{-1}$ d.w.							
Crop cycle								
Winter	50.8	27.5	3.46	3.27	2.74	7.91	68.1	11.6
Spring	55.0	25.9	3.25	3.58	2.68	8.84	59.5	15.5
	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
Mulch treatment								
Biodegradable	52.7	26.9	3.68 a	4.04 a	2.87 a	8.22	68.8 a	13.1
Photoselective LDPE	54.5	26.9	3.18 b	3.07 c	2.66 b	8.67	64.5 a	13.9
Standard LDPE	52.5	26.7	3.29 b	3.57 b	2.64 b	8.38	67.9 a	12.6
Non-mulched	52.0	26.4	3.25 b	3.02 c	2.66 b	8.21	53.9 b	14.7
	n.s.	n.s.				n.s.		n.s.

Within each column, n.s. no statistically significant difference, * significant difference at $p \leq 0.05$; means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

3.3. Leaf Antioxidants

As for antioxidants (Table 5), the spring harvested leaves of perennial wall rocket showed higher concentration of phenols and ascorbic acid as well as of lipophilic and hydrophilic antioxidant activities compared to the winter grown leaves.

With regard to the comparison between the mulches, the biodegradable and photoselective LDPE films showed the highest values of the antioxidant compounds and activities in *D. tenuifolia* leaves; however, the effect of the photoselective LDPE material was not significantly different from the standard LDPE referring to phenols content and lipophilic antioxidant activity.

In the present research the ascorbic acid content in perennial wall rocket leaves was 36.7 mg 100 g^{-1} on average, compared to the average content of 9 mg 100 g^{-1} reported in literature [2]. In this respect, the recommended daily intake for vitamin C is 30–60 mg [46] and, therefore, 123 g per day of wall rocket leaves produced in the present investigation are needed to fulfill the daily requirement of this antioxidant.

Consistently with the findings of the present research, Morra et al. [47] found that polyphenols and other antioxidant compounds increased in strawberry fruits grown in soil mulched with MaterBi 20 µm thick biodegradable film in comparison to LDPE. Unlike the results of our investigation, Sartore et al. [41] reported that the total polyphenols concentration in lettuce leaves did not differ

between the two protein hydrolyzate-based mulches tested (with the addition of ESO or PEG-ESO), but under both materials the content was 17% lower compared to LDPE; ascorbic acid showed the same trend and was 11% lower for the two spray treatments than for the LDPE mulch.

In previous research [48] the antioxidants showed a positive correlation with the antioxidant activity, whereas their correlation with the temperature was positive in *Solanum lycopersicum* [49] but negative in *Lactuca sativa* and *Citrullus lanatus* [49,50]. Indeed, stressing temperature levels enhance the activity of phenylalanine ammonium-lyase (PAL) which is the main enzyme involved in phenolic biosynthesis, catalyzing the production of trans-cinamic acid starting from L-phenylalanine [51], thus promoting cell acclimation [52]. On the other hand, the oxidation of o-diphenols to o-diquinons caused by polyphenols oxidases (PPO) or peroxidases (POD), as well as the monophenols hydroxylation are the consequences of physiological disorders due to thermal stress [53]. In this respect, the accumulation of soluble phenolics is a mechanism of adaptation to extreme temperatures [49] and the content of these antioxidants is affected by both farming practices and environment [54]. In addition, phenolics counteract the degrading enzyme activity of ascorbic acid [55] which exerts essential functions such as antioxidant, photosynthetic enzyme regulating, phytohormonal [56].

Table 5. Mean values of antioxidant content and activity of perennial wall rocket leaves as affected by crop cycle and mulch treatment.

	Polyphenols mg gallic acid·100 g^{-1} d.w.	**Ascorbic Acid mg·100 g^{-1} f.w.**	**Lipophilic Antioxidant Activity mmol trolox eq·100 g^{-1} d.w.**	**Hydrophilic Antioxidant Activity mmol ascorbic acid eq·100 g^{-1} d.w.**
Crop cycle				
Winter	175	17.0	6.2	6.2
Spring	408	56.4	20.0	8.0
	*	*	*	*
Mulch treatment				
Biodegradable	316 a	43.9 a	14.5 a	8.1 a
Photoselective LDPE	303 ab	40.0 a	13.7 ab	7.7 a
Standard LDPE	290 b	32.5 b	13.0 b	6.4 b
Non-mulched	258 c	30.4 b	11.2 c	6.2 b

Within each column, * significant difference at $p \leq 0.05$; means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

4. Conclusions

The effect of starch-based biodegradable mulch was assessed in research carried out in southern Italy on three crop cycles of *Diplotaxis tenuifolia*. The biodegradable film did not differ from both standard (LDPE) and photoselective plastic mulch in terms of yield but resulted in the best overall performances with regard to quality and chemical composition. The eco-compatible material also led to higher antioxidants content and activity, but only in comparison to LDPE. Indeed, the biodegradable mulch led to soil temperature increase, but never over the optimal threshold of perennial wall rocket requirement, which occurred in some days under both the standard and photoselective plastic mulch. Further, the biodegradable mulch is an environmentally friendly and cost-effective tool available for sustainable management of crop systems.

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References

1. Brusco, M.L. *Rucola Della Piana del Sele a un Passo dall'IGP*; FreshPlaza: Tholen, The Netherlands, 2018. Available online: www.freshplaza.it/article/99765/Rucola-della-Piana-del-Sele-a-un-passo-dallIGP (accessed on 2 April 2019).
2. Caruso, G.; Parrella, G.; Giorgini, M.; Nicoletti, R. Crop systems, quality and protection of *Diplotaxis tenuifolia*. *Agriculture* **2018**, *8*, 55. [CrossRef]
3. Jansen, M.; van den Noort, R.E.; Tan, M.; Prinsen, E.; Lagrimini, L.M.; Thorneley, R.N.F. Phenol-oxidizing peroxidases contribute to the protection of plants from ultraviolet radiation stress. *Plant Physiol.* **2001**, *126*, 1012–1023. [CrossRef] [PubMed]
4. Cho, H.Y.; Kleeberger, S.R. Nrf2 Protects against airway disorders. *Toxicol. Appl. Pharmacol.* **2010**, *244*, 43–56. [CrossRef] [PubMed]
5. Caruso, G.; Conti, S.; La Rocca, G. Influence of crop cycle and nitrogen fertilizer form on yield and nitrate content in different species of vegetables. *Adv. Hortic. Sci.* **2011**, *25*, 81–89.
6. Amalfitano, C.; Del Vacchio, L.; Somma, S.; Cuciniello, A.; Caruso, G. Effects of cultural cycle and nutrient solution electrical conductivity on plant growth, yield and fruit quality of "Friariello" pepper grown in hydroponics. *Hortic. Sci.* **2017**, *44*, 91–98. [CrossRef]
7. Amalfitano, C.; Gomez, L.D.; Frendo, P.; De Pascale, S.; Pepe, O.; Simister, R.; Ventorino, V.; Agrelli, D.; Borrelli, C.; McQueen-Mason, S.J.; et al. Plant–Rhizobium symbiosis, seed nutraceuticals, and waste quality for energy production of *Vicia faba* L. as affected by crop management. *Chem. Biol. Technol. Agric.* **2018**, *5*, 15. [CrossRef]
8. Conti, S.; Villari, G.; Amico, E.; Caruso, G. Effects of production system and transplanting time on yield, quality and antioxidant content of organic winter squash (*Cucurbita moschata* Duch.). *Sci. Hortic.* **2015**, *183*, 136–143. [CrossRef]
9. European Commission. Brussels, Belgium, 16 January 2018. Available online: ec.europa.eu/environment/circular.../plastics-strategy-swd.pdf (accessed on 2 April 2019).
10. Lanorte, A.; De Santis, F.; Nolè, G.; Blanco, I.; Loisi, R.V.; Schettini, E.; Vox, G. Agricultural plastic waste spatial estimation by Landsat 8 satellite images. *Comput. Electron. Agric.* **2017**, *141*, 35–45. [CrossRef]
11. Scarascia-Mugnozza, G.; Sica, C.; Picuno, P. The optimisation of the management of agricultural plastic waste in Italy using a geographical information system. *Acta Hortic.* **2008**, *801*, 219–226. [CrossRef]
12. Blanco, I.; Loisi, R.V.; Sica, C.; Schettini, E.; Vox, G. Agricultural plastic waste mapping using GIS. A case study in Italy. *Resour. Conserv. Recycl.* **2018**, *137*, 229–242. [CrossRef]
13. Kasirajan, S.; Ngouajio, M. Polyethylene and biodegradable mulches for agricultural applications: A review. *Agron. Sustain. Dev.* **2012**, *32*, 501–529. [CrossRef]
14. Briassoulis, D.; Giannoulis, A. Evaluation of the functionality of bio-based plastic mulching films. *Polym. Test.* **2018**, *67*, 99–109. [CrossRef]
15. Briassoulis, D.; Mistriotis, A. Key parameters in testing biodegradation of bio-based materials in soil. *Chemosphere* **2018**, *207*, 18–26. [CrossRef]
16. Kapanen, A.; Schettini, E.; Vox, G.; Itavaara, M. Performance and environmental impact of biodegradable films in agriculture: A field study on protected cultivation. *J. Polym. Environ.* **2008**, *16*, 109–122. [CrossRef]
17. Tachibana, Y.; Maeda, T.; Ito, O.; Maeda, Y.; Kunioka, M. Utilization of a biodegradable mulch sheet produced from poly (lactic acid)/Ecoflex/modified starch in mandarin orange groves. *Int. J. Mol. Sci.* **2009**, *10*, 3599–3615. [CrossRef] [PubMed]
18. Sun, T.; Li, G.; Ning, T.Y.; Zhang, Z.M.; Mi, Q.H.; Lal, R. Suitability of mulching with biodegradable film to moderate soil temperature and moisture and to increase photosynthesis and yield in peanut. *Agric. Water Manag.* **2018**, *208*, 214–223. [CrossRef]
19. Bastioli, C.; Bellotti, V.; Gilli, G. The Use of agricultural commodities as a source of new plastic materials. In Proceedings of the Biodegradable Packagings and Agricultural Films, Paris, France, 10–11 May 1990; pp. 1–36.
20. Martín-Closas, L.; Bach, M.A.; Pelacho, A.M. Biodegradable mulching in an organic tomato production system. *Acta Hortic.* **2008**, *767*, 267–274. [CrossRef]

21. Schiattone, M.I.; Viggiani, R.; Di Venere, D.; Sergio, L.; Cantore, V.; Todorovic, M.; Perniola, M.; Candido, V. Impact of irrigation regime and nitrogen rate on yield, quality and water use efficiency of wild rocket under greenhouse conditions. *Sci. Hortic.* **2018**, *229*, 182–192. [CrossRef]
22. Rouphael, Y.; Giordano, M.; Cardarelli, M.; Cozzolino, E.; Mori, M.; Kyriacou, M.C.; Bonini, P.; Colla, G. Plant- and seaweed-based extracts increase yield but differentially modulate nutritional quality of greenhouse spinach through biostimulant action. *Agronomy* **2018**, *8*, 126. [CrossRef]
23. Rouphael, Y.; Colla, G.; Giordano, M.; El-Nakhel, C.; Kyriacou, M.C.; De Pascale, S. Foliar applications of a legume-derived protein hydrolysate elicit dose-dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars. *Sci. Hortic.* **2017**, *226*, 353–360. [CrossRef]
24. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178. [CrossRef]
25. Kampfenkel, K.; Van Montagu, M.; Inzé, D. Extraction and determination of ascorbate and dehydroascorbate from plant tissue. *Anal. Biochem.* **1995**, *225*, 165–167. [CrossRef]
26. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
27. Bonasia, A.; Lazzizera, C.; Elia, A.; Conversa, G. Nutritional, biophysical and physiological characteristics of wild rocket genotypes as affected by soilless cultivation system, salinity level of nutrient solution and growing period. *Front. Plant Sci.* **2017**, *8*. [CrossRef]
28. Ibarra, L.; Zermeño, A.; Munguía, J.; Quezada, M.A.R.; de la Rosa, M. Photosynthesis, soil temperature and yield of cucumber as affected by colored plastic mulch. *Acta Agric. Scand.* **2008**, *58*, 372–378. [CrossRef]
29. Cirujeda, A.; Aibar, J.; Anzalone, A.; Martín-Closas, L.; Meco, R.; Moreno, M.M.; Pardo, A.; Pelacho, A.M.; Rojo, F.; Royo-Esnal, A.; et al. Biodegradable mulch instead of polyethylene for weed control of processing tomato production. *Agron. Sustain. Dev.* **2012**, *32*, 889–897. [CrossRef]
30. Moreno, M.M.; Cirujeda, A.; Aibar, J.; Moreno, C. Soil thermal and productive responses of biodegradable mulch materials in a processing tomato (*Lycopersicon esculentum* Mill.) crop. *Soil Res.* **2016**, *54*, 207–215. [CrossRef]
31. Brault, D.; Stewart, K.A.; Jenni, S. Growth, development, and yield of head lettuce cultivated on paper and polyethylene mulch. *HortScience* **2002**, *37*, 92–94. [CrossRef]
32. Zhao, H.; Xiong, Y.C.; Li, F.M.; Wang, R.Y.; Qiang, S.C.; Yao, T.F.; Mo, F. Plastic film mulch for half growing-season maximized WUE and yield of potato via moisture-temperature improvement in a semi-arid agroecosystem. *Agric. Water Manag.* **2012**, *104*, 68–78. [CrossRef]
33. Ngouajio, M.; Auras, R.; Fernandez, R.T.; Rubino, M.; Counts, J.W.; Kijchavengkul, T. Field performance of aliphatic–aromatic copolyester biodegradable mulch films in a fresh market tomato production system. *HortTechnology* **2008**, *18*, 605–610. [CrossRef]
34. Rangarajan, A.; Ingall, B. *Biodegradable Mulch Product Testing*; Research Report; Cornell University, Department of Horticulture: Ithaca, NY, USA, 2006.
35. Moreno, M.M.; Moreno, A. Effect of different biodegradable and polyethylene mulches on productivity and soil thermal and biological properties in a tomato crop. *Sci. Hortic.* **2008**, *116*, 256–263. [CrossRef]
36. Ham, J.M.; Huitenberg, G.J.; Lamont, W.J. Optical properties of plastic mulches affect the field temperature regime. *J. Am. Soc. Hortic. Sci.* **1993**, *118*, 188–193. [CrossRef]
37. Teasdale, J.R.; Abdul-Baki, A.A. Soil temperature and tomato growth associated with black polyethylene and hairy vetch mulches. *J. Am. Soc. Hortic. Sci.* **1995**, *120*, 848–853. [CrossRef]
38. Bristow, K.L. The role of mulch and its architecture in modifying soil temperature. *Aust. J. Soil Res.* **1988**, *26*, 269–280. [CrossRef]
39. Chandra, R.; Rustgi, R. Biodegradable polymers. *Progr. Polym. Sci.* **1998**, *23*, 1273–1335. [CrossRef]
40. Gu, X.B.; Li, Y.N.; Du, Y.D. Biodegradable film mulching improves soil temperature, moisture and seed yield of winter oilseed rape (*Brassica napus* L.). *Soil Tillage Res.* **2017**, *171*, 42–50. [CrossRef]
41. Sartore, L.; Schettini, E.; de Palma, L.; Brunetti, G.; Cocozza, C.; Vox, G. Effect of hydrolyzed protein-based mulching coatings on the soil properties and productivity in a tunnel greenhouse crop system. *Sci. Total Environ.* **2018**, *645*, 1221–1229. [CrossRef]
42. Waterer, D. Evaluation of biodegradable mulches for production of warm-season vegetable crops. *Can. J. Plant Sci.* **2010**, *90*, 737–743. [CrossRef]

43. Ertani, A.; Schiavon, M.; Nardi, S. Transcriptome-wide identification of differentially expressed genes in *Solanum lycopersicum* L. in response to an alfalfa-protein hydrolysate using microarrays. *Front. Plant Sci.* **2017**, *8*, 1159. [CrossRef] [PubMed]
44. León, A.S.; Viña, S.Z.; Frezza, D.; Chavaz, A.; Chiesa, A. Estimation of chlorophyll contents by correlations between SPAD-502 meter and chroma meter in butterhead lettuce. *Commun. Soil Sci. Plant Anal.* **2001**, *38*, 2877–2885. [CrossRef]
45. Verdial, M.F.; Santos de Lima, M.; Morgor, A.F.; Goto, R. Production of iceberg lettuce using mulching. *Sci. Agric.* **2001**, *58*, 737–740. [CrossRef]
46. National Research Council (US). *Recommended Dietary Allowances*, 10th ed.; National Academies Press: Washington, DC, USA, 1989; p. 285.
47. Morra, L.; Bilotto, M.; Cerrato, D.; Coppola, R.; Leone, V.; Mignoli, E.; Pasquariello, M.S.; Petriccione, M.; Cozzolino, E. The Mater-Bi® biodegradable film for strawberry (*Fragaria* × *ananassa* Duch.) mulching: Effects on fruit yield and quality. *Ital. J. Agron.* **2016**, *11*, 731. [CrossRef]
48. Chaieb, N.; Gonzalez, J.L.; Lopez-Mesas, M.; Bouslama, M.; Valiente, M. Polyphenols content and antioxidant capacity of thirteen faba bean (*Vicia faba* L.) genotypes cultivated in Tunisia. *Food Res. Int.* **2011**, *44*, 970–977. [CrossRef]
49. Rivero, R.M.; Ruiz, J.M.; Garcia, P.C.; López-Lefebre, L.R.; Sánchez, E.; Romero, L. Resistance to cold and heat stress: Accumulation of phenolic compounds in tomato and watermelon plants. *Plant Sci.* **2001**, *160*, 315–321. [CrossRef]
50. Boo, H.O.; Heo, B.G.; Gorinstein, S.; Chon, S.U. Positive effects of temperature and growth conditions on enzymatic and antioxidant status in lettuce plants. *Plant Sci.* **2011**, *181*, 479–484. [CrossRef]
51. Dixon, R.A.; Paiva, N.L. Stress-induced phenylpropanoid metabolism. *Plant Cell* **1995**, *7*, 1085–1097. [CrossRef] [PubMed]
52. Leyva, A.; Jarillo, J.A.; Salinas, J.; Martinez-Zapater, J.M. Low temperature induces the accumulation of phenylalanine ammonia-lyase and chalcone synthase messenger-RNAs of *Arabidopsis thaliana* in a light-dependent manner. *Plant Physiol.* **1995**, *108*, 39–46. [CrossRef] [PubMed]
53. Martínez-Téllez, M.A.; Lafuente, M.T. Effect of high temperature conditioning on ethylene, phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase activities in flavedo of chilled ‹Fortune› mandarin fruit. *J. Plant Physiol.* **1997**, *150*, 674–678. [CrossRef]
54. Wang, S.Y.; Zheng, W.; Galletta, G.J. Cultural system affects fruit quality and antioxidant capacity in strawberries. *J. Agric. Food Chem.* **2002**, *50*, 6534–6542. [CrossRef] [PubMed]
55. Altunkaya, A.; Gökmen, V. Effect of various anti-browning agents on phenolic compounds profile of fresh lettuce (*L. sativa*). *Food Chem.* **2009**, *117*, 122–126. [CrossRef]
56. Barth, C.; De Tullio, M.; Conklin, P.L. The role of ascorbic acid in the control of flowering time and the onset of senescence. *J. Exp. Bot.* **2006**, *57*, 1657–1665. [CrossRef]