



Article

Modulation of Light and Nitrogen for Quality-Traits Improvement: A Case Study of Altino Sweet Pepper

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Abstract: Local varieties are known to cope more efficiently with stressful and/or low-input conditions than cultivated ones by activating secondary metabolisms and, hence, are supposed to have higher nutraceutical potential. In this work, we investigate the effects of N and light, supplied at optimal and sub-optimal levels, on the fruit yield and quality of a local ecotype of sweet pepper, Altino, grown in the Abruzzo Region (Southern Italy). In 2017, two open-field experiments were carried out, comparing increasing N rates (0, 100 and 200 kg N ha⁻¹, 0_N, 100_N and 200_N, respectively) and different percentages of shading and/or manipulations of the transmitted solar radiation, obtained through photosensitive nets (red net, RN; black net, BN; unshaded Control). Both N and light were preconditions to obtain stable yields in terms of both fruit number and fruit weights. However, BN significantly reduced the number of sunscalded fruits (0.39 vs. 3.38 and 2.59 fruit plant⁻¹ for BN, Control and RN, respectively), leading to lower waste. N deficiency favoured higher total polyphenol (TPC) and flavonoid (TFC) contents in ripened fruits; on the other hand, shading significantly reduced TPC (−12.4%) in immature and TFC (−18.2%) in red fruits. The variations in nutraceuticals were also evaluated in terms of the variations of the single phenolic acids in fruits during ripening; the most interesting results were associated with light treatments. Further research should be directed to the in-depth study of nutrition regimes, in combination with other photosensitive nets applications (i.e., pearl nets), that could be suitable for the Altino genotype to enhance the yield and nutraceutical potentials of its fruits.

Keywords: N management; light availability; Altino ecotype; sweet pepper; nutraceutical traits; fruit quality; yield performances



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1. Introduction

A landrace is defined as a dynamic population of a cultivated plant characterized by its own distinct identity, often genetically diverse and locally adapted, selected and associated with specific farmers' practices of seed selection and crop/field management [1,2]. Italy, due to its geographical and historical compliance, includes a significant richness of cultivated biodiversity and local varieties such as Altino, an ecotype of sweet pepper (*Capsicum annuum* L.). This crop is one of the most cultivated and economically important within the Mediterranean Basin [3], and the Italian germplasm includes a variety of highly diverse landraces having specific morphological traits [4]. The Altino ecotype is typical of the Abruzzo region (Southern Italy), in the province of Chieti, and it is known for its air- or sun-dried fruits, commonly used in many typical culinary dishes and as a spice after grinding [5].

Generally, local varieties are characterized by good adaptation and rusticity, including resistance to both biotic and abiotic environmental factors, and are prone to sustainable cultivation systems under low-input conditions [6]. In addition, environmental constraints could eventually translate into modification in terms of phytochemical's profiles and nutraceutical potentials. Indeed, plant stress-response mechanisms are correlated with the increased content of bioactive molecules; consequently landraces, coping more efficiently with environmentally stressful conditions than cultivated varieties, are generally characterized by higher nutraceutical potential [7].

In such context, we report data on yield and quality (nutraceutical) performances of Altino ecotype in response to N and light availability as a section of our complex study [8] involving morphological and physiological adaptation to the environment, as well as the activation/modulation of secondary metabolism. N availability is essential for normal plant growth and development, although high N fertilizer rates often result in increased levels of N losses and environmental pollution [9]; moreover, high N levels lead to lower secondary metabolites and vitamin contents [10]. Hence, N management has become a priority issue, and local varieties required a specific approach, leading to lower chemical inputs.

On the other hand, the availability of solar radiation can be modulated through the use of photoselective nets, which also provides physical protection against hail, wind, and bird- and insect-transmitted virus diseases [11]. Moreover, it has been shown that photoselective nets are also involved in the biosynthesis of bioactive compounds in many crop species [12,13], including sweet peppers [14,15]. The Altino ecotype could take advantage of this protection practice, in terms of sunburn damages [16], providing a valid justification for the high added value of local production (i.e., DOP, IGP, Slow Food praesidium).

The assumptions at the base of our study were: (i) N and light are involved in the biosynthesis of secondary metabolites; (ii) both factors can be easily modulated in open fields under low-input systems, thus optimizing yield and quality characteristics (harvest and post-harvest); (iii) adaptive responses to the environment and crop management of local varieties are still poorly studied, and (iv) the N and light availabilities significantly influence the growth and physiological performances of the Altino ecotype [8]. On these bases, our study was aimed at evaluating both the separate and cumulative effects of light and N, supplied at optimal and suboptimal levels, on the yield and nutraceutical characteristics of the fruits of the Altino sweet pepper.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

This study used data as a part of a complex study that investigated the growth and physiological responses of the sweet pepper (red-skinned fruit) ecotype "Altino", already described in Stagnari et al. [8], to which we recommend referring to obtain any further information. Seeds obtained from a keeper farmer located at Altino (CH), in the Abruzzo region (Central Italy), were used. As a local variety, the collection was firstly subjected to two cycles of phenotypic selection (2015 and 2016) to obtain a generation of self-fertilization sufficiently stable for the typical characters indicated for the ideotype [8]. Two phenotypes (named 97 and 99) were selected and used to establish two experiments (from 15 May to 22 September, 2017), named Exp_1 and Exp_2, using seeds of the two phenotypes 97 and 99, for Exp_1 and Exp_2, respectively. Experiments were carried out at the experimental fields of the Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics (CREA-OF), located in Monsampolo del Tronto (AP) (latitude 42°52'59.1" N, longitude 13°48'01.9" E), in the coastal area of the Marche Region (Central Italy) (see also [8]).

In both experiments, pepper seeds were sown on 15 May 2017, in a nursery substrate; 22 days after sowing, seedlings (4 leaf stage) were transplanted in the open field in twin rows with a spacing of 0.4 m between plants and rows and 1.6 m between twin rows (to ensure the setting-up of the experimental treatments), following a split-plot design with three replications. For both Exp_1 and Exp_2, experimental treatments (nine) concerned

three nitrogen fertilization rates (main treatment; N_rates) in combination with three shade conditions (secondary treatment; Sh), covering a total of 20 pepper plants for each “N_rates x Sh” combination, grown in a single twin row (see [8]). N_rates were obtained by applying urea at 0, 100 and 200 kg N ha⁻¹ (0_N, 100_N and 200_N, respectively). For each N treatment, subplots were obtained by splitting the total area into three subplots and shading conditions were accomplished using different colored shading nets (Sh treatments: red net, RN; black net, BN), in addition to an unshaded control (Control). Nets were wrapped around 1.7 m-height tunnels, opened at both ends, and placed over the crop. Red and black nets were provided by Agrintech S.r.l. (Iridium[®] Rosso and Monotex 30, respectively; Eboli, Italy). Photosynthetically active radiation (PAR) intensity, relative humidity and air temperatures under nets were continuously monitored starting from 9 days after transplanting (DAT) with specific sensors connected to a data logger (EM50 Data Collection System; Decagon Devices Inc., Pullman, WA, USA). The amount of shade (%) was determined by comparing the daily average PAR values of nets with the daily average PAR values of the Control treatment; the amount of scattered light in the PAR range was measured as indicated by Shahak et al. [17]. Radiance (from 325 to 1075 nm; ±2 nm) was measured with the HandHeld 2 Pro Portable Spectroradiometer (FieldSpec, ADS Inc., Boulder, CO, USA). The analysis of the light properties were as follows: irradiance total daily quantum input (DQI), 45.6 mol m⁻² day⁻¹, 34.9 mol m⁻² day⁻¹ and 27.3 mol m⁻² day⁻¹ for Control, RN and BN, respectively; shade, 23.8% for RN and 40.4% for BN; scattering, 15.4% and 12.1% for RN and BN, respectively; daytime mean temperatures, 30.0 °C for Control, 30.5 °C for RN and 29.7 °C for BN; night mean temperatures, 19.9, 19.8 and 19.5 °C for Control, RN and BN, respectively; relative humidity, 58.4% for Control, 57.8% for RN and 59.6% for BN (see [8]).

Potassium (80 kg P₂O₅ ha⁻¹) and phosphorus (150 kg P₂O₅ ha⁻¹) fertilization was provided during soil preparation. Black plastic mulch and a drip irrigation system on twin rows were used. Plant protection measures were adopted to keep crops free from fungal diseases, using when necessary, CUPROXAT SDI (Nufarm Italy, Milano, Italy), at the dose of 2 L ha⁻¹. No insecticide treatment was carried out. Weeds were mechanically and manually removed between twin rows and in the rows, respectively.

2.2. Yield and Morphological Traits

In both Exp_1 and Exp_2, the yield was monitored in three randomly selected plants for each experimental unit. Fruits were harvested along with three successive timing at 77 and 93 DAT, plus a further collection at 108 DAT, before crop termination. Fruits were counted and divided into marketable (ripe and free of visible defects) and unmarketable fruits. The unmarketable fruits included misshapen, undersized, sunscalded and green (only at 108 DAT) fruits. Before fresh weight (FW, g) determination, marketable fruits were characterized for their length (L, cm) and equatorial diameter (D, cm) using a manual caliper; the number of seeds per fruit (Seed), as well as the single seed dry weight (Seed DW, mg), were also measured after placing the seeds in an oven at 70 °C for 72 h.

At 83 DAT, fruits were sampled in a further two plants per experimental unit and were divided into green (immature, with up to 90% green color; G_fru), early ripening (with 10–90% green color; RI_fru) and red (mature, with up to 90% red color; R_fru) fruits. The fruits were thoroughly cleaned and cut, and the seeds and placenta were removed. Sub-samples were selected and frozen, freeze-dried, finely ground and homogenized and then were kept at –20 °C until analysis (total polyphenols content, TPC; total flavonoid content, TFC; vitamin C, Vit C; antioxidant capacity, TEAC/ABTS; single phenolic acids, PAs).

2.3. Pigments

Pigment concentrations in fruits—chlorophyll (Chla), Chlb and carotenoids (Car)—were determined, following the method described by Lichtenthaler and Buschmann [18]. The results were expressed in µg g⁻¹ of dry weight (DW).

2.4. Spectrophotometric Determination of TPC, TFC and Antioxidant Capacity

The extraction of polyphenols and flavonoids was carried out as described by Gouveia and Castilho [19].

The TPC was evaluated using the Folin–Ciocalteu reagent following the method described by Gouveia and Castilho [20]. The samples (50 μ L) were added to 1.25 mL of Folin–Ciocalteu (dilution, 1:10) and 1.0 mL of a 7.5% Na_2CO_3 solution. After 30 min at room temperature under dark conditions, TPC was determined at 765 nm using a Beckman DU640B spectrophotometer (Beckman Coulter, Brea, CA, USA). Results were expressed as mg gallic acid equivalents (GAE) per 100 g^{-1} dry weight (DW).

Total flavonoid content (TFC) was calculated following the procedure described by Gouveia and Castilho [19] and expressed as mg of rutin equivalents (RUE) 100 g^{-1} DW. Extract sub-samples (100 μ L) were mixed with 1.5 mL of methanol, 2.8 mL of water, 100 μ L of potassium acetate (1 M) and 100 μ L of aluminum chloride (10% in methanol). TFC was determined after 30 min at room temperature, reading the reaction mixture at 415 nm, using a Beckman DU640B spectrophotometer.

For the antioxidant capacity (AOC) determination, Trolox equivalent antioxidant capacity—with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)—TEAC/ABTS assay (ABTS)—was used following Re et al. [21], modified as described by Gouveia and Castilho [22]. The absorbance at 734 nm was evaluated, and results were expressed as mmol Trolox equivalent (TE) 100 g^{-1} DW.

2.5. Vitamin C Content

Ascorbic acid was determined according to the 2,6-dichlorophenolindophenol (DIP) dye method as described by Ghasemnezhad et al. [23], with some modifications. Dry samples (250 mg) were homogenized with 5 mL of 3% metaphosphoric acid (*w/v*) and centrifuged. A total of 3 mL of supernatant were titrated against standard DIP dye, which was already standardized against standard ascorbic acid. Results were expressed in mg ascorbic acid 100 g^{-1} DW.

2.6. Determination of the Polyphenol Profile by High-Performance Liquid Chromatography (HPLC)

2.6.1. Chemicals

Methanol (high-performance liquid chromatography (HPLC grade) and acetonitrile (HPLC grade) were purchased from Carlo Erba (Milano, Italy). 4-hydroxybenzoic acid (4-OHBA), vanillic acid (VA), caffeic acid (CaA) and trans-ferulic acid (FA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All standards were prepared as a stock solution at 1 mg mL^{-1} in methanol and stored at $-20\text{ }^\circ\text{C}$ in dark conditions.

2.6.2. Sample Extraction

The extraction of free phenolic acids (PAs) was achieved using finely homogenized freeze-dried material and following the procedure of Zhang et al. [24], starting from 0.25 g of each sample. After sonication with methanol 80% in H_2O , samples were centrifuged at $2000\times g$ for 10 min, and the supernatant was filtered through nylon 0.45 μm filters. All the extraction procedures were performed under dim light to avoid sample degradation by photo-oxidation.

2.6.3. PAs Identification and Quantification

Identification and quantification of the PAs were carried out on 10 μ L of each sample extract according to the procedure described by Benincasa et al. [25]. The identification of PAs was based on their retention times and UV–VIS spectra compared with those of standard solutions eluted using the same chromatographic conditions. The calibration curves (i.e., the peak area versus concentration) were linear in the range of concentration between 0.1 and 10 mg L^{-1} . The lines of regression calculated were used for the quantitative analysis of PAs in the extracts; the results are expressed as mg kg^{-1} DW. Accuracy, evaluated through the recovery of PAs added to samples, was found to be more than 94% for all of

the PAs detected. The within-assay precision (repeatability) never exceeded 7%. Limits of detection, set based on a signal-to-noise ratio (S/N) of 3, were 0.40 mg kg⁻¹ for 4-OHBA, VA, CaA and 0.8 mg kg⁻¹ for FA.

2.7. Statistical Analysis

A three-factor analysis of variance (ANOVA) was applied to test (F-test) the effects of the experiment (Exp) and treatments (N_rates and Sh). Since no significant effects of 'Exp' or 'Exp × N_rates' or 'Exp × Sh' or 'Exp × N_rates × Sh' interactions were detected, we have performed a split-plot ANOVA to evaluate the effects of N_rates (main factor) and Sh (secondary factor). ANOVA assumptions were tested through graphical methods. The statistical analyses were performed using R software [26].

3. Results

3.1. Yield and Yield Components

The yield of pepper was significantly influenced by both N availability and shade conditions. N availability increased total yield (442.5 vs. 362.3 g plant⁻¹ for N fertilized—averaged over 100_N and 200_N- and 0_N, respectively), as well as total fruits number (23.3 vs. 18.6 plant⁻¹ for N fertilized—averaged over 100 and 200 kg N ha⁻¹—and unfertilized plants, respectively). These differences disappeared when considering only marketable yield since the number of unmarketable fruits significantly declined under 0_N treatments (Table 1). Conversely, N nutrition seemed to not affect fruit morphology, as well as MFW (Table 2).

Table 1. Yield (total, marketable and unmarketable; g plant⁻¹) and yield parameters (num fruits plant⁻¹) as recorded in pepper plants subjected to different N fertilization rates (N_rates; 0_N: 0 kg N ha⁻¹; 100_N: 100 kg N ha⁻¹; 200_N: 200 kg N ha⁻¹) and shading levels (Sh; accomplished using: no cover, Control; red photoselective net, RN; black photoselective net, BN) in 2017^a. Unmarketable yields and number of fruits included both sunscalded fruits and fruits characterized by other physiological or not-physiological disorders, green and undersized fruits.

Effects	Yield (g Plant ⁻¹)				Fruit Number (Num Plant ⁻¹)			
	Control	RN	BN	<i>o.m.</i>	Control	RN	BN	<i>o.m.</i>
Total								
0_N	480.9	457.1	362.3	433.4	22.4	19.7	13.9	18.6
100_N	549.0	583.7	443.8	525.5	27.9	25.1	16.1	23.0
200_N	563.1	576.7	441.1	527.0	28.6	24.9	17.0	23.5
<i>o.m.</i>	531.0	539.1	415.7		26.3	23.2	15.7	
F-test								
N_rates		<i>n.s.</i> (28.8)				** (1.0)		
Sh		** (39.1)				** (1.4)		
N_rates × Sh		<i>n.s.</i> (67.8–62.4)				<i>n.s.</i> (2.5–2.3)		
Marketable								
0_N	364.5	372.0	303.4	346.6	16.5	15.7	11.5	14.5
100_N	399.5	435.3	391.1	408.6	19.8	18.2	14.0	17.3
200_N	421.3	441.5	376.9	413.2	20.2	18.1	14.6	17.6
<i>o.m.</i>	395.1	416.3	357.2		18.8	17.3	13.4	
F-test								
N_rates		<i>n.s.</i> (25.7)				<i>n.s.</i> (1.0)		
Sh		<i>n.s.</i> (31.9)				** (1.3)		
N_rates × Sh		<i>n.s.</i> (55.2–51.9)				<i>n.s.</i> (2.2–2.0)		
Unmarketable								
0_N	116.4	85.1	58.9	86.8	5.9	4.0	2.4	4.1
100_N	149.5	148.4	52.7	116.8	8.1	6.9	2.1	5.7
200_N	141.8	135.1	64.1	113.7	8.4	6.8	2.4	5.9
<i>o.m.</i>	135.9	122.9	58.6		7.5	5.9	2.3	
F-test								
N_rates		** (5.4)				* (0.5)		
Sh		** (14.1)				** (0.6)		
N_rates × Sh		<i>n.s.</i> (24.3–20.6)				<i>n.s.</i> (1.0–0.9)		

^a analysis of variance (ANOVA) at 5% level of probability: N fertilization rates (N_rates); Sh levels (Sh). *o.m.*: overall means. * $p < 0.05$; ** $p < 0.01$; *n.s.* = not significant. In brackets: standard error of differences between means (SED); for the interaction, SED1 is for comparing means at different levels of Sh and the same level of N_rates; SED 2 is for comparing all other means.

Shading with black nets significantly reduced total yield and fruit number per plant, as well as the number of unmarketable fruits and, consequently, the unmarketable yield (Table 1). In particular, regardless of N conditions, BN sharply decreased the number of sunscalded fruits (0.39 vs. 3.38 and 2.59 fruit plant⁻¹ for BN, Control and RN, respectively; Figure 1). Although BN significantly reduced the number of marketable fruits per plant (13.4 vs. 18.8 and 17.3 fruit plant⁻¹ for BN, Control and RN, respectively), marketable yield was not affected (357.2, 395.1 and 416.3 g plant⁻¹ for BN, Control and RN, respectively; Table 1), and a significant increase of MFW was also observed (21.2, 24.4 and 26.7 for Control, RN and BN, respectively; Table 2). Indeed, shade influenced fruit length, and BN recorded the highest values (Table 2). On the contrary, RN data on yield and yield components were not significantly different from Control (Table 1 and Figure 1).

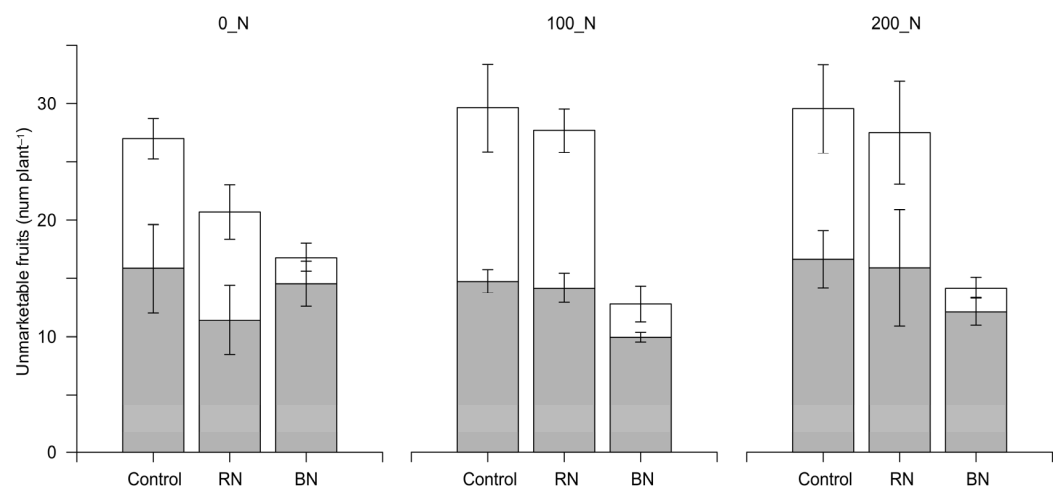


Figure 1. Number of unmarketable fruits (num plant⁻¹), as recorded in pepper plants subjected to different N fertilization rates (N_rates; 0_N: 0 kg N ha⁻¹; 100_N: 100 kg N ha⁻¹; 200_N: 200 kg N ha⁻¹) and shade levels (Sh; accomplished using: no cover, Control; red photosselective net, RN; black photosselective net, BN) in 2017. White chart: sunscalded fruits; gray chart: fruits characterized by other disorders. Data are means \pm standard errors, over Exp_1 and Exp_2 ($n = 6$).

Light availability seemed to be crucial for seed development: fruits yielded from BN plants showed a lower seed number, while N did not induce any effects (Table 2).

3.2. Fruit Pigments

The amounts of Chl (Chl A, Chl B and Chl TOT) and Car in pepper fruits were recorded from green, early ripening and red fruits and are reported in Figure 2 and Table 3. Shade significantly enhanced Chl A and Chl B contents (and, consequently, Chl TOT as the sum of Chl A and Chl B) in fruits, as recorded in the immature ones (Chl TOT: 947.3, 710.8 and 773.9 $\mu\text{g g}^{-1}$ for BN, Control and RN, respectively). Despite Car content in red fruits being promoted by full light availability, especially at the lowest N doses, the differences were always insignificant (Figure 2 and Table 3).

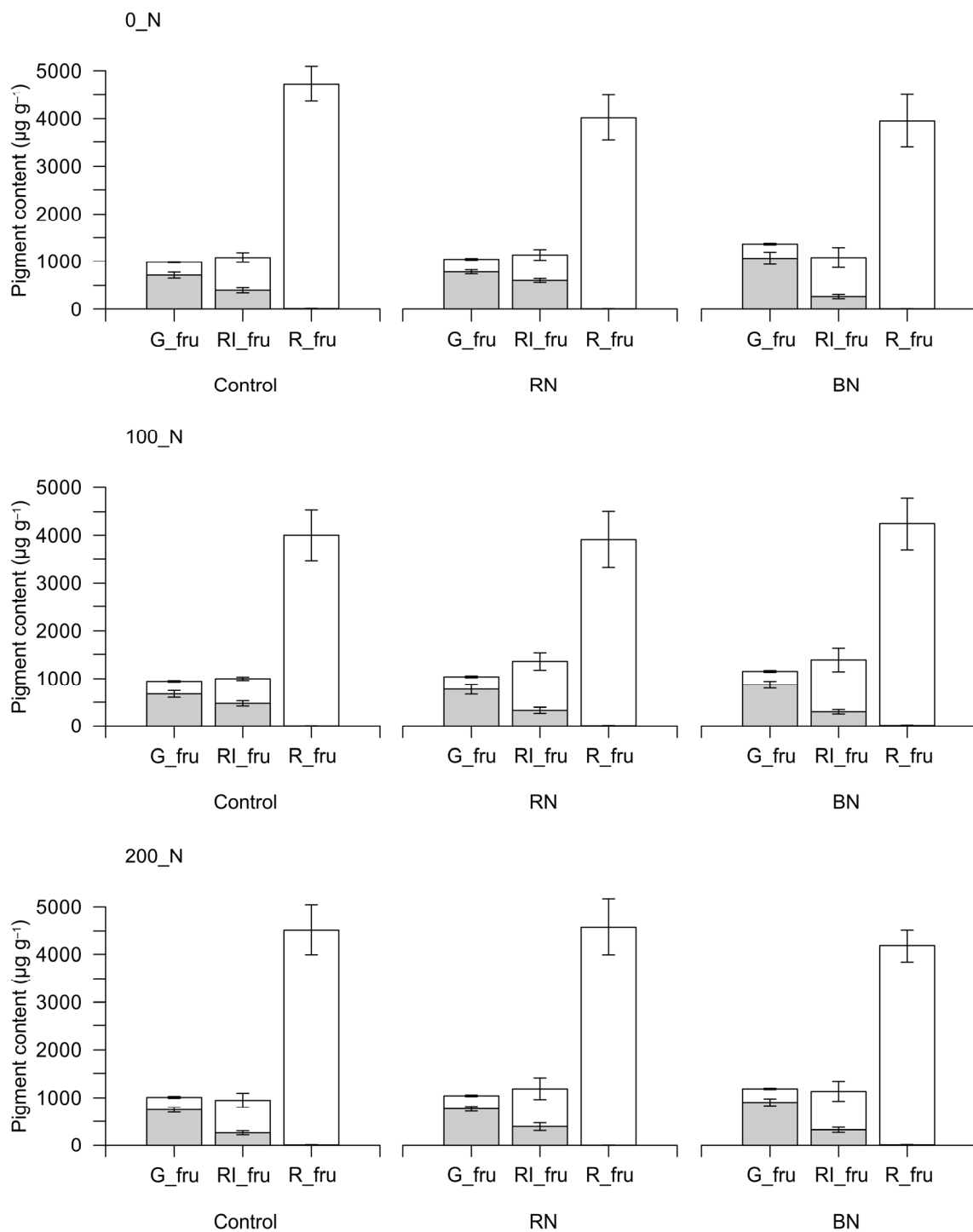


Figure 2. Pigment content ($\mu\text{g g}^{-1}$ dry weight), as determined in pepper fruits from plants subjected to different N fertilization rates (0_N: 0 kg N ha⁻¹; 100_N: 100 kg N ha⁻¹; 200_N: 200 kg N ha⁻¹) and shade levels (accomplished using: no cover, Control; red photosensitive net, RN; black photosensitive net, BN) at harvest (83 days after transplanting, DAT), in 2017. Light gray chart: total chlorophyll (Chl TOT) content (Chl A + Chl B); white chart: carotenoids (Car) content. G_fru: green fruits; RI_fru: early-ripening fruits; R_fru: red fruits. Data are means \pm standard errors, over Exp_1 and Exp_2 ($n = 6$).

Table 3. Overall means and F-test from the analysis of variance (ANOVA ^a) performed on pigment content [Chlorophyll (Chl) A, Chl B, Chl TOT (Chl A + Chl B) and Carotenoids (Car); $\mu\text{g g}^{-1}$ dry weight] data. Pigments were analyzed on pepper fruits from plant subjected to different N fertilization rates (N_rates; 0_N: 0 kg N ha⁻¹; 100_N: 100 kg N ha⁻¹; 200_N: 200 kg N ha⁻¹) and shade levels (Sh; accomplished using: no cover, Control; red photosensitive net, RN; black photosensitive net, BN) at harvest (83 days after transplanting, DAT), in 2017.

Overall Means	Chl A	Chl B	Chl TOT	Car
Green fruits				
0_N	629.6	221.1	850.7	280.5
100_N	571.7	202.5	774.2	271.1
200_N	594.0	213.1	807.1	271.9
Control	533.1	177.7	710.8	268.5
RN	572.6	201.3	773.9	267.7
BN	689.6	257.7	947.3	287.3
N_rates	n.s. (70.3)	n.s. (20.0)	n.s. (89.7)	n.s. (10.2)
Sh	** (37.8)	** (18.9)	** (55.8)	n.s. (12.9)
N_rates × Sh	n.s. (65.4–88.2)	n.s. (32.8–33.4)	n.s. (96.6–119.4)	n.s. (22.3–20.9)
Early-ripening fruits				
0_N	312.1	111.7	423.9	675.5
100_N	271.1	98.6	369.7	875.3
200_N	225.7	101.5	327.2	760.4
Control	283.0	97.6	380.6	628.2
RN	300.8	139.5	440.4	784.6
BN	225.1	74.8	299.9	898.4
N_rates	n.s. (60.9)	n.s. (9.4)	n.s. (67.6)	n.s. (200.3)
Sh	* (27.4)	** (14.3)	** (37.3)	n.s. (111.7)
N_rates × Sh	** (47.5–72.2)	* (24.8–22.4)	** (64.6–85.7)	n.s. (193.4–255.0)
Red fruits				
0_N	1.7	2.8	4.5	4228.3
100_N	3.1	4.1	7.2	4045.8
200_N	3.0	4.5	7.5	4418.6
Control	2.4	4.0	6.3	4409.4
RN	1.9	2.1	4.0	4167.2
BN	3.4	5.3	8.8	4116.0
N_rates	n.s. (1.9)	n.s. (2.2)	n.s. (3.9)	n.s. (335.2)
Sh	n.s. (1.8)	n.s. (3.8)	n.s. (5.0)	n.s. (416.8)
N_rates × Sh	n.s. (3.1–3.2)	n.s. (5.9–5.3)	n.s. (8.6–8.1)	n.s. (721.8–678.1)

^a analysis of variance (ANOVA) at 5% level of probability: N fertilization rates (N_rates); Sh levels (Sh). * $p < 0.05$; ** $p < 0.01$; n.s. = not significant. In brackets: standard error of differences between means (SED); for the interaction, SED1 is for comparing means at different levels of Sh and the same level of N_rates; SED 2 is for comparing all other means.

3.3. Phenolics and Vitamin C in Fruits

The dynamic of polyphenols accumulation (TPC and TFC) in pepper fruits was recorded at different ripeness fruit stages, from green to red, without separating the endocarp, mesocarp and epicarp (Table 4). The role of N nutrition emerges at the end of fruit ripening, when nitrogen stress (i.e., 0_N treatment) induced higher (although not significantly higher) TPC and TFC in red fruits (TPC: +4.4% on average; TFC: +8.7% on average (Table 4).

In general, a clear trend showing a decrease in TPC and TFC content, as shade changed, was observed. However, BN showed significantly lower TPC values with respect to Control and RN only in G_fru and RI_fru (G_fru: 989.6, 927.4 and 866.5 mg GAE 100 g⁻¹ for Control, RN and BN, respectively; RI_fru: 932.3, 921.4 and 847.3 mg GAE 100 g⁻¹ for Control, RN and BN, respectively); conversely, significant differences in terms of TFC were recorded only in R_fru (332.1, 293.7 and 271.6 mg RUE 100 g⁻¹ for Control, RN and BN, respectively; Table 4). The observed differences translated into a reduction in ABTS values in green and early-ripening fruits harvested from plants grown under the black net (Table 4).

Table 4. Total phenolic content [TPC, mg gallic acid equivalent (GAE) 100 g⁻¹ dry weight (DW)], total flavonoid content [TFC, mg rutin equivalents (RUE) 100 g⁻¹ DW], vitamin C [mg ascorbic acid 100 g⁻¹ dry weight (DW)] and Trolox-equivalent antioxidant capacity [with 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS)—mmol Trolox equivalents (TE) 100 g⁻¹] as determined in pepper fruits from plants subjected to different N fertilization rates (N_rates; 0_N: 0 kg N ha⁻¹; 100_N: 100 kg N ha⁻¹; 200_N: 200 kg N ha⁻¹) and shade levels (Sh; accomplished using: no cover, Control; red photosensitive net, RN; black photosensitive net, BN) at harvest (83 days after transplanting), in 2017^a.

Effects	TPC (mg GAE 100 g ⁻¹)				TFC (mg RUE 100 g ⁻¹)				Vit C (mg 100 g ⁻¹)				ABTS (mmol TE 100 g ⁻¹)			
	Control	RN	BN	<i>o.m.</i>	Control	RN	BN	<i>o.m.</i>	Control	RN	BN	<i>o.m.</i>	Control	RN	BN	<i>o.m.</i>
Green fruits																
0_N	968.3	958.6	887.9	938.3	388.2	347.4	337.5	357.7	126.6	129.81	86.7	114.4	1.79	1.48	1.52	1.60
100_N	981.2	871.2	866.7	906.4	383.5	328.3	369.4	360.4	123.7	88.72	91.1	101.2	1.88	1.61	1.62	1.70
200_N	1019.1	952.5	845.0	938.9	430.9	389.2	284.0	368.1	82.4	143.64	91.7	105.9	2.03	1.67	1.31	1.67
<i>o.m.</i>	989.6	927.4	866.5		400.9	355.0	330.3		110.9	120.7	89.8		1.90	1.59	1.48	
F-test																
<i>N_rates</i>		<i>n.s.</i> (58.6)				<i>n.s.</i> (45.4)				<i>n.s.</i> (22.3)				<i>n.s.</i> (0.17)		
<i>Sh</i>		* (39.1)				<i>n.s.</i> (42.5)				<i>n.s.</i> (13.5)				<i>n.s.</i> (0.18)		
<i>N_rates</i> × <i>Sh</i>		<i>n.s.</i> (67.7–80.6)				<i>n.s.</i> (73.7–75.4)				<i>n.s.</i> (23.4–29.4)				<i>n.s.</i> (0.32–0.31)		
Early-ripening fruits																
0_N	871.4	860.8	807.7	846.6	305.6	284.4	291.5	293.8	107.0	138.6	139.2	128.3	2.01	1.92	1.77	1.90
100_N	992.5	954.1	851.2	932.6	369.8	275.6	323.6	323.0	108.7	104.6	120.9	111.4	2.26	1.86	1.84	1.98
200_N	933.0	949.4	883.2	921.8	328.4	338.4	279.1	315.3	141.1	138.3	131.8	137.1	2.04	2.04	1.87	1.98
<i>o.m.</i>	932.3	921.4	847.3		334.6	299.5	298.1		118.9	127.2	130.7		2.10 A	1.94	1.82	
F-test																
<i>N_rates</i>		<i>n.s.</i> (29.2)				<i>n.s.</i> (16.8)				<i>n.s.</i> (11.5)				<i>n.s.</i> (0.12)		
<i>Sh</i>		** (24.6)				<i>n.s.</i> (19.3)				<i>n.s.</i> (10.1)				* (0.11)		
<i>N_rates</i> × <i>Sh</i>		<i>n.s.</i> (42.6–45.4)				<i>n.s.</i> (33.4–32.0)				<i>n.s.</i> (17.5–18.3)				<i>n.s.</i> (0.17–0.18)		
Red fruits																
0_N	1069.0	1006.1	1011.1	1028.7	358.8	310.3	278.5	315.8	160.6	156.6	160.6	159.2	2.54	2.39	2.28	2.40
100_N	944.6	948.6	1041.5	978.2	282.0	285.9	282.6	283.5	130.2	139.7	145.0	138.3	2.13	2.03	2.22	2.12
200_N	1036.9	957.3	985.1	993.1	355.7	284.8	253.7	298.1	151.9	139.4	147.7	146.3	2.26	2.11	2.20	2.19
<i>o.m.</i>	1016.8	970.7	1012.5		332.1	293.7	271.6		147.6	145.2	151.1		2.31	2.17	2.23	
F-test																
<i>N_rates</i>		<i>n.s.</i> (25.6)				<i>n.s.</i> (17.6)				<i>n.s.</i> (17.9)				<i>n.s.</i> (0.19)		
<i>Sh</i>		<i>n.s.</i> (30.8)				* (18.4)				<i>n.s.</i> (8.6)				<i>n.s.</i> (0.08)		
<i>N_rates</i> × <i>Sh</i>		<i>n.s.</i> (53.4–50.6)				<i>n.s.</i> (31.9–31.4)				<i>n.s.</i> (15.0–21.7)				<i>n.s.</i> (0.13–0.22)		

^a analysis of variance (ANOVA) at 5% level of probability: N fertilization rates (*N_rates*); Sh levels (*Sh*). * $p < 0.05$; ** $p < 0.01$; *n.s.* = not significant. In brackets: standard error of differences between means (SED); for the interaction, SED1 is for comparing means at different levels of Sh and same level of *N_rates*; SED 2 is for comparing all other means.

No significant differences in terms of Vit C content were observed in response to both N and radiation availability (Table 4).

Interestingly, significant differences in terms of phenolic acids were found, starting from the beginning of ripening, and are attributable exclusively to shade (Table 5).

Table 5. Overall means and F-test from the analysis of variance (ANOVA ^a) performed on phenolic acid content ($\mu\text{g g}^{-1}$ dry weight) data: caffeic acid (CaA), trans-ferulic acid (FA), 4-hydroxybenzoic acid (4-OHBA) and vanillic acid (VA). Phenolic acids were analyzed on pepper fruits from plant subjected to different N fertilization rates (N_rates; 0_N: 0 kg N ha⁻¹; 100_N: 100 kg N ha⁻¹; 200_N: 200 kg N ha⁻¹) and shade levels (Sh; accomplished using: no cover, Control; red photosensitive net, RN; black photosensitive net, BN) at harvest (83 days after transplanting, DAT), in 2017.

Overall Mean	CaA	FA	4-OHBA	VA
Green fruits				
0_N	6.83	6.82	11.30	14.35
100_N	6.75	6.39	10.34	13.58
200_N	7.20	7.03	12.44	13.93
Control	7.04	6.86	15.12	14.5
RN	6.85	6.88	11.21	13.4
BN	6.91	6.51	7.74	14.0
N_rates	<i>n.s.</i> (0.52)	<i>n.s.</i> (0.56)	<i>n.s.</i> (1.68)	<i>n.s.</i> (1.70)
Sh	<i>n.s.</i> (0.71)	<i>n.s.</i> (0.67)	** (1.86)	<i>n.s.</i> (1.59)
N_rates × Sh	<i>n.s.</i> (1.23–1.13)	<i>n.s.</i> (1.16–1.10)	<i>n.s.</i> (3.23–3.12)	<i>n.s.</i> (2.76–2.82)
Early-ripening fruits				
0_N	6.20	10.66	11.50	12.95
100_N	6.48	11.30	12.55	17.41
200_N	6.65	11.21	13.31	13.30
Control	9.13	17.36	16.81	17.99
RN	5.66	9.13	12.40	14.69
BN	4.54	6.68	8.14	10.98
N_rates	<i>n.s.</i> (1.45)	<i>n.s.</i> (3.86)	<i>n.s.</i> (2.00)	<i>n.s.</i> (2.45)
Sh	** (1.07)	** (1.90)	** (1.22)	* (2.14)
N_rates × Sh	<i>n.s.</i> (1.86–2.10)	<i>n.s.</i> (3.30–4.70)	<i>n.s.</i> (2.12–2.65)	<i>n.s.</i> (3.71–3.90)
Red fruits				
0_N	6.19	11.32	10.89	17.77
100_N	6.55	16.19	9.64	19.54
200_N	6.07	11.94	9.64	18.86
Control	7.48	17.38	12.45	20.04
RN	6.39	14.78	10.93	20.71
BN	4.94	7.29	6.78	15.42
N_rates	<i>n.s.</i> (0.31)	<i>n.s.</i> (1.19)	<i>n.s.</i> (2.59)	<i>n.s.</i> (1.05)
Sh	** (0.46)	** (1.77)	** (1.49)	** (1.18)
N_rates × Sh	<i>n.s.</i> (0.80–0.72)	<i>n.s.</i> (3.07–2.77)	<i>n.s.</i> (2.58–3.33)	<i>n.s.</i> (2.05–1.97)

^a analysis of variance (ANOVA) at 5% level of probability: N fertilization rates (N_rates); Sh levels (Sh). * $p < 0.05$; ** $p < 0.01$; *n.s.* = not significant. In brackets: standard error of differences between means (SED); for the interaction, SED1 is for comparing means at different levels of Sh and the same level of N_rates; SED 2 is for comparing all other means.

In particular, CaA registered a reduction of 50.3 and 33.9% in RI_fru and R_fru, respectively, sampled from BN plants with respect to Control, as well as FA, whose values were 2.6- and 2.4-fold lower in BN rather than Control (Figure 3); no significant differences concerning unshaded Control were registered in RN treatments. Additionally, 4-OHBA significantly decreased in both early-ripening and red fruits as shading went on (RI_fru: 16.8, 12.4 and 8.1 $\mu\text{g g}^{-1}$ DW for Control, RN and BN, respectively; R_fru: 12.5, 10.9 and 6.8 $\mu\text{g g}^{-1}$ DW for Control, RN and BN, respectively; Figure 3). For vanillic acid, the greatest differences from Control treatments were recorded in the RI_fru, with values of 18.0, 14.7 and 11.0 $\mu\text{g g}^{-1}$ DW for Control, RN and BN, respectively (Figure 3).

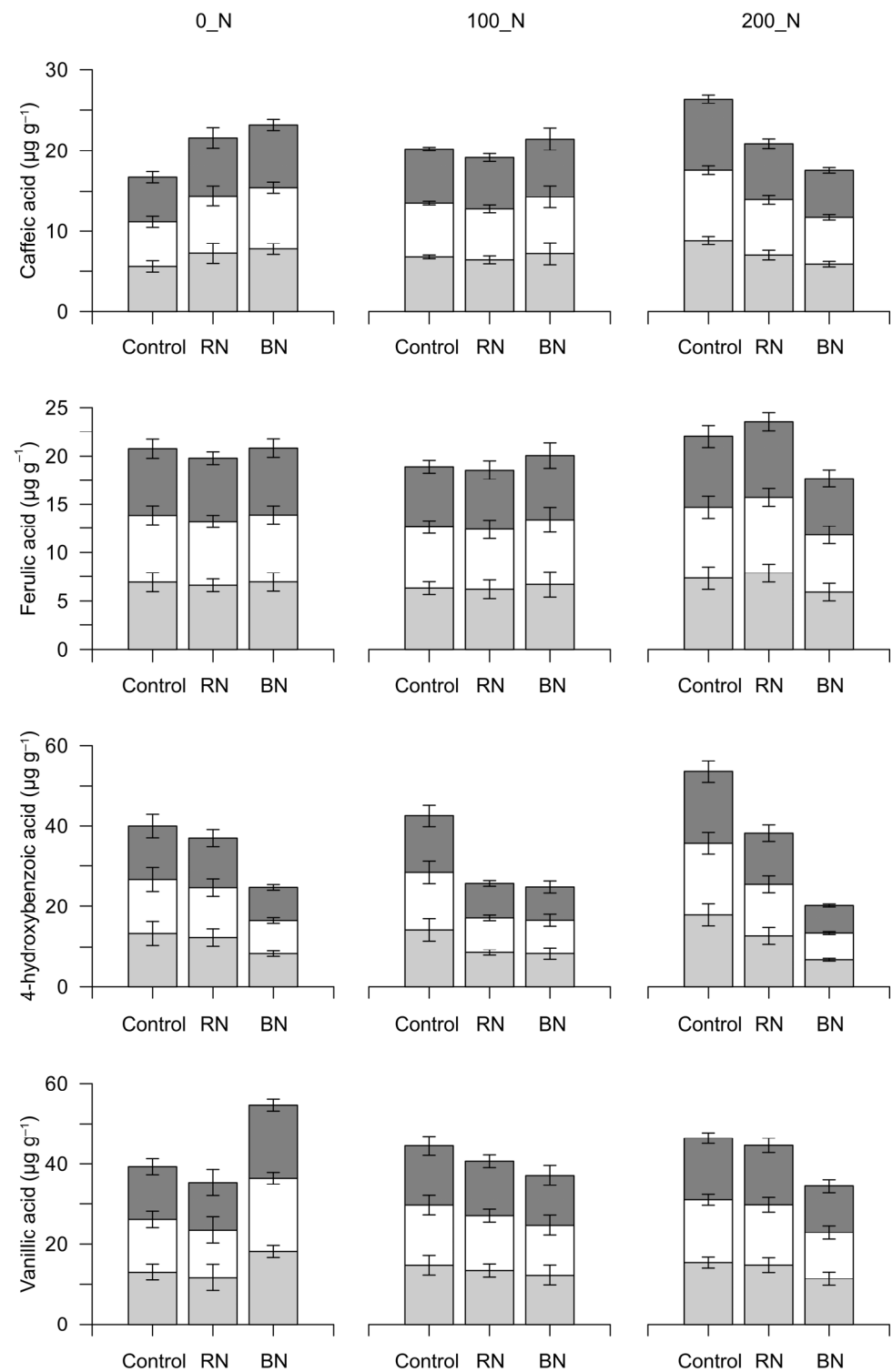


Figure 3. Phenolic acid content ($\mu\text{g g}^{-1}$ dry weight), as determined in pepper fruits from plants subjected to different N fertilization rates (first column, 0_N: 0 kg N ha $^{-1}$; second column, 100_N: 100 kg N ha $^{-1}$; third column, 200_N: 200 kg N ha $^{-1}$) and shade levels (accomplished using: no cover, Control; red photosensitive net, RN; black photosensitive net, BN) at harvest (83 days after transplanting, DAT), in 2017. Light gray chart: green fruits, G_fru; white chart: early-ripening fruits, RI_fru; deep gray chart: red fruits, R_fru. Data are means \pm standard errors, over Exp_1 and Exp_2 ($n = 6$).

4. Discussion

Local varieties, such as the Altino ecotype, are supposed to have traits favorable to sustainable and low-input crop management systems. This work brings some useful information on the adaptation of such landrace to modified environments in terms of nitrogen and light availability, also involving quality/nutraceutical traits. It is worth pointing out that, even if data on fruit quality and characterization are yet available for this ecotype [5,27], no investigation on yield and antioxidant responses as tolerance mechanisms to cope with the growing environment were accomplished.

Our results exerted a clear effect of both N and light availability—two essential growth factors—on crop yield, and especially a stronger influence of the quantity and quality of radiation transmitted to the canopy in terms of nutraceutical traits. We confirmed the primary role of N in pepper yield [28,29], as it was involved principally in flowering and fruit-setting and, consequently, in terms of fruit number, rather than fruit size. As evaluated in our previous work [8], the stimulating effect of N in crop vegetation growth translated into higher production, as has been extensively observed in various Pepper spp. genotypes [30–32]). We found that 100_N is confirmed to be the N fertilization rate to assure optimal yields [8].

On the other hand, our study demonstrates that, to decrease total yield, it is necessary to reduce light at the highest levels and that the application of photoselective nets can be usefully adjusted to obtain quality results without affecting crop performances. In addition, it should be remarked that the effects of photoselective net applications depend on: (i) the color, type of polymer, weave, porosity and knitting density of the nets, which influence the quantity and quality of the direct and diffuse radiation under the cover; (ii) species and specific light requirements. For instance, D'Egidio et al. [33] recorded a slightly enhanced yield of storage roots of red beet grown under a 50% shade net; additionally, Caruso et al. [34] found a yield increase—due to the higher number of produced fruits, under 30% on average—from light extinction. Higher shade levels (up to 79%) induced a markable reduction in the yield of rocket plants [35], while a 36.2% leaf dry weight constriction was observed in lettuce plants grown under 85% shade [36]. In our study, we found that, although a 40.4% PAR reduction lowered total fruit production in the Altino ecotype, the marketable yield remained unchanged. Such results found explication in the increase in L and MFW—although at the expense of fruit number, as well as in the reduction of the number of unmarketable and, especially, sunscalded fruits—as already observed in other studies [11,37]. The latter represents an enormous advantage for this local variety, characterized by fruit with an upright bearing and that are more sensitive to physiological disorders related to excessive light exposure during fruit development.

The reduction in total fruit number was due to the reduction of fruit set—also accompanied by a lower seed number per fruit—and to the increase of flower and/or bud abortion rates. Pepper normally exhibits high rates of organ abortion due to numerous factors, especially low light intensity [38,39]. Severe shade is involved in curtain pollen germination and/or tube growth but is more likely in extremely low light environments, and the abscission of nearby organs is associated with the very low assimilation rates of the leaves [40,41].

The availability of N and light was investigated due to their key regulatory role for genes and gene products related to photosynthetic pigments, including carotenoids. Our previous study indicates that high N availability stimulates Chl accumulation in Altino leaves [8] but not in immature fruits. Differently, shaded conditions (e.g., BN treatments; [8]) induce high Chl TOT (and Chl A and Chl B) both in leaves and green, immature fruits. High levels of light deficiency also seems to inhibit Car biosynthesis, leading to lower concentration in mature pepper fruits, confirming previous works [42,43]. It is known that chloroplasts are gradually replaced by chromoplasts during fruit ripening, translating into the replacement of chlorophylls with carotenoids, capsanthin and capsorubin, which give the pepper fruit a red color [44]. Our work highlights the role of light in increasing the accumulation of carotenoids and flavonoids (i.e., higher Car in R_fru in Control) and in

accelerating the degradation of chlorophyll in immature fruits (i.e., lower Chl in G_fru in Control) during ripening [45,46].

Moreover, N and light are also directly involved in secondary metabolism, and their modulation could produce significant modifications in the nutraceutical values of harvested fruits. del Amor et al. [47] found high levels of TPC under relatively limited N supply, while other authors highlighted differences in phytochemical content related to an appropriate ammonium–nitrate ratio [48] or to other agricultural practices (organic vs. conventional) [49] or the use of biostimulants [50]. We did not observe any significant difference in terms of total phenolics, flavonoids, Vit C and antioxidant activity as a consequence to N nutrition management, in any of the stages of fruit ripening, confirming previous works on both sweet and hot peppers [10,51]. Conversely, we observed a reduction of TPC and ABTS in green and early-ripened fruits under light scarcity; the effects were appreciable at the highest shading levels, with no apparent influence of the quality of the transmitted radiation (e.g., RN treatments with high levels of diffuse radiation, greater light absorption around 350–550 nm with respect to the other spectral bands; [8]). Our results match with literature bringing out the detrimental effects of the black net on some nutraceutical parameters, i.e., ascorbic acid, total phenolic content, flavonoid content and the antioxidant scavenging activity of sweet pepper [14]. Medium-low shading levels (RN) seems to also be contradictory towards phenolic acid accumulation (i.e., caffeic, trans-ferulic, 4-hydroxybenzoic and vanillic acids) in the mature fruits of the Altino ecotype, beginning early in ripening (RI_fru).

It is worth pointing out that we identified a higher number of PAs in Altino pepper extract but found relatively low concentrations of both PAs and TPC than those detected by Della Valle et al. [27]. It was probably related to the extraction methods and to the use of the fresh product rather than whole sundried peppers.

In conclusion, this study represents the first attempt to include agronomic practices within sustainable management systems of local varieties, such as the Altino ecotype, to induce the nutraceutical and/or quality amelioration of marketable yields adding value to such typical products of Abruzzo Region (Southern Italy). The crop copes with both low N and PAR environments through a modification of yield and fruit quality. Low-light availability from black net coverage assure less fruit waste, leading to longer and heavier fruits, although with worse nutraceutical traits. The amount of photosynthetically active radiation necessary to assure adequate quality performance should range between 874 and 670 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Control and RN, respectively). Further studies, involving nets of different colors (i.e., pearl nets), could find a justification based on the limited effect of RN on all the investigated variables, as well as on the literature results encouraging the use of photoselective covers thanks to the positive effects on the harvest and post-harvest of many horticultural crops, including sweet pepper.

Nutrition is confirmed to be essential to obtaining adequate yields in pepper—with 100 kg N ha⁻¹ being the rate to reach adequate production standards—and further efforts should be directed to the in-depth study of fertilization rates and forms (both for macro and micronutrients), including biological formulations, that could be suitable for this pepper genotype (i.e., local variety, lack of a formal crop improvement).

Lastly, two cycles of phenotypic selection were enough to characterize and stabilize the Altino ecotype for its uniformity (please refer to Stagnari et al. [8] for further explanations), as confirmed by the insignificant differences that emerged between the two selected genotypes used in the two experiments.

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