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Pre-Harvest Bagging of Table Grapes Reduces Accumulations of Agrochemical Residues and Increases Fruit Quality

Leonardo Paul Luca [1,†], Francesco Scollo [1,†], Gaetano Distefano [1,*], Filippo Ferlito [2], Stefania Bennici [1], Ilaria Inzirillo [1], Alessandra Gentile [1], Stefano La Malfa [1] and Elisabetta Nicolosi [1]

1 Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Valdisavoia 5, 95123 Catania, Italy; leonardo.luca@phd.unict.it (L.P.L.); fscollo@unict.it (F.S.); stefania.bennici@unict.it (S.B.); alessandra.gentile@unict.it (A.G.); stefano.lamalfa@unict.it (S.L.M.); elisabetta.nicolosi@unict.it (E.N.)
2 Council for Agricultural Research and Economics, Research Centre for Olive, Fruit and Citrus Crops, Corso Savoia 190, 95024 Acireale, Italy; filippo.ferlito@crea.gov.it
* Correspondence: gaetano.distefano@unict.it; Tel.: +39-095-4783407
† These authors equally contribute to the work.

Abstract: Since ancient times, table grapes (*Vitis vinifera* L.) have been one of the most important fruit crops from the standpoint both of the producer (regional economics) and the consumer (healthy eating). In recent decades, much effort has been devoted to the development of this crop in order to improve fruit quality and yield; however, these advances have also entailed considerable increases in the use of agrochemicals. Unfortunately, as is now coming to light, the increased agrochemical use has had deleterious effects on the environment and has also had significant negative effects on human health and wellbeing. Our research investigates the effects of pre-harvest fruit bagging on key fruit quality traits and also on the accumulation of agrochemical residues in the fruit. Two prevalent white table grape cultivars were used, 'Italia' (late ripening) and 'Vittoria' (early ripening). They were bagged with three different materials: (1) paper, (2) parchment (a cellulose-based material), and (3) a non-woven fabric (felted polypropylene fibers). The bags were placed on grape clusters at phenological state BBCH 75 until harvest, and the bagged clusters were then compared with the unbagged control clusters. Qualitative traits and agrochemical residuals were assessed at harvest for two consecutive years, 2021 and 2022). The results show that the parchment protection bags positively affected some key fruit quality traits, with bigger and better-colored berries than the unbagged controls. Compared with the unbagged controls, all bagging treatments greatly reduced the levels of agrochemical residues, analyzed using GC-MS/MS and HPLC-MS/MS. For cv. 'Italia', in 2021 residues fell from 0.733 mg/kg (unbagged control) to 0.006 mg/kg (bagged), and in 2022 from 0.201 mg/kg (unbagged control) to 0.008 mg/kg (bagged); for cv. 'Vittoria', in 2021 residues fell from 0.201 mg/kg (unbagged control) to 0.008 mg/kg (bagged), and in 2022 from 0.077 mg/kg (unbagged control) to 0.046 mg/kg (bagged). The study shows the benefits of pre-harvest fruit bagging on grape berry quality and underscores the pivotal role bags can play in minimizing agrochemical residue accumulations on the fruit. The study marks the taking of a crucial step towards more sustainable and safer practices in the table grape production industry.

Keywords: *Vitis vinifera*; fruit texture; skin color; agrochemical residues; morphological traits

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1. Introduction

In many parts of the world, table grape (*Vitis vinifera* L.) production accounts for an essential source of income for growers, while the fruit accounts for a significant portion of the fresh fruit market. According to the Food and Agriculture Organization of the United Nations (FAO), table grapes are one of the most valuable fruit crops in international trade, with global production estimated at around 27.3 million metric tons in 2018 [1]. The cultivation of grapes has a very long history, with grapes being one of the first species domesticated by humankind [2]. The close and lasting interconnection between grapes and people and their high economic value has resulted in continuing efforts to improve both the plant's agronomic traits and the fruit's qualitative traits [3]. Over the last hundred years or so, protection of our agricultural crops against biotic stressors has in the main been achieved through the use of agrochemicals. The use of these has now become the normal way to limit the yield/quality losses caused by weeds, pests and diseases [4]. Nowadays, however, we are coming to realize that agrochemical use, while still widespread and essential for high-efficiency production, is also associated with significant negative effects in the environment and also with negative effects on human health for the consumers [5–7]. Thus, exposure to agrochemicals or to their residues in food is associated with a wide range of medical diseases, including cancer, diabetes mellitus, respiratory illnesses, neurological disorders and various reproductive (sexual/genital) syndromes [7,8]. Notwithstanding the now strict regulations around agrochemical use, the safety limits imposed probably still underestimate the health risks involved in their use [9]. The continuing rise in agrochemical use worldwide is now recognized to be causing alarming levels of ecosystem pollution and human food poisoning [10]. This has prompted the development of new strategies to help limit fruit and vegetable contamination by agrochemical residues, as part of the so-called 'good agricultural practices' (GAPs) [11]. For table grapes, genetic improvement strategies have till now focused on improving fruit quality traits; however, interest is now refocusing onto the selection of pest- and disease-resistant genotypes that allow reductions in the use of the range of agrochemical plant protection products [12–14]. Alternative plant protection strategies are also being developed that use physical rather than chemical means. Among these is the bagging of fruits during their last growth stages before harvest [15], the aim being to limit crop losses due to abiotic and biotic factors, including abiotic (e.g., mechanical damage) and biotic (e.g., insect pests, birds and microbial pathogens). In the United States, China, and Australia, pre-harvest bagging is now used widely to improve fruit quality in many fruit crop species, including apple, pear, grape and peach. In particular, it has been shown that bagging improves fruit skin color and also reduces a number of physiological and pathological disorders [16,17]. The bag creates a microenvironment for the developing fruit which inhibits chlorophyll production and stimulates anthocyanin accumulation [18]. Fruit bagging is also an effective way of reducing accumulations of agrochemical residues as the bag material itself presents a physical barrier between the outside world (whole-canopy sprays) and the fruit [19–21]. Of course, the benefits of bagging (reduced chemical residues and improved fruit quality) depend on numerous variables, including: (i) the bag material, (ii) the timing of the bagging, (iii) the external environmental conditions, and (iv) the phenological stages during which the bags are applied [22–26]. Our study explores the effects of pre-harvest fruit bagging of two white table grape *V. vinifera* cultivars, the early-ripening 'Vittoria' and the late-ripening 'Italia'. We use bags of three different materials: (1) paper, (2) a cellulose-based material (parchment) and (3) a non-woven polypropylene material. Bags were placed on the clusters from phenological stage BBCH 75 through to harvest. Control clusters were without bags. The influences of the different bags on fruit quality traits and on the levels of agrochemical residues on the berries at commercial harvest were evaluated.

2. Materials and Methods

2.1. Plant Material

The experiment was carried out over two growing seasons, 2021 and 2022, on two commercial white-berry table grape cultivars, 'Italia' and 'Vittoria' (the vines being in their fourth and fifth production years, respectively). Both grape cultivars were grafted on 140 Ru rootstocks. The vines are located in Mazzarrone (Catania, Sicily, Italy 37.0831 N, 14.5995 E, 270 m above sea level). The local table grape growers adopted the I.G.P. "UVA DI MAZZARRONE" procedural guidelines and the training system was the *Tendone* horizontal trellis (a uniform horizontal-plane canopy management at about 2 m above the ground. Each vine was pruned to two branches, with two fruit-bearing shoots per branch.

Plants were spaced 2.8 × 2.8 m apart, and each season 3000 m^3/ha of drip irrigation was provided (Reg. EEC No 2081/92, Ministero Delle Politiche Agricole, 6 November 2000, Italy).

2.2. Bagging Treatments

Three bags were evaluated, each of a different material, with unbagged controls. Bags were: (1) parchment (cellulose-based material), (2) paper, and (3) non-woven (composed of polypropylene fibers). Bags were all of the same dimensions (30 × 40 cm) and were applied approximately 120 days after anthesis at BBCH fruit development stage 75. The control clusters were not bagged (Figure S1). A randomized complete block design was used, consisting of three replicates per treatment (twelve vines per cultivar). Fifteen bunches per treatment were harvested, prior to commercial ripening and at commercial ripening, based on the evaluation of the berry's skin, which should be elastic and not plastic, and on-field measurements of berry sugar content, deemed acceptable from 15°Brix upwards (PAL-BX/ACID Meter, Atago Co. LTD, Tokyo, Japan). The 'Vittoria' was harvested in the second week of August 2021 and in the third week of August 2022, while the 'Italia' was harvested in the first week of October 2021 and the last week of October 2022. The bags covered the bunches for 90 days in 'Vittoria' and for 120 days in 'Italia'.

2.3. Agrochemical Treatments

Agrochemical treatments were carried out according to need, in compliance with the regulations in force at the time. Table 1 reports all the treatments, classified by the commercial name of the product, the active ingredients, and the target organisms for which they were employed. The mechanism of action of the various active substances is also reported: (i) S (systemic): the molecules are absorbed by the plant and translocated via the phloem and xylem systems; (ii) L.S. (loco-systemic): cytotropic compounds with possible translaminar translocation; and (iii) C (contact): the active substances exert their action without penetrating the plant tissues. The plants were treated using a trailing sprayer (Agrimaster 696 ultra, Bologna, Italy) and the plant protection products were diluted according to the manufacturer's instructions, in 600 L of water for each hectare treated. Finally, we report the pre-harvest interval (PHI), the shortest possible interval from the last pesticide treatment to the harvest. All data refer to the Italian ministerial labels and the information provided by the manufacturers.

Table 1. List of agrochemicals used after applying bagging treatments during harvest seasons 2021 and 2022.

Commercial Name	Active Compound	Target Organism	Action Modes	PHI (Days)	Rate per Hectare (600 L of Water)	Italia 2021	Vittoria 2021	Italia 2022	Vittoria 2022
Vertimec EC	Abamectin (1.84%)	Insecticide Acaricide Nematocide	L.S.	4	1 L	16 July	16 July	Not used	Not used
Vitene Ultra SC	Cymoxanil (20.83%)	*Plasmopara viticola*	S.	28	1 L	Not used	Not used	13 August 15 September 29 September	Not used
Topas 10 EC	Penconazole (10.1%)	Fungicide	S.	14	0.6 L	16 June 30 June	16 June 30 June	Not used	Not used
Epik SL	Acetamiprid (46.7%)	Insecticide Acaricide	S.	14	2 L	12 June 28 July	12 June 28 July	13 August 2 September	13 August 2 September
Karathane Star	Meptyldinocap (35.71%)	*Erysiphe necator*	C.	21	0.5 L	12 June 30 June	12 June 30 June	Not used	Not used
Reboot	Cymoxanil (33%) Zoxamide (33%)	*Plasmopara viticola*	L.S.	28	0.5 kg	12 June	12 June	21 August	Not used

Table 1. *Cont.*

Commercial Name	Active Compound	Target Organism	Action Modes	PHI (Days)	Rate per Hectare (600 L of Water)	Italia 2021	Vittoria 2021	Italia 2022	Vittoria 2022
Radiant pro	Spinetoram (12%)	Insetticide	C.	7	0.25 L	30 June	30 June	13 July 15 September	Not used
Cymbal	Cymoxanil (44%)	*Plasmopara viticola*	L.S.	28	0.5 kg	30 June 16 July 28 July 12 August	30 June 16 July 28 July 12 August	16 June 13 July	16 June
Prosper 300 CS	Spiroxamine (30.6%)	*Erysiphe necator*	S.	35	1.2 L	16 July 28 July 12 August	16 July 28 July 12 August	16 June 29 June 13 July 28 July 21 August 15 September 29 September	Not used
Topas 200EW	Penconazole (19%)	*Erysiphe necator*	S.	14	0.25 L	28 July 12 August	28 July 12 August	16 June 29 June 13 July 28 July 13 August 21 August 2 September 15 September	16 June 29 June 21 August
CoStar WG	*Bacillus thuringiensis* (18%)	*Lobesia botrana* *Eupoecilia ambiguella* *Cryptoblabes gnidiella*	C.	/	0.75 kg	12 August	12 August	16 June 29 June 28 July 21 August 29 September	16 June 29 June 21 August
Enviromite FL	Bifenazate (43.55%)	Acaricide	C.	14	0.35 L	Not used	Not used	22 April 8 June 28 July	Not used
Tiovit Jet	Sulfur (80%)	*Erysiphe necator*	C.	/	2 kg	Not used	Not used	22 April	Not used
Kop-Twin	Copper Hydroxide (8.9%) Tribasic Copper Sulphate (13.3%)	*Plasmopara viticola* *Guignardia bidwellii*	C.	21	1 L	Not used	Not used	2 September	Not used
Sercadis SC	Fluxapyroxad (26.6%)	*Erysiphe necator*	S.	35	0.15 L	Not used	Not used	2 September	Not used
Switch	Cyprodinil (37.5%) Fludioxonil (25%)	*Botrytis cinerea* *Aspergillus* spp. *Penicillium* spp.	S.	7	1 kg	Not used	Not used	15 September	Not used

List of agrochemicals employed after the application of the bags. The dates of the treatments are reported as day/month (e.g., 15/09 is 15 September). PHI (Pre-Harvest Interval, the minimum time between the last pesticide application and when the crop can be harvested). Mode of action abbreviation: S. (Systemic), C. (Contact), L.S. (Loco-Systemic). All data reported refer to the product labels.

2.4. Morphological, Colorimetric and Texture Analyses

At harvest, the weights of the fifteen bunches per treatment, were assessed with the length, weight and number of both the berries and the shot berries. Additionally, fifteen berries per bunch were randomly selected to acquire data for the berry skins with a Minolta CR410 colorimeter (Minolta Camera Co., Osaka, Japan). The descriptive color coordinates adopted were L* (from black to white), a* (from green to red) and b* (from blue to yellow). Finally, the CIRG2 (Color Index Red Grape 2) index was adopted to enable the distinction between cultivars that belong to the same "green-yellow" group [27]. This index uses the CIELab coordinates H (Hue angle), C (Chroma) and L (Light intensity) as factors according to the following Formula (1):

$$\text{CIRG2} = (180 - \text{H})/(\text{L} \times \text{C}) \quad (1)$$

To examine the mechanical properties of the berries, texture analysis was carried out using TA.XT-Texture Analyzer (Stable Micro System, Godalming, UK). Two probes were used, the P2/N to perform a puncture test and the SQ11 probe to perform a compression test on two sets of berries. The puncture test was conducted at a speed of 1 mm/sec and a compression of 5 mm; the puncture test enables assessment of the mechanical properties of the berry skin. The compression test involved a single compression of the entire berry, with a deformation of 25% and a test speed of 1.5 mm/sec [28].

2.5. Chemical Analyses

A digital refractometer (RX-5000, Atago Co. LTD, Tokyo, Japan) was used to measure the total soluble solid content (TSS) in each sample of juice made from 10 berries, and expressed as °Brix. The titratable acidity (TA) of the juice was assessed employing potentiometric titration with 0.1 N NaOH up to a pH of 8.1, and is represented as mg/L of tartaric acid equivalent per 100 mL. By using the Folin–Ciocalteu reagent (FCR) assay [29], the total phenolic content of the fruit was determined and is represented as mg of gallic acid equivalent (GAE) per 100 g of berries. To inactivate polyphenol oxidases, 50 g of berries were milled in liquid N_2 and then collected in a flask with 100 mL water-methanol (2:8, v:v) and 2 mmol sodium fluoride. After centrifugation, the supernatant was withdrawn, and 1 mL was mixed with 5 mL of commercial FCR reagent (previously diluted with water 1:10 v:v) and 4 mL of 7.5% sodium carbonate. The mixture was stirred for two hours at room temperature, avoiding exposure to light. The absorbance of the resulting blue solution was measured at 740 nm using a spectrophotometer (UV-Vis Cary 100 Scan model, Varian Inc., Palo Alto, CA, USA). The pH value of the grape juice was measured using a Mettler DL25 pH meter (Mettler-Toledo International Inc., Columbus, OH, USA).

2.6. Agrochemical Residual Analyses

The assay for agrochemical residual detection was carried out in accordance with the EFSA (European Food Safety Agency) guidelines by applying the QuEchERS method UNI EN 1562:2018 [30]. The technique first required an extraction/separation procedure with acetonitrile. Fruits were first homogenized (Blixer 4 V.V, Robot Coupe, Vincennes, France) and 10 g were collected. Then 100 µL of standard (Mix 5 mg/L of triphenyl phosphate and 5 mg/L of polychlorinated biphenyl No 18) and 10 mL of acetonitrile were added. The tubes were mixed for 1.5 min at 1500 rpm (Geno/Grinder 2010, SPEX Sample prep, Metuchen, NJ, USA). The extraction salts (4 g $MgSO_4$ and 1 g NaCl) were added to the tubes, which were agitated again for 1.5 min at 1500 rpm, and centrifuged at 3000× g rpm for 5 min. Then 6 mL of supernatant (acetonitrile phase) was sampled, and the purification was carried out using a kit as indicated by the manufacturer (dSPE Purification Kit, QuE-Lab, Bari, Italy). The tubes were agitated for 1.5 min and centrifuged at 3000× g rpm for 5 min. The supernatant was collected and filtered at 0.45 µm with a PTFE (polytetrafluoroethylene) filter (Sigma-Aldrich, St. Louis, MI, USA). For GC-MS/MS (Gas Chromatography tandem Mass Spectrometry) analyses 500 µL of the extract was combined with 20 µL of analyte protectant (5 mg/mL sorbitol, 10 mg/mL gluconic-D-lactone, 5 mg/mL shikimic acid, 0.2 g/mL 3-ethoxy-1,2-propanol) and 25 µL of polychlorinated biphenyl No 18. The experiments were performed on a GC-MS/MS, equipped with a triple quadrupole mass analyzer (TSQ 9610, ThermoFisher Scientific, Waltham, MA, USA). For liquid chromatography analyses 500 µL of the extract was mixed with 500 µL of solution A (containing H_2O, HCOOH 0.05%, $HCOONH_4$ 2 mM) plus 500 µL of solution B (containing CH_3OH, HCOOH 0.05%, $HCOONH_4$ 2 mM). Liquid chromatographic analyses were conducted using an HPLC-MS/MS (High Performance Liquid Chromatography tandem Mass Spectrometer) with a triple quadrupole mass analyzer (TSQ Quantum Access MAX, ThermoFisher Scientific, Waltham, MA, USA). The quantity of analyte (C_{Pest}) was calculated using the TraceFinder software version 5.1 SP1 (ThermoFisher Scientific, Waltham, MA, USA) from the intercept (b) and gradient (a) parameters defined by the calibration line, analyzed in the same batch of samples, using the following Formulas (2 and 3):

$$C_{Pest} = (\ Area_{Pest}/Area_{ISTD}) - (a/b) \text{ for the GC-MS/MS} \tag{2}$$

$$C_{Pest} = (Area_{Pest} - a)/b \text{ for the HPLC-MS/MS} \quad (3)$$

The final concentration of the compound investigated in the sample was calculated using the following Formula (4):

$$C_{Pest} = C_{Pest}\ (ug/L) \times V_{tot}\ \text{acetonitrile (L)/Sample weight (g)} \quad (4)$$

The concentrations of the agrochemical residuals detected were expressed in mg/kg. Only values above the LOD and LOQ (Limit Of Detection, Limit Of Quantification) are reported, and calculated for each active compound based on the instrument's performance.

2.7. Statistical Analyses

One-way ANOVA tests were conducted for the two cultivars. In addition, a principal components analysis of averaged and normalized data of qualitative variables was performed. Furthermore, a correlation study was conducted using Pearson's *r* test. A previous validation of the normal distribution of the data utilized was conducted using the Shapiro–Wilk test, considering both cultivars together. All statistical analyses and plots were performed using R software (version 4.2.3, 15 March 2023, R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Berry Quality Traits

Statistically significant differences were not found for the effects of the treatments on bunch weight, number of berries per bunch, or the number of shot berries per bunch for either cultivar (Table 2). However, significant differences (p-value ≤ 0.01) were observed for single-berry measurements (weight, width and length). Notably, both cultivars treated with parchment bags showed significantly higher weight and length values compared to the control group; 'Italia' 8.25 g and 26.2 mm long and 'Vittoria'11.5 g and 32.2 mm long. A reduction in berry width was observed in 'Italia', with 19.9 mm for the paper bag treatment and 20.9 mm for the non-woven bag treatment. The same trend was also observed for 'Vittoria', where width decreased significantly compared with the control for both the paper, 21.4 mm, and non-woven treatments, 21.5. These values were not significantly different from one another (p-value ≤ 0.01). Other treatments showed either no effect or only minor effects compared with the controls (Table 2).

Table 2. Results of morphological analyses after application of bagging treatments (control, paper, parchment and non-woven) on grape clusters.

Cultivar	Treatment	Bunch Weight [g]	Berry Weight [g]	Berry Length [mm]	Berry Width [mm]	Berries Number	Shot Berries Number
Italia	Control	894.26 ± 241.54	7.82 ^{b} ± 1.52	25.5 ^{b} ± 2.2	21.6 ^{a} ± 1.63	159.4 ± 42.79	30.5 ± 18.4
	Non-woven	949.45 ± 259.73	7.64 ^{b} ± 1.17	25.7 ^{ab} ± 1.85	20.9 ^{b} ± 1.59	149.5 ± 41.83	40.4 ± 40.9
	Paper	975.2 ± 250.94	6.65 ^{c} ± 1.45	25.2 ^{b} ± 2.07	19.9 ^{c} ± 1.72	161.6 ± 37.02	41.4 ± 30.5
	Parchment	1067.5 ± 251.02	8.25 ^{a} ± 1.44	26.2 ^{a} ± 2.6	21.6 ^{a} ± 1.84	155.3 ± 46.30	41.4 ± 37.2
	Significance	N.S.	***	***	***	N.S.	N.S.
Vittoria	Control	908.35 ± 244	10.6 ^{b} ± 2.79	30 ^{b} ± 3.33	22.7 ^{a} ± 2.32	101.5 ± 27.1	7.45 ± 8.65
	Non-woven	938.7 ± 167	9.58 ^{c} ± 1.86	28.2 ^{c} ± 2.74	21.4 ^{b} ± 1.74	117.3 ± 27.2	13.8 ± 20.7
	Paper	972.7 ± 237	10.1 ^{bc} ± 2.25	30.1 ^{b} ± 3.08	21.5 ^{b} ± 2.07	120.9 ± 30.5	11.4 ± 11.4
	Parchment	946.75 ± 144	11.5 ^{a} ± 3.41	31.2 ^{a} ± 4.04	22.7 ^{a} ± 2.57	98.8 ± 22.9	10.8 ± 16.1
	Significance	N.S.	***	***	***	N.S.	N.S.

Data are expressed as means ± standard deviations. CIRG2 (Color Index Red Grape 2). An ANOVA one-way analysis was carried out combined with a Tukey's honest significant difference (HSD) test to generate compact letters for the different cultivars. Significance *** (p-value ≤ 0.01).

For the colorimetric analyses, the L* values were generally lower in the treated samples for both cultivars; in particular, the lowest value was for the paper bag treatment both for the 'Italia' (40.8) and for the 'Vittoria' (41.2) cultivar. The values of the a* coordinate (green-to-red) were also lower in both cultivars treated with the parchment bag, −3.44 for

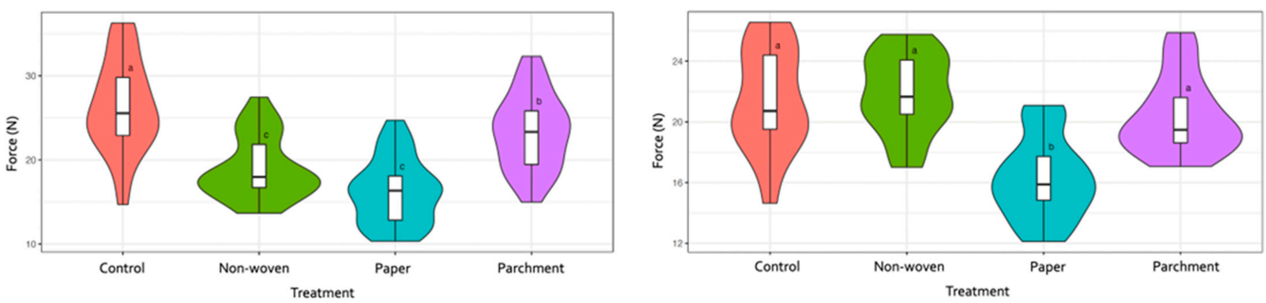
'Italia' and −6.88 for 'Vittoria', and for the paper bag treatment the value was −6.82, for the 'Vittoria' cultivar. Analysis of the b* (blue-to-yellow) coordinate revealed the parchment and paper treatments to be the lowest in both cultivars, 12.5 and 12.4 for 'Italia' and 13.1 and 13.7 for 'Vittoria', respectively. The CIRG2 results indicate that in both cultivars the controls were lower than the treatments, with 0.115 for 'Italia' and 0.092 for 'Vittoria'; the paper treatments were the highest, with 0.11 for 'Italia' and 0.146 for 'Vittoria', the latter also having the parchment treatment among the highest values, together with paper, with 0.147 (Table 3).

Table 3. Results of colorimetric analyses after application of bagging treatments (control, paper, parchment, and non-woven) on grape clusters.

Cultivar	Treatment	L*	a*	b*	CIRG2
Italia	Control	43.4 ^{a} ± 2.79	−4.56 ^{a} ± 2.09	14.9 ^{a} ± 3.47	0.115 ^{c} ± 0.03
	Non-woven	42 ^{b} ± 2.62	−3.64 ^{a} ± 1.62	13.1 ^{b} ± 2.72	0.135 ^{b} ± 0.03
	Paper	40.8 ^{c} ± 2.7	−3.51 ^{a} ± 1.5	12.5 ^{b} ± 2.4	0.146 ^{a} ± 0.03
	Parchment	41 ^{c} ± 2.71	−3.44 ^{b} ± 1.38	12.4 ^{b} ± 2.53	0.147 ^{a} ± 0.04
	Significance	***	***	***	***
Vittoria	Control	43.9 ^{a} ± 3.63	−7.38 ^{a} ± 1.38	14.3 ^{b} ± 2.45	0.092 ^{c} ± 0.02
	Non-woven	42.9 ^{b} ± 3.18	−7.25 ^{ab} ± 1.63	15.1 ^{a} ± 3.2	0.095 ^{bc} ± 0.02
	Paper	41.2 ^{b} ± 3.82	−6.82 ^{b} ± 1.92	13.1 ^{c} ± 2.83	0.11 ^{a} ± 0.03
	Parchment	42.5 ^{c} ± 2.79	−6.88 ^{b} ± 1.45	13.7 ^{bc} ± 2.26	0.10 ^{b} ± 0.02
	Significance	***	**	***	***

Data are expressed as means ± standard deviations. CIRG2 (Color Index Red Grape 2). An ANOVA one-way analysis was carried out combined with a Tukey's honest significant difference (HSD) test to generate compact letters for the different cultivars. Significance *** (p-value ≤ 0.01); ** (p-value ≤ 0.05).

Texture analyses using the puncture test revealed no significant differences within the treated groups in either cultivar, resulting in no treatment effects on the mechanical properties of the berry skin (p-value > 0.05). The mean values for the control were 4.38 N for the 'Vittoria' and 3.99 N for the 'Italia'. In contrast, a significant difference emerged from the compression tests; a lower compressive strength value was recorded for the paper bag treatment in both cultivars, with a mean strength of 16.2 N. As regards the other treatments, in 'Italia', the non-woven treatment was statistically equal to the paper treatment, with a mean value of 19 N; the parchment treatment, with a mean value of 23.3 N, had an intermediate effect, and the control had the highest value, with a mean of 26.3 N (p-value ≤ 0.01). In 'Vittoria', the non-woven treatment, with a mean of 21.9 N, and the parchment treatment, with a mean of 20.4 N, showed no significant differences from the control in each case, which had a mean of 21.5 N (p-value ≤ 0.01) (Figure 1).



(a) (b)

Figure 1. Boxplot of the compression force (N) after application of bagging treatments (control, paper, parchment, and non-woven) on grape clusters. In the left panel (**a**) are the 'Italia' treatments and in the right panel the 'Vittoria' treatments (**b**). An ANOVA one-way analysis was carried out, combined with a Tukey's honest significant difference (HSD) test to generate compact letters (p-value ≤ 0.01, for both cultivars).

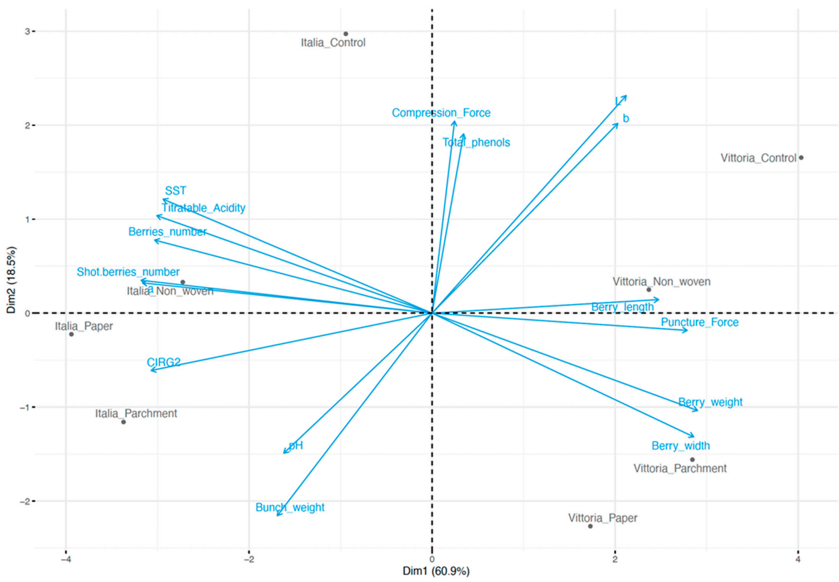
Chemical analyses of the fruits of the two cultivars showed no differences across treatments (bag materials) except for the total phenol concentration in 'Italia', where the paper bag showed the highest concentration (28.6 mg GAE/L) and the parchment bag the lowest (19.6 mg GAE/L)(p-value $\leq$ 0.05). There was a slight difference in pH for 'Vittoria', but this not practically significant (Table 4).

Table 4. Chemical analyses of grape clusters vs. bagging treatments (control, paper, parchment and non-woven).

Cultivar	Treatment	TSS [°Bx]	pH [pH]	Titratable Acidity [mg/L]	Total Phenols [mg GAE/L]
Italia	Control	18 ± 0.83	4.23 ± 0.15	3.73 ± 2.47	23.3^{ab} ± 4.77
	Non-woven	17.2 ± 4.11	4.3 ± 0.05	3.75 ± 2.55	24.3^{ab} ± 1.87
	Paper	17.7 ± 4.05	4.28 ± 0.03	3.73 ± 2.49	28.6^{a} ± 3.98
	Parchment	17.3 ± 4.03	4.35 ± 0.79	3.69 ± 2.52	19.6^{b} ± 3.33
	Significance	N.S.	N.S.	N.S.	**
Vittoria	Control	14.2 ± 0.32	4^{b} ± 0.11	3.97 ± 2.69	36.9 ± 9.93
	Non-woven	14.9 ± 2.32	4.3^{a} ± 0.12	3.79 ± 2.68	21.3 ± 6.65
	Paper	14.3 ± 0.11	4.21^{ab} ± 0.02	3.76 ± 2.55	18.1 ± 3.33
	Parchment	14.3 ± 0.49	4.35^{a} ± 0.19	3.61 ± 2.42	18 ± 4
	Significance	N.S.	**	N.S.	N.S.

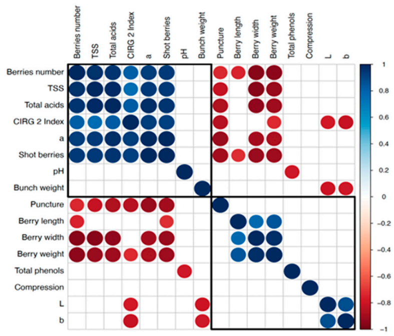
Data expressed as means ± standard deviations. TSS (Total Soluble Solid) expressed as Brix (°Bx). GAE (Gallic Acid Equivalent). An ANOVA one-way analysis was carried out combined with a Tukey's honest significant difference (HSD) test to generate compact letters for the different cultivars. Significance ** (p-value $\leq$ 0.05); N.S. (not significant, p-value > 0.05).

The PCA revealed treatment differences between the two cultivars. A large difference was observed between the two controls vs. the various treatments. Principal component 1 (Dim 1, 60.9% total variance) was related to berry morphological values. Colorimetric properties, expressed as L* and b*, together with bunch weight, dominated in principal component 2 (Dim 2, 18.5% total variance), displaying itself as the most influential component in determining the variance between control and treatments in both cultivars (Figure 2a). As expected, the correlation results (Figure 2b) show that all the highest correlation variables were those associated with the morphological analyses of the berries: positive correlations were found for berry length, berry width and berry weight, and these same variables were negatively correlated with berry number and shot berries. The berry-number and shot-berry-number variables correlated negatively with the puncture test values.



(a)

Figure 2. *Cont.*



(**b**)

Figure 2. The left panel (**a**) is represented as a bi-plot the principal component analyses of variables and individuals. The X axis shows the principal component 1 (Dim 1, which explains 60.9% variability) and the Y axis the principal component 2 (Dim 2, which explains 18.5% variability). The right panel (**b**) represents a correlation matrix, and the color gradient from red to blue via white is related to the correlation coefficient: dark blue is 1, white is 0, and dark red is −1.

3.2. Agrochemical Residue Detection

The agrochemical analysis results for 'Italia' are shown in Table 5. Here, for both years (2021 and 2022) the unbagged controls show significantly higher concentrations of agrochemical residues than the bagging treatments. The highest agrochemical residue concentrations were for the crop protectant 'Acetamiprid', with 0.33 mg/kg in the 2021 unbagged control and 0.161 mg/kg in 2022. Compared with the bagged fruit, in 2021 the unbagged 'Italia' fruit also showed significantly higher concentrations of Penconazole (0.14 mg/kg), Spiroxamine (0.14 mg/kg) and Zoxamide (0.078 mg/kg). The bagging treatments with the lowest concentrations of residues for 'Italia' were parchment in 2021 (0.006 mg/kg) and paper in 2022 (0.008 mg/kg). For cv. 'Vittoria' (Table 6), the results are broadly similar, with the unbagged controls having the highest total concentrations of residues, 0.206 mg/kg in 2021 and 0.077 mg/kg in 2022. For 'Vittoria', the highest agrochemical residue was for Acetamiprid in the unbagged control fruit with 0.19 mg/kg (2021) and 0.074 mg/kg (2022). The lowest total agrochemical residues were in 2021 for the paper bagged fruit (0.07 mg/kg) and in 2022 for the parchment (0.044 mg/kg) and the non-woven bagged fruit (0.046 mg/kg).

Table 5. Results of agrochemical analyses of the fruit of 'Italia' grapes in 2021 and 2022 for three bagging treatments (1) paper, (2) parchment, and (3) non-woven vs. an unbagged control.

Agrochemicals Detected	M.R.L	Italia 2021				Italia 2022			
(mg/kg)		Non-Woven	Paper	Parchment	Control	Non-Woven	Paper	Parchment	Control
Acetamiprid	0.5	0.014	0.005	N.D.	0.33	0.014	0.003	0.001	0.161
Ametoctradin	6	N.D.	N.D.	N.D.	0.008	N.D.	N.D.	N.D.	N.D.
Cymoxanil	0.05	0.006	0.007	0.006	0.006	N.D.	N.D.	N.D.	0.01
Fenhexamid	15	0.011	N.D.	N.D.	0.017	N.D.	N.D.	N.D.	N.D.
Meptyldinocap	1	N.D.	N.D.	N.D.	0.005	N.D.	N.D.	N.D.	N.D.
Metrafenone	7	N.D.	N.D.	N.D.	0.009	N.D.	N.D.	0.016	N.D.
Penconazole	0.5	0.026	0.022	N.D.	0.14	N.D.	0.001	N.D.	N.D.
Spinosad	0.5	N.D.	0.012	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Spiroxamine	0.6	0.01	N.D.	N.D.	0.14	0.009	0.0038	0.003	0.039
Zoxamide	5	0.005	N.D.	N.D.	0.078	N.D.	N.D.	N.D.	N.D.
Total		0.072	0.046	0.006	0.733	0.023	0.008	0.02	0.201

N.D. (Not detected). M.R.L. (Maximum Residue Levels) according to European Commission REGULATION (EU) 2019/88 of 18 January 2019—G.U.C.E.L. 22–24 January 2019.

Table 6. Results of agrochemical analyses of the fruit of 'Vittoria' grapes in 2021 and 2022 for three bagging treatments (1) paper, (2) parchment, and (3) non-woven vs. an unbagged control.

Agrochemicals Detected	M.R. L	Vittoria 2021				Vittoria 2022			
(mg/kg)		Non-Woven	Paper	Parchment	Control	Non-Woven	Paper	Parchment	Control
Acetamiprid	0.5	0.131	0.07	0.179	0.19	0.046	0.068	0.044	0.074
Cymoxanil	0.05	N.D.	N.D.	N.D.	0.002	N.D.	N.D.	N.D.	0.001
Metrafenone	7	N.D.	N.D.	N.D.	0.012	N.D.	N.D.	N.D.	N.D.
Penconazole	0.5	N.D.	N.D.	N.D.	N.D.	0.02	N.D.	0.001	N.D.
Spiroxamine	0.6	N.D.	N.D.	N.D.	N.D.	0.004	0.001	0.001	0.002
Zoxamide	5	0.001	0.001	0.001	0.002	N.D.	N.D.	N.D.	N.D.
Total		0.132	0.071	0.18	0.206	0.07	0.069	0.046	0.077

N.D. (Not detected). M.R.L. (Maximum Residue Levels) according to European Commission REGULATION (EU) 2019/88 of 18 January 2019—G.U.C.E.L. 22–24 January 2019.

4. Discussion

The effect of pre-harvest bagging on fruit quality is strongly influenced by the material of which the bag is made and by the treatment period [31]. Parchment is a cellulose-based breathable material that resists high temperatures [32]. In both cultivars studied here, this increased berry size—length, width and weight. The other two bag materials, paper and non-woven, did not show any beneficial effects on these morphological traits, with berries generally being smaller than in the unbagged controls. Overall, pre-harvest bagging did not much affect the whole bunch. For colorimetric traits, the positive effects of bagging were confirmed. And, once again, the bag material strongly influenced the result [17,24]. The parchment bag, especially, improved results in both grape cultivars. The L* and a* values were the lowest, resulting in darker and greenish-colored berries. The b* coordinate was lower in both cultivars for the paper and parchment-bag treatments. The CIRG2 index analyses revealed that the parchment and paper bags markedly improved fruit color, distinguishing them clearly from the control fruit and from the non-woven bagged fruit, which were a lighter bright-green and slightly yellowish color.

A trend for a decrease in fruit quality with bagging was noticed in the berry texture profile. Although the puncture test did not show significant effects of bagging on skin strength in either cultivar, the compression test showed that in both cultivars the paper bagging treatment resulted in a lowering of compressive strength—berries were softer or less firm. In 'Italia', a negative effect of bagging on berry firmness was also noted in the other two bagging treatments—the effects of the non-woven bags on fruit firmness (19 N) were similar to those of the paper bags (16.1 N), while fruit firmness with the parchment bags (23 N) was only lightly lower than in the unbagged controls (26.3 N). This negative effect of bagging on fruit firmness can probably be attributed to a changed microclimate within the bags [33]. While we did not measure this, we surmise that the humidity was higher in the paper bags, due to the tendency of paper to absorb and retain water [34]. The fruit of the later-ripening 'Italia' were harvested 30 days later than that of 'Vittoria'. Hence, seasonal differences (i.e., the weather) could well have affected the berries' textural properties. Chemical traits such as pH, total soluble solids, titratable acidity and total polyphenols were unaffected by bagging in both cultivars. The PCA analysis of the qualitative trait variables confirmed how the controls deviated from the three bagging treatments. The variance was mainly attributable to the morphological and colorimetric berry traits, which were strongly positively correlated. A negative correlation was observed between titratable acidity and TSS vs. berry size.

The agrochemical residue analyses confirmed that fruit bagging reduces accumulations of all crop protection chemicals. The bag acts as a physical barrier between the various chemicals applied and the fruit, thereby reducing their accumulation in the fruit [22–24]. We note that this study was conducted in a real production scenario and followed the guidelines and methodologies specified by the EFSA (European Food Safety Authority) for identifying and quantifying agrochemical residues (UNI EN 1562:2018). Fruit bagging of 'Italia' lowered the concentrations of total agrochemical residues in 2021 from 0.73 mg/kg for the unbagged controls to 0.006 mg/kg for the parchment bags and in 2022 from 0.2 mg/kg for the unbagged controls to 0.008 mg/kg for the paper bags. The effects for 'Vittoria' were similar but less marked, but in both years the unbagged controls showed the highest concentrations of total agrochemical residues. The apparent cultivar difference was most likely attributable to the different durations of bagging for the two cultivars. The 'Italia' fruit was bagged for 30 days longer than the 'Vittoria' fruit. The meteorological data for the two years, acquired at the Mazzarrone weather station (Catania, Sicily, Italy), were analyzed, and no significant 'weather events' were recorded (Figure S2). The predominant agrochemical residue found in both cultivars and the most persistent in 'Vittoria' was Acetamiprid, a systemic neonicotinoid insecticide. It is applied to the canopy, from where it enters the plant's vascular systems and is translocated all around the plant [35]. The main and most important degradation pathway for Acetamiprid is likely photolysis, in particular by UV-light [36,37]. Acetamiprid has a half-life in the plant of 1.84 to 2.25 days [38]. It would seem reasonable that both the bag material and the bagging period would affect the degradation of Acetamiprid, and so played roles in limiting residue accumulations in the berries. Further investigations will be required to confirm this interpretation and so understand the effects of Acetamiprid's degradation mechanisms on fruit following pre-harvest bagging.

5. Conclusions

This study confirms the results of previous work on the effects of pre-harvest bagging on improving the fruit quality traits of table grapes. Moreover, the bagging material and also the timing of bagging will have significant effects on agrochemical residue levels on the fruit.

Our agrochemical residue analyses allowed a fine assessment of the effectiveness of pre-harvest bagging in limiting agrochemical accumulations in the context of real-world agronomy. The mitigation of agrochemical residue accumulations was more pronounced in the late-ripening cultivar 'Italia' than in the early ripening 'Vittoria', an effect which we attribute to the longer duration of bagging in 'Italia'. Our research represents an initial evaluation of the effects of pre-harvest bagging on limiting the accumulation of crop protection chemicals. In future studies we will seek to further understand the pathways of agrochemical degradation and of residue accumulation as they are affected by pre-harvest bagging.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13101933/s1, Figure S1: The different pre-harvest bagging treatments using bags of different materials on the cultivar 'Italia': (a) parchment, (b) non-woven, (c) paper and (d) unbagged control. Figure S2: Time charts of key meteorological data in 2021 and 2022 during the pre-harvest bagging treatments. Graphs (a) and (c) indicate the temperatures (°C): maximum (TMAX), average (TMEAN) and minimum (TMIN) over the years 2021 and 2022. Graphs (b) and (d) show values of the relative humidities (%): maximum (HUMAX), average (HUMEAN) and minimum (HUMIN) for the years 2021 and 2022. All data were acquired from our meteorological station located in Mazzarrone (Catania, Italy).

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