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Research Paper

Effect of two reflective materials on the physiological and production behaviour of bergamot (*Citrus bergamia* Risso et Poiteau) plants

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ABSTRACT

The Mediterranean basin area is characterised by photosynthetic photon flux density, which is often well above the saturation level point of the leaf. This phenomenon has been exacerbated by global warming, giving rise to reactive oxygen species in chloroplasts, which can damage tissues (photodamage) and reduce photosynthetic potential.

Citrus bergamia Risso et Poiteau (bergamot) was successfully cultivated in Southern Italy for the high-quality essential oil extracted from the flavedo fruit and freshly consumed for its numerous health properties.

This study aimed to use two reflective materials, caolin and calcium carbonate, to improve tree productivity. The experiment was conducted over two years in southern Italy. A randomised block design was used in this study. At harvest, fruit samples were randomly detached.

The results showed that bergamot trees treated with reflective materials significantly increased the average yield per plant compared to that of the control plants, but the effect was stronger with caolin and calcium carbonate. The number of fruits at harvest was similar between the treatment and control trees; therefore, there was no effect of the two reflective materials on decreased fruit pre-harvest, but the change in yield was attributable to an increase in fruit size. This can be attributed to the higher performance of the leaves covered by reflective materials. Regarding maturation, the indices were not influenced by the treatment, whereas ascorbic acid was higher in the fruit of the treated trees. Essential oil production was improved by this treatment. This improves both the essential oil yield, used as a base for the worldwide perfume industry, and the nutraceutical parameters for fresh consumption, justifying the increased costs of applying reflective material on the tree.

1. Introduction

As the Mediterranean basin area is characterized by levels of photosynthetic photon flu density (PPFD), often well above saturating levels, crops of agricultural interest undergo photon pressure in excess of their photosynthesizing capacity. It is well-known that net carboxylation increases linearly with increasing incident PPFD until a steady state (saturation point) is reached, at which point photosynthesis does not increase, and the Benson cycle reaches maximum speed. The saturation point varies depending not only on the growth conditions but also on the plant species. In *Vitis vinifera*, the saturating light level is between 750 and 1000 μmol photons m^{-2} s^{-1} (Escalona et al., 1999); in the apple tree, saturation is reached at widely varying light intensities, between 400 and 1,000 μmol photons m^{-2} s^{-1} (Cheng et al., 2001); in peach and *Actinidia deliciosa* trees, the saturation point is indicated between 700 and 900/1000 μmol photons m^{-2} s^{-1} (Kappel and Flore, 1983; Greer and Halligan, 2001). Finally, in the genus *Citrus*, saturation point is approximately 400 μmol photons m^{-2} s^{-1} (Kriedemann, 1968). Excess electrons in an excited state give rise to reactive oxygen species (ROS) that can damage chloroplast tissues (photodamage) and reduce its photosynthetic potential (Pandhair and Sekhon, 2006). Leaves have evolved photoprotective mechanisms that include photorespiration, the water-water cycle, non-photochemical quenching (NPQ), cyclic electron transport around PSI, and the glutathione-ascorbate cycle. Despite these protective mechanisms, the photon pressure can be so high that it exceeds the protective capacity, damaging the Antenna complex of PSII (LHCII), core complex of PSII, and core complex of PSI (Niyogi, 1999). Specifically, in LHCII, the oxidation of the antenna pigments and thylakoid proteins (Knox and Dodge, 1985) whereas the core complex contains impairment of P680 and especially the destruction of reaction centre proteins, especially D1 protein. Lipid oxidation and complete inactivation of the reaction centre are also found (Aro et al., 1993;

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Barber and Andersson, 1992). In PSI, excess ROS result in the destruction of iron-sulphur centres, inactivation and degradation of various enzymes. The damage from excess light can be repaired at the expense of photosynthetic carbon, all of which results in lower photosynthetic efficiency. In recent years, the phenomenon has been exacerbated because of "global warming" causing negative effects on agricultural activity, including the citrus industry. Regarding the citrus species, the *Citrus bergamia* Risso et Poiteau (bergamot) is successfully cultivated in Souther Italy, the main cultivation area in the world, which produces high-quality bergamot. Essential oils are also extracted from the flavedo fruits. However, in recent years, it has been increasingly appreciated by consumers for its numerous health properties of the nutraceutical components, such as the polyphenolic fraction of juice, and albedo. Agronomic strategies to limit the damage caused by thermal and radiative excesses, e.g. nets, are effective because nets are effective (Cohen et al., 1997; Sabir et al., 2020; Gullo et al., 2021) but require considerable expenditure. Reflective material is sunscreen equipment distributed on leaves. It creates a thin film of microparticles capable of selectively reflecting sun's rays, allowing visible light to pass through. However, much of the infrared (IR) and ultraviolet (UV) radiation allows the leaf to cool under summer stress. Therefore, this study aimed to use two biological products classified as reflective materials on Citrus bergamia Risso et Poiteau to counteract the negative effects of high solar radiation and to evaluate the behaviour of the trees in relation to physiological and productive parameters for an important citrus species, such as bergamot.

2. Materials and methods

2.1. Plant material and treatment

The experiment was conducted over two years, in 2022 and 2023, in a Bergamot orchard in Reggio Calabria, Italy. Bergamot trees (Citrus bergamia Risso et Poiteau), cv. "Fantastico", grafted onto sour orange (*Citrus aurantium* L.) were planted in the 2008 year. The plants were spaced 5 *m* × 5 m apart (417 plants ha^{-1}). Each tree subjected to stable drip irrigation was trimmed into a global shape. Orchards were cultivated under an organic farming regime (EC Regulation 848/2018). The climate of this area is Mediterranean, a subtype of temperate climate, according to the classification provided by Koppen–Geiger, characterised by rainfall concentrated in the winter and dry and very hot summers (Csa: C "Temperate" – S "Dry Summer" – A "Hot Summer") (Kottek et al., 2006). The average maximum temperature was reached in July (Graph 1). The precipitation was mainly concentrated in the autumn–winter period (Graph 1).The reflective materials (RM), Kaolin (Ka) (Surround WP, Serbios Srl, Rovigo, Italy, UE) and calcium carbonate (CC) (Purshade, Serbios Srl, Rovigo, Italy, UE) were applied monthly at concentrations of 50 g.L^{-1} and 67 g.L^{-1} (indicated by the producer), respectively, starting from the phenological stage of fruit growing. Trees sprayed with reflective materials, caolin, and calcium carbonate (4 L) were compared with trees sprayed with water only (control trees, Ctr) (Photo 1). The treatments were arranged in a randomised block design with 27 trees: three blocks of nine trees per treatment with an equal canopy volume. Each treatment was separated by a row of trees used as a buffer. For each tree, 4 L of the suspension was used. Preliminary tests showed satisfactory distribution and coverage of the leaves. For each tree at harvest, (270 DAFB) samples were randomly detached; 15 fruits per treatment were sampled (15 fruits × 9 trees × 3 treatments = 405 fruits–135 fruits per treatment).

2.1.1. Leaf temperature, brightness and SPAD index

The leaf temperature was monitored every minute with a fine–wire thermocouple (GMR Struments, Firenze, Italy) connected to a data logger (Spectrum Technologies, Inc. Aurora, Illinois, USA). The thermocouples were pressed against the abaxial leaf surface. and was held in place using lightweight clips. Leaf reflection (whiteness) was measured using spectrophotometer (CR700; Minolta Cor., Ramsey, New Jersey, USA). Whiteness measurements (L values ranged from 0 to 100, where black=0 and white=100) adaxial leaf surfaces were recorded before treatment when the leaf was clean and after treatment when the individual leaf was coated with kaolin or calcium carbonate.

2.2. Ecophysiological measurements

Leaf ACO_2, stomatal conductance (gs), leaf-to-air vapour pressure deficit (LAVPD), leaf transpiration, internal CO_2 partial pressure (Ci), and water use efficiency (WUE; ACO_2/Transpiration) was measured for mature leaves in the outer, intermediate, and inner layers of each tree (three leaves × three plants × three layers × three blocks); the measurement was obtained using a portable photosynthetic system (6400

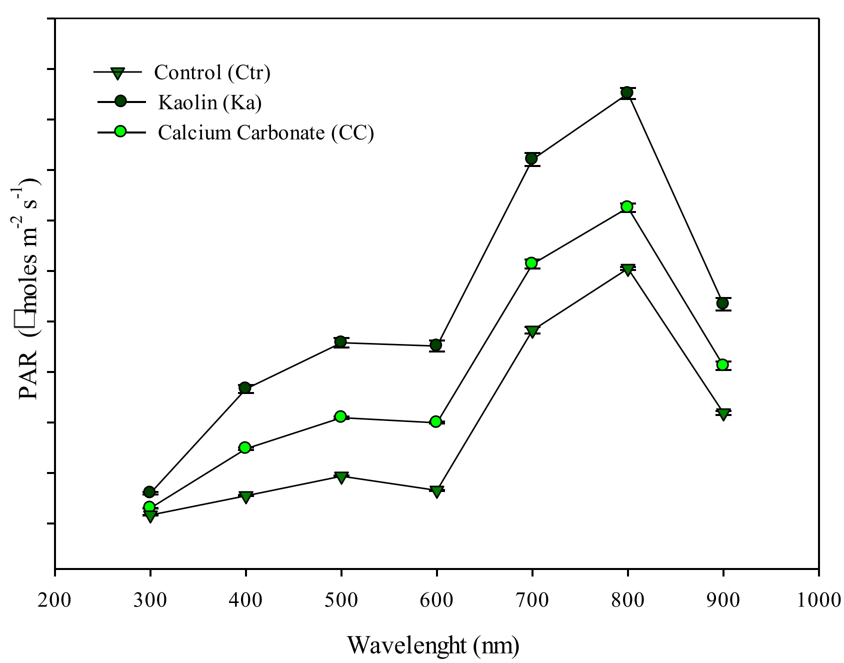


Fig. 1. Thermo-pluviometric regime of the 2022–2023 biennium of the area of interest.

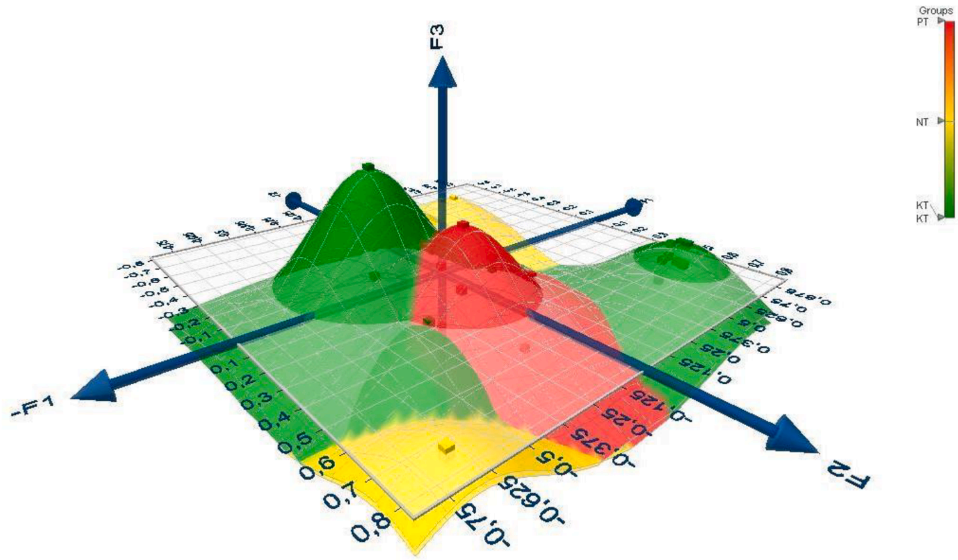


Fig. 2. Calcium Carbonate and kaolin on the leaves after treatment and leaves of control plants.

XT; LI-COR, Lincoln, Nebraska, USA) and was carried out on a clear sunny summer day (from 11:00 to 13:00), under optimal weather conditions (average CO_2 partial pressure of 38 Pa. average saturing PFD 1600 micromol.m^{-2}.s^{-1}) during the last week of summer months in both years. Photoinhibition was also estimated using a chlorophyll fluorometer (6400–40; LI-COR, Lincoln, Nebraska, USA), and measurements were taken on the same leaves used during gas exchange measurements and after dark adaptation for 30 min using leaf clips; therefore, the same leaves of outer layers used for the measurement were detached, and after washing with distilled water, the SPAD 502 (Spectrum Technologies Inc., Aurora, Illinois, USA) index was measured. The PAR spectrum was measured at midday (12:00 h) using a spectroradiometer (Mod. PS-300 Spectroradiometer Apogee Instruments Inc., Logan, Utah, USA). We used an average of four points for each layer of each of the three plants per treatment per block.

2.3. Fruit quality attribute

2.3.1. Harvest

The yield per tree (in weight and number of fruits) was determined during harvesting. Observations of fruit parameters were carried out at the "Colture Arboree". Laboratory of the AGRARIA Department (Mediterranean University of Reggio Calabria). The fruit was transported one hour after harvesting in an air-conditioned van at 20 °C. The fruits were used to detect biometric, maturation index, and nutraceutical parameters.

2.3.2. Biometric and maturation index

Transversal and longitudinal diameters, circumference, relative length and fresh weight were measured using a calibre and an electronic balance (Mettler-Toledo, Grelfensee, Switzerland). The skin and pulp colours were determined in terms of CIELab and HSB colour space using a Minolta CM700d photocolorimeter (Minolta, Osaka, Japan) equipped with a target mask with an 8 mm section. The fruit juice was obtained from each half-fruit (before weighing), and the juice yield was calculated (%). Then, the juice was used to evaluate total soluble solids (TSS) using a handle refractometer (Atago PAL-1, Tokyo, Japan) and ascorbic acid (AA) using a procedure based on the reduction of 2,6 diclorophenolindophenol (IFUMA 17). Titratable acidity (TA) was determined by a potentiometric titrator (Titralab AT1000 series, HACH, Loveland, Colorado, USA). A 25 mL aliquot of juice was titrated with an aqueous solution of sodium hydroxide (NaOH 0.5 N) until neutralised. Potassium acid phthalate ($KHC_8H_4O_4$) was used as the primary standard for standardisation of the base (NaOH). Titratable acidity (TA) was expressed as a percentage of citric acid monohydrate. The other half-fruit, preventively weighed, was used to determine the Dry Matter Content of each layer of fruit (flavedo, albedo, and pulp) using a dehydrator (Binder EED240, Tuttlingen, Germany) at 70 °C until a constant dry weight was reached. Dry matter content is expressed as follows:

$$\% DMC = (\text{dry weight})/(\text{fresh weight}) * 100$$

2.4. Nutraceutical parameters

Total polyphenols content (TPC) and total antioxidant capacity (TAC) analyses were performed. These analyses were performed on pulp, flavedo, and albedo. For each block, five fruits for graft combination were placed in polyethene bags and frozen at −80 °C, until the analysis of TAC, and TPC. The bergamot samples were homogenised using an Ultraturrax blender (20,000 rpm; T 25 Basic, IKA Werke, Germany). The TPC and TAC were analysed separately using a Lambda 35 spectrophotometer (Perkin Elmer Corporation, USA). Before measuring the TPC and TAC, standard curves were prepared for each test. TPC (mg gallic acid equivalents g^{-1} FW) was determined using Folin-Ciocalteu method (Slinkard and Singleton, 1997). The TAC was determined usingthe following equation: the modified TEAC assay and expressed as μmol Trolox equivalents g^{-1} FW (Pellegrini et al., 1999; *Re* et al., 1999). The TEAC assay included both the hydrophilic and lipophilic contributions (Scalzo et al., 2005) of the bergamot samples. The Total Flavonoid Content (TFD) of the samples was measured using a colorimetric method (Zhishen et al., 1999; Dewanto et al., 2002). The methanolic extract (250 μL) was mixed respectively with 1.25 mL distilled water and 75 μL of 5% $NaNO_2$ solution, then allowed to mix for 6 min. After the addition of 150 μL of 10% $AlCl_3$ solution and mixing for 5 min, the reaction was initiated by adding 1 M NaOH (0.5 mL), and the total volume was increased to 2.5 mL with distilled water. The absorbance of each sample was measured at 510 nm using a spectrophotometer. UV/Vis spectrophotometer (Lambda 35, Perkin Elmer Corporation, USA). Total Flavonoid Content was expressed as μg (+)-catechin equivalents g-1 FW. The preparation of samples for Total Flavonols Content (TFL) determination was done according to the aluminium chloride colorimetric technique: 0.5 mL of each extract was mixed with 0.5 mL aluminium chloride (2%), and then 1.5 mL of potassium acetate (5%) were added. After 150 min, the absorbance was determined at 440 nm. The calibration curve was plotted by different concentrations of quercetin equivalents (Miliauskas et al., 2004).

2.5. Determination of essential oil yield and analysis of the volatile fraction

2.5.1. Glands oil count

On portions of peel, the number of gland oil (n cm^{-2}) was determined using the "Counting" tool of the Adobe Photoshop® CS6 Extended software (Adobe, San Jose, CA, USA) (Saad et al., 2008). The images were acquired in TIFF format (Tagged Image File Format) using a single-lens reflex digital camera (Nikon D5500, Tokyo, Japan) equipped with a 24.2 effective megapixel CMOS APS-C sensor over an area of 23.2 × 15.4 mm.

2.5.2. Essential oil

Bergamot essential oil is a heterogeneous and complex mixture of numerous chemicals that make up the volatile fraction, which is approximately 95%, and the non volatile fraction, which is the remaining 5%; it has a clear appearance and a colour ranging from dark green to yellow, depending on the degree of ripeness of the fruit. Extraction of the essential oil from the bergamot samples was performed by hydrodistillation using a Clevenger apparatus for 3 h The volatile fraction was analysed by gas chromatography; 1 μl of sample diluted 1/20 with hexane was injected into a Shimadzu GC/17A, equipped with an FID detector. Gas chromatographic analyses were performed using a GC 17/A with a Split-Splitless injector at a temperature of 250 °C and QP 5050 mass spectrometer as detector (Shimadzu) (interface temperature 270 °C). Helium was used as the carrier. Separation of the compounds was performed on an SE52 column (30 m X 0.25 mm I.D., 0.25 Dm film) from Mega (Milan, Italy). The column placed at 40 °C for 10 min, was heated in increments of 3 °C/min to 150 °C, then remained in isotherm for 5 min before being raised in increments of 30 °C/min to 250 °C/min temperature at which it remained for 10 min. The interface temperature was set to 270 °C. The ionisation energy was set to 70 eV Compounds were identified by comparing their mass spectra with those of standard compounds, NIST 11, and Wiley 2009 libraries and by comparing linear retention indices (LRIs) with those reported in the literature. LRIs were calculated for all volatile compounds using the retention times of the homologous series of aliphatic hydrocarbons from C5 to C24 under the same chromatographic conditions used for sample analysis, according to the IUPAC Gold Book (Russo et al., 2012).

2.6. Statistical analysis

The data were statistically processed using SPSS software (version 22.0; IBM Corp, Armonk, NY, USA) and a two-way analysis of variance. Tukey's test was used to discriminate averages that showed a significant ANOVA effect. Principal Component Analysis (PCA) was performed on the main biometric fruit parameters, maturation, and physiological indices to assess the relationships between the variables and different treatments. PCs with eigenvalues greater than one 1 (Dunteman, 1989) and loadings greater than |0.75| were selected, values indicating a significant correlation between the original variables and the extracted components (Matus et al., 1996). The dataset was used to make a 3-dimensional graph with Excel using the XLSTAT add-on statistical software. The data corresponded to the outputs (row and column points) of the correspondence analysis.

3. Results

The yield per tree changed between two years and was 10% lower in the first year to the second year. No interaction was observed between the treatment and year. The average yield over two years, expressed as weight, was 14% higher under Ka than under Ctr (Table 1). No significant differences were observed between CC and Ctr groups (Table 1). As the number of fruits per tree was not influenced by treatments over two years (Table 1), the factor that influenced the weight yield per tree must be searched for in the change in fruit weight (see below). Leaf temperature was lower in the treated trees. The temperature dropped about 4 °C in Ka and 3 °C in CC compared to Ctr (Table 1). The actions of the reflective materials were confirmed using the lightness parameters. Indeed, it was significantly higher in the treated trees than in the Ctr trees, and the highest value was recorded for Ka. Finally, the SPAD index increased with reflective materials; indeed, the values were 22% and 17% higher those of Ctr with Ka and CC, respectively (Table 1). The treatments had statistically significant effects on fluorescence parameters. The maximum quantum efficiency (Fv'/Fm') was significantly higher in both two treatments compared to the control without showing any difference between them. These treatments positively affect the quantum efficiency of PSII (PhiPS2). In this case, the values for the Ka and CC were also similar significantly between them and 75% higher than those Ctr (Table 2). The improvement in light absorbed by the leaf and used to fuel the photochemical reactions of the light phase of the photosynthetic process in treated trees was confirmed by other parameters such as photochemical quenching (qP). The control tree showed the significantly lowest values compared to the CC and Ka, because the absorbed energy was mostly dissipated through non-photochemical

Table 1
Effect of treatment with reflective materials [Kaolin (Ka) and Calcium Carbonate (CC)] compared to control tree on production yield, number of fruits, and some leaf parameters (Temperature, Lightness, and SPAD index), in Citrus bergamia Risso et Poiteau tree of the Fantastic cv. average of two years (2022–2023).

Treatment	Yield (Kg. $tree^{-1}$)	Number of fruits (number. $tree^{-1}$)	Leaf temperature °C	Lightness	SPAD Index
Ctr	*60.211 ±2.141b*	*324.12 ±15.91ns*	*28.24±0.94a*	*37.41 ±0.55c*	*62.11 ±2.15b*
CC	*62.315 ±1.721ab*	*286.33 ±16.09*	*20.35±0.62b*	*48.35 ±1.42b*	*74.11 ±1.08a*
Ka	*69.182 ±1.448a*	*312.22 ±15.12*	*19.33±0.45b*	*59.23 ±1.98a*	*76.22 ±1.23a*
Year (A)	*ns*	*ns*	*ns*	*ns*	*ns*
Treatment (B)	*	*ns*	*	*	*
Interaction (AxB)	*ns*	*ns*	*ns*	*ns*	*ns*

Analysis of Variance (two-way ANOVA) with $N = 15$; (A = Year; B: Treatment; AxB: interaction between year and treatment); Means in the same column followed by different letters are significantly different ($P \leq 0.05$) using Tukey's test. NS not significant.

Table 2
Effect of treatment with reflective materials [Kaolin (Ka) and Calcium Carbonate (CC)] compared to untreated tree (Ctr) on maximum efficiency of photosystem II photochemistry (Fv'/Fm'), non-photochemical quenching (qN), photochemical quencing (qP) and PSII quantum efficiency (PhiPS2) in the leaf of Citrus bergamia tree; average of two production season (2022–2023).

Treatment	Fv'/Fm'	qN	qP	PhiPS2
Ctr	0.261 ±0.011b	1.536 ±0.039a	0.344 ±0.020b	0.088 ±0.008b
CC	0.354 ±0.021a	1.421 ±0.007b	0.475 ±0.019a	0.178 ±0.008a
Ka	0.378 ±0.029a	1.412 ±0.014b	0.492 ±0.021a	0.188 ±0.003a
Year (A)	*	*	*	*
Treatment (B)	*	*	*	*
Interaction (AxB)	*	*	ns	ns

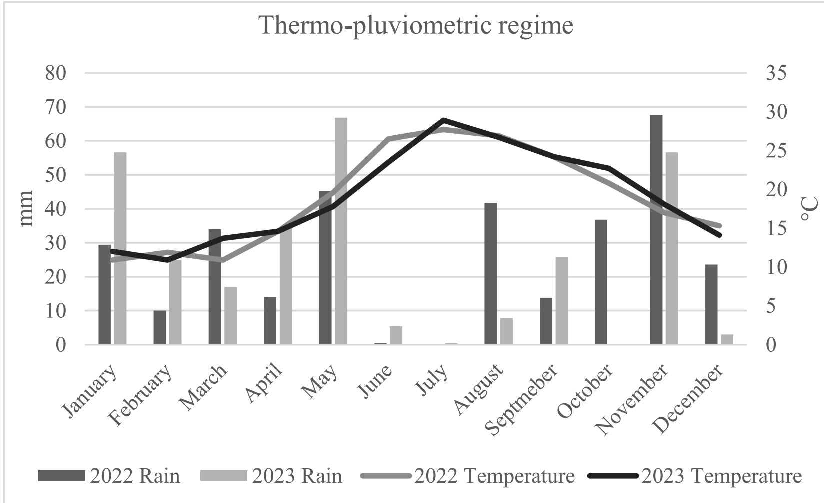
Analysis of Variance (two-way ANOVA) with $N = 15$; (A = Year; B: Treatment; AxB: interaction between year and treatment); Means in the same column followed by different letters are significantly different ($P \leq 0.05$) using Tukey's test. NS not significant.

processes (qN) (Table 2). Therefore, photosynthesis appears to be influenced by the distribution of reflective materials. Indeed, the net assimilation (Pn) showed that the reflective materials improved the photosynthetic performance by up to 90% over Ctr. However, better results were obtained with Ka which was 22% higher that with CC (Table 3). Other gas exchange parameters such as gs, VpdL, Tr, Ci confirmed the improved performance tree. Finally, the WUE was higher in the treated trees (Table 3). The light spectra confirm that reflective materials increased the share of photosynthetically active radiation in the inner layers of the canopy; the average values were 101 μmol photons m^{-2} s^{-1} and 111 μmol photons m^{-2} s^{-1} in the canopy of the Ka and CC respectively while the PAR was lower in Ctr, 42 μmol photons m^{-2} s^{-1} (Fig. 1). All these factors affect the fruit characteristics. The Ka fruit reached an average fresh weight of approximately 215 g (±4.45) 13% significantly and 10% higher than the Ctr, while no difference was observed between CC and Ctr treatments, respectively. A similar trend was also recorded for other biometric parameters, such as polar and equatorial diameters and circumference. However, treatment with shielding products did not affect the relative length; therefore, the treatments did not change the shape of the bergamot fruit compared to that of the Ctr (Table 4). Difference statistically significant was detected in juice yield expressed as a percentage value; indeed, the Ka showed a yield decrease of approximately 7% compared to the Ctr (Table 4), but the juice yield in grams did not change among the treatments. Differences in dry matter content were statistically significant among the three fruit layers. Indeed, the flavedo and albedo in the Ka showed a 10% and 13% significant increases compared to the Ctr respectively (Table 4). The dry weight of the pulp was also higher in Ka, reaching average values over 10% than Ctr. However, the alternative reflective material showed no statistically significant differences compared to Ctr for all layers of fruit (Table 4). The recorded biometric values changed between the two years, but no interaction between year and treatment was observed. The tinte (°hue) showed statistically significant differences among treatments, and we observed extended variability for this parameter, as shown by the standard error (Table 5). The flavedo with Kaolin showed a tendency to turn yellow earlier than the Ctr flavedo. The hue value did not change in the flavedo fruits of CC compared to that of the alternative solutions (Table 5). The other colour components, lightness and chroma, showed no statistically significant differences between the different theses (Table 5). Regarding the maturation indexes, titratable acidity (TA), expressed as % citric acid monohydrate, showed no statistically significant differences between the different treatments. Total Soluble Solids (TSS), an important index that can define internal and sensory qualities, also showed no statistically significant differences, although the value increased with reflective materials and was higher in Ka (Table 5); the TSS/TA ratio, showed no statistically significant differences between the different treatments compared to that of the Ctr group (Table 5). Both treatments showed higher ascorbic acid content than the Ctr; in particular, the increase was 12% and 7% higher in the fruit of Ka and CC, respectively, compared with the Ctr group (Table 6). However, in the TPC and TAC in different layers of the fruit, the differences between treatments appeared to be statistically significant. Among the three layers of fruit, the greatest contribution to TPC was from the albedo, 50%, 30% from the flavedo, and 20% from the pulp. For all three layers, the TPC were significantly higher in the treatment group than in the Ctr group (Table 6). In the pulp, the TPC was 54 and 11% significantly higher in the Ka and CC, respectively, compared to years in the Ctr group (Table 6). The Total Antioxidant Capacity followed a trend similar to that of TPC. Indeed, the treatments increased the TAC in all fruit layers. In particular, the highest TAC was also observed in the albedo, followed by the flavedo, whereas the lowest value was recorded in the pulp (Table 6). Finally, no effect was observed regarding the TFD and TFL, and no differences were observed between layers. The results of treatments on essential were focalized over 57 molecules. The essential oil of bergamot is mainly composed of 3 major groups of compounds: monoterpene hydrocarbons, sesquiterpene, alcohols, and monoterpene esters. The first group includes 14 molecules, which reported different concentrations in the three treatments 30. 061% (±8.77) in the Ctr, 19.66% (±4.39) in the CC thesis and 35.76% (±4.58) in the Ka; the second group includes 11 molecules, 51.19% (±8.56) in the Ctr thesis, 65.45% (±3. 55) in the CC and 44.67% (±5.31) in the KA; finally, monoterpene esters include 8 molecules, 17.43% (±0.26) in the Ctr, 13.80% (±0.25) in the CC and 18.26% (±0.14) in the Ka; two monoterpene aldehydes (0.68%, 0.69%, and 0.60%), ten sesquiterpene hydrocarbons (0.41%, 0.19%, and 0.46%), two monoterpenes oxides (0.015%, 0.010%, and 0.011%) in Ctr, CC, and Ka theses, respectively. Finally 5 aliphatic aldehydes and 3 aliphatic esters. The monoterpene ester linalyl acetate showed the highest concentrations in the Ka (15.51±0.01) and the lowest concentrations in the CC (Table 7); limonene also showed the highest values in the Ka, 23% higher than in the Ctr thesis, whereas the CC also showed significantly lower values than previously reported for the Ctr (Table 7). Another compound that defines the quality of bergamot essential oil is linalool, which showed higher concentrations in the CC thesis, compared with the Ctr thesis and Ka groups (Table 7). The ratio of linalyl acetate to linalool was calculated as follows: results: 0.32 (±0.02) in Ctr, 0.19 (±0.01) in CC and 0.40 (±0.03) in Ka (Table 7). Production of essential oils and the number of oleiferous vesicles was influenced by treatment with the reflective material. In fact, the CC gave yields of 284.84 gr/100 kg (±1.52), 19% higher than the Ctr, but the Ka treatment reported also higher values (16%) compared to Ctr (Table 7) without significant difference between treatments; this result can be attributed to the effect of oleiferous vesicles number. Indeed, no differences between treatments were recorded, but the number detected in treatment was 7% group was significantly higher than that in the Ctr group (Table 7). Principal component analysis (PCA) provided a broader view of the effect that screening products had on the main carpometric and quality parameters of the production of the different theses. Through this multivariate technique, new variables can be extracted and correlated with the original variables. The first three principal components explained about 70% of the total variance, PC1 34%, PC2 22% and PC3 13.5% (Table 8). PC1 was positively correlated with the juice yield expressed in g (0.84), average fruit weight (0.81), circumference (0.81), and equatorial diameter (0.80), polar diameter (0.78), chroma (0.70),

Table 3
Effect of treatment with reflective materials [Kaolin (Ka) and Calcium Carbonate (CC)] compared to untreated tree (Ctr) on net CO_2 assimilation rates (Pn), stomatal conductance (gs), Vapor pressure deficit (VpdL), Transpiration (E), internal CO_2 partial pressure (Ci,) and Water User Efficiency (WUE) in the leaf of Citrus bergamia tree; average of two production season 20,022–2023.

Treatment	Pn (μmol CO_2 m^{-2} s^{-1})^1	gs (mol H_2O m^{-2} s^{-1})	VpdL (kPa)	Trmmol (mmol H_2O m^{-2} s^{-1})	Ci (μmol mol^{-1})	WUE μmol CO_2 mmol H_20
Ctr	6.12±0.61c	0.062±0.022ns	2.48±0.48ns	1.48±0.24ns	188.91±28.12ns	0.044±0.006ns
CC	9.11±0.44b	0.068±0.015	2.88±0.58	1.86±0.25	141.42±29.33	0.044±0.014
Ka	12.06±0.43a	0.082±0.011	3.10±0.54	2.08±0.15	132.82±28.45	0.039±0.007
Year (A)	*	*	*	*	*	*
Treatment (B)	*	ns	ns	ns	ns	ns
Interaction (AxB)	ns	ns	ns	ns	ns	ns

Analysis of Variance (two-way ANOVA) with $N = 15$; (A = Year; B: Treatment; AxB: interaction between year and treatment); Means in the same column followed by different letters are significantly different ($P \leq 0.05$) using Tukey's test. NS not significant.



Graph 1. Light spectrum trend in the inner canopy layer of Citrus bergamia Risso et Poiteau, Cv Fantastico, treated with caolin and purshade, compared to the Ctr canopy.

Table 4
Effect of treatment with reflective materials [Kaolin (Ka) and Calcium Carbonate (CC)] compared to untreated tree (Ctr) on main fruit morphobiometric parameters (Fresh weight, polar diameter, equatorial diameter, relative length, circumference, juice content and dry weight) on *Citrus bergamia* Risso et Poiteau fruit sampled at harvest; average of two production season 2022–2023.

Treatment	Fresh Weight (g)	Polar diameter (mm)	Equatorial diameter (mm)	Relative Lenght	Circumference (cm)	Juice content (g)	Juice content (%)	Dry Matter Content (%)		
								Exocarp	Mesocarp	Endocarp
Ctr	190.38 ±5.23b	68.15±0.58b	74.88±0.61b	1.08 ±0.02ns	23.95±0.31b	82.25 ±4.45ns	40.15 ±0.89ab	23.12 ±0.45b	23.88 ±0.38b	9.81 ±0.51b
CC	195.19 ±5.41b	69.45 ±0.85ab	75.06±0.82b	1.09±0.01	23.78±0.25b	91.15 ±4.78	42.51 ±0.82a	24.11 ±0.46b	24.81 ±0.35b	9.84 ±0.34b
Ka	215.89 ±4.45a	71.56±0.61a	78.89±0.84a	1.08±0.02	24.93±0.19a	91.12 ±3.22	36.55 ±2.89b	25.38 ±0.72a	26.88 ±1.41a	10.11 ±0.29a
Year (A)	*	*	*	ns	*	*	ns	ns	ns	ns
Treatment (B)	*	*	*	ns	*	ns	*	*	*	*
Interaction (AxB)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Analysis of Variance (two-way ANOVA) with $N = 15$; (A = Year; B: Treatment; AxB: interaction between year and treatment); Means in the same column followed by different letters are significantly different ($P \leq 0.05$) using Tukey's test. NS not significant.

Table 5
Effect of treatment with reflective materials [Kaolin (Ka) and Calcium Carbonate (CC)] compared to untreated tree (Ctr) on main colour indices [Lightness (L*), Chroma and °Hue], determined on the peel of *Citrus Bergamia* Risso et Poiteau fruits, Fantastico cv., sampled at harvest; average of two production season 2022–2023.

Treatment	TSS (Brix)	TA (%)	TSS/TA	Lightness	Chroma	°Hue
Ctr	8.25±0.09ns	4.31±0.07ns	1.94±0.04ns	54.85±0.41ns	54.22±0.41ns	192.55±0.22a
CC	8.31±0.12	4.38±0.06	1.94±0.054	54.57±0.37	54.50±0.35	191.45±0.32ab
Ka	8.41±0.06	4.45±0.05	1.93±0.05	54.18±0.42	54.59±0.42	191.05±0.34b
Year (A)	ns	ns	ns	ns	ns	ns
Treatment (B)	ns	ns	ns	ns	ns	*
Interaction (AxB)	ns	ns	ns	ns	ns	ns

Analysis of Variance (two-way ANOVA) with $N = 15$; (A = Year; B: Treatment; AxB: interaction between year and treatment); Means in the same column followed by different letters are significantly different ($P \leq 0.05$) using Tukey's test. NS not significant.

colour coordinate b* (0.70), PC2 was negatively correlated with lightness (−0. 67) and chromatic coordinate a* (−0.52) and, positively correlated, with hue (0.72) while PC3 was negatively correlated with relative length (−0.59) and total acidity (−0.71), and positively correlated with the TSS/TA ratio (0.90) (Table 8). Generally, the first two components show significant variance. Therefore, only the The PC1 and PC2 components were interpreted. Overall, the fruits from the Ka treatment was placed in the F1 factor, 0.563 in the CC treatment, and in the Ctr in the F2 factor, respectively 0.50 and 0.532 (Table 8). This dataset was used to make a 3-dimensional graph (Fig. 2).

4. Discussions

The results showed that *Citrus bergamia* Risso et Poiteau (Bergamot) trees treated with Reflective Materials (RM) increased significantly the average yield per plant, compared with the control plants, but the effect was stronger with Ka than with CC. Similar results were reported by Spiers et al. (2003) for *Vaccinium corymbosum* plants, and Saour and

Table 6
Effect of treatment with reflective materials [kaolin (Ka) and calcium carbonate (CC)] compared to untreated tree (Ctr) on main quality indices such as SST, TA and SST/TA of *Citrus bergamia* Risso et Poiteau fruits, cv. Fantastic, sampled at harvest; average of two production seasons 2022–2023.

Treatment	TPC			TAC			TFD			TFL			AA
	mg Exocarp	GAE g^{-1} Mexocarp	FW Endocarp	µmoli Exocarp	trolox g^{-1} Mexocarp	FW Endocarp	mg Exocarp	quercitin g^{-1} Mexocarp	FW Endocarp	mg Exocarp	quercitin g^{-1} Mexocarp	FW Endocarp	mg/100 ml Endocarp
Ctr	3.68±0.22b	6.15±0.21b	1.56±0.22b	9.42±0.48b	12.01±0.55b	4.21±0.15b	16.42±0.54ns	7.71±0.51ns	0.622±0.04ns	1.08±0.08ns	0.34±0.22b	0.14±0.23ns	37.45±0.61b
CC	4.48±0.24a	7.55±0.24a	1.85±0.61a	11.54±0.44a	13.22±0.74a	4.58±0.41a	14.65±0.61	6.35±0.41	0.625±0.02	1.12±0.05	0.46±0.31a	0.14±0.22	38.17b±0.85a
Ka	4.28±0.24a	7.58±0.49a	2.38±0.21a	11.28±0.61a	13.51±0.88a	4.81±0.38a	16.22±0.85	7.21±0.21	0.619±0.10	1.15±0.11	0.39±0.28a	0.13±0.18	40.95b±0.91a
Year (A)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns	ns
Treatment (B)	*	*	*	*	*	*	ns	ns	ns	ns	*	ns	*
Interaction (AxB)	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*

Analysis of Variance (two-way ANOVA) with $N = 15$; (A = Year; B: Treatment; AxB: interaction between year and treatment); Means in the same column followed by different letters are significantly different ($P \leq 0.05$) using Tukey's test. NS not significant.

Makee (2003) for *Olea europea*, Glenn et al. (2001) for *Malus communis*, Puterka et al. (2000) also studied *P. communis*. We measured the number of fruits harvested. It was similar between the treatment and control trees; therefore, RM had no effect on pre-harvest dropped fruit; however, the change in yield is attributable to an increase in fruit size. This can be attributed to the higher performance of the leaf-covered RM. The reducing excess light from the outermost layer of the canopy is due to the reflective power of RM. This reduces PAR and the negative effects of high leaf temperatures induced by the infrared component of light such as observed with RM on Grapefruit, *Carya illinoensis, Amygdalus communis, Juglans regia* L, *Punica granatum* and Table vitis (Nakano and Uehara 1996; Jifon and Syvertsen 2003; Lombardini et al. 2004) Rosati et al., 2006; Melgarejo et al., 2004; Bedrech and Farag, 2015). A plausible explanation for the decrease in temperature is the change in the ratio of the of the light reflected from the treated leaf to the amount of light received by it (albedo): confirmed by the higher lightness (L*) of the leaves covered with RM, with better results with Ka. In contrast, the fluorescence and gas-exchange dynamics measurements conducted showed an increase in maximum quantum efficiency (Fv'/Fm'). According to a report on grapefruit and grapevine plants (Jifon and Syvertsen (2003): Dinis et al., 2016; Correia et al., 2012), quantum efficiency of PSII (PhiPS2), a fluorescence index, which allowed us to trace the amount of light absorbed by leaves and used to fuel the photochemical reactions of the light phase of the photosynthetic process such as that confirmed by photochemical quenching (qP). The control theory reported in lowest values (0.344 ± 0.020) over the course of the measurements, compared to the CC (0.475 ± 0.01) and Ka (0.492 ± 0.02) theses because some of the absorbed energy was dissipated through non-photochemical processes (qN) (Table 3). The photosynthetic process seems, therefore, to be influenced by the distribution of RM based on observations on apples (Glenn et al., 2001; 2003). Furthermore, light spectra reconstructed with the spectroradiometer confirm how the shielding products increased the share of photosynthetically active radiation in the inner layers of the canopy (Fig. 1). The treatment with RM, therefore, increased the share of scattered light in the different layers of the canopy (Rosati et al., 2006). Doraiswamy and Rosenberg (1974) reported that caolin increases the share of the reflected PAR radiation (albedo) by much as 87–312%, depending on the form of cultivation, weather conditions, and time of day. In addition, photosynthetic performance was supported by a high SPAD value. Smirnoff (1993) reports how a reduction in chlorophyll concentration can be considered a clear sign of oxidative stress attributable to a high rate of degradation of chlorophyll pigments or a reduction in their synthesis(Gussakovsky et al., 1993). Furthermore, a decrease in light excess from the upper layer is fully compensated by albedo diffuse radiation penetrating the canopy interior better than direct radiation, according to reports by several authors (Spitters, 1986; Sinclair et al., 1992; Cottrell et al., 2002; Gullo et al., 2020). Then, in the upper layer there was a decreased of excess PAR, UV, and infrared radiation, whereas the inner layers, there was an increase in the radiation of each waveband and the leaves responded to photosynthesis improved compared to no treated tree; however, in our experiment, the effect of caolin was stronger than that of the CC. Maturation indices (TSS, TA, and TSS/TA) ratio was not influenced by the treatment (Table 7). The recorded values were comparable to those observed for cv. Fantastico (Calvarano et al., 1996). Finally, with regard to nutraceutical aspects, it is known that the antioxidant capacity of fruits takes into account the synergistic activity carried out by several biomolecules present in varying amounts, depending on the type of fruit. These biomolecules certainly include polyphenols, chlorophyll pigments (e.g. chlorophyll a, chlorophyll *b*, and carotenoids) and ascorbic acid. In some fruits, such as kiwifruit, ascorbic acid accounts for approximately 40% of the total antioxidant capacity (Tavarini et al., 2008), and in citrus fruits, it is present at high concentrations, although several pre- and post-harvest factors can influence the content. Klein and Perry (1982) reported the quality and quantity of the light radiation as follows: average temperatures can influence the chemical composition

Table 7
Effect of treatment with reflective materials [kaolin (Ka) and calcium carbonate (CC)] compared to the untreated tree (Ctr) on the number of glandular cavities, essential oil yield and major essential oil components in fruits of Citrus bergamia Risso et Poiteau, cv. Fantastico, sampled at harvest; average of two production seasons 2022–2023.

Treatment	Glandular cavities (number cm^{-2})	Essential oil yield (g 100 kg^{-1})	linalool %	linalyl acetate %	limonene %	linalyl acetate/linalool %
Ctr	265.33±3.48b	238.66±0.98c	44.983±8.07b	14.517±0.01b	17.101±7.74b	0.323±0.02b
CC	285.00±2.52a	284.84±1.51a	58.351±3.34a	11.245±0.03c	10.395±3.81c	0.193±0.01c
Ka	286.00±4.00a	277.45±1.64b	38.917±4.74c	15.510±0.01a	21.027±3.85a	0.399±0.03a
Year (A)	*	*	*	*	*	*
Treatment (B)	*	*	*	*	*	*
Interaction (AxB)	ns	ns	ns	ns	ns	ns

Analysis of Variance (two-way ANOVA) with $N = 15$; (A = Year; B: Treatment; AxB: interaction between year and treatment); Means in the same column followed by different letters are significantly different ($P \leq 0.05$) using Tukey's test. NS not significant.

Table 8
The eingenvalues of the model and correlations between variables and factors.

Eingenvalues	F1	F2	F3
Eigenvalue	5.445	3.543	2.164
Variability (%)	34.032	22.146	13.524
Cumulative %	34.032	56.178	69.702
Correlation variables/factors			
L (10°/D65)*	0.6176	**−0.6794**	−0.0611
a (10°/D65)*	0.2955	**−0.5199**	0.0881
b (10°/D65)*	**0.6995**	−0.6780	0.0024
Chroma	**0.6982**	−0.6675	−0.0029
°Hue	−0.6563	**0.7286**	−0.0554
Fresh Weight	**0.8173**	0.4971	0.1787
Polar Diameter	**0.7833**	0.4552	−0.1951
Equatorial Diameter	**0.8038**	0.5309	0.1876
Circumference	**0.8117**	0.5204	0.1837
Relative Lenght	0.0379	−0.0451	**−0.5982**
Yield Juice	**0.8428**	0.4483	0.1193
TSS	−0.0496	0.1581	0.5348
TA	0.2438	0.2296	**−0.7108**
TSS/TA	−0.2277	−0.1302	**0.9066**
AA	−0.3020	0.2652	−0.1219
Ka	**F1 (0.563)**		
CC - Ctr	**F2 (0.500 – 0.532)**		

of different crops. Harris (1975) reported that fruits exposed to light radiation have a higher vitamin C content than in the shaded or ripened fruits in the most inner layers of the canopy. Instead, our results showed higher AA in the fruits of the treated trees, which is consistent with the results of Nagy (1980) showed that *C. paradisi* and *Citrus unshiu* fruit contain more vitamin C when developed in milder temperature conditions. Finally, the time of ripening plays a central role in determining production quality. In some fruit species, such as Prunus armeniaca cv. Tilton, and Prunus persica cv. Elberta, and Carica papaya, cv. Solo and Zubeckis (1962) and Wenkam (1979) reported that ascorbic acid content increases with the degree of ripeness, whereas in citrus fruits immature fruits contain the highest concentration of vitamin C content that tends to decrease with ripening because of the increase in fruit size (Nagy, 1980). However, the Total Polyphenol Content (TPC) and Total Antioxidant Capacity (TAC) in different layers of the fruit, the differences between treatments appeared to be statistically significant. Regarding the production of essential oil, as well as the number of oleiferous vesicles, these last were influenced by the treatment with shielding products; in fact, the RM treatments resulted in higher yields than control trees. This can be attributed to the higher number of oleiferous vesicles found in flavedo fruit of RM treatments. The PCA showed how the fruits of the three treatments had distinctive characteristics by placing them in the two different factors: Ka in F1, CC and Ctr in F2.

5. Conclusion

This study suggests that in warm areas, such as Southern Italy, where Citrus bergamia is cultivated, reflective materials, such as kaolin, could improve leaf performances such as the carbon uptake potential in the outer and inner canopy layers, respectively. The treated tree has improved yield production, fruit quality, such as fruit size, and nutraceutical parameters. Regarding the production of essential oil treatments gave higher yields than the control tree. This can be attributed to the higher number of oleiferous vesicles in the flavedo fruits treated with reflective material (RM). In conclusion, it was possible to determine the adoption of appropriate cultivation techniques, such as the use of reflective materials, would make it possible to contrast, in the short to medium term, the effects of global warming improving the physiological and productive performance of *Citrus bergamia*. The improvement in essential oil yields is used as a base for the worldwide perfume industry. The increase of nutraceutical parameters, for fresh consumption, justify the increased costs to apply reflective material on trees important for their production.

Photo 1. Three-dimensional representation of the 3 factors extracted from principal component analysis.

CRediT authorship contribution statement

Antonio Dattola: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gregorio Gullo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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