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Global warming threatens the world production of bergamot essential oil

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ABSTRACT

Rationale: Global warming has the potential to impact on the olfactory features of bergamot essential oil, which is a key component of perfumes, ointments and juices.
Objectives: The present study aimed at evaluating the hypothesis of a possible correlation between the chemical compositional characteristics of bergamot essential oil and climatic conditions over a twenty-year period (1999–2019) in Calabria. This Southern Italy region is responsible for ~95 % of the worldwide production of bergamot oil.
Materials and methods: Unlike the vast majority of studies on stress tolerance that focus on a single stress condition, this study faced the challenge to evaluate the complex effects of a combination of different abiotic stress causes. It was found that the impoverishment of the olfactory qualities of bergamot essential oil reported for specific years closely correlate with the combination of heat waves and droughts. Chemically, the effect was attributed to dramatic compositional changes and to the massive accumulation of monoterpenes, in particular D-Limonene, in the fruit peel.
Conclusions: The data thus predict that, without a carefully planned increase of irrigation, the world bergamot industry may soon be jeopardized.

1. Introduction

Bergamot (*Citrus Bergamia* Risso et Poit) is a plant that produces a citrus fruit of color between green and yellow widely used as an aromatic ingredient in food, infusions, perfumes, and cosmetics. Oil from the fruit peel and extracts from the fruit juice are used in medicine. The origin of bergamot is controversial. For some authors (Nicolosi et al., 2000; Li et al., 2010), bergamot is a hybrid between cedar (*Citrus medica*) and bitter orange (*Citrus aurantium*). More recently, bergamot was suggested to be a hybrid between lemon (*C. limon*) and bitter orange (*C. aurantium* L.) based on cytoplasmic studies (Curk et al., 2016). Bergamot blooms in April and May. The ripe fruits are harvested manually from November to March not to damage the utricles containing the precious essential oil. This accumulates in oil glands located in the fruit peel (exocarp) and appears as a clear liquid of yellow-green color >93 % consisting of volatile or semi-volatile terpenoids (Di Giacomo, 1999; Dugo and Mondello, 2010).

More than 95 % of the world production of cold-pressed bergamot essential oil is concentrated in the province of Reggio Calabria, in Southern Italy. Since the bergamot industry contributes significantly to Calabrian regional export, the quality of this important essence is crucial for the economy of the region but also for the international use of the essential oil in perfumes and fragrances. The bergamot cultivar is so important that, since 1999, the Calabrian production of Reggio Calabria bergamot oil is protected by the Protected Designation of Origin (PDO) granted by the European Union in March 2001 as "Bergamotto di Reggio Calabria - Olio essenziale" (Commission Regulation (EC) No 509/2001 of 15 March 2001).

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Bergamot is cultivated in coastal areas (mostly along the Ionian Sea coasts), on saline soils and in a typically Mediterranean ecosystem, characterized by a semi-arid climate, with strong summer drought in conjunction with high environmental temperatures and a chronic unavailability of water for irrigation. Despite this, bergamot has found its ideal habitat in Calabria, as evidenced by the high yields in fruit production in this area (Nesci, 1994; Caruso et al., 1994). Harsh conditions are well known to impact on the agricultural productivity of bergamot crop (Rhodes et al., 1999; Mittler, 2006; Selmar, 2008; Servillo et al., 2018) and result in changes in the compositional characteristics of the oil (Nowak et al., 2010; Kleinwachter and Selmar, 2015). These changes are due to inhibition of photosynthesis and reduction of CO_2 assimilation by the plants, caused by closure of the leaf stomata (Chaves, 1991; Cornic, 2000; Galmés et al., 2007). Accordingly, it was reported that the bergamot production campaign 1998/1999, that was particularly arid, resulted in a strong effect on the quality of the essential oil (Gionfriddo et al., 2000). July 1998 was in facts characterized by an exceptional heat wave with temperatures close to 46 °C in the bergamot cultivation area, subjecting the plants to strong vegetative stress. A similar phenomenon had occurred in 1982/1983 (Mammì and De Leo, 1984). While these reports set out an important precedent, solid evidence of a correlation between climatic variations and bergamot oil quality could only be established through a systematic analysis.

In the present study, it is reported a comprehensive collection of the analytical compositional data of Reggio Calabria bergamot oil collected from 1999 to 2019. The oil quality described in detailed reports (Di Giacomo, 1972) was compared with the standards described by the International Standard Organization (ISO, 3520: 1998). This organ has standardized internationally the ranges of variability and chemical-physical parameters of the bergamot essential oil. It was then verified whether variations of these values could correlate with the climatic data in the bergamot production area over 20 years. A clear correlation was found between parameters known to determine the oil olfactory quality and extreme climate conditions, such as heat waves and droughts (European Environment Agency (EEA, 2012). The data provide an important reference that demands prompt and efficient response to stabilize the risks that the climatic changes could have on the bergamot essential oil production.

2. Materials and methods

2.1. Input data: SSEA reports on bergamot essential oil

Approximately 1150 analytical reports relative to bergamot essential oils were issued by the experimental station for citrus essences and derivatives (SSEA) from 1999 to 2019 and integrated with other ca. 180 reports, relative to samples directly collected by SSEA staff in the province of Reggio Calabria. All chemical-physical parameters were obtained according to ISO methodologies: The relative density at 20 °C (d^{20}_{20}) and the refractive index (n^{t}_{D}) were set according to ISO 279: 1998 and ISO 280: 1998 respectively. Determination of optical rotation (α^{t}_{D}) at 20 °C and of esters as % of linalyl acetate were set according to the ISO 592: 1998 and ISO 709: 2001 procedures. Quantitative residue evaluation on evaporation was expressed as mass percentage, obtained by eliminating the volatile fraction of the oil by heating on a boiling water and weighing of the residue, according to ISO, 4715: 1978. The CD value and maximum absorption by UV spectrophotometric analysis was evaluated according to the ISO, 4735: 1981 procedure.

The only exception was for the volatile fraction which was analyzed by GC/MS with an internal SSEA protocol (Siano and Cautela, 2012), similar to the ISO 7609 procedure by gas chromatography on capillary columns (International Standard ISO, 7609: 1985). Analysis of the volatile fraction was obtained with a GC Thermofisher Trace 2000 interfaced with a spectrometer model GCQ plus (Thermofisher, Monza, Italy) (Siano and Cautela, 2012). The analysis was carried out using a DB-5 column (Restek Co., Cernusco sul Naviglio, Italy) (30 m length, 0.25 mm inner diameter, and thickness of the film 0.25 µm) using the following temperature ramp: 10 min at 70 °C, from 70 to 120 °C with a ramp of 3 °C/min, from 120 to 220 °C with an increase of 4 °C/ min; from 220 to 280 °C with ramp of 15 °C/min, 280 °C for 10 min. Helium at a constant flow of 1.5 mL/min was used as the carrier gas. 0.2 µl of bergamot essential oil, diluted 1:10 (v/v) with acetone, was injected in 1:100 split mode and thermostated at 250 °C. The GC/MS interface was thermostated at 280 °C. An electronic impact of 70 eV in positive ion mode was used for the ionization phase. Mass scans between 70 and 400 *m/z* were recorded and analyzed using the Excalibur software (ThermoFischer, Monza, Italy). The percentage composition was calculated from the gas chromatographic peaks without the use of correction factors. Identification of the components was carried out by comparing the mass spectra with that of a standard reference mixture kindly obtained by Dr Ehrenstorfer (LGC Standard Italy, Sesto San Giovanni, Italy).

2.2. Input data: Bergamot study site and climate data analysis

The area examined is located in the Southern Italy province of Reggio Calabria, located between latitudes 37 ° 55′ to 38 ° 25′N and longitudes 17 ° 37' to 16 ° 35′E (Official Journal of the European Communities C193 11.07.2000) (Fig. 1). The bergamot cultivation area is ca. 900 km^2 and extends over a coastal strip of about 150 km from the Strait of Messina (Scilla, 38 ° 15'N; 15 ° 43'E) to the northern Ionian border of the province of Reggio (Monasterace, 38 ° 26'N; 16 ° 35'E). The strip of land interested in the cultivation of bergamot is 10 km away from the sea and has an altitude <300 m above sea level.

The daily climatic data for the period January 1979-December 2019 (maximum and minimum temperatures, relative humidity, rainfall, wind speed and solar radiation) were obtained from the Bova weather station (ID. T379159) (37.936 N and 15.938E) located at 90 m from mean sea level. The data were extracted from the database of the National Centers for Environmental Prediction-Climate Forecast System Reanalysis (NCEP-CFSR) (http://globalweather.tamu.edu) and integrated with measurements acquired by stations responsible for the collection, processing and dissemination of Climatic data of Environmental Interest (SCIA) (http://www.scia.isprambiente.it/) and from the historical archive of the "Multi-Functional Center Risks" (Centro Regionale Funzionale Multirischi, 2021) of the Calabria Region ARPACAL (www.cfd.calabria.it). The distribution of the maximum temperatures was obtained from the SCIA web portal (Sistema nazionale per la raccolta, l'elaborazione e la diffusione di dati Climatologici di Interesse Ambientale (SCIA), 2021), elaborated using a geostatistical algorithm (Kriging) with spatial interpolation on a 5 km resolution grid (Pellicone et al., 2019).

The reference potential evapotranspiration was calculated from climatic data recovered by the Hargreaves-Samani method (Hargreaves and Samani, 1985), expressed in mm/day. The bioclimatic index of aridity was calculated as the ratio between mean monthly precipitation (P) and mean monthly potential evapotranspiration according to the FAO - United Nations Environment Programme (United Nations Environment Programme (UNEP, 1992).

2.3. Statistical data analysis

All analytical data used in this study were statistically evaluated through variance analysis (ANOVA). The chemical-physical parameters extracted from the analytical reports from 1999 to 2019 were compared using the Tukey-Kramer HSD test (Haynes, 2013). The statistical study was performed using the Analyze It version 5.65 software (Leeds, United Kingdom).

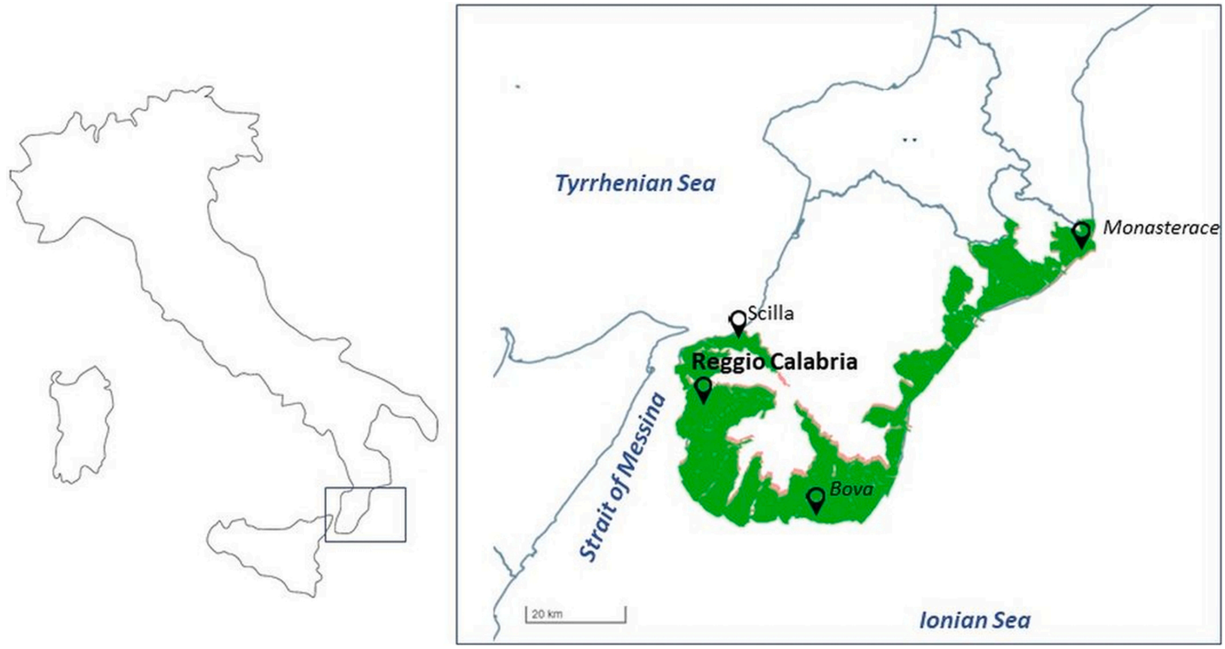

Fig. 1. Localization of the Bergamot production area. Left panel: the position of Calabria within the map of Italy. Right panel: area of production of Bergamot essential oil PDO "Bergamotto di Reggio Calabria Olio essenziale".

3. Results

3.1. Bergamot essential oils: chemical-physical parameters

The variations in numerous chemical and physical parameters of the bergamot essential oil were analyzed. All parameters fell within the variability limits set by the ISO 3520:1998 standard. The average relative density in the period 1999–2019 fluctuated within 0.876−0.883. 53 % of the 1999 samples had a density lower than the minimum ISO limit, while in 2008, 2009 and 2013 the samples that differed from the lower limit were less (about 35 %). For all the other years, the density did not vary significantly ($p < 0.05$) (Fig. 2a).

The average optical rotation was higher in 1999–2001, 2004; 2007–2010 and 2013. Years 2007 and 2010 in particular were characterized by a distribution in which the values of the upper quartile exceeded the maximum ISO limit (Fig. 2b). The refractive index (Fig. 2c) did not undergo significant variations over the years and oscillated between the minimum value (1.4448) in 2006 and the maximum value (1.4701) in 2007 and 2009.

The average ester content, expressed as % linalyl acetate, oscillated between 30 % (ester index value 88) in 1999 and 41 % in 2012 (ester index value 117) (Fig. 2d). Although the average content in esters fell within the ISO limits, about 30 % of the samples analyzed in 1999, 2004, 2008 and 2009 and 2013 had an ester index below the minimum ISO limits. The 1999 vintage differed significantly from all other vintages for lower ester values ($p < 0.05$). while a significantly higher ester content was observed in 2012, 2013 and 2014. The CD (Fig. 2e) and residue on evaporation (Fig. 2f) values were within the ISO reference limits (0.760–1.180 and 4.50 %–6.40 % respectively) with no significant differences among vintages.

3.2. Chemical and compositional variations of BEO in response to climate change

The value distribution of the optical rotation at 20 °C of the essential oil was then analyzed with respect to the reference ISO value of +32 ° set as the maximum limit for genuine bergamot essences (International Standard ISO 3520:1998). The relationship between the terpene content and the optical rotation was clarified by the SSEA researchers Maria and Ignazio Calvarano (Calvarano, 1963; Calvarano and Calvarano, 1964), who found that an increase in the total terpene content causes a corresponding increase in optical rotation and a concomitant increase of D (+)-limonene. It was also noticed that the essences characterized by high optical rotation always corresponded to a lower content of oxygenated monotorpenes, mainly alcohols (linalool, nerol and α-terpineol) and esters. This composition results in a poorer olfactory "bouquet" (Calvarano, 1965).

In addition to 1998, also the 1999, 2000, 2001, 2004, 2008, 2009 and 2013 vintages had average optical rotation levels above +32 °. From a chemical point of view, the increase in optical rotation could be related either to a decrease in optically inactive substances or, more probably, to a variation in the ratios between the individual monoterpenic hydrocarbons contained in the terpene fraction. Among the main monoterpenic hydrocarbons identified and measured in bergamot essences (Gionfriddo et al., 2000), only (+)limonene and (-)β-pinene contribute significantly to the optical rotation value. (+)Limonene alone represents 50 %–60 % of the total terpenes and has an optical rotation of +105 ° while (-)β-pinene represents only 8 %–10 % of the total terpenes with an optical rotation of −19 °. All the other monoterpenic hydrocarbons are mostly optically inactive.

The sum of monoterpenes exceeded the total by 60 % for the years in which the optical rotation was +32 °, while the average of these compounds in the other years ranged between 50 % (2015) and 58 % (2006 and 2018) (Fig. 3 and Table 1). The sum of alcoholic compounds did not exceed 9% in 1998; 1999; 2000; 2001; 2004; 2008; 2009; 2013, with minimum values of 5% in 2013. In other years, the average content of alcohols oscillated between 8.2 % (2016) and 10.9 % (2002). An impoverishment of the ester content was observed in years with optical rotation >+32 °, with a maximum content of 28 % in 2000, while this class of compounds oscillated between 30 % and 35 % in vintages conforming to the ISO 3520:1998 specification. No significant differences between the non-standard vintages and those compliant with the ISO specifications were observed for minor components (carbonyl and sesquiterpene compounds).

3.3. Bergamot climatic site study: general aspects

To gain a detailed climatic representation of the bergamot oil

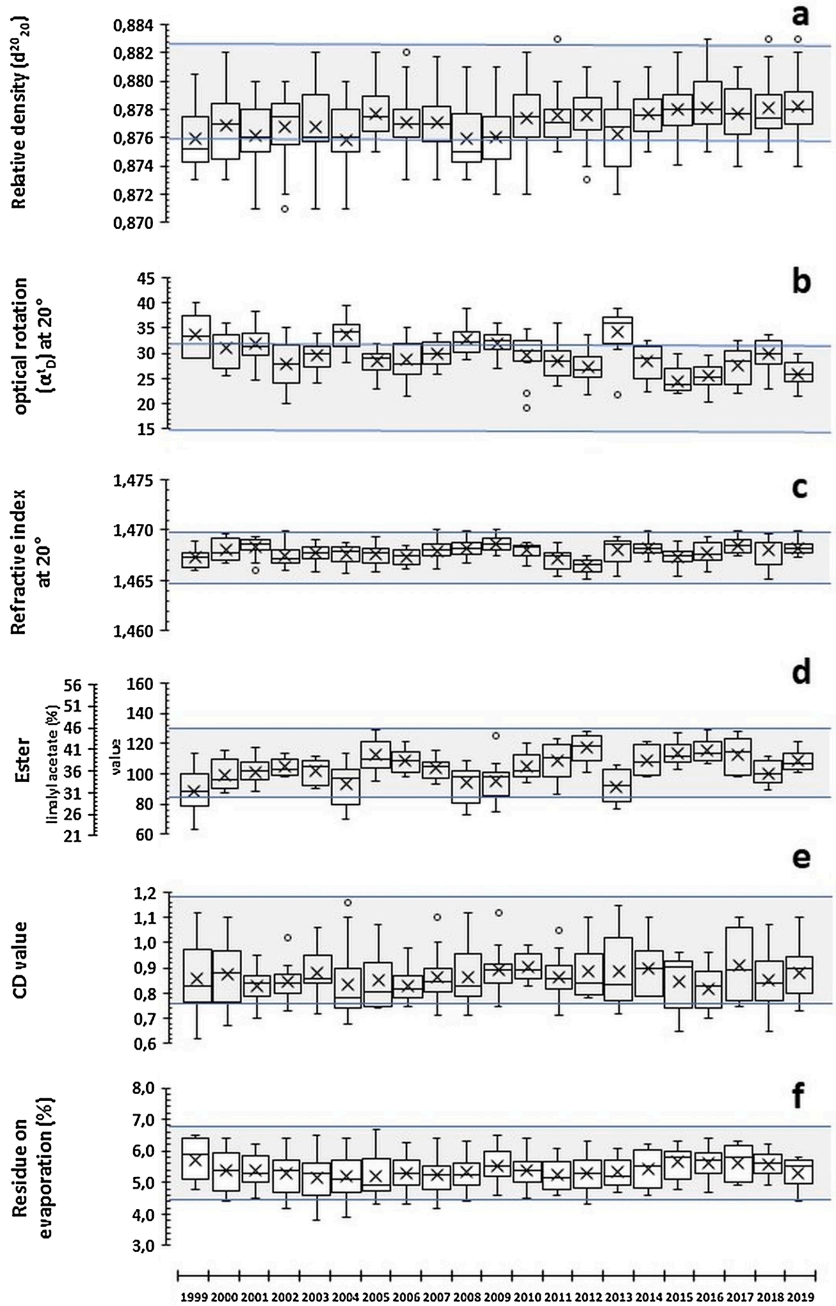

Fig. 2. Boxplot representation of (**a**) Relative density at 20 °C (d^{20}_{20}); (**b**) Optical rotation (α^t_D) at 20 °C; (**c**) Refractive index (n^t_D); (**d**) Ester Index as Linalyl acetate and Ester value; **(e)** Residue on evaporation and **(f)** CD value. Boxes represent the mean as a cross, the median line, and 1st and 3rd quartiles of the physical-chemical parameters. Variations between the years was estimated with a one-way-ANOVA considering all the combinations together.

production area, the climatic data (precipitations, minimum and maximum temperatures) by month were then analyzed together with derived data such as the potential evapotranspiration and the dryness index recorded by the Bova weather station over the period 1979–2019 (Fig. 4). The average annual distribution of rainfall resulted highly irregular, with about 80 % of the rain occurring in the September to March period, while in May-August the production area was characterized by reduced rainfall with average values below 5 mm. The average temperatures were between 24 and 30 °C on the rise with the average maximal temperature below 35 °C.

The evapotranspiration, i.e. the maximum amount of water that can evaporate from the soil and be transpired by the vegetation per surface unit per month, tended to increase in May (≥50 mm of evaporated and/or transpiring water), June (≥65 mm) and July (≥75 mm). July resulted to be the month with lower annual rainfall and higher average temperature. It is also the most critical month for the bergamot cultivation: in this period, the aridity index (i.e. the ratio between precipitation and potential evapotranspiration) reached values close to 0.2, that is what is expected for an arid or semi-arid climate according to the United Nations Environment Programme definition (United Nations Environment Programme (UNEP, 1992). The climatic situation of the bergamot production area tended to normalize to more humid climatic conditions (aridity index ≥ 0.7) only between the end of September and the beginning of December, due to the increase of rainwater compared with the potential evapotranspiration over the same period.

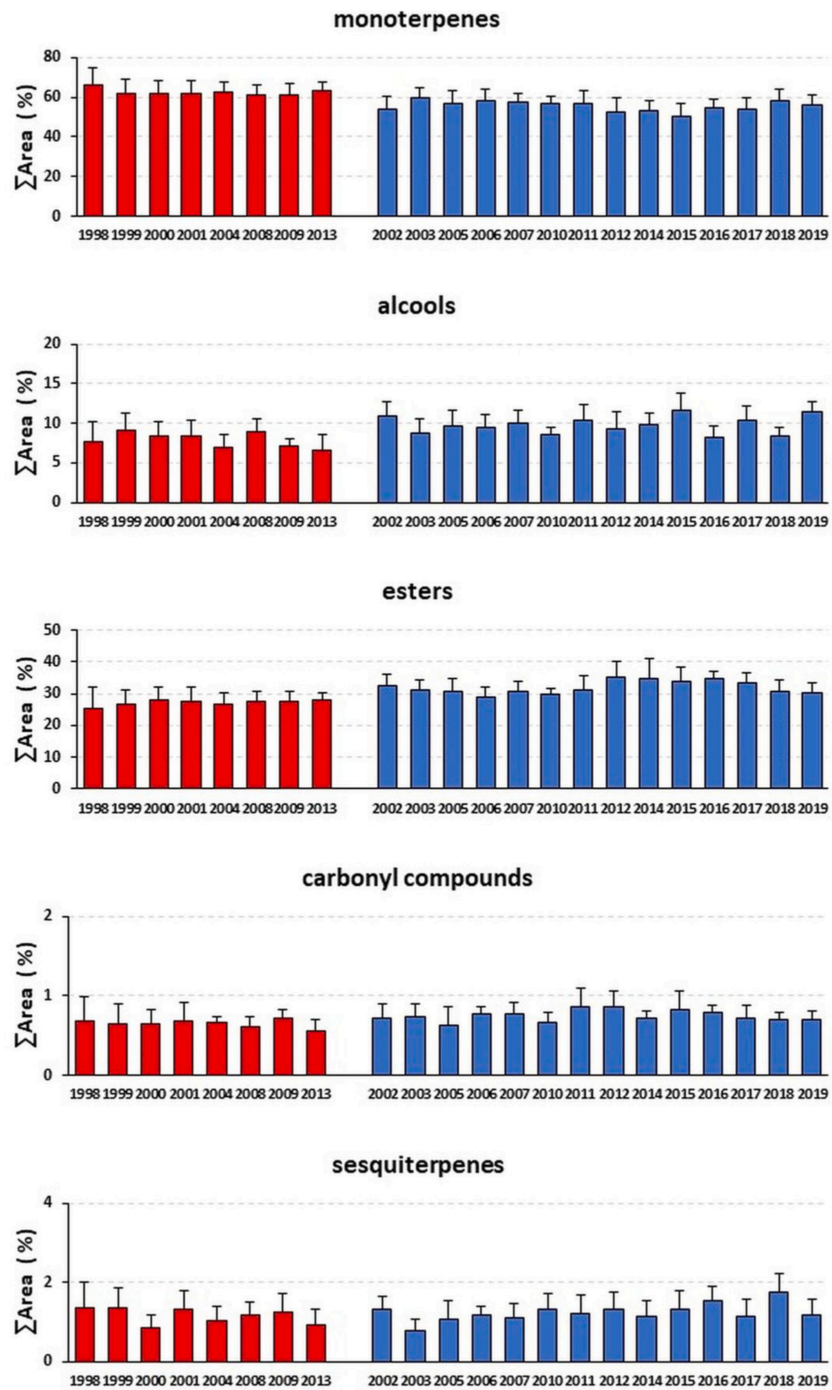

Fig. 3. Sum of Area percentage (mean ± std dev) of the volatile compound classes quantified by GC-FID in BEO during 1998–2019. White bars represent production years characterized by Optical Rotation exceeds ISO, 3520: 1998 standard, blue bars represent production years with Optical rotation is within ISO, 3520: 1998 specification.

3.4. Impact of climatic changes and optical rotation correlation

The geo-climatic picture of the bergamot production area becomes clearer if the values of the maximum temperatures and average daily rainfall recorded in the period 1998–2019 are analyzed for the months of June, July and August, which are critical for the citrus cultivation. Numerous vintages had maximum temperatures above 40 °C (Fig. 5a). The vintages 1999, 2000, 2001, 2005, 2007, 2008, 2009, 2012, 2013 and 2016 were all characterized by temperature excursions similar to those found in 1998 with outliers above the third quartile (more days with Tmax greater than 35 °C) while the remaining vintages were largely characterized by outliers below the first quartile (Fig. 5b).

Analysis of the distribution of average daily precipitation, in millimeters of rain, for the months of June-August recorded in the 1998–2019 period showed low levels of rainfall (Fig. 5c) with 1998, 2000, 2001, 2004, 2008, 2009, 2011, 2013 and 2014 being drier (average daily rainfall <0.5 mm) to be compared with 2005, 2006, 2015, 2016, 2017 and 2018 with average daily rainfall >1 mm. In 2010 and 2012, the average daily rainfall was about 1 mm of rain in July and <0.5 mm of rain in June and August.

The average optical rotation >+32 ° measured for these years was then compared with controls. This analysis confirmed that the

Table 1

Range of variability and average values of bergamot essential oil relevant volatile compounds according to ISO, 3520: 1998 and analyzed by GC-FID over the period 1998-2019. The volatile components and the Sum of Area % of volatile compound classes quantified by GC-FID in BEO during 1998-2019 are reported in Table S1 of Supplementary material.

	Volatile compounds (%)													
	β-pinene		Limonene		γ-terpinene		Linalool		Linalyl acetate		Geranial		β-bisabolene	
	min-max	mean ± SD[a]	min-max	mean ± SD[a]	min-max	mean ± SD[a]	min-max	mean ± SD[a]	min-max	mean ± SD[a]	min-max	mean ± SD[a]	min-max	mean ± SD[a]
1998	5.00−7.50	6.00 ± 0.80	42.0−49.00	47.00 ± 3.70	5.00−8.80	8.00 ± 0.82	6.0−11.0	7.50 ± 2.00	20.00−32.00	24.00 ± 4.00	0.20−0.40	0.34 ± 0.10	0.30−0.80	0.62 ± 0.20
1999	5.15−6.75	5.95 ± 0.46	40.63−48.03	44.33 ± 3.86	7.14−8.78	7.96 ± 0.77	7.10−11.10	9.10 ± 1.41	21.43−29.77	25.77 ± 2.93	0.26−0.46	0.36 ± 0.04	0.31−0.71	0.51 ± 0.08
2000	5.79−7.05	6.42 ± 0.59	40.17−49.83	45.00 ± 4.98	5.86−6.63	6.24 ± 0.42	6.50−9.91	8.20 ± 1.54	23.54−30.47	27.00 ± 2.58	0.29−0.43	0.36 ± 0.03	0.41−0.69	0.55 ± 0.04
2001	5.19−6.79	5.99 ± 0.63	40.04−47.44	43.74 ± 4.83	6.18−7.82	7.00 ± 0.39	6.27−10.27	8.27 ± 1.71	21.44−29.94	25.34 ± 3.47	0.27−0.47	0.37 ± 0.07	0.22−0.62	0.42 ± 0.14
2002	4.11−5.37	4.74 ± 0.53	34.31−43.97	39.14 ± 2.82	6.10−6.87	6.49 ± 0.59	8.91−12.32	10.61 ± 1.48	27.62−34.55	31.09 ± 2.75	0.31−0.45	0.38 ± 0.04	0.36−0.64	0.50 ± 0.06
2003	5.71−6.94	6.32 ± 0.54	37.76−43.52	40.64 ± 2.53	7.34−8.13	7.73 ± 0.59	6.96−10.26	8.61 ± 1.52	26.81−33.15	29.98 ± 2.73	0.29−0.40	0.35 ± 0.05	0.37−0.58	0.47 ± 0.09
2004	5.18−6.55	5.86 ± 0.62	42.27−47.67	44.97 ± 2.88	6.90−7.99	7.45 ± 0.40	5.36−8.43	6.89 ± 1.65	22.60−28.28	25.44 ± 3.17	0.35−0.38	0.37 ± 0.06	0.39−0.47	0.43 ± 0.11
2005	5.19−6.79	5.99 ± 0.69	35.55−42.95	39.25 ± 2.70	6.58−8.22	7.40 ± 0.55	7.38−11.38	9.38 ± 1.54	25.57−33.57	29.57 ± 2.84	0.25−0.45	0.35 ± 0.01	0.23−0.63	0.43 ± 0.04
2006	6.10−7.02	6.56 ± 0.52	35.97−43.69	39.83 ± 2.39	6.58−8.12	7.35 ± 0.56	8.01−10.83	9.42 ± 1.28	24.79−30.65	27.72 ± 3.50	0.33−0.41	0.37 ± 0.07	0.34−0.50	0.42 ± 0.09
2007	5.20−6.43	5.82 ± 0.61	38.52−43.38	40.95 ± 2.65	6.31−7.36	6.84 ± 0.50	8.26−11.37	9.82 ± 1.49	25.42−31.35	28.38 ± 3.17	0.22−0.34	0.28 ± 0.05	0.42−0.64	0.53 ± 0.08
2008	5.80−7.11	6.42 ± 0.62	40.97−46.55	43.85 ± 2.43	6.82−7.76	7.22 ± 0.52	6.99−10.18	8.64 ± 1.56	23.07−29.08	26.24 ± 2.96	0.30−0.37	0.35 ± 0.06	0.42−0.56	0.53 ± 0.11
2009	5.93−7.56	6.75 ± 0.94	40.25−45.49	42.87 ± 3.98	7.10−8.16	7.63 ± 0.84	6.01−7.83	6.92 ± 2.24	22.97−28.67	25.82 ± 5.19	0.32−0.37	0.35 ± 0.04	0.26−0.51	0.38 ± 0.11
2010	5.21−6.14	5.68 ± 0.78	39.13−42.56	40.84 ± 2.75	6.46−7.21	6.84 ± 1.19	7.46−9.34	8.40 ± 1.11	27.20−29.42	28.31 ± 5.10	0.26−0.32	0.29 ± 0.02	0.37−0.49	0.43 ± 0.13
2011	5.42−7.02	6.22 ± 0.46	36.18−43.58	39.88 ± 1.72	6.07−7.71	6.89 ± 0.38	8.10−12.10	10.10 ± 0.94	26.13−34.01	30.13 ± 1.11	0.26−0.46	0.36 ± 0.03	0.27−0.67	0.47 ± 0.06
2012	4.16−5.96	5.06 ± 0.86	33.75−42.85	38.30 ± 4.61	5.82−7.44	6.63 ± 0.69	7.07−11.17	9.12 ± 1.77	28.81−38.38	33.59 ± 4.44	0.34−0.42	0.38 ± 0.05	0.34−0.56	0.45 ± 0.06
2013	4.20−5.37	4.79 ± 0.90	44.05−48.55	46.30 ± 4.55	6.75−7.14	6.94 ± 0.81	4.76−8.31	6.53 ± 2.05	24.33−28.33	26.23 ± 4.78	0.25−0.35	0.30 ± 0.04	0.37−0.56	0.46 ± 0.11
2014	4.60−6.17	5.38 ± 0.34	35.41−40.33	37.87 ± 4.70	5.68−7.89	6.78 ± 0.69	8.20−11.14	9.67 ± 1.12	25.97−37.53	31.75 ± 4.06	0.30−0.35	0.33 ± 0.02	0.35−0.62	0.49 ± 0.07
2015	4.34−5.94	5.14 ± 0.78	31.76−39.16	35.46 ± 2.46	5.97−7.61	6.79 ± 1.10	9.54−13.54	11.54 ± 1.47	28.36−36.36	32.36 ± 5.78	0.19−0.39	0.29 ± 0.03	0.25−0.65	0.45 ± 0.13
2016	4.60−6.00	5.30 ± 0.71	34.10−38.10	36.10 ± 2.97	6.46−7.26	6.86 ± 0.35	6.55−9.55	8.05 ± 1.86	31.53−35.53	33.53 ± 2.05	0.37−0.43	0.40 ± 0.04	0.36−0.56	0.46 ± 0.05
2017	3.90−5.40	4.70 ± 0.59	35.30−41.00	39.00 ± 2.25	5.83−7.05	6.65 ± 0.19	8.15−11.65	10.15 ± 1.78	28.00−34.00	32.00 ± 2.00	0.22−0.35	0.32 ± 0.05	0.22−0.52	0.42 ± 0.10
2018	4.87−6.50	5.68 ± 0.57	38.38−43.62	41.00 ± 1.41	6.78−7.85	7.32 ± 0.16	7.46−9.28	8.37 ± 1.50	25.95−31.65	28.80 ± 1.45	0.29−0.35	0.32 ± 0.04	0.30−0.54	0.42 ± 0.05
2019	5.30−6.65	5.83 ± 0.81	37.38−42.54	39.92 ± 2.62	6.51−7.63	7.10 ± 0.53	9.71−12.14	11.23 ± 0.91	25.96−31.53	28.68 ± 2.85	0.26−0.34	0.31 ± 0.03	0.40−0.61	0.48 ± 0.12

[a] SD: Standard Deviation. The numbers of samples analyzed varied over the years and were 48 in 1999, 62 in 2000, 70 in 2001, 65 in 2002, 85 in 2003, 55 2004, 75 in 2005, 46 in 2006, 60 in 2007, 81 in 2008, 70 in 2009, 65 in 2010, 69 in 2011, 71 in 2012, 44 in 2013, 68 in 2014, 75 in 2015, 64 in 2016, 53 in 2017, 59 in 2018, and 47 in 2019.

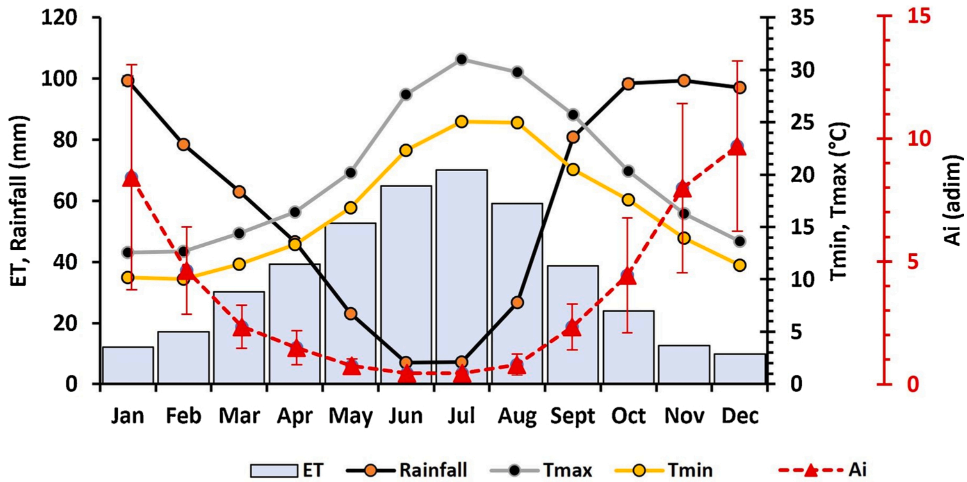

Fig. 4. Climatic characteristics of the bergamot production area. Monthly average values of evapotranspiration (ET), rainfall aridity index minimal and maximal temperatures within the 1999–2019 period.

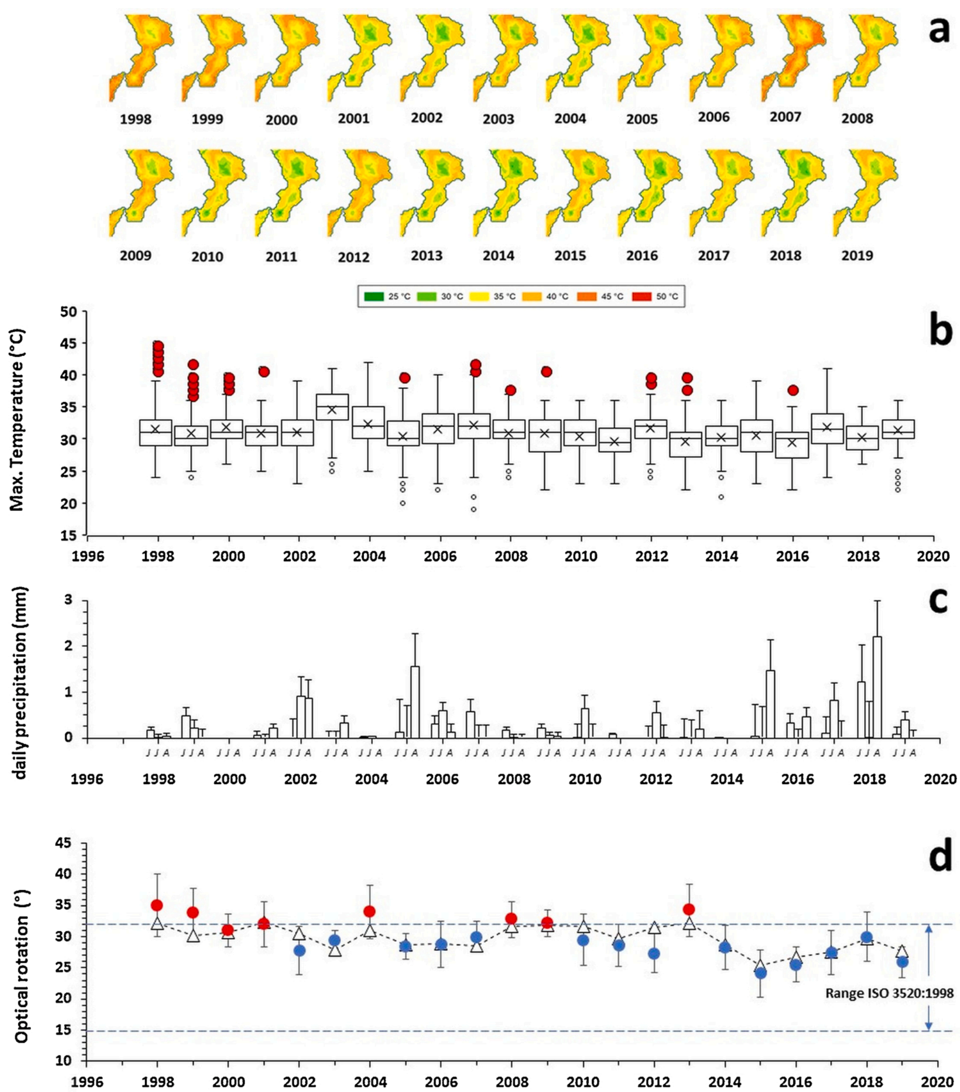

Fig. 5. Temporal trends and variability of daily maximum temperature and precipitation in the BEOs Production Area during the summer months (June, July, August) and correlation with essential oil optical rotation. (d) Optical rotation (average ± std dev) in samples of essential oil (red circles). The white triangles are the values estimated by regression analysis (*optical rotation* = *31.7–3.44 aridity index*$_{June}$ − *3.00 aridity index*$_{July}$ + *0.012 aridity index*$_{August}$).

monoterpene fraction in the essential oils was between 44 % and 68 %. The levels of oxygenated derivatives (alcohols, carbonyl compounds and esters) were between 30 % and 54 % while the sesquiterpene fraction (β-caryoffilene, trans-d-bergamottene and β-bisabolen) had average values close to 1 % (Di Giacomo, 1999; Dugo and Mondello, 2010). To understand how and to what extent the meteorological variability of the summer months may have impacted on the optical rotation, a multiple linear regression analysis was estimated correlating the values of the optical rotations with the relative aridity indices, calculated for the months of June, July and August (Fig. 5d). This showed a significant relationship ($p < 0.001$) between the optical rotation and the dryness index of the months of June-August and explains the ca. 62 % variability of the optical rotation observed over the years. Sample regression coefficients, negative in the months of June (−3.44, $p < 0.001$) and July (−3.00; $p < 0.001$), indicated that, as precipitation increased, rotation decreased, while this is not statistically affected from rainfall in August (0.012, $p = 0.197$). The June and July months were thus those with greater impact on the optical rotation of the essential oil.

4. Discussion

In the present study, the effects of stressors on the quality of the bergamot essential oil were analyzed by correlating the oil metabolite composition with climatic conditions in different years. The vast majority of studies focusing on plant stress tolerance have typically considered a single stress condition. This is however unrealistic since, under field conditions, several abiotic stress situations are likely to co-occur, constituting a unique stress condition that is not a simple additive combination of the effects of individual stressors. The presence of high temperatures during spring and summer was considered (primary stress). High temperatures were combined with high evapotranspiration levels and paucity of seasonal rain (secondary stress). Drought and high temperatures represent the most frequent combination of abiotic stress that occurs in natural environments (Mittler, 2006). Most of the citrus trees grown in Calabria are largely managed without irrigation, leaving the trees more vulnerable to the long dry season, which can reach 90 days without rain in some areas. The quality of the bergamot fruits, from which the essential oil is obtained, strongly depends on the stress of drought during the ripening period.

Collectively, the data confirmed that the particular aridity observed in the 1998/1999 season was only the beginning of an irreversible trend towards "abnormal" characteristics of the essential oil (Gionfriddo et al., 2000). The compositional changes found in some of the essential oils produced within the last twenty years can be interpreted on the basis of the studies conducted by Selmar and collaborators on plants exposed to drought stress (Selmar, 2008; Selmar and Kleinwächter, 2013a, b; Selmar et al., 2017). These studies have clarified the chemical and biochemical-physiological bases of compositional differences found in spices and medicinal plants grown in conditions of drought stress as compared with the same plants grown in moderate climates and/or in the absence of drought. Overproduction of secondary metabolites, that are highly reduced compounds, like isoprenoids, phenols or alkaloids, that accumulate in specialized cell structures of organs and tissues most exposed to stressors is functionally linked to the biochemical dissipation of the energy surplus induced by drought stress. The process occurs through an increase in all the biosynthetic processes that consume $NADPH + H^+$ and aims at restoring a new "physiological stability" or even cellular homeostasis. Since water imbalance often occurs in conjunction with high irradiation and temperatures (Cornic, 2000; Mittler, 2006), the molecular response mechanisms described by Selmar and coworkers can be used to mimic the combination of at least two different abiotic stresses such as high temperatures and droughts (Selmar and Kleinwächter, 2013b).

The increase in the biosynthesis of monoterpene hydrocarbons, such as the monocyclic d-limonene terpene, found massively in the essential oils of outlier years, supports a role of the production of these metabolites in the attempt to maintain the $NADP^+$/NADPH ratios at values compatible with an adjusted metabolism capable of allowing plants to acclimatize. The model proposed by Selmar and coworkers is consistent with the accumulation of secondary non-hydrophobic metabolites to counteract abiotic stress phenomena. It has for instance been reported that proline and its *N*-methylated derivatives accumulate in response to a wide range of abiotic stresses in the cytoplasm of citrus and other plants (Nolte et al., 1997; Rhodes et al., 1999; Servillo et al., 2011a, 2011b). This accumulation is commonly attributed to the ability of proline and its *N*-methylated derivatives to mediate osmotic adjustments. An even small increase in the biosynthesis of this amino acid has a large impact on the level of reduction of the cellular $NADP^+$ pool (Hare and Cress, 1997), allowing regulation and maintenance of the $NADP^+$/NADPH ratios at values compatible with a metabolism close to normal also in adverse conditions. Hare and Cress (1997) have reported that the biosynthetic pathway of proline from glutamate, although short, involves a high consumption rate of NADPH under abiotic stress, that favors the nitrogen metabolism and the demand for increased production of secondary metabolites, contributing significantly to the dissipation of the stress-related energy surplus.

Recent climate projections from the International Panel for Climate Change (Intergovernmental Panel on Climate Change (IPCC, 2016) predict that water scarcity will increase in the near future in many regions of the globe. Climate changes in recent decades are unequivocal and will likely continue in the near future. In addition to the already naturally high temperatures in the southernmost regions, water remains above all a scarce resource in the Mediterranean regions and even more so in the Ionian coastal areas of Calabria. Therefore, the ongoing climate changes pose a major problem for citrus crops and put at risk the whole sector that is expected to maintain its yield/quality standards also under adverse conditions. To contain the effects due to abiotic stress on the quality of the bergamot essential oil, it will be necessary to intervene by rationally increasing the irrigation of the bergamot groves, especially in summer, to counteract an increase in evapotranspiration and drought that affect stress conditions. This is the only weapons to counteract the "out of standard" phenomena of bergamot essential oils.

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Domenico Cautela: Visualization, Investigation, Writing - original draft. **Annalisa Pastore:** Conceptualization, Writing - review & editing. **Giovanna Ferrari:** Validation, Formal analysis. **Bruna Laratta:** Validation, Formal analysis. **Nunzia D'Onofrio:** Resources, Methodology. **Maria Luisa Balestrieri:** Resources, Methodology. **Luigi Servillo:** Supervision. **Domenico Castaldo:** Conceptualization, Methodology.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2021.113986.

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