The Journal of Agricultural Science

cambridge.org/ags

Climate Change and Agriculture Research Paper

Cite this article: Cataldo EC, Salvi LS, Paoli FP, Fucile MF, Masciandaro GM, Manzi DM, Masini CMM, Mattii GBM (2021). Effects of natural clinoptilolite on physiology, water stress, sugar, and anthocyanin content in Sanforte (*Vitis vinifera* L.) young vineyard. *The Journal of Agricultural Science* **159**, 488–499. https://doi.org/10.1017/S0021859621000915

Received: 13 July 2021
Revised: 16 October 2021
Accepted: 3 November 2021

Keywords:
Grapevine; soil management; sustainable agriculture; zeolite

Author for correspondence:
E. C. Cataldo,
E-mail: eleonora.cataldo@unifi.it

Effects of natural clinoptilolite on physiology, water stress, sugar, and anthocyanin content in Sanforte (*Vitis vinifera* L.) young vineyard

E. C. Cataldo[1], L. S. Salvi[1], F. P. Paoli[1], M. F. Fucile[1], G. M. Masciandaro[2], D. M. Manzi[3], C. M. M. Masini[3] and G. B. M. Mattii[1]

[1]Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, 50019 Sesto Fiorentino (FI), Italy; [2]CNR, IRET, Via Moruzzi, 1, 56124 Pisa (PI), Italy and [3]DN360 Piazza d'Ancona, 3, 56127 Pisa (PI), Italy

Abstract

In the Mediterranea area, major effects of climate change are a modification in rainfall patterns, an increase in temperature with an intensify in tropical nights, and an increase in incoming radiations, especially UV-Bs. Despite the various adaptation strategies, grapevines are sensitive to altered climatic conditions. This paper aims to assess the benefits of applying a new sustainable product to the soil that can implement farmers' resources to adapt to this changing situation. Zeowine was realized by combining the properties of zeolite, which has excellent potential in many sectors such as in agriculture, with the organic substance of a compost obtained on a company scale from the reuse of waste processing grapes, pomace and stalks. The effects of two different soil management (Z – Zeowine, 30 t/ha dose and C – Compost, 20 t/ha dose) on vine physiology and berry compositions in Sanforte grapevines (new plantation) were studied during the 2019–2020–2021 growing seasons in the San Miniato area, Italy. The following physiological parameters of grapevines were measured: leaf gas exchange, leaf temperature, stem water potential and chlorophyll fluorescence. The results showed that Z increased single leaf photosynthesis, reduced leaf temperature and water stress. In addition, phenolic and technological parameters were studied. The Z-treated vines had higher sugar content and total and extractable anthocyanin content as well as berry weight. These results suggested that the application of zeolites added to compost in the vineyard to the soil can be a valid tool to mitigate the effects of climate change.

Introduction

Climate change can affect agriculture in several aspects (Cline, 2008). An increase in intensity and frequency of many extreme climate events, a decrease in rainfall and frequent heat waves can be identified such as characteristics of climate change (Houghton, 2005; Gourdji *et al.*, 2013). In fact, the global climate scenario will be distinguished by a rise in greenhouse gases amount, a rise in temperature and changes in the precipitation diagrams (Marín *et al.*, 2021). With excessive increase temperatures, warming leads to reduce crop yields because plants increase the speed of their development, producing less in the process (Hedhly *et al.*, 2009; Chen *et al.*, 2020). On the one hand, although the increase of atmospheric carbon dioxide (CO_2) concentration can enhance photosynthesis, as a carbon source and reduce transpiration rates (Burkart *et al.*, 2011; Deryng *et al.*, 2016), on the other, the higher temperatures also affect the ability of plants to use and obtain moisture (Viciedo *et al.*, 2021). In fact, high temperatures lead to a decline in stomatal conductance (gs) characteristic of upsurging water stress (Trahan and Schubert, 2016).

The water availability for the grapevine is influenced by the combination of water deficit and high summer temperatures, especially from the point of view of berry ripening; the resulting wines show imbalances due to high alcohol content and low polyphenolic complexity (Santos *et al.*, 2020; Savoi *et al.*, 2020). Hence, the water status evaluation, in grapevine, acquires enormous importance especially in warm producing terroir, where appropriate vineyard soil management may represent a sustainable approach to achieve a balanced grape quality (Buesa *et al.*, 2021; Cataldo *et al.*, 2021*a*).

The benefits of the contribution made by compost, used as soil improvers, in herbaceous crops, fruit growing and viticulture are widely described in the literature (Martínez-Blanco *et al.*, 2013). A good organic matter content ensures better cultivation conditions in many ways, for example, the effects are on workability, water retention, density, porosity, permeability and slow release of nutrients (Johnston, 1986). Due to the application of natural products to the soil or plant, farmers and winegrowers should be able to mitigate the harmful impact of climate change by a suitable adaptation strategy (Rosenzweig *et al.*, 1994; Paudel and Hatch, 2012). It is in this context that the use of zeolite in soil management assumes greater importance (Ramesh and Reddy, 2011). Zeolite [Greek words ζέω, 'boil' and λίθος, 'stone', 'boiling stones' (Polat *et al.*, 2004)], are interesting and versatile minerals that are vital for several ranges of industries due to their particular and unique chemical and structural properties (Van Speybroeck *et al.*, 2015). They are aluminosilicate solids, natural or synthetic origin, bearing a negatively charged framework of micropores into which molecules may be adsorbed for environmental decontamination and to catalyse chemical reactions (Bacakova *et al.*, 2018).

Due to their ability to perform cation exchange, zeolite applications were found in various industries, such as in the pharmaceutical industry, petrochemical industry (Rhodes, 2010; Bish and Ming, 2018). Zeolites have many properties, some of these are of interest for agricultural purposes: high CEC, high water holding capacity in the free channels, and high adsorption capacity (Hedström, 2001). In agriculture, some of the important applications are water treatment (Margeta *et al.*, 2013), gas adsorption (Mofarahi and Gholipour, 2014), aquaculture (Nomura *et al.*, 2017), animal husbandry (Ilić *et al.*, 2011), absorption of heavy metals (Tahervand and Jalali, 2017) and also for odour control (Halim *et al.*, 2010). Zeolites can also absorb up to 55% water, later this water is used by the plants for their metabolic activities (Pisarovic *et al.*, 2003). In grapevine, foliar applications of chabasite-rich zeolitites were able to control simultaneously grey mould, sour rot and grapevine moth and improve the composition of grapes and wines (Calzarano *et al.*, 2019, 2020).

However, until now the advantages of applying zeolite in vineyards to the soil have not been studied. This research is an outcome for the need to find sustainable products that can support the performance of new plantations as a result of climate change, without the help of irrigation. The present investigation examines the effects of a new product called Zeowine (made by the synergy of compost and zeolite) compared with only compost on Sanforte new plantations vines, in local Mediterranean conditions, especially this study evaluates its effects on ecophysiological parameters, yield, sugar and anthocyanin content.

Materials and methods

Study region, climatic conditions, experimental design and settings

This study was carried out in the viticultural Chianti area, in the San Miniato county (PI) (coordinates Lat. 43°40′55.1″N and Long. 10°53′13.8″E), Tuscany, Italy, located at an elevation of 190 m a.s.l. facing North-East exposure, at the Cosimo Maria Masini estate. The San Miniato climate is Mediterranean, semi-arid, with a mean annual precipitation of 800 mm and a mean annual temperature of 14.5°C. According to Italian legislation, a decree of the President of the Republic n. 412 of 26 August 1993 (Table 1) the climatic classification of the municipality of San Miniato is D, 1513 degree days (GG).

An automated weather station (Ecotech, Germany) located at 80 metres from the vineyard, was used to record total rainfall (mm) and maximum, mean and minimum air temperature (°C). Trials were conducted during 2019, 2020 and 2021 growing seasons in the newly planted experimental vineyard. Soil horizons present a clay loam texture with the following average characteristics: 20.2% silt, 27.9% clay and 51.8% sand; organic matter 1.79% (USDA classification); pH (H_2O) 7.7. The newly planted vineyard of the red cv. Sanforte (*Vitis vinifera* L.), grafted on 1103 P rootstock, was planted during 2018, with a spacing of 2.3 m between rows × 0.8 m between plants (∼5434 vines/ha) and located in an area with a 5.5% slope. Vines were planted without irrigation aid; at the time of plantation 90 kg/ha of biodynamic compost was applied. The experimental plot was arranged with a complete randomized block design, consisting of six blocks (four rows each) and one factor (soil treatment). Two soil treatments were applied: C commercial compost (20 tons per hectare) and Z Zeowine (30 tons per hectare). Zeowine is a product that is derived from the composting of waste from the wine supply chain (stalks, grape pulp, pomace, etc.) with the addition of 30% zeolite during the initial phase of the process. The treatments were applied in February 2019 by using a manure spreader to guarantee its uniform application over the entire soil surface and integrated into the soil at a depth of 30 cm. As following the main characteristics of the applied compost and zeolite (clinoptilolite type) used for the preparation of Zeowine are reported (Doni *et al.*, 2021):

Table 1. Climatic zones of the Italian territory according to the degree days (GG)

Climatic zones	From (GG)	To (GG)
A	0	600
B	601	900
C	901	1400
D	1401	2100
E	2101	3000
F	3001	$+^{\infty}$

- Clinoptilolite: 68.30% SiO_2, 2.80% K_2O, 0.75% Na_2O, 12.30% Al_2O_3, 1.30% Fe_2O_3, 0.15% TiO_2, 3.90% CaO, 0.90% MgO, 12.50% loss on ingnition, 5.10 Si/Al, 130 CEC cmolc/kg.
- Compost: <0.5 E.C. (dS/m), 8.32 pH, 28.00% Total Organic Carbon, 4.00% Total Nitrogen, 7 C/N, 3.90% Humic Acids, 2.20% Fulvic Acids.

From the two central rows of each block, two homogeneous vines (total 12 vines per soil treatment) were randomly tagged for leaf gas exchange, water potential and grape composition assessments. Ecophysiological measurements were carried out during the three vegetative seasons (2019–2020–2021), while grape sampling was conducted in the 2020 season (third year of planting of the vineyard) and 2021 (fourth year of planting of the vineyard) as 2019 was without production.

Stomatal conductance and leaf temperature, net photosynthesis and water use efficiency, midday stem water potential, leaf chlorophyll fluorescence and content

During three seasons, from flowering to maturity, between 10 and 12 a.m., leaf temperature and leaf gas exchange (stomatal conductance (gs), transpiration rate (E) and net photosynthesis (Pn)) was measured using Ciras 3, a portable infrared gas analyser (PP Systems, Amesbury, MA, USA), on 12 healthy and fully developed leaves per treatment, in the median portion of a primary shoot (12 replicates, one each tagged vine). Measurements were taken once a day on the following dates: flowering (9 July 2019, 2 July 2020, 28 June 2021), fruit set (17 July 2019, 13 July 2020, 5

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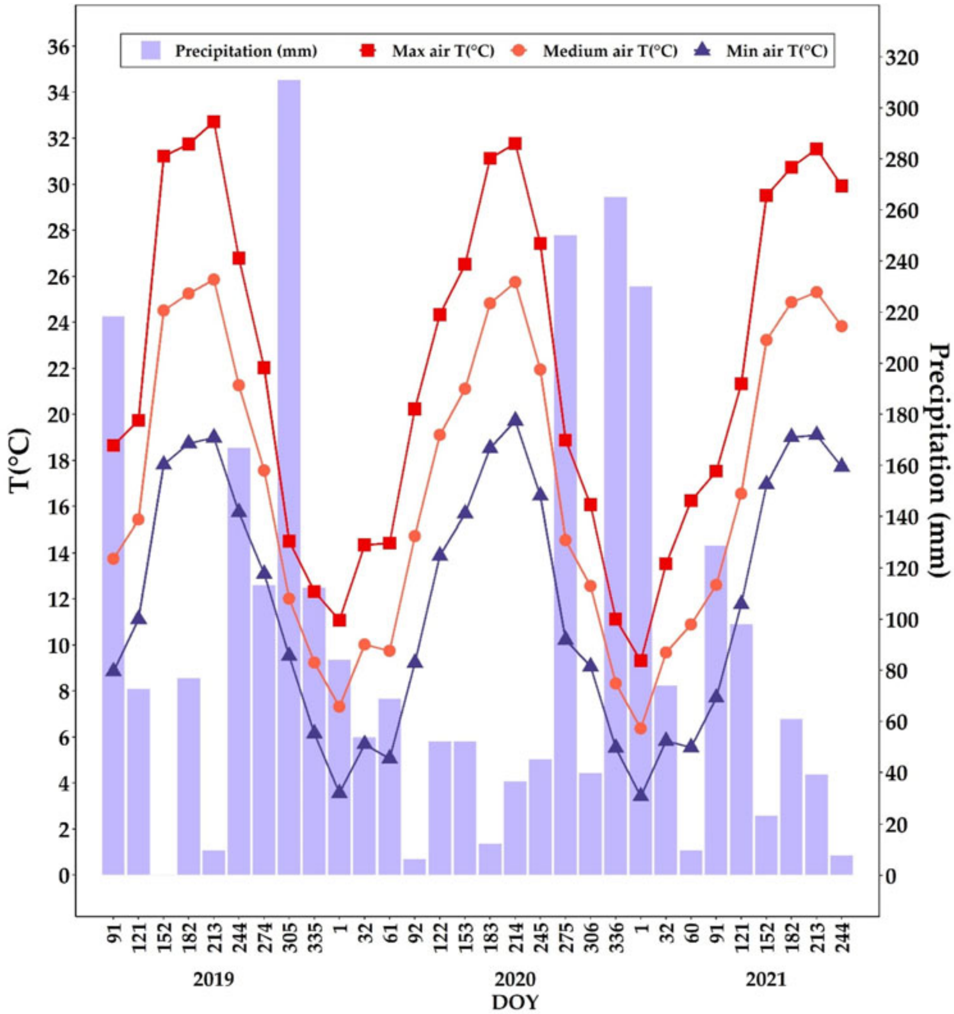

Fig. 1. Colour online. Vineyard Microclimate. Monthly total rainfall (mm) and mean, maximum, minimum temperature (°C) of 2019, 2020 and 2021. The data refer to the following months: April 2019–December 2019 (91–335 DOY), January 2020–December 2020 (1–336 DOY) and January 2021–September 2021 (1–244 DOY).

July 2021), pre veraison (22 July 2019, 21 July 2020, 12 July 2021), veraison (6 August 2019, 1 August 2020, 29 July 2021), mid maturation (19 August 2019, 17 August 2020, 18 August 2021), full maturation (26 August 2019, 3 September 2020, 31 August 2021). The photosynthesis/transpiration ratio, extrinsic water use efficiency (eWUE), was calculated. Setting the leaf chamber flow under the same conditions as Cataldo *et al.* (2021*b*) measurements were performed: saturating photosynthetic photon flux of 1300 μmol/m^{2}s, ambient CO_2 concentration ∼400 ppm and ambient temperature.

Using a pressure chamber (model 600, PMS Instrument Co., Albany, OR, USA), midday-stem water potential (Ψstem, MPa) of dark-adapted leaves (over a 60-min period) was determined on 10 fully expanded leaves per treatment (Ritchie and Hinckley, 1975). Measurements were conducted between 12 noon and 1:00 p.m., from flowering to the ripening phase, in the median portion of a primary shoot (10 replicates, one each tagged vine). Measurements were taken once a day on the following dates: flowering (9 July 2019, 2 July 2020, 28 June 2021), fruit set (17 July 2019, 13 July 2020, 5 July 2021), pre veraison (22 July 2019, 21 July 2020, 12 July 2021), veraison (6 August 2019, 1 August 2020, 29 July 2021), mid maturation (19 August 2019, 17 August 2020, 18 August 2021), full maturation (26 August 2019, 3 September 2020, 31 August 2021).

In the hottest and driest period, from pre-veraison to the ripening, using Handy-PEA® tool (Hansatech Instruments, UK), Chlorophyll a fluorescence transient of dark-adapted leaves was recorded with a saturating flash of actinic light at 3000 μmolm/m^{2}s for 1 s. Briefly, the maximum quantum yield of photosystem II (PSII) was calculated as the ratio $F_v/F_m = (F_m - F_0)/F_m$ where F_v represents the variable fluorescence and F_m represents the maximal fluorescence of dark-adapted (over a 30-min period) leaves (Maxwell and Johnson, 2000).

Measurements were taken once a day on the following dates on 12 healthy and fully developed leaves per treatment, in the median portion of a primary shoot (12 replicates, one each tagged vine): pre veraison (22 July 2019, 21 July 2020, 12 July 2021), veraison (6 August 2019, 3 August 2020, 29 July 2021), mid maturation (19 August 2019, 14 August 2020, 18 August 2021), full maturation (26 August 2019, 3 September 2020, 31 August 2021).

A 502 SPAD device (Konica Minolta Inc., Japan) was used to measure chlorophyll content in leaves. Measurements were taken once a day on the following dates on 12 healthy and fully developed leaves per treatment, in the median portion of a primary shoot (12 replicates, one each tagged vine): pre veraison (22 July 2019, 21 July 2020, 12 July 2021), veraison (6 August 2019, 3 August 2020, 29 July 2021), mid maturation (19 August 2019, 14 August 2020, 18 August 2021), full maturation (26 August 2019, 3 September 2020, 31 August 2021).

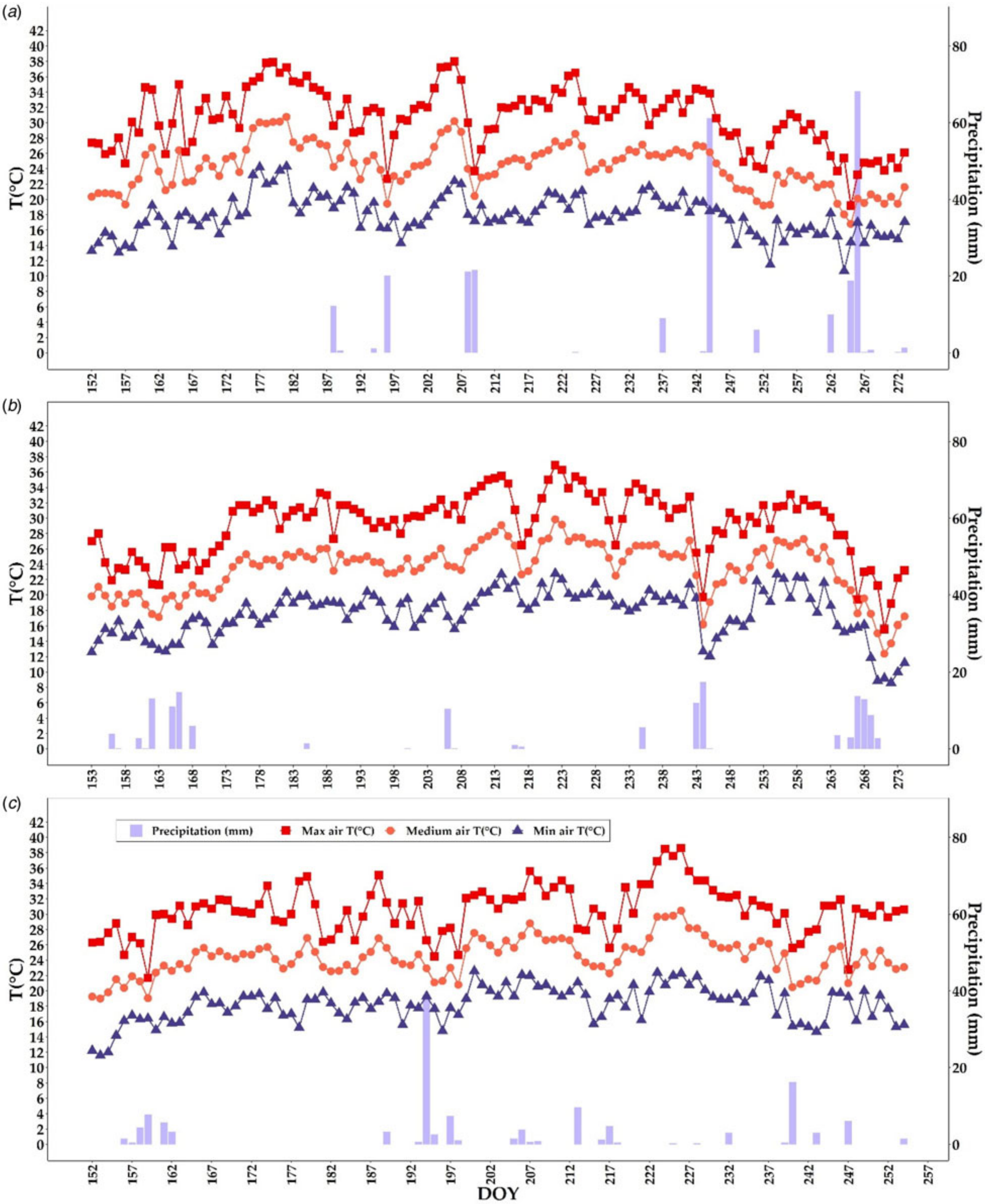

Fig. 2. Colour online. Vineyard Microclimate. Daily total rainfall (mm) and mean, maximum, minimum temperature (°C) of 2019 (*a*), 2020 (*b*) and 2021 (*c*). All data refer to the hottest central months of each year (from June to September). The days are expressed in Day of the Year (DOY) as follows: June 2019 (152–181), July 2019 (182–212), August 2019 (213–243), September 2019 (244–273) and June 2020 (153–182), July 2020 (183–213), August 2020 (214–244), September 2020 (245–274) and June 2021 (152–181), July 2021 (182–212), August 2021 (213–243), September 2021 (244–255).

Berry composition

During 2020–2021 seasons, from veraison to harvest (3 August 2020, 17 August 2020, 3 September 2020, 8 September 2020 and 29 July 2021, 18 August 2021, 31 August 2021, 10 September 2021), a 100-berry sample was collected mixing berries from the tagged vines of each block of both Zeowine and

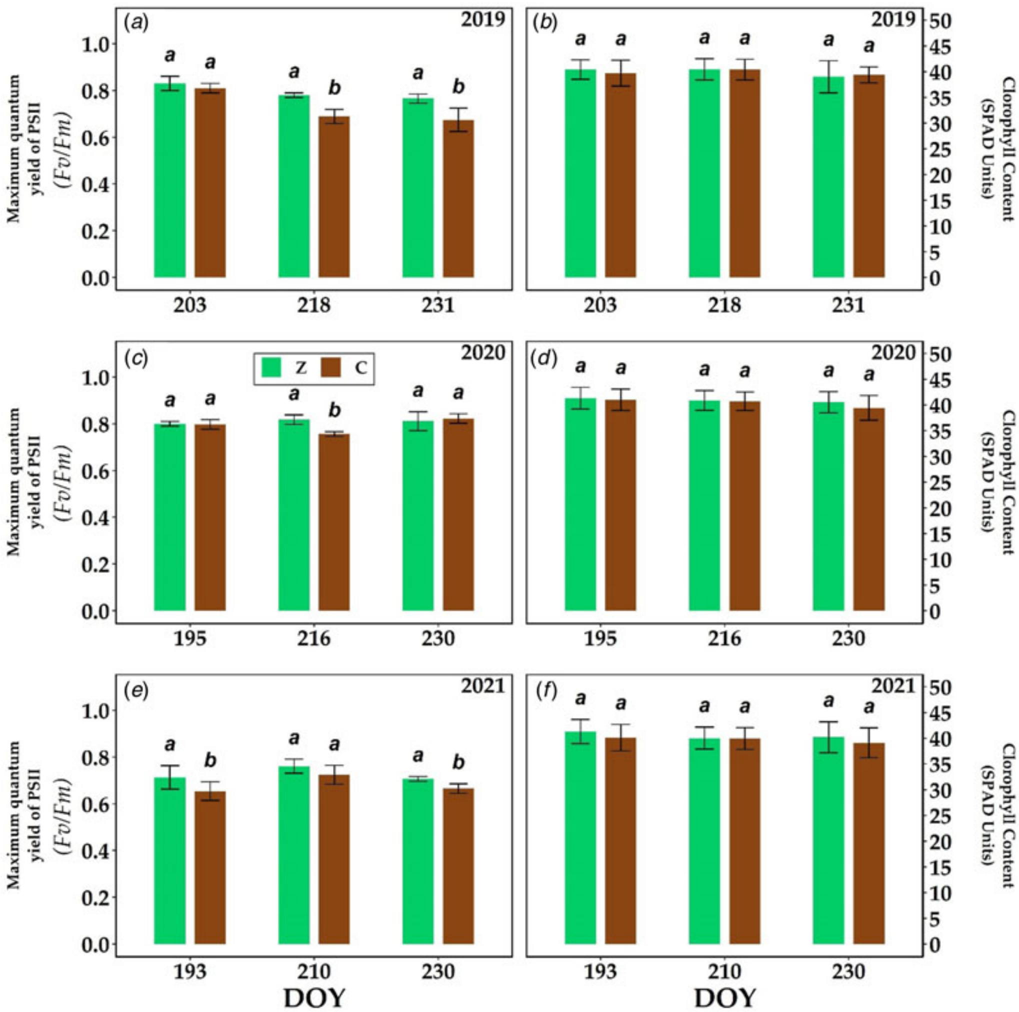

Fig. 3. Colour online. Maximum quantum yield of PSII (F_v/F_m) ((*A*), 2019; (*C*), 2020; (*E*), 2021) and chlorophyll content (SPAD Units) ((*B*), 2019; (D), 2020; (*F*), 2021) in *Vitis vinifera* with two different soil management: Zeowine (Z, green column) and Compost (C, brown column). The days are expressed in Day of the Year (DOY): 22 July 2019 (203), 21 July 2020 (195), 12 July 2021 (193); 6 August 2019 (218), 3 August 2020 (216), 29 July 2021 (210); 19 August 2019 (231), 14 August 2020 (230), 18 August 2021 (230). Different letters within the same parameter indicate significant differences. Data (mean ± s.e., $n = 12$) were subjected to one-way ANOVA (LSD test, $P \leqslant 0.05$).

Compost vines (12 samples of 100-berry in total per treatment) to perform technological analyses and determine the optimal maturity level to harvest (ripening curves). Each sample was weighed with a digital scale (model ES2201, Artiglass, Due Carrare, PD, Italy) and immediately juiced. Sugar content (°Brix) was measured using a refractometer (PCE-Oe Inst., Lucca, Italy); pH was measured using a portable pH meter (PCE-Oe Inst., Lucca, Italy) and must g/L tartaric acid (titratable acidity) was determined on a 10 mL for each 100-berry sample by manual glass burette using 0.1 M NaOH to an endpoint of pH 7.0. Moreover, a duplicate 100-berry sample was picked mixing berries from the tagged vines of each block of both Zeowine and Compost vines (12 samples of 100-berry in total per treatment), was processed for phenolic maturity parameters, such as extractable and total polyphenols and anthocyanins (mg/l) (Ribéreau-Gayon *et al*., 2021) with the Yves-Glories method (Glories, 1984*a*, 1984*b*). Briefly, the samples were read with the spectrophotometer (Hitachi U-2000, Chiyoda, Japan) at 520 nm for anthocyanins and 280 nm for polyphenols. In addition, as described by the method, the following solutions were used: aqueous solution of HCl at pH 1, an aqueous solution of tartaric acid at pH 3.2, solution of ethanol hydrochloride EtOHCl, 2% solution of HCl and an aqueous solution of SO_2.

Statistical analysis

Data from each season 2019, 2020 and 2021 were separately analysed by means of one-way ANOVA with soil treatments as the main factor ($P \leqslant 0.05$). In addition, mean values were separated by Fisher's least significant difference (LSD). *P* value adjustment was performed with the Holm method ($P \leqslant 0.05$). All statistical analyses were performed using R and RStudio (Boston, MA, USA) (Allaire, 2012).

Results

Vineyard microclimate

Climate resulted as typical of the Mediterranean region, although some differences in rainfall pattern were detected in the three

Table 2. Physiological parameters

	Pn (μmol CO_2/m^2 s)		eWUE (μmol CO_2/mmol H_2O)	
Survey date	Z	C	Z	C
9 July 2019	5.85 ± 1.12a	4.91 ± 0.99a	1.16 ± 0.31a	1.05 ± 0.44a
17 July 2019	7.26 ± 1.37a	6.82 ± 1.52a	1.48 ± 0.25a	1.78 ± 0.12a
22 July 2019	7.32 ± 2.04a	5.71 ± 1.96b	2.00 ± 0.69a	1.82 ± 0.76a
6 August 2019	9.36 ± 1.75a	8.10 ± 1.23b	2.54 ± 0.87a	2.06 ± 0.59b
19 August 2019	8.22 ± 1.55b	9.90 ± 0.97a	2.13 ± 0.64b	2.61 ± 0.43a
26 August 2019	9.72 ± 2.10a	3.80 ± 0.87b	2.51 ± 0.58a	1.53 ± 0.32b
2 July 2020	9.40 ± 2.23a	8.10 ± 1.76a	2.29 ± 0.43a	2.04 ± 0.22a
13 July 2020	12.42 ± 2.34a	12.32 ± 2.80a	3.42 ± 0.89a	3.08 ± 0.91a
21 July 2020	13.47 ± 2.99a	10.75 ± 2.55b	3.04 ± 0.21a	2.22 ± 0.65b
1 August 2020	10.14 ± 2.67a	9.56 ± 1.71a	2.06 ± 0.42a	2.21 ± 0.23a
17 August 2020	11.99 ± 1.41a	5.60 ± 2.05b	4.68 ± 0.99a	3.47 ± 0.62b
3 September 2020	7.30 ± 1.77a	3.77 ± 1.45b	2.52 ± 0.48a	1.22 ± 0.30b
28 June 2021	11.36 ± 3.20a	9.58 ± 2.58b	5.79 ± 0.56a	4.84 ± 0.21b
5 June 2021	16.42 ± 4.60a	11.28 ± 3.67b	5.29 ± 0.32b	6.19 ± 0.80a
12 July 2021	11.50 ± 2.43a	7.33 ± 1.31b	1.37 ± 0.40a	1.04 ± 0.12a
29 July 2021	8.04 ± 2.50a	5.55 ± 2.42b	1.47 ± 0.23a	1.18 ± 0.28a
18 August 2021	7.63 ± 2.87a	4.64 ± 1.30b	3.86 ± 0.76a	3.57 ± 0.43a
31 August 2021	4.68 ± 1.78a	2.08 ± 0.99b	2.52 ± 0.30a	1.12 ± 0.22b

Net photosynthesis (Pn), water use efficiency (eWUE) of Vitis vinifera treated with two different soil management methods: Zeowine (Z) and Compost (C). Different letters within the same parameter indicate significant differences. Data (mean ± s.e., $n = 12$) were subjected to one-way ANOVA (LSD test, $P \leqslant 0.05$)

different years of research (Fig. 1). The 2019 year (657.8 mm rain/growing season) was characterized by an even distribution of rain especially during spring and autumn with a dry period in June and August. The 2020 year (454.6 mm rain/growing season) was characterized by an even distribution of rain especially autumn whit a dry period in April and July. Whereas the 2021 year (458.2 mm rain/growing season) was characterized by an even distribution of rain especially spring with a dry period in June and July. Maximum temperatures exceeded 35°C on the following days (Fig. 2): from 25 to 30 June 2019 (176–181 DOY), from 1 to 3 July 2019 (182–184 DOY), from 23 to 26 July 2019 (204–207 DOY), 11 and 12 August 2019 (223–224 DOY), from 30 July to 1 August 2020 (212–214 DOY), from 8 to 10 August 2020 (221–223 DOY) and 12 August 2020 (225 DOY), 7 July 2021 (188 DOY), 26 July 2021 (207 DOY) and from 11 to 15 August 2021 (223–227 DOY).

Stomatal conductance and leaf temperature, net photosynthesis and water use efficiency, midday stem water potential, leaf chlorophyll fluorescence and content

As reported in Figs 3(*A*)–(*F*), no significant differences were noted in chlorophyll content (maximum quantum yield of PSII) in leaves of *Vitis vinifera* between treatments (Zeowine and Compost), while chlorophyll a fluorescence reported differences especially in the warmer period (6–19 August 2019, 3 August 2020, 12 July 2021 and 18 August 2021).

On the hottest days, significant differences in physiological parameters (Pn and WUE) between Z and C during the three study seasons (2019, 2020 and 2021) were found; Zeowine showed higher rates of photosynthesis *v.* C treatment (Table 2).

Treatment C showed in all seasons, values of leaf temperature higher than treatment Z. On the hottest days, the Z treatment tended to record values of superior stomatal conductance (Fig. 4).

Significant differences in stem water potential between soil treatments were found (Fig. 5). Leaf water potential values reflect seasonal trends; peaks of increased water stress were recorded in July 2019, August 2020 and July/August 2021 after the driest and hottest months (June 2019, July 2020 and July 2021).

Berry composition

Table 3 shows the composition of Sanforte berries under two different soil management approaches in 2020 year (first year of production of the vineyard) and in 2021 year (second year of production of the vineyard) in terms of technological maturity. During the 2020 season, significant differences at full veraison, mid-maturation and harvest were noted in sugar content and during the 2021 season, significant differences at mid-maturation, full-maturation and harvest were noted in sugar content: Z had the highest values than C treatment. In the 2020 harvest, the C treatment detected higher acidity values, while in the 2021 harvest no difference was noted in acidity values.

As shown in Table 4, the greatest differences in phenolic maturity were found in the composition of extractable and total anthocyanins. As for the 2020 season, at full maturation and harvest, Z berries showed significantly higher extractable and total anthocyanin content compared to C berries. The lowest values in total

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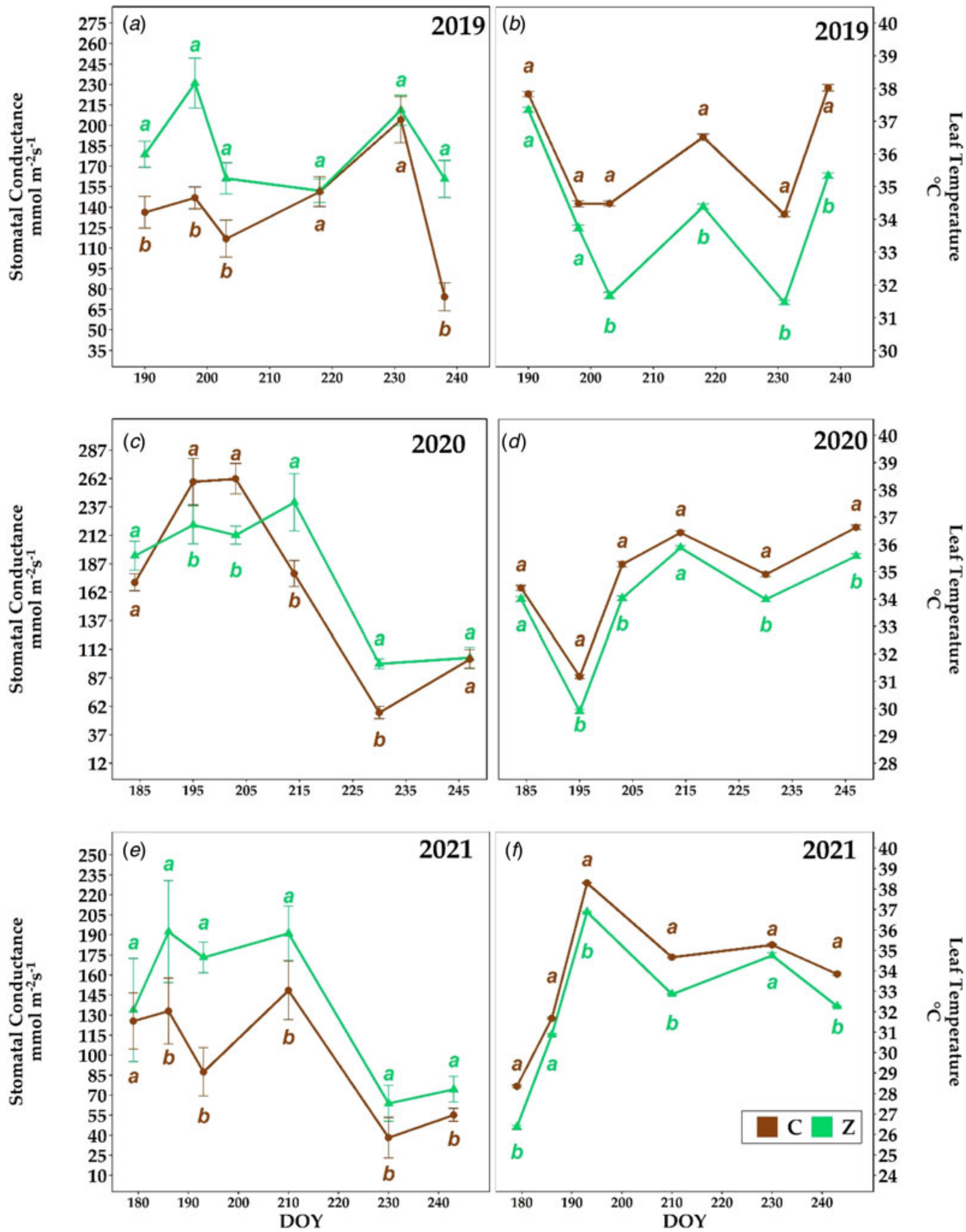

Fig. 4. Colour online. Stomatal conductance (gs, mmol m-2s-1), ((*A*), 2019; (*C*), 2020; (*E*) 2021) and leaf temperature (°C) ((*B*), 2019; (*D*), 2020; (*F*), 2021) in *Vitis vinifera* with two different soil management: Zeowine (Z, green line) and Compost (C, brown line). The days are expressed in Day of the Year (DOY): 9–17–22 July 2019 (190–198–203), 6–19–26 August 2019 (218–231–238) and 2–13–21 July 2020 (184–195–203), 1–17 August 2020 (214–230), 3 September 2020 (247) and 28 June 2021 (179), 5–12–29 July 2021 (186–193–200), 18–31 August 2021 (230–243). Different letters within the same parameter indicate significant differences. Data (mean ± s.e., $n = 12$) were subjected to one-way ANOVA (LSD test, $P \leqslant 0.05$).

anthocyanins were recorded for the C treatment at the three different stages. No differences in total polyphenols at harvest were found. At full maturation, no differences in extractable polyphenols were found, while at harvest C berries showed significantly higher extractable polyphenol content compared to the Z treatments.

As for the 2021 season, at mid-, full-maturation and harvest Z berries showed significantly higher extractable and total anthocyanin content compared to C berries. At harvest, Z berries showed significantly higher extractable and total polyphenol content compared to the C treatments.

In both seasons, significant differences were found for the production parameters at harvest (Table 5). Treatment Z, in general, had a higher weight of the bunches and yield per plant compared to the C treatment.

Discussion

To focus on climate change, the main objectives of soil management are to maintain an environment that favours the development of the vegetative apparatus, the accumulation of organic

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matter, the absorption of water and the use of nutrients; management that causes drop-in soil skills to reduce these aspects (Smith and Powlson, 2007). However, proper soil management can be expected to restore ecosystem functions that have been degraded (Komatsuzaki and Ohta, 2007).

This study highlights the importance of soil management in the Mediterranean area through the application of a new zeolitic-based product against the problems of climate change. Chlorophyll a fluorescence (F_v/F_m, an indicator of photo-oxidative stress; Baroli *et al*., 2004; Lichtenthaler *et al*., 2005) reported differences especially on the hottest days (6–19 August 2019, 3 August 2020 and 18 August 2021). The photo-oxidative shock inhibited photosystem II (PSII) efficiency, as suggested by the reduction of F_v/F_m ratios (Pietrini *et al*., 2005). We hypothesize, that in the C treatment, the extreme temperature-induced photo-oxidative stress as could be derived from increased expression of reactive oxygen species (ROS) scavengers and an increased pool size of the xanthophyll cycle pigments (Jaghdani *et al*., 2021). Consequently, in C treatment, a robust inhibitory effect on photosynthetic capacity and net CO_2 assimilation (Pn) was reported during that period, as a typical impact of PSII deficiency (Pintó-Marijuan and Munné-Bosch, 2014). Gaseous exchanges were affected in zeolite-treated vines; Zeowine showed higher rates of photosynthesis *v*. C treatment (De Smedt *et al*., 2017). The photosynthesis trend during the seasons of both treatments reflected that temperature directly influenced the photosynthesis rate by stimulating the activity of photosynthetic enzymes and the electron transport chain (ETC) (Slot and Winter, 2017). At low temperatures, the Pn rate increased proportionally with the temperature until it reached an optimum (Long, 1983). The higher-summer temperatures reduced C photosynthesis (i.e. during 2020, on August 3th maximum temperatures above 30°C led to the following photosynthesis values: C treatment 3.77 µmol CO_2/m^2s and Z treatment 7.30 µmol CO_2/m^2s). In addition, an increase in the air temperature for the C treatment indirectly led to increased leaf temperature, which could stimulate water loss by transpiration and elevate vapour pressure deficit (VPD) (Yang *et al*., 2012). Probably the effect on leaf temperature was mediated by water availability, as it was observed from stem water potential data. As with barley and corn seedlings (Krutilina *et al*., 2000), the application of zeolite to the vineyard soil was found to increase photosynthetic activity.

In contrast to what was observed by Steiman *et al*. (2007) the WUE of zeolite treated plants was usually higher to compost vines, suggesting that zeolites did increase water consumption with increasing CO_2 fixation (Chaves *et al*., 2004). Due to zeolitic ability to retain water (Sepaskhah and Barzegar, 2010), plants treated with clinoptilolite (Zeowine) showed significantly lower leaf temperatures in both seasons than zeolite-free composting plants. The following reductions were recorded during 2019: −1.25% on June 21st, −2.14% on July 9th, −8.14% on July 17th, −5.81% on July 22nd, −7.90% on July 31st, −7.11% on August 19th. The following reductions were recorded during 2020: −1.11% on May 22nd, −3.79% on June 9th, −3.49% on June 22nd, −1.58% on July 2nd, −2.77% on July 13rd, −2.85% on August 3rd. Instead, the following reductions were recorded during 2021: −7.00% on June 28th, −2.51% on 5th July, −3.69% on 12 July, −5.17% on 29 July, −1.47% on 18th August and −4.66% on 31st August. Probably the lower transpiration rates of compost-treated plants may explain the higher leaf-air temperature that was observed (De Smedt *et al*., 2015).

In all seasons the Sanforte cultivar recorded valuable stomatic conductance values, reflecting its anisohydric-conservative behaviour (drought-tolerance) under stress conditions, keeping stomas always open (Rogiers *et al*., 2012), despite the fall in water potential for compost treatment.

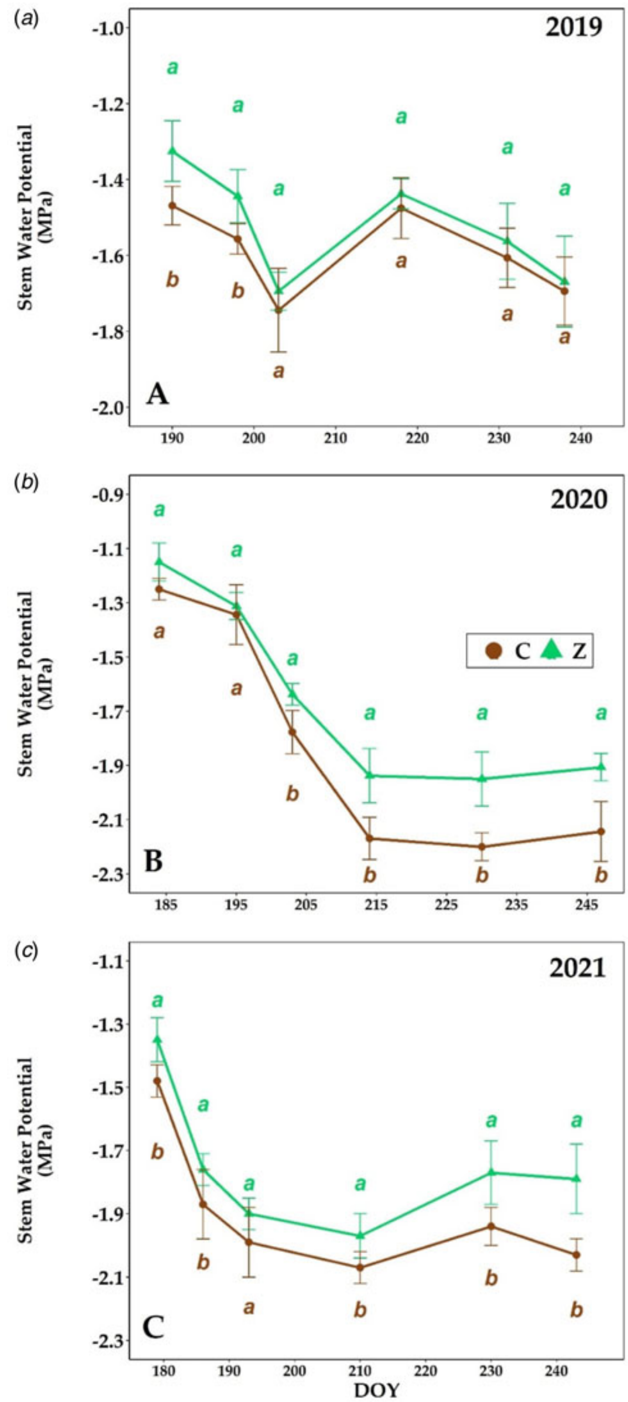

Fig. 5. Colour online. Physiological parameters. Stem water potential (*ψ*, MPa), ((*A*), 2019; (*B*), 2020; (*C*), 2021) in Vitis vinifera with two different soil management: Zeowine (Z, green line) and Compost (C, brown line). The days are expressed in Day of the Year (DOY): 9–17–22 July 2019 (190–198–203), 6–19–26 August 2019 (218–231–238) and 2–13–21 July 2020 (184–195–203), 1–17 August 2020 (214–230), 3 September 2020 (247) and 28 June 2021 (179), 5–12–29 July 2021 (186–193–200), 18–31 August 2021 (230–243). Different letters within the same parameter indicate significant differences. Data (mean ± S.E., $n = 12$) were subjected to one-way ANOVA (LSD test, $P \leqslant 0.05$).

https://doi.org/10.1017/S0021859621000915 Published online by Cambridge University Press

Table 3. Technological maturity

	Sugar content (°Brix)		TA (mg/l Tartaric acid)	
Survey date	Z	C	Z	C
3 August 2020	17.00 ± 0.06a	16.00 ± 0.09b	10.80 ± 0.02a	10.40 ± 0.04b
17 August 2020	22.05 ± 1.00a	22.80 ± 0.08a	8.60 ± 0.03b	8.90 ± 0.02a
3 September 2020	22.25 ± 0.04a	21.05 ± 0.06b	7.12 ± 0.06a	7.00 ± 0.06a
8 September 2020	27.25 ± 0.05a	25.85 ± 0.03b	6.52 ± 0.02b	6.65 ± 0.05a
29 July 2021	17.40 ± 0.01a	16.80 ± 0.03a	12.60 ± 0.02b	13.8 ± 0.05a
18 August 2021	20.20 ± 0.04a	19.10 ± 0.03b	10.20 ± 0.04b	11.20 ± 0.03a
31 August 2021	24.80 ± 0.07a	23.40 ± 0.05b	8.10 ± 0.04b	9.20 ± 0.06a
10 September 2021	26.12 ± 0.02a	25.68 ± 0.08b	7.70 ± 0.02a	7.66 ± 0.07a
	pH		Berry weight (g)	
Survey date	Z	C	Z	C
3 August 2020	2.95 ± 0.01a	2.90 ± 0.01a	1.45 ± 0.05a	1.92 ± 0.07b
17 August 2020	3.21 ± 0.01a	3.14 ± 0.01b	1.71 ± 0.04b	2.03 ± 0.02a
3 September 2020	3.29 ± 0.02a	3.29 ± 0.01a	2.25 ± 0.04a	1.97 ± 0.08b
8 September 2020	3.41 ± 0.02a	3.39 ± 0.02b	1.95 ± 0.02a	1.59 ± 0.05b
29 July 2021	2.91 ± 0.01a	2.89 ± 0.01a	1.78 ± 0.03a	1.80 ± 0.05a
18 August 2021	3.01 ± 0.01a	2.99 ± 0.02a	1.73 ± 0.04a	1.25 ± 0.02b
31 August 2021	3.12 ± 0.02b	3.22 ± 0.01a	1.40 ± 0.06a	1.11 ± 0.03b
10 September 2021	3.40 ± 0.01a	3.36 ± 0.01b	1.60 ± 0.05a	1.22 ± 0.07b

Sugar content (°Brix), titratable acidity (TA), pH and berry weight of Sanforte berries treated with two different soil managements: Zeowine (Z) and Compost (C). Data (mean ± s.e., $n = 12$) were subjected to one-way ANOVA. Different letters within the same parameter and row indicate significant differences (LSD test, $P \leqslant 0.05$)

In our study the synergy of compost and zeolite positively affected water stress; due to the zeolitic skill of adsorption and release water (Polat *et al*., 2004), the Zeowine application showed less negative water potential values during the most syccitous period in all years (2019, 2020 and 2021). In fact, several studies on species other than the grapevine also reported that water deficit stress was mitigated by soil applications of zeolite such as in *Aloe vera* L. (Hazrati *et al*., 2017), in Trigonella foenum-graecum (Baghbani-Arani *et al*., 2017), in *Oryza sativa* L. (Zheng *et al*., 2018), in *Hordeum vulgare* L. (Ahmed *et al*., 2017) and in *Cucumis sativus* L. (Mohabbati *et al*., 2018). Zeolite increased the water-holding capacity of the soil and improved soil quality in the root zone (AL-Busaidi *et al*., 2011).

During the 2020 season, significant differences at full veraison, mid-maturation and harvest were noted in sugar content, while during the 2021 season, significant differences at mid-, full-maturation and harvest were noted in sugar content: Z had the highest values than C treatment. At the time of harvest, Zeowine increased the sugar content of 5.50% in 2020 and 2.00% in 2021. We hypothesize that the higher sugar content of Zeowine-treated plants was due to their higher rate of photosynthesis (Medrano *et al*., 2003). A close link between photosynthesis (Rubisco activity) and vine carbohydrate metabolism was found and it was observed that the photosynthesis rate (Pn) was directly related to the rate of the sugar metabolic process (Mao *et al*., 2018). Moreover, we hypothesize that this correlation was due to the young age of the plants; in fact, as there were few stored carbohydrates, berry sugar accumulation was more sensitive to photosynthesis. Vines subjected to zeolite foliar applications (chabasite rich-zeolitite) in addition to significantly reducing grey mould and sour rot infections, increased sugar and alcohol content. In addition, these effects have been linked to the reduction of the leaf temperature, in this case, due to zeolite ability to reflect infrared radiation (Calzarano *et al*., 2020). However, it cannot be excluded the zeolite capacity to absorb carbon dioxide, determining its increase near the stomata and net photosynthesis increase (De Smedt *et al*., 2017). This aspect deserves more and deeper investigation.

A 23% (2020) and 31% (2021) increase in the weight of the harvest berry for zeowine treatment was also observed; the ability of zeolite to improve the radical water microclimate led to more hydrated and larger berries (Baeza *et al*., 2007).

Regarding phenolic maturity, the greatest differences were found in the composition of extractable and total anthocyanins. At full maturation and harvest, Z berries showed significantly higher extractable and total anthocyanin content compared to C berries (2020). The lowest values in total anthocyanins were recorded for the C treatment at the three different stages during the seasons. At mid-, full-maturation and harvest Z berries showed significantly higher extractable and total anthocyanin content compared to C berries (2021). The higher Brix degree of Z treatment may explain the increased accumulation of anthocyanins in the berries (sugar/anthocyanin relationship) (Hernández-Hierro *et al*., 2014). In fact, it was demonstrated that differences in the anthocyanin extractability were highly influenced by the ripeness degree and also, by the soluble solids contents (Hernández-Hierro *et al*., 2012). During the 2020 season, no differences in total polyphenols at harvest were found. At full maturation, no differences in extractable polyphenols

https://doi.org/10.1017/S0021859621000915 Published online by Cambridge University Press

Table 4. Phenolic maturity

	Total anthocyanin (mg/l)		Extractable anthocyanin (mg/l)	
Survey date	Z	C	Z	C
3 August 2020	505.75 ± 15.06a	498.75 ± 22.54a	264.25 ± 12.80a	262.50 ± 20.10a
17 August 2020	848.75 ± 31.45a	803.25 ± 13.90b	379.75 ± 24.66a	350.55 ± 11.74a
3 September 2020	965.45 ± 17.75a	810.49 ± 24.31b	570.61 ± 28.90a	460.00 ± 24.89b
8 September 2020	1042.2 ± 22.87a	844.34 ± 32.78b	610.45 ± 25.76a	485.13 ± 27.87b
29 July 2021	297.50 ± 21.77a	269.50 ± 11.57a	122.50 ± 12.74a	113.75 ± 10.21a
18 August 2021	810.25 ± 33.80a	586.25 ± 15.90b	375.00 ± 20.70a	241.50 ± 18.29b
31 August 2021	938.00 ± 25.55a	745.50 ± 23.56b	350.00 ± 18.33a	290.50 ± 16.33b
10 September 2021	715.05 ± 18.49a	632.80 ± 31.43b	312.9 ± 23.78a	252.00 ± 22.15b
	Total polyphenol (mg/l)		Extractable polyphenol (mg/l)	
Survey date	Z	C	Z	C
3 August 2020	2188.81 ± 40.01a	2041.55 ± 48.65b	2046.63 ± 25.65a	1977.66 ± 27.32a
17 August 2020	1725.22 ± 37.80b	2011.09 ± 33.12a	1724.20 ± 30.13b	1864.97 ± 12.54a
3 September 2020	2025.25 ± 39.45b	2156.90 ± 35.15a	1783.87 ± 28.34a	1708.34 ± 29.04a
8 September 2020	1940.31 ± 42.57a	1976.26 ± 39.76a	1535.61 ± 22.10b	1613.36 ± 23.05a
29 July 2021	2003.47 ± 45.31a	1894.31 ± 35.50b	1835.91 ± 37.41a	1488.10 ± 20.50b
18 August 2021	1757.21 ± 35.25a	1543.95 ± 32.22b	1455.09 ± 34.21a	1401.78 ± 24.37a
31 August 2021	1635.34 ± 38.32a	1450.01 ± 37.41b	1137.74 ± 28.16b	1312.92 ± 28.25a
10 September 2021	1540.90 ± 40.75a	1338.81 ± 39.55b	1434.27 ± 27.72a	1183.95 ± 28.50b

Total anthocyanin (Tot. Anth.), extractable anthocyanin (Extr. Anth.), total polyphenol (Tot. Polyp.) and extractable polyphenol (Extr. Polyp.) content of Sanforte berries treated with two different soil managements: Zeowine (Z) and Compost. Data (mean ± s.e., $n = 12$) were subjected to one-way ANOVA. Different letters within the same parameter and row indicate significant differences (LSD test, $P \leqslant 0.05$)

Table 5. Production parameters

	N° cluster/vine		Cluster weight (kg)		Yield/vine (kg)	
Survey date	Z	C	Z	C	Z	C
8/9/2020	5.26 ± 0.06a	4.80 ± 0.04b	0.19 ± 0.06a	0.15 ± 0.08b	0.98 ± 0.03a	0.88 ± 0.04b
10/9/2021	6.00 ± 0.03a	6.60 ± 0.07a	0.21 ± 0.07a	0.18 ± 0.07b	1.25 ± 0.05a	0.88 ± 0.03b

Cluster weight (kg), yield/vine (kg) and a number of cluster/vine of Sanforte cv. With two different soil management: zeowine (Z) and compost. Different letters within the same parameter indicate significant differences. Data (mean ± s.e., $n = 12$) were subjected to one-way ANOVA (LSD test, $P \leqslant 0.05$)

were found, while at harvest C berries showed significantly higher extractable polyphenol content compared to the Z application. During the 2021 season, significant differences in polyphenols at harvest were found; Z berries showed significantly higher extractable and total polyphenol content compared to the C application. Increases in total anthocyanins, but also in total polyphenols and colour intensity, were recorded in wine obtained from vines treated with zeolite leaf applications (Calzarano *et al.*, 2019). Again, the leaf temperature reduction effect may have been decisive, for these results, because linked to higher biosynthesis of phenolic compounds (Conde *et al.*, 2016; Movahed *et al.*, 2016). Considering the promising results obtained by zeolite leaf applications, and in this study, by zeolite soil applications, their synergic use may be desirable, with a view to environmentally friendly crop management.

Significant differences were found for the production parameters at harvest. Treatment Z had generally increased the weight of bunches, yield by vine (2020, 2021) and their number (2020). In fact, an increased concentration of CO_2 (CO_2 adsorption/desorption zeolite skill; Liu *et al.*, 2017) provides a higher crop production (Kimball, 1986).

Conclusions

Based on the findings of this experiment, it could be concluded that the deleterious effects of global warming, in a new grapevine plant, can be reduced with Zeowine soil improver. The zeolite skill to hold water and exchange nutrients, gave the vines the strength to improve their performance and carry out better production than the compost treatment. The features of zeolite combined with compost could be one of the best solutions to make a stand against drought problems. Therefore, it is in this scenario that the application of Zeowine in the vineyard to the soil can be a valid tool to mitigate the effects of climate change.

However, further investigations are needed, given the few studies carried out on the application of zeolites in vineyards.

Acknowledgements. The authors gratefully acknowledge the technical assistance of Filippo Rossi (GMR, Strumenti). The study was carried out within the framework of the LIFE EU project LIFE ZEOWINE LIFE17 ENV/IT/000427 'ZEOlite and WINEry waste as an innovative product for wine production'.

Financial support. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Conflict of interest. The authors declare there are no conflicts of interest.

Ethical standards. Not applicable.

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