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Adaptation Strategies to Climate Change in Vineyard: innovation in vine training and pruning system, and cover crops

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SUMMARY

Abbreviations

PAR Photosynthetically active radiation
LLN Leaf layer number
YAN Yeast assimilable nitrogen
TA Total acidity
TSS Total soluble solid
DOY Day of the year
SMPH Semi-Minimal-Pruned hedge training system
VSP Vertical shoot positioned training system
HC High Cane
GY Guyot
T1 Grass-legume mixture (*Trifolium repens, Lotus corniculatus, Lolium perenne, Festuca rubra)*
T2 Legume cover crop (*Trifolium alexandrinum)*
T3 Natural covering
N Nitrogen

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Abstract

Climate change is a developing threat which has a serious impact on the regions historically suited to viticulture. There is experimental evidence that grapes are unbalanced, they tend to contain high sugar, high pH levels and low acid.

In the years 2020, 2021 and 2023, vines trained through the traditional Guyot system were compared to those trained and pruned by means of an innovative system, called High Cane.

The innovation, consisted in increasing the distance of the cane from the soil, without changing the height of the trunk. It led to an increase in the number of buds and in shoots per vine and to a modification of the canopy architecture which resulted low and thick, with a higher yield and consequently a lower total leaf area-to-yield ratio.

At the harvest, the High Cane vines showed a reduction in total soluble solids compared to Guyot vines (-17%, -3%, -10% in 2020, 2021 and 2022 respectively), a significant reduction in pH, which was associated with an increase in total acidity (+15%, +9%, +19% in 2020, 2021 and 2022 respectively).

In October 2020, 2021, and 2022, we used three types of cover crop: a grass-legume mixture (T1), a cover crop of *Trifolium alexandrinum* (T2) and a spontaneous natural covering (T3) in vineyard. We compared their effects on yield and we register qualitative parameters.

Firstly, the vines of T1 and T2 theses showed a higher vigor and yield (6.84, 6.41 and 4.09 kg/vine in 2021 for T1, T2 and T3 respectively, 5.15, 5.80 and 2.73 kg/vine in 2022 for T1, T2 and T3 respectively).

Secondly, the *Trifolium alexandrinum* and the grass-legume mixture vines showed a lower total soluble solids content compared to natural covering vines (-3.85 and -3.36 °Brix, respectively for T1 and T2 in 2021, -4.14 and -5.09 °Brix respectively for T1 and T2 in 2022), and a significant reduction in pH, with values of 3.01, 3.05, 3.13 in 2021 and 2.97, 2.97, 3.08 in 2022 for T1, T2 and T3 respectively. These values were associated with an increase in total acidity, equal to + 0.58 g/l and + 0.52 g/l in 2021 for T1 and T2 compared to T3 and + 0.36 g/l and + 0.60 g/l in 2022 for T1 and T2 in comparison with T3. Even the YAN content in must improved in T1 and T2 in both years.

Riassunto

Il cambiamento climatico sta causando non pochi problemi nelle regioni storicamente vocate alla viticoltura. Frequentemente le uve tendono ad essere squilibrate, caratterizzate da elevati contenuti zuccherini, alti livelli di pH e bassi contenuti di acidi.

Nelle stagioni 2020, 2021 e 2023, viti allevate a Guyot sono state messe a confronto con viti allevate e potate con un sistema innovativo, denominato High Cane.

L'innovazione, che consiste nell'aumentare la distanza del capo a frutto dal suolo, senza modificare l'originaria altezza del tronco, ha portato ad un aumento del numero di gemme e dei germogli per ceppo e ad una modifica dell'architettura della chioma, più bassa e spessa, caratterizzata da una maggiore produzione e di conseguenza da un minor rapporto tra superficie fogliare e produzione.

Alla vendemmia le viti HC hanno mostrato una riduzione del contenuto di solidi solubili rispetto alle viti Guyot (-17%, -3%, -10% rispettivamente nel 2020, 2021 e 2022), una significativa riduzione del pH, associata a un aumento dell'acidità totale (+15%, +9%, +19% rispettivamente nel 2020, 2021 e 2022).

A partire dalla semina, effettuata ad ottobre 2020, nelle stagioni 2021 e 2022, sono state confrontate tre tipologie di gestione del suolo in vigneto, un miscuglio di graminacee e leguminose (T1), una coltura di copertura di *Trifolium alexandrinum* (T2) e un inerbimento naturale spontaneo del vigneto (T3).

Le viti delle tesi T1 e T2 hanno mostrato una maggiore vigoria ed una maggiore produzione per ceppo, con valori significativamente più elevati.

Anche i parametri qualitativi delle bacche ne hanno risentito, alla vendemmia, le viti delle tesi T1 e T2 hanno mostrato un contenuto di solidi solubili inferiore rispetto alle viti della tesi T3 (-3,85 e -3,36 °Brix, rispettivamente per T1 e T2 nel 2021, -4,14 e -5,09 °Brix rispettivamente per T1 e T2 nel 2022), e una significativa riduzione del pH, con valori rispettivamente di 3,01, 3,05, 3,13 nel 2021 e 2,97, 2,97, 3,08 nel 2022 per T1, T2 e T3. A questi valori è stato associato un aumento dell'acidità totale, pari a + 0,58 g/l e + 0,52 g/l nel 2021 per T1 e T2 rispetto a T3 e + 0,36 g/l e + 0,60 g/l nel 2022 per T1 e T2 rispetto a T3. Anche il contenuto di APA nel mosto è migliorato in T1 e T2 rispetto a T3 in entrambi gli anni di prova.

General introduction

Human activities are increasing the concentration of greenhouse gases (especially carbon dioxide). They are responsible for global warming. The IPPC 2022 reported that extreme weather events such as heatwaves, wildfires, torrential rains, flooding and droughts are the serious effects of rising temperature.

According to ERA5 dataset, the Copernicus Climate Change Service (C3S) reported that the last eight years were the warmest ones; in 2022 the average global temperature was 0.3°C higher than the reference period of 1991–2020 and even 1.2°C higher than that in the reference period 1850–1900 often taken as representative of the pre-industrial level.

Previously the IPCC 2021 report claimed that the increase in global surface temperature was 1.09 [0.95 to 1.20]°C in the period 2011–2020 above the period 1850–1900.

Friedlingstein reported that in 2022 the concentration of CO_2 in the atmosphere reached 417.2 ppm, more than 50 % above pre-industrial levels, around 278 ppm (Friedlingstein et al., 2022) whereas the C3S declared that it was the highest one of the last two million years.

Agriculture could play an important role to uptake carbon from atmosphere, fix it in soil and plant tissues. In this way orchards could be strategic crop, not only for their growth but also for the managing of soil in the interrow.

Global warming has also deeply affected viticulture, particularly in the Mediterranean region. The scientific literature agree that the climate will experience a reduction in rainfall and widespread warming (Lionello and Scarascia, 2018).

As a consequence, on the one hand musts are unbalanced, they have high sugar content (Keller, 2010; Jones, 2012), high pH and low acidity (Neethling et al., 2012; Orduna, 2010), at risk of microbiological instability. On the other hand, consumers prefer wines with a moderate alcohol content (Deroover et al., 2021).

The international scientific literature has already reported various adaptation strategies to solve the above-mentioned problems (Gutierrez-Gamboa et al., 2020; Santos et al., 2020; Palliotti et al., 2014). In particular three strategies aim at delaying the berry ripening (Palliotti et al., 2014), (1) changing the establishment of the vineyards; (2) changing plant material and (3) adapting different viticultural techniques. The latter are very interesting since they could be applied into established vineyards. The adaptation strategies are divided into flexible, that could be implemented during the growth season, and into non-flexible, that must be programmed before the beginning of the vegetative cycle.

The flexible techniques are shoot trimming (Bondada et al.,2016; Herrera et al., 2015; Martínez de Toda, Sancha, & Balda, 2013), post-veraison apical defoliation to the cluster zone (Palliotti et al., 2013a; Lanari et al., 2013), shading nets (Chorti et al., 2010; Palliotti et al., 2014), late irrigation (Novello & de Palma, 2013; Silvestroni et al., 2020) or cooling irrigation (Paciello et al. 2016) and use of antitranspirant sprays (Palliotti et al., 2013 b).

As far as regards the antitraspirant sprays, Silvestroni et al., 2020 found out that the use of di-1-p-menthene, sprayed post-veraison, caused a temporary reduction in photosynthesis and consequently a reduction in berry total soluble solids concentration. Moreover, in high crop-load vines, the di-1-p-menthene delayed TSS accumulation more than the high crop load did.

The non-flexible technique are minimal pruning (Zheng et al., 2017), crop load modifications (Kovalenko et al. 2022; Soltekin et al., 2022; Schafer et al., 2021; Molitor et al. 2019b), late winter pruning (Gatti et al., 2016b; Frioni et al., 2016), the forcing regrowth (Poni et al., 2020; Martinez-Moreno et al., 2019; Gu et al., 2012) and mulching strategies (Fraga & Santos, 2018; Bavougian and Read, 2018).

Several studies reported a delay in berry ripening caused by the improved vine yield (Kovalenko et al. 2022; Soltekin et al., 2022; Schafer et al., 2021; Molitor et al. 2019b). Molitor et al. used the semi minimal pruned hedge training system (SMPH) on Pinot blanc in Luxembourg and observed that yield levels in non-thinned SMPH treatments were 74% higher compared to vertical shoot positioned training system (VSP), and total soluble solids (TSS) at harvest 2.2 brix lower than in VSP (Molitor et al. 2019). In a study on Gewurztraminer vines, Kovalenko et al. found out a significative reduction in TSS in high crop load vines compared to light crop load (50% of high crop), high TA and lower pH (Kovalenko et al. 2022).

Since late winter pruning cannot be used for varieties with low fertility of basal buds, a new strategy was used over a period of three years in order to delay berry ripening in the Italian, white-berried Verdicchio variety. It is called High Cane system and consists in:

- improving the distances between the soil and the cane of the vines without modifying the original height of the spurs for the renewal of canes;
- improving the number of buds per vine and so per linear meter, and consequently yield;

- reducing the height of the canopy and so the Total Leaf Area, significantly reducing the TLA-to-yield ratio;
- positioning the fruiting zone more distant from the soil, probably in a zone with a different microclimate.

Living mulches and cover crop in vineyard are the best ways to combine strategies to face climate change and sustainable management of the vineyard.

This is the perfect response to the increasing number of consumers who are willing to pay more for products which promote sustainability (Toth et al., 2020), biodiversity and ecosystem services (Garcia et al., 2018).

For this reason, we started a trial in October 2020. It was based on three theses:

- an entire natural covering of the vineyard, managed with two-three mowing per year;
- an annual cover crop of *Trifolium alexandrinum* L. in the inter-row, managed with mowing, whereas the underrow was managed with a mechanical weed control;
- a sown interrow covering with a grass-legumes mixture consisting of *Lolium perenne* L., *Festuca rubra* L. (*Graminaceae)*, *Trifolium repens* L., and *Lotus corniculatus* L. (*Leguminose)*. The underrow was managed with a mechanical weed control.

The goals of the trial were to evaluate the effects of cover crops on grape quality and yield; to observe whether a nitrogen-providing cover crop could increase the vigor of the vines; to delay berry ripening.

CHAPTER 1

Innovation of vine training and pruning system as adaptation strategy to face global warming

1.1 Abstract

Global warming has serious effects on regions historically suited to viticulture. Grapes are often unbalanced, they have by high sugar contents, high pH levels and low acidic content. Beside consumers prefer fresh and fruity wines with a moderate alcohol content. Therefore, the need to find effective solutions led research activities to test innovative management techniques.

In the years 2020, 2021 and 2022, the traditional Guyot training system vines was compared to vines trained and pruned with an innovative system, called High Cane.

The innovation consisted in increasing the distance of the cane from the soil without changing the original height of the spur for the renewal of the cane. It led to an increase in the bud number and in shoot per vine and to a modification of the canopy architecture which is low and thick and is characterized by a higher yield and, consequently, by a lower Total Leaf Area-to-yield ratio.

At harvest, the High Cane vines showed a reduction in total soluble solids compared to Guyot vines (-17%, -3%, -10% in 2020, 2021 and 2022 respectively), a significant reduction in pH which was associated with a significant increase in total acidity (+15%, +9%, +19% in 2020, 2021 and 2022 respectively).

The innovation in training and pruning system is a possible non-flexible strategy which can be applied at the beginning of the vegetative cycle in varieties with low basal buds' fertility, and can slow down the grape ripening.

Keywords: Climate change, sugar accumulation, total acidity, grape ripening

1.2 Introduction

The recent IPCC special reports (IPCC, 2021) show that temperature of the last decade (2011-2020) exceed those of around 6500 years ago. Marked effects related to climate variability are expected for the next decades, especially in very vulnerable areas such as the Mediterranean region. It is a "hot spot" of the climate change of the 21st century, since in this area temperature exceed the global average rate by 20% and there is a trend towards a reduction in rainfall (Lionello and Scarascia, 2018). The increase of air temperature has impacted winegrowing areas worldwide, leading to shorter growth seasons and advanced phenological phases with repercussions effects on the berry ripening and harvest dates (Palliotti *et al*. 2014).

Musts are rich in soluble solids, poor in acidity, and have high pH, all factors which lead to wine microbiological instability. But consumers require fresh and fruity wines with a moderate alcohol content (Seccia and Maggi, 2011). Therefore, recent research aims at identifying and evaluating techniques which can slow down the phenology and grape ripening (Palliotti *et al*., 2014).

Shoot trimming (Caccavello *et al*., 2019, Filippetti *et al*. 2011), late irrigation (Santos *et al*., 2020), post-veraison leaf removal apical to the bunch zone (Poni *et al.*, 2013), application of shading nets (Ghiglieno *et al*., 2020, Martínez-Lüscher *et al*., 2020), post-veraison antitranspirant sprays (Silvestroni *et al.*, 2020, Palliotti *et al*., 2013), management techniques based on the use of growth regulators (Bottcher *et al.*, 2010), early harvest (Kontoudakis *et al*., 2011), are some of the flexible techniques which can be applied even in late season when critical conditions occured.

Moreover, crop load modification or late winter pruning are non-flexible tecniques, as they are applied at the beginning of the growing season. Late winter pruning has been evaluated on varieties characterized by high basal bud fertility and has shown important results on yield and berry composition (Gatti *et al*., 2016; Palliotti *et al*., 2017; Silvestroni *et al*., 2018). Crop load modification with a calibrated increase in vine yield could also delay berry ripening process (Kovalenko et al. 2022; Soltekin et al., 2022; Schafer et al., 2021; Molitor et al. 2019b).

Schafer et al. used Semi-Minimal-Pruned hedge training system (SMPH) and reported that it led to higher crop load (+61%) and a poorer leaf area to fruit weight-ratio if compared to the traditional vertical shoot positioned training system (VSP), and it delayed ripening process (Schafer et al. 2021). Soltekin et al. found higher values for total soluble solids (TSS) in the lower crop level regimen, compared to the high crop level regimen, for both Merlot and Cabernet Sauvignon, during a two years study under semiarid climate conditions (Soltekin et al. 2022).

Other adaptation strategies to face climate change are training and pruning systems. They can be applied at the beginning of the vine growing season, even for varieties with low basal buds' fertility, which are cultivated with renewed cane systems, where late pruning cannot be used.

The aim of this investigation is to test the effects of a modified vine training and pruning system suitable for varieties with low fertility of the basal buds.

1.3 Materials and methods

1.3.1 Plant material, experimental conditions and experimental design

The trial was carried out over three consecutive seasons, namely 2020, 2021 and 2022, in a 4-years-old hillside vineyard (~20% slope) situated near the town of San Paolo di Jesi in the Marche region-Italy (latitude: 43°27'N; longitude: 13°09'E, elevation 190 m above sea level).

The vines were planted in 2017 with certified virus free-cuttings of cv Verdicchio (clone VLVR20) grafted onto 420A rootstock, oriented north-northeast to south-southwest and planted at 1.10 m vine spacing and 3 m row spacing resulting in a density of 3030 vines/ha. Grapevines were cane pruned in winter and vertically shoot positioned. The vineyard was rainfed and conducted under certified organic farming.

Pest and disease management programs were carried out according to local practices determined by field scouting and weather conditions. During the two years of trial, usually at mid-June, shoots were mechanically trimmed when their growth exceeded the top wires.

The study was conducted on 48 vines organized into three randomized blocks with 16 vines each. Each block was divided in two plots of 8 vines each. In one plot the vines were trained with the High Cane system (HC) and compared to the vines of the other plot, trained with the traditional Guyot training system (GY).

The traditional Guyot training system cane were set at 0.9 m aboveground with two pairs of catch wires and a single catch wire providing trellising extending 0.9 m above the cane.

The innovative High Cane system canes were set at 1.1 m aboveground, maintaining the same height of the spur for the renewal of the cane, and so the same height of the trunks, of the traditional Guyot training system (0,7 m aboveground), with two pairs of catch wires providing trellising extending 0,7 m above the canes. In this way the HC training system reduced the height of the canopy and improved the number of buds per vine, positioning the fruiting zone higher up with possible repercussions on its microclimate (Figure 1).

No shoot thinning or bunch removal were performed.

In the years 2020, 2021 and 2022, the mean and maximum daily temperature and rainfall data were recorded on the Civil Protection site of Marche region (Meteo-Hydro-Pluviometric Regional Information System). It has a meteorological station 4,5 km far from the vineyard. Growing degree-day (GDD, base 10 °C) accumulated from 1st April to harvest was calculated.

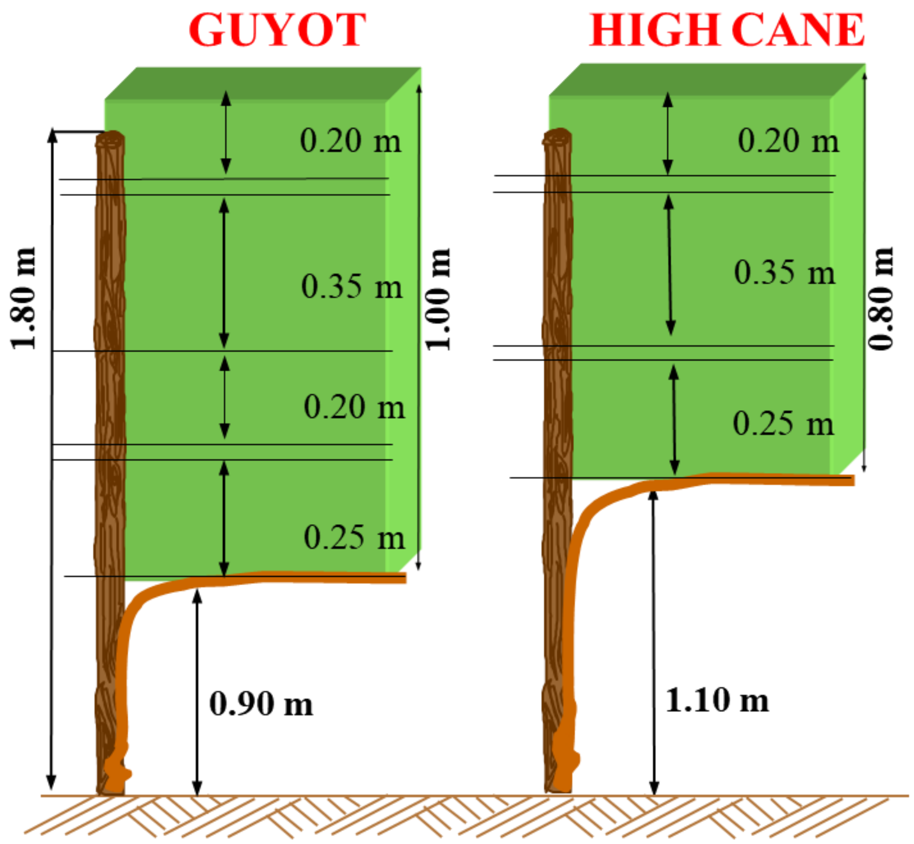

Figure 1. Schematic representation of guyot (GY) and high cane (HC) training system. The black lines represent the location of the catch wires.

1.3.2 Vine growth and canopy measurements

Each year the total leaf area (TLA) and canopy density (expressed as leaf layer number, LLN) were determined via Point Quadrat Analysis (Smart and Robinson 1991). On 15th and 13 th September, respectively in 2020 and 2021 and on 26 th August in 2022, the TLA and LLN were estimated using 100-120 insertions, according to the full height of the canopy, at 10 cm intervals with a thin metal rod following a sampling grid. The metal rod simulated the sunlight ray and, each contact with a canopy component, represented the sunlight interceptions.

Each year of the trial, the annual vine growth was assessed by counting and weighing the canes on all vines, and the Ravaz index (Ravaz, 1903). It is commonly used to evaluate the balance between vine growth and yield, was calculated as the ratio of yield to pruning mass.

1.3.3 PAR measurements

During the two-year trial, at full canopy development and under saturating light [photosynthetically active radiation (PAR) > 1200 µmol photons/ (m^2 s)], the photosynthetically active radiation (PAR) inside the canopy of both G and HG vines was measured. The measures were taken with an AccuPAR PAR/LAI Ceptometer Model LP-80 (Decagon Devices, inc.), to evaluate the effects of the different training system on the exposure to light in the fruiting zone and on the canopy microclimate. In 2020, measurements were carried out, at midday, by inserting the instrument perpendicularly and internal to the vine canopy at the cane level, positioning it in the middle of this, as schematized in figure 2. In the 2021 and 2022 seasons, measurements were deepened examining the active radiation, not only at the cane level, but also at 30, 60, 90 cm over the cane level, with the recording of 10 PAR measurements for each insertion (Figure 2).

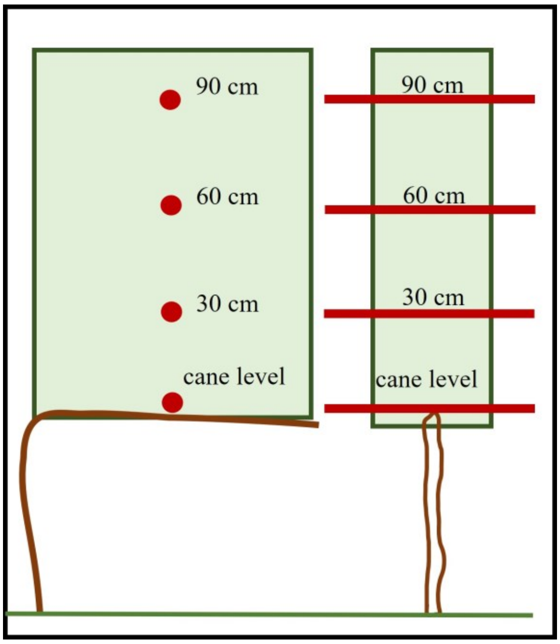

Figure 2. Schematic representation of PAR measurements on different levels of vine canopy. The red dots and lines represent the location of the AccuPAR ceptometer sensor.

1.3.4 Vine yield and grape composition

For each year of the study, after the veraison (indicatively at the end of July), the total soluble solids [TSS (°Brix)], pH, titratable acidity (TA), tartaric and malic acid were assessed on 100 berries weekly, until the harvest. Vines were harvested on 16 th September 2020 [Day of the year (DOY 260)], 8 th September 2021 (DOY 251) and 29 th August 2022 [Day of the year (DOY 241)] when the TSS began to level off.

Grapes were picked individually and the total number of bunches per vine was counted and weighed. Mean individual bunch weight was calculated as the ratio of total bunch weight per vine (yield) and the total number of bunches per vine.

At harvest, 100 berries per vine were collected and weighed to determine the berry fresh weight. The berries were crushed, and the juice was used to determine TSS, pH, titratable acidity (TA), tartaric and malic acid. The TSS were measured using a temperature-compensating Maselli LR-01 digital refractometer (Maselli Misure, Parma, Italy). Must pH was analysed with a Crison two decimal pH meter (Crison Instruments, Barcelona, Spain) by means of a glass electrode; TA with a Crison Titrator

(Crison Instruments) using 0.25 N NaOH to a pH 7.00 endpoint, expressed as g/L of tartaric acid equivalent. The tartaric acid concentration was measured by means of 'colorimetric method' based on the reaction between tartaric and vanadium acid resulting in an orange colour, measured by spectrophotometry at 500 nm; the malic acid concentration through an enzymatic kit (Enzyplus-Raisio, Raisio, Finland).

1.3.5 Statistical analysis

The results were tested with Statistical version 4.3 (StatSoft,Tulsa, OK, USA) for homogeneity of variance and subjected to ANOVA. The graphical representations were obtained using the Sigma Plot version 10 (SPSS, Chicago, IL, USA). In each year, data of TLA and LLN, PAR, grape composition at harvest and yield components were tested using mean separation calculated by applying the Student–Newman–Keuls test at $P \leq 0.05$.

In the figures concerning the evolution of berry mass, TSS, must pH and TA are shown as mean values SE.

1.4 Results

1.4.1 Environmental conditions

In summer, the average temperature was higher in 2021 (24.93°C) than in 2020 (23.81°C) and 2022 (24.52°C). The growing degree days (GDD, base 10°C) accumulated from April to harvest were 2006 in 2020, 2121 in 2021 and 2287 in 2022 (Table1). A drought event occurred in summer 2021 and 2022, whereas rain was high and well distributed throughout the 2020 growing season. Total rainfall from April to harvest was very low in 2021 (94 mm) and 2022 (154 mm), whereas it was quite high in 2020 (397 mm).

The distribution of rainfall in 2020 probably led to constant and adequate water availability in the soil during the growing season.

In the drier season 2021, only 94 mm of rainfall fell from budbreak to harvest, the period encompassing fruit set, veraison and berry ripening. In summer 2022, there were 85 days with daily maximum air temperature higher than 30 °C and 16 with daily maximum air temperature higher than 35 °C. In 2021 we registered respectively 65 and 23 days, while in 2020 we registered respectively 51 and 7 days (Table1). To sum up 2021 and 2022 seasons were characterised by severe summer drought.

Table 1. Weather variables and growing degree-days from April to October in Verdicchio vines.

year	Rain (mm/year)	Rain from budbreak-harvest (mm)	Average summer Temperature (°C)	Number of day T>30°C	Number of day T>35°C	GDD April-October
2020	865	397	23.81	51	7	2006
2021	754	94	24.93	65	23	2121
2022	702	154	24.52	85	16	2287

Daily average and maximum temperature and precipitation data were collected from the site of Protezione Civile Regione Marche. *GDD*, Growing degree-days (daily temperature base 10 °C). Data are from 1st April to 31st October in 2020, in 2021 and in 2022.

1.4.2 Canopy architecture and total leaf area

The vine canopy development was strongly influenced by the seasonal meteorological evolution. In fact, regardless of the theses, the high temperatures in 2021 led to a reduced development of TLA, compared to the 2020 growing season. On the contrary the 2022 season showed intermediate values (Table 2).

Table 2. Total leaf area at harvest in Verdicchio Guyot vines and High Cane vines.

year		Shoot/vine (n°)	TLA/shoot (m^2)	TLA/vine (m^2)
2020	GY	13 b	0.31 a	5.2 a
	HC	16 a	0.26 b	4.48 a
2021	GY	11 b	0.26 a	2.88 a
	HC	14 a	0.16 b	2.22 a
2022	GY	12 b	0.28 a	3.47 a
	HC	16 a	0.16 b	2.59 b

Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *TLA*, total leaf area. *GY*, Guyot vines, *HC*, High Cane vines.

According to three years-study, HC vines registered a significantly higher shoot number per vine. However, the shoots of the HC vines exhibited a lower TLA for shoot, whereas the TLA per vine was significantly different only in 2022 between the two theses (Table 2). Over three years, the mean height of the canopy was significantly lower in the HC vines. Indeed, the registered values of canopy thickness were higher in HC vines, especially in the distal part of the canopy (Table 3).

Table 3. Canopy architecture in GY and HC vines.

year		Canopy height (cm)	Thickness in the distal canopy level (cm)	Thickness in the fruiting zone level (cm)
2020	GY	133 a	41 b	37 a
	HC	126 b	67 a	38 a
2021	GY	126 a	28 b	29 b
	HC	98 b	41 a	38 a
2022	GY	124 a	32 b	32 b
	HC	107 b	48 a	45 a

Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *GY*, Guyot vines, *HC*, High Cane vines.

1.4.3 PAR measurements

In 2020, the photosynthetically active radiation (PAR) measures were carried out only in the fruiting zone, to evaluate the bunch shading and protection effect from the sunburn damages. Compared to GY vines, the HC ones showed lower PAR in the fruiting zone, reaching very low values between 20 cm to the right and 20 cm to the left from the center of the cane (represented by 0), between about 70 and 80 µmol photons $m^{-2} s^{-1}$. On the contrary, in GY vines, PAR resulted in around 200 µmol photons $m^{-2} s^{-1}$ (Figure 3).

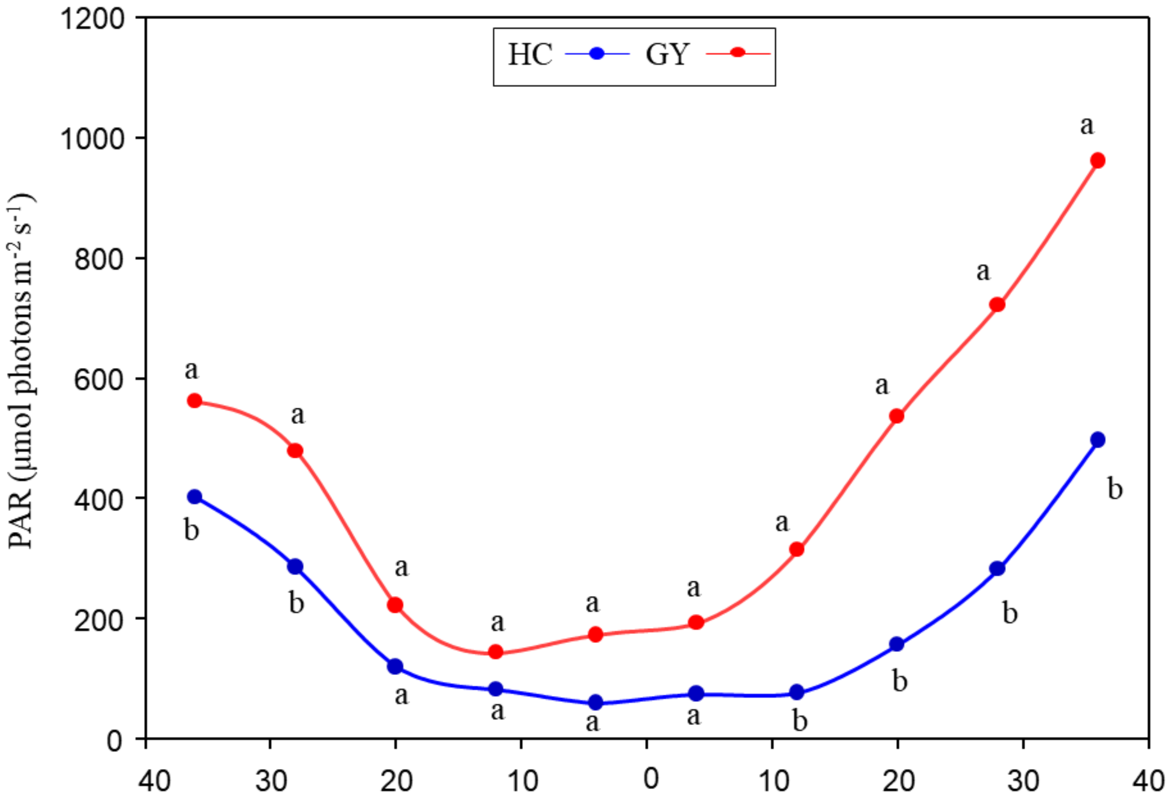

Figure 3. Vine canopy photosynthetically active radiation at cane level, in the 2020 season.

Mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *GY*, Guyot vines, *HC*, High Cane vines.

In 2021 and 2022, the PAR measures were carried out in four levels of the canopy: at the cane level and at the distance of 30, 60, 90 cm from the cane. In both years, at all canopy levels, the HC vines training system showed a lower PAR compared to GY vines (Figure 4 and 5).

In 2021the differences between HC and GY were statistically significant starting from the sides of the canopy and decreased progressing towards the centre of the cane, between 20 cm to the right and 20 cm to the left from the centre of the cane (represented by 0). Their values passed from 1400 µmol photons m^{-2} s^{-1} to values below 60 µmol photons m^{-2} s^{-1} for the GY vines and from 400 to values below 600 µmol photons m^{-2} s^{-1} for the HC vines, at cane level, where the differences cancelled out (Figures 4, A).

The same trend was shown by the PAR at 30, 60 and 90 cm above cane level where the PAR registered significant differences from the sides of the canopy towards the center, where the PAR reached values not significantly different, below 200 µmol photons m^{-2} s^{-1} or 300 µmol photons m^{-2} s^{-1} for GY and

HC vines, respectively at the level of 30 and 60 cm above the cane and between 20 cm to the right and 20 cm to the left from the center of the cane (Figures 4, B and C).

The PAR of G and HG vines, measured at 90 cm from the cane, showed statistically different trends both on the right side and on the left side of the cane except for point 0, where the PAR was the same (Figures 4, D).

The same trend was recorded both in 2022 and in 2021. Remarkable differences concerned the sides of the canopy were recorded, between 20 cm to the right and 20 cm to the left from the centre of the cane (represented by 0), whose values passed from 1000 µmol photons m^{-2} s^{-1} to values below 200 µmol photons m^{-2} s^{-1}, at cane level, for the GY vines and from 400 to values close to 0 µmol photons m^{-2} s^{-1}, at cane level, for the HC vines (Figures 5, A).

The same trend was shown by the PAR at 30, 60 and 90 cm above cane level, where the PAR showed big differences from the sides of the canopy towards the centre, where the values were not so different, close to 200 µmol photons m^{-2} s^{-1} or between 200 and 600 µmol photons m^{-2} s^{-1} for HC and GY vines, respectively at the level of 30 and 60 cm above the cane and between 20 cm to the right and 20 cm to the left from the center of the cane (Figures 5, B and C).

The PAR of GY and HC vines, measured at 90 cm from the cane, showed statistically different trends both on the right and on the left side of the cane with the exception of two points, point 20 starting from the left, and point 10 starting from the right, where the PAR had the same values (Figures 5, D).

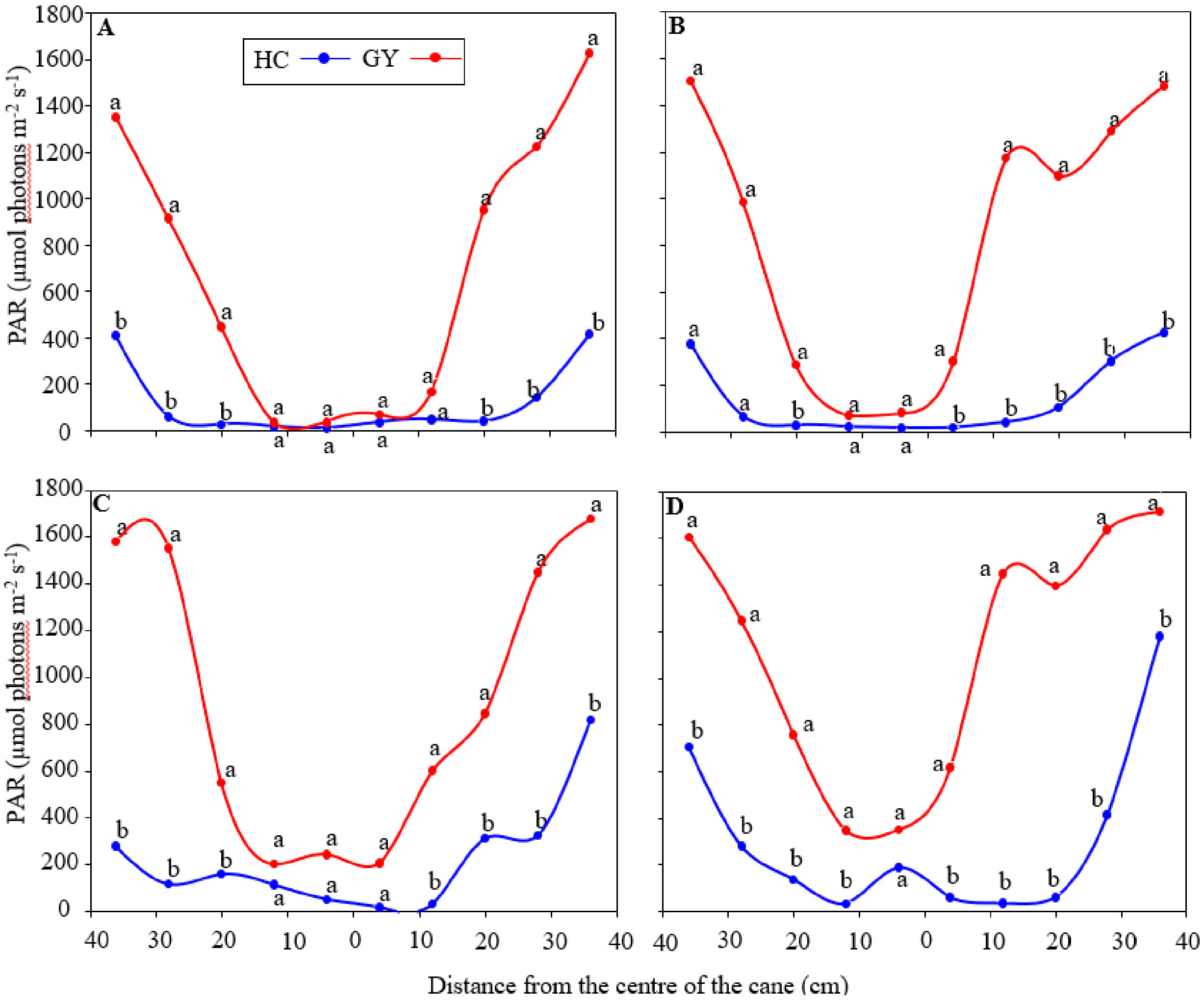

Figure 4. Vine canopy photosynthetically active radiation at cane level (A), at 30 cm (B), at 60 cm (C) and at 90 cm (D) above the cane, in the 2021 season.

Mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *GY*, Guyot vines, *HC,* High Cane trained vines.

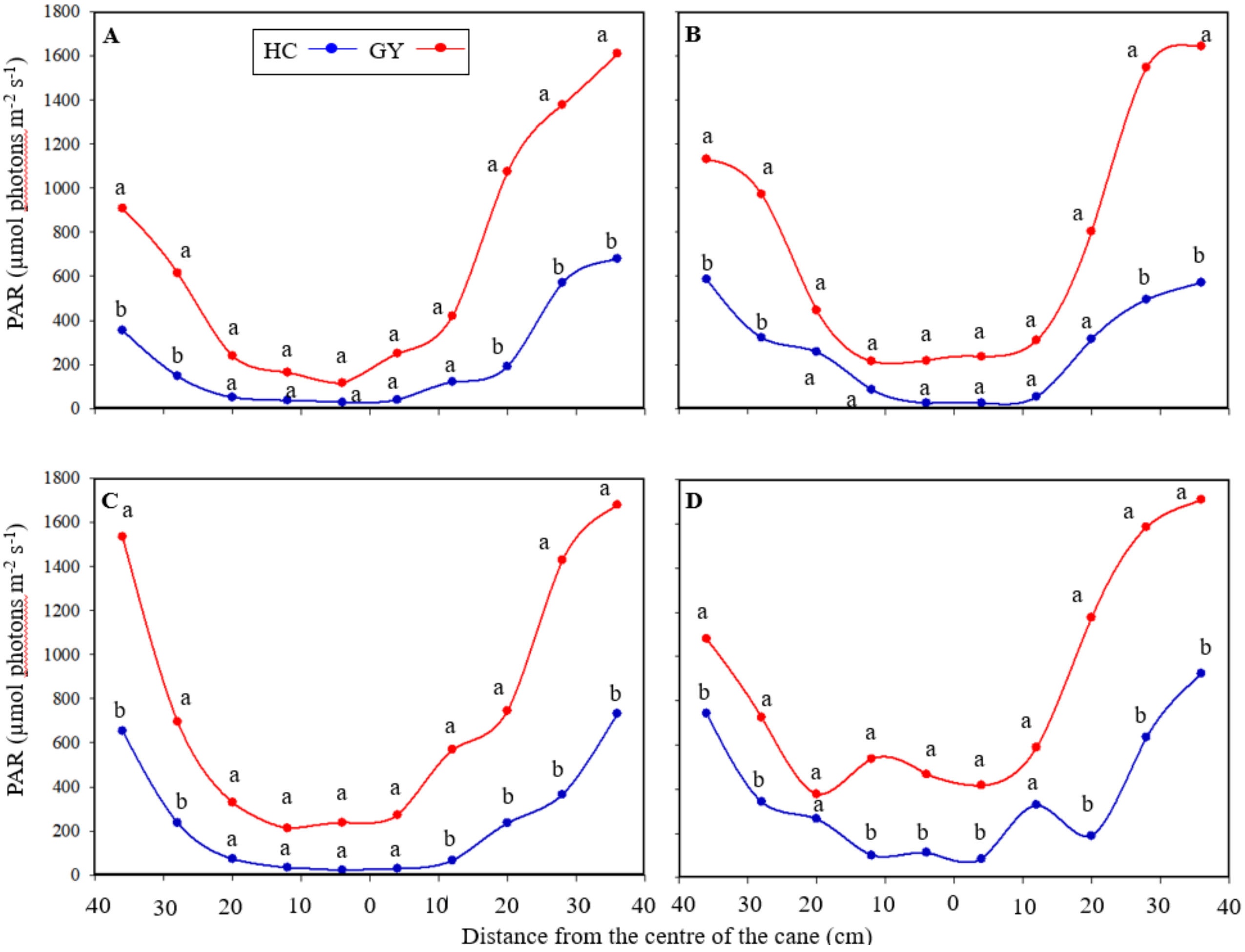

Figure 5. Vine canopy photosynthetically active radiation at cane level (A), at 30 cm (B), at 60 cm (C) and at 90 cm (D) above the cane, in the 2022 season.

Mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *GY*, Guyot vines, *HC*, High Cane trained vines.

1.4.4 Berry ripening and grape composition at harvest

The HC system affected berry development only in the first year, with values much lower than the GY (2.04 vs 2.19 g in 2020).

In the warmer seasons 2021 and 2022 (Table 1), the berry weight showed similar values between the theses, the values were lower than those of 2020 season, regardless of the theses (Figure 6 and Table 5).

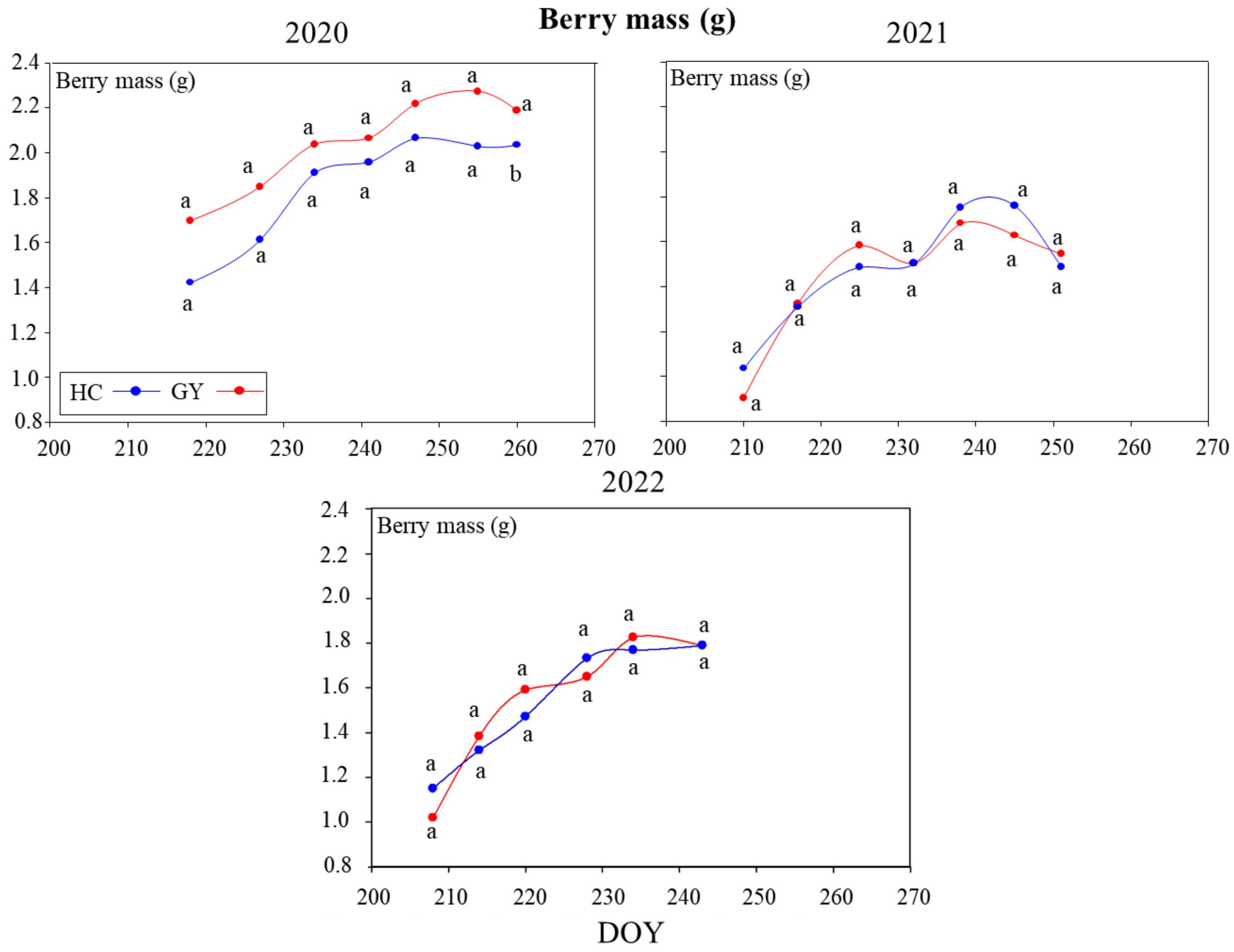

Figure 6. Evolution of berry mass in 2020, 2021 and 2022.

Mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *G*Y, Guyot vines, *HC,* High Cane vines. (n = 100 berries per treatment, at harvest n = 100 berries per vine)

At harvest, if compared with HC, G vines showed a higher TSS concentration in 2020 and 2022.

No remarkable differences were recorded in 2022 (Figure 7 and Table 4).

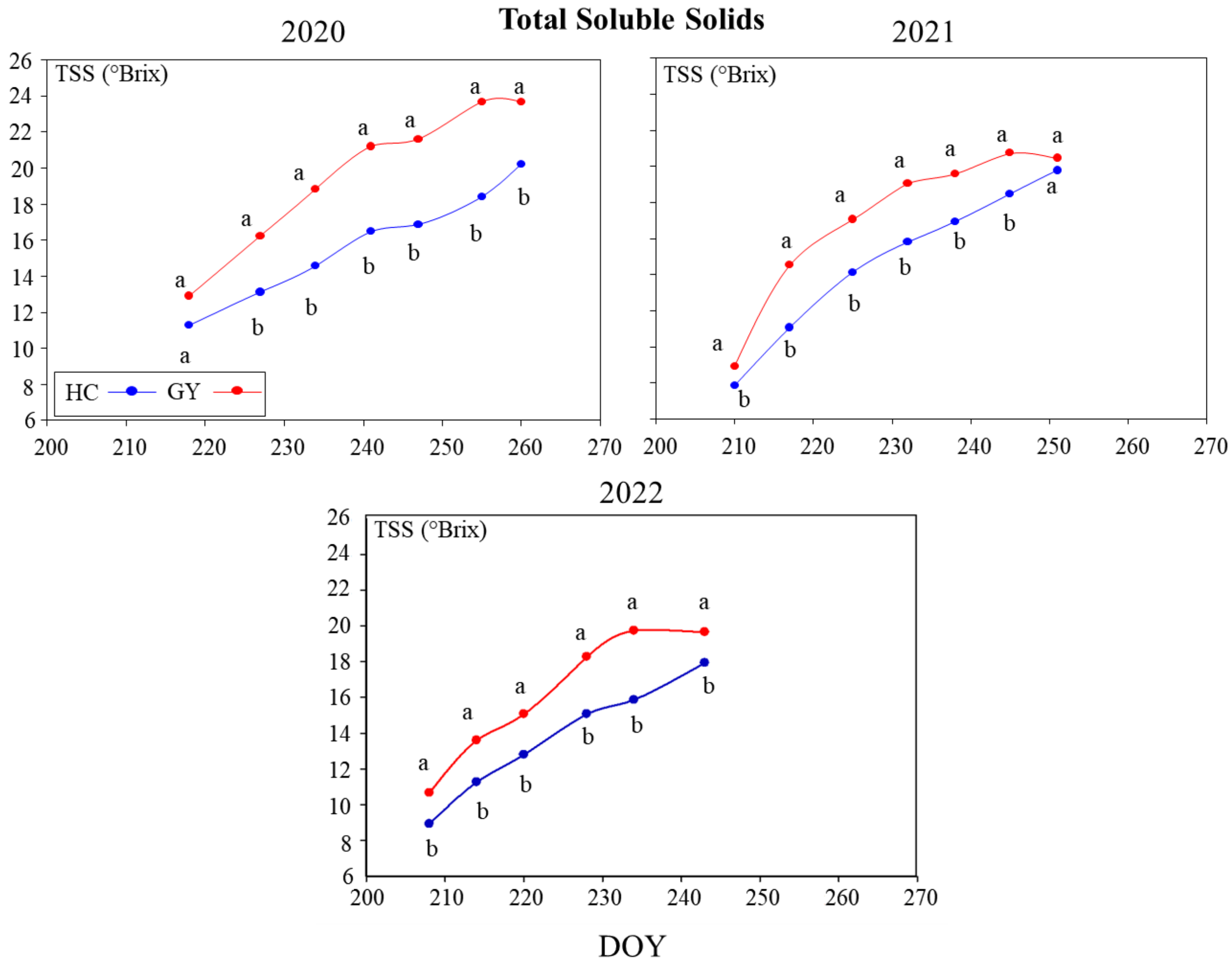

Figure 7. Evolution of Total Soluble Solid (TSS) in 2020, 2021 and 2022.

Mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *GY*, Guyot vines, *HC*, High Cane vines. (n = 100 berries per treatment, at harvest n = 100 berries per vine).

Similarly, the HC system affected the berry pH. It was lower in all samplings and showed considerable differences at harvest in the course of three years in comparison with GY vines (Figure 8 and Table 4).

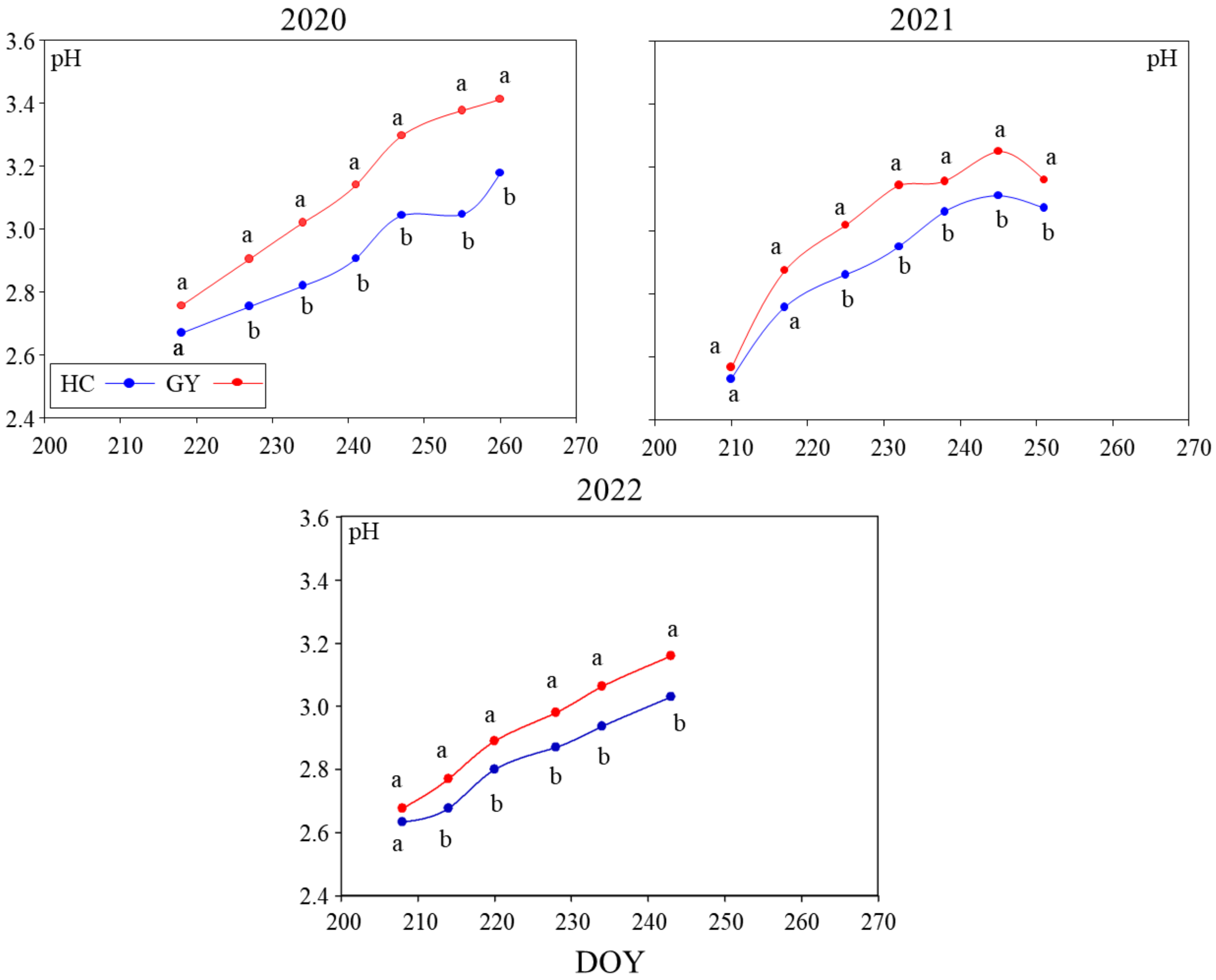

Figure 8. Evolution of pH in 2020, 2021 and 2022.

Mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *GY*, Guyot trained vines, *HC*, High Cane trained vines. (n = 100 berries per treatment, at harvest n = 100 berries per vine)

Furthermore, in the three-year study the TA evolution in HC berries was delayed (Figure 9 and Table 4). It showed higher values throughout the berry ripening phase and was statistically different at harvest, with an increase of +15%, +9% and +19% in 2020, 2021 and 2022 respectively (Figure 9 and table 4).

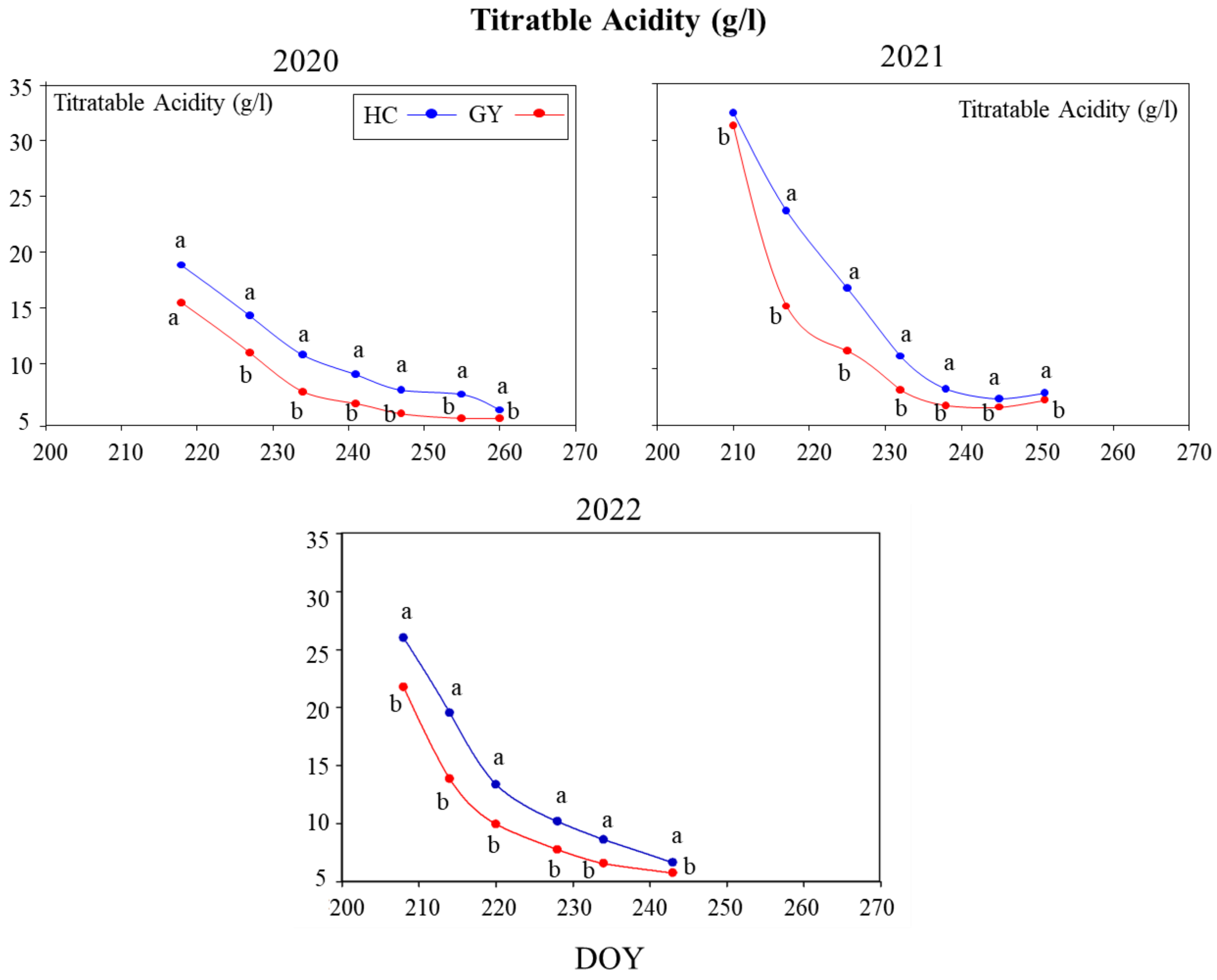

Figure 9. Evolution of Titratable Acidity (TA) in 2020, 2021 and 2022.

Mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *GY*, Guyot vines, *HC*, High Cane vines. (n = 100 berries per treatment, at harvest n = 100 berries per vine)

However, at harvest, only in the third-year remarkable differences in tartaric acid and malic acid content were observed. In 2022 HC and GY showed values of tartaric acid equal to 8.18 g/l and 6.87 g/l respectively, and of malic acid equal to 0.66 g/l and 0.37 g/l respectively. Within the year, the TA resulted higher in the warmer season 2021, characterized by higher tartaric acidic values. Malic acid values were lower than the cooler season 2020 (Table 4).

Finally, at harvest, in the HC vines the yeast assimilable nitrogen (YAN) values were much lower than those of the GY vines in all years (Table 4).

Table 4. Must composition in GY and HC vines at harvest.

	thesis	TSS (°Brix)	pH	TA (g/l)	Tartaric acid (g/l)	Malic acid (g/l)	YAN (mg/l)
2020	GY	23.68 a	3.41 a	5.07 b	6.17 a	0.70 a	158.67 a
	HC	20.21 b	3.18 b	5.84 a	6.93 a	0.73 a	82.83 b
2021	GY	20.46 a	3.16 a	7.22 b	10.31 a	0.47 a	126.73 a
	HC	19.78 a	3.07 b	7.85 a	10.21 a	0.53 a	85.17 b
2022	GY	19.65 a	3.16 a	5.73 b	6.87 b	0.37 b	102.38 a
	HC	17.93 b	3.03 b	6.62 a	8.18 a	0.66 a	74.96 b

Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *TSS*, total soluble solids, TA, titratable acidity. *GY*, Guyot vines, *HC*, High Cane trained vines.

1.4.5 Vine yield and vine size

Total yield per vine was higher, in all years, in HC vines, with considerable differences in 2020 and 2022 (Table 5). The HC vines showed a higher bunch number per vine than the GY vines, 13 vs 7 in 2020, 17 vs 14 in 2021 and 21 vs 15 in 2022, respectively (Table 5).

Regardless of the treatment, in the hot season 2021, the bunch mass values were lower than 2020 in all the vines, whereas they were intermediate in 2022 (Table 5).

Table 5. Yield parameters in GY and HC vines at harvest.

	thesis	Yield/vine (kg)	Bunches/vine (N)	Bunch mass (g)	Berry mass (g)
2020	GY	2.93 b	7 b	420 a	2.19 a
	HC	5.54 a	13 a	417 a	2.04 b
2021	GY	3.79 a	14 b	270 a	1.55 a
	HC	4.36 a	17 a	264 a	1.49 b
2022	GY	4.87 b	15 b	343 a	1.79 a
	HC	6.20 a	21 a	303 a	1.79 a

Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *GY*, Guyot vines, *HC,* High Cane vines.

The HC vines always showed the lower total leaf area to yield ratio compared to GY ones (Table 6). This was due to the higher yield induced by the improved number of buds leaved with the pruning and the reduced canopy height.

In 2020 and 2022, the pruning mass was the same in the two theses. On the contrary the HC showed significantly lower values in 2021 (0.8 vs 1.02 kg, respectively).

The Ravaz index (yield-to-pruning mass) pointed out higher values in HC vines over the three years (Table 6).

Table 6. Vegetative and pruning features recorded in GY and HC vines.

	thesis	Leaf area/yield (m^2/kg)	Canes (N/vine)	Pruning wood (kg/vine)	Yield/pruning mass (kg/kg)
2020	GY	1.71 a	13 b	1.27 a	2.56 b
	HC	0.79 b	16 a	1.10 a	6.17 a
2021	GY	0.81 a	11 b	1.02 a	3.95 b
	HC	0.58 b	14 a	0.80 b	5.66 a
2022	GY	0.82 a	12 b	1.09 a	4.85 b
	HC	0.43 b	16 a	0.93 a	7.07 a

Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *GY*, Guyot vines, *HC,* High Cane vines.

1.5 Discussion

Unlike the drought summers in 2021 and 2022, the regular distribution of rainfall in 2020, led to a consistent and adequate water availability in the soil throughout the growing season, thus improving the vegetative growth of the vines.

It is well known that a leaf area-to-yield ratio around 0.8 and 1,2 m^2/kg is appropiate for full grape ripening (Kliewer and Dokoozlian, 2005). Therefore, if we reduce that ratio, we will delay technological maturation and musts will have lower soluble solid content (Stoll et al., 2010) and maybe lower pH and higher acidity.

The HC vines showed a ripening delay. It was probably due to the combined effect of the reduction in leaves (source) as yield (sink) increased. Several articles reported a ripening delay linked to high crop load (Silvestroni et al., 2020), or to the reduction of TLA and of TLA to yield ratio (Stoll et al., 2010; Molitor et al., 2019).

Thanks to a greater distance of the cane from the soil, without changing the height of the poles, the HC system reduced the height of the canopy and the total leaf area for shoot and for vine.

Moreover, maintaining the same height of the spur for the renewal of the cane, the HC vines showed a larger number of buds. The number of shoots and bunches per vine and per linear meter was higher. Consequently, the canopy had a different architecture: it was thicker, and it could shade the bunches from solar radiation better.

In addition, the increased distance of the cane from the soil could also reduce the amount of heat radiated from the ground to the vine and affect the microclimate of the fruiting zone and the bunch temperature.

As a consequence, in HC vines, the reduction in TLA directly exposed to sunlight could reduce the water losses from the vine into the atmosphere, thus improving the resilience of the vines during the heat waves.

The higher number of bunches in HC vines led to a higher yield, with remarkable differences in 2020 and 2022 but not in 2021, because of the hot season.

The lower TSS in the berries which showed big differences of 3.47 °Brix in 2020 and 1.72 °Brix in 2022, could be a consequence of the reduction of the leaf area-to-yield ratio (Kliewer and Dokoozlian, 2005), due to the cumulative effects of the reduced TLA of the canopies and the high crop load.

The lower values of TSS determined in the HC vines confirmed the results of several studies.

Stoll et al. (2010) observed a delay in the ripening speed in vines with reduced leaf area and reduced leaf area to crop weight that recorded lower TSS.

Somkuwar et al. (2018), studied the effect of different canopy modifications on growth, yield and quality in Tas-A-Ganesh grapevine and observed that as bunch load increased, TSS was lower while total acidity was higher.

Horak et al. (2021), studied three varieties (‘Rhine Riesling’, ‘Pinot Gris’, ‘Sauvignon Blanc’) and remove three different amount of leaves from the canopy (0%, 40%, and 70%). They found positive connections between leaf area and TSS and noticed that the size of the leaf area affected the accumulation of sugar in the grapes.

Schafer et al. (2021) used the semi minimal pruned hedge training system (SMPH) and observed high crop load, poor leaf area to fruit weight-ratio compared to vertical shoot positioned training system (VSP) which resulted in a TSS decrease.

Must pH and TA showed differences between HC and GY: lower pH values and higher TA in HC vines thanks to a delay in berry ripening. The increased crop load, reduced TLA, reduced TLA to yield ratio and shading effects were the major factors of delay in berry ripening.

Silvestroni et al. (2020) registered lower TSS and pH in high crop load Sangiovese vines compared to medium crop load vines.

Candar et al. (2019), studied the influence of canopy microclimate on Merlot grape composition and comparing the effect of no lateral shoot presence (100% removal), half lateral shoot (50% removal) and full lateral shoot. They observed higher total acidity and lower pH in presence of half and full lateral shoot, proving a strong relationship between temperature and berry quality. In fact, high temperature reduced organic acid concentration in berry (Kliewer, 1973).

In a two-year study on early shading and defoliation on Aglianico vines, Basile et al. (2015) observed that berry composition was not affected negatively by 50%-to-90% shading, whereas defoliations and 10%- 30% shading caused a decrease in titratable acidity and an increase in pH of berry juice at harvest.

Kovalenko et al. (2022), studied Gewurztraminer vines and found a significative reduction in TSS in high crop load vines compared to light crop load (50% of high crop), high TA and lower pH.

In a study on Merlot and Cabernet Sauvignon with different crop loads, Soltekin et al. (2022) observed TSS and pH decrease and total acidity increase in higher crop load vines.

Furthermore, Friedel et al. (2015) reported that a major shading in the fruiting zone improved the total acidity and the malic acid content and decreased must pH.

In our study, we found higher acidity and lower pH coupled with higher canopy thickness and reduced PAR in the fruiting zone. Regarding malic acid content, we found higher values in HC vines only in the third year of the trial.

The YAN was lower in HC vines in all seasons. It was probably due to the high crop load, as reported by Reeve et al. (2016), who found a lower YAN content in vine of Pinot noir with full crop level as compared to half crop level.

Concerning vine growth, HC vines showed lower pruning weight and higher Ravaz index values (Ravaz 1903).

Kovalenko et al. (2022) noticed higher Ravaz index and lower pruning weight in high crop load compared to medium crop load vines. The values of Ravaz index for the high crop load were between 5 kg/kg and 10 kg/kg, which are considered ideal values (Smart et al., 1990), whereas in medium crop vines they were lower than 4 kg/kg, thus revealing that plants invested a bigger amount of energy in shoot growth rather than in cluster development.

We registered the same trend in our study: a good balance between yield (kg of grapes per vine) and vine vigor (annual pruning mass) only in HC vines; GY vines showed high total leaf area compared to low yield (Smart et al., 1990).

As far as pruning mass regards, Kovalenko et al. (2022) observed lower pruning weight in high crop load vines, as compared to medium crop load. The same trend was recorded in our study, but in both cases the pruning wood had a good vigor level (Smart and Smith, 1988).

1.6 Conclusions

The innovative pruning and training system based on the increase of the distance of the cane from the soil without modifying the height of the trunk improved the crop load of vines. It also reduced total leaf area and changed the canopy architecture. All these factors delate grape ripening and so musts showed lower sugar content, lower pH and higher total acidity.

Different factors influenced the reduction in the TSS and pH of HC grapes: the decrease of the TLA-to-yield ratio; the decrease in bunch exposure to direct solar radiation due to the greater thickness of the canopy; the improved distance from the soil that maybe changed the microclimate of the fruiting zone.

The combined effects of a thicker canopy and the reduction of the TLA-to-yield ratio are effective techniques for a higher total acidity.

So High Cane training and pruning system helped reduce sugar and pH in berries and increase total acidity and yield.

Moreover, a greater distance of the cane from the ground provided both less heat to the bunches with a consequent higher acidity in must and a lower risk of frost damage in spring.

HC training and pruning system provides high quality grapes and, therefore, is a good response to the current consumer demand.

CHAPTER 2

Use of cover crops in vineyard, effects on yield and quality

2.1 Abstract

The general increase in temperatures, the frequent heat waves, and the prolonged droughts, are affecting grapevine yield and berry qualitative parameters. Reaching grape full ripening is no longer a problem, but the high pH and the low acidity of the grapes promote microbiological instability in the resulting musts. Moreover, for organic viticulture, also the yeast assimilable nitrogen content in the musts results low, with evident problems in the fermentation process.

Furthermore, sustainability is a concept we cannot ignore, we must prevent soil erosion and recreate the biodiversity we have lost in it. The repeated and shallow tilling of vineyards should be avoided and actions to improve soil fertility must be implemented. In this way, the use of cover crops in the vineyard is a possible solution, that could increase soil organic carbon, the YAN content in the must, the vine vigor, and could also delay the ripening process, as well as reduce erosion, and provide ecosystem services.

Starting the seeding in October 2020, in the 2021 and 2022 seasons, three types of cover crops were compared in the vineyard and their effects on grape yield and quality were measured. The three theses were a grass-legume mixture (T1), a cover crop of *Trifolium alexandrinum* (T2) and a natural covering of the vineyard (T3). The thesis of the natural covering showed a high percentage of soil covering and a high dry matter yield of mowing compared to the other thesis, moreover, it showed a low percentage of legumes species, and it was characterized by a high percentage of grass species especially in the second year of trial.

As expected, the cover crops containing legumes species improved the vigor of the vine as well as the yield (6.84, 6.41 and 4.09 kg/vine in 2021 for T1, T2 and T3 respectively, 5.15, 5.80 and 2.73 kg/vine in 2022 for T1, T2 and T3 respectively).

Also qualitative parameters of berries were affected at harvest, the *Trifolium alexandrinum* and the grass-legume mixture vines showed a lower total soluble solids content compared to natural covering vines (-3.85 and -3.36 °Brix, respectively for T1 and T2 in 2021, -4.14 and -5,09 °Brix respectively for T1 and T2 in 2022), and a significant reduction in pH, with values of 3.01, 3.05, 3.13 in 2021 and 2.97, 2.97, 3.08 in 2022 for T1, T2 and T3 respectively. These values were associated with an increase in the total acidity, equal to + 0.58 g/l and + 0.52 g/l in 2021 for T1 and T2 respect to T3 and + 0.36 g/l and + 0.60 g/l in 2022 for T1 and T2 respect to T3. Also, the YAN content in the must was improved in T1 and T2 compared to T3 in both years of trials.

The use of cover crops containing legumes species could be considered a possible strategy to slow down the grape ripening process as well as a strategy to improve YAN content and provide ecosystem services in the vineyard.

Keywords: sustainability, biodiversity, global warming, adaptation strategies, N supply, sugar accumulation, total acidity, pH, grape ripening

2.2 Introduction

Viticulture is a key socio-economic and cultural sector, and the majority of areas cultivated with vines are historically located in temperate climate regions. In addition to the direct economic aspect, there are other indirect benefits provided by viticulture, such as ecosystem services (Garcia et al., 2018), and aspects related to land management, such as landscapes and suggestive places that attract tourists, in other words conceptualized as terroir wine tourism (Marlowe and Lee, 2018).

In this way we noticed that a growing number of consumers prefer products that are linked with the concept of sustainability (Toth et al., 2020). For what concerns terroir tourism, a strategy focused on organic farming practices will contribute to the quality of life of local individuals and tourists and it would also help the promotion of organic vineyards and of a more sustainable production in the world of wine (Marlowe and Bauman, 2019).

Besides, climatic change has affected viticulture worldwide, in our territory we registered a general increase of temperature, hot extremes, heavy precipitation events and drought (IPCC 2022). It was reported that high temperatures in the growing season promoted a decrease in the grape acidity (Leolini et al., 2019; Schultz and Jones, 2010; Duchene et al., 2005), an increase in grape pH (Neethling et al., 2012; Orduna, 2010), and a high sugar content and so probable alcohol (Santos et al. 2020; Palliotti et al. 2014; Jones and Davis, 2000). Furthermore, increased summer dryness led to a yield reduction, because of warming and drought, and also it led to a high propensity of N deficit in Northern/Central Europe (Fraga et al, 2016). So, the characteristics of fruity aroma, the acidity and the moderate alcohol content, are expected to be negatively influenced by high ripening temperatures (Molitor et al., 2019a).

A possible adaptation strategy that could be used to solve the above-mentioned problems, and a great way to implement biodiversity and sustainability, essential factors especially in organic viticulture, is the use of cover crops in the vineyard (Abad et al., 2021 a; Abad et al., 2021 b; Garcia et al., 2018; Guerra & Steenwerth, 2012).

Cover crops could be classified as inflexible adaptation strategies to climate change, since they are usually planted before the beginning of the vegetative vines cycle, and they could cover soil for several seasons.

Cover crops provide several positive services but also some disservices in the vineyard (Garcia et al., 2018).

For what concerns soil gas emission, on the one hand viticulture contributes to greenhouse gas emission, but on the other could play a primary role in carbon sequestration. Conservative management practices such as natural covering or cover crop, with no soil cultivation, led to a net carbon sequestration of about – 421 gC m^{-2} (Tezza et al., 2019), actively contributing to climate change mitigation.

Another positive service , exerted by cover crops, is the protection of soil from water and wind erosion (Le Bissonnais et al., 2004; Novara et al., 2011); the protection from water erosion could be performed in summer, when strong storms occur (Bagagiolo et al., 2018; Biddoccu et al., 2015), or in autumn-winter when the highest soil losses occur (Ferreira et al., 2018, Novara et al., 2013). Moreover, this protection depends on cover duration, since a perennial cover crop presented a higher protection from erosion compared to a temporal cover crop (Ruiz-Colmenero et al., 2011). As for the cover composition, for example an annual *Vicia faba* cover crop has a lower effect on erosion reduction than a legume mixture or a grass-legume mixture (Novara et al., 2011).

Other services are the improving of soil structure and porosity (Ruiz-Colmenero et al., 2013; Ferrero et al., 2005) and consequently the improving of water infiltration (Garcìa-Diaz et al., 2018; Gaudin et al., 2010), the leaching reduction (Thorup-Kristensen et al., 2003), and also the better trafficability in vineyards.

Regarding soil fertility, the cover crop could provide services or disservices depending on the species used. Generally, cover crops increase soil organic carbon (Belmonte et al. 2016; Messiga et al., 2015); legume species increase soil nitrogen content (Tribouillois et al., 2016; Fourie et al., 2007) and yeast assimilable nitrogen content in berries (Fourie et al., 2007), while grass species decrease soil nitrogen (Gontier et al., 2014; Mattii et al., 2005) and YAN (Giese et al., 2015; Ripoche et al., 2011; Palliotti et al., 2007).

Generally speaking, cover crops compete with vines for water and nutrients, decreasing yield (Gomez, 2017) and reducing the vine vigor (Muscas et al., 2017), furthermore the reduction in the vine vigor could decrease the botrytis incidence (Morlat and Jacquet 2003). However annual cover crops, especially legumes like Trifolium spp. (Messiga et al., 2016; Susaj et al., 2013) could increase yield, while permanent cover crops, in particular grasses, could decrease yield (Palliotti et al., 2007; Mattii et al., 2005). Also, a greater reduction in yield occurred when the cover crop affected the whole inter-row soil surface (Reeve et al., 2016).

Regarding qualitative parameters, such as total soluble solids, total acidity and pH, several studies did not show any variation (Donkò et al., 2017; Varga et al. 2012; Giese et al., 2015). In other studies grapes grown under a permanent cover crop showed an increase of total soluble solid concentration, often linked to yield reduction (Wheeler et al., 2015; David et al., 2001), but when the permanent cover crop was constituted of legumes species showed a TSS reduction (Peng et al., 2022; Nauleau 1997).

Moreover, some studies showed a reduction in total acidity under spontaneous cover crops (Lopez et al., 2011; Lopes et al., 2008).

The aim of this work was to evaluate the effects on vegetative growth, grapevine yield and on berry quality parameters of T1 (grass-legume mixture) and T2 (*Trifolium alexandrinum* cover crop), compared to T3 (entire natural covering).

2.3 Materials and methods

2.3.1 Plant material, experimental conditions and experimental design

The trial was carried out over two consecutive seasons, namely 2021 and 2022, in a 9-years-old hillside vineyard (~20% slope) sited near the town of San Paolo di Jesi in the Marche region of east-central Italy (latitude: 43°27’N; longitude: 13°09’E, elevation 190 m above sea level).

The vines were planted in 2012 with certified virus free-cuttings of cv Verdicchio (clone VLVR20) grafted onto 1103 Paulsen rootstock, oriented north-northeast to south-southwest and spaced at 1.10 m x 3 m, resulting in a density of 3030 vines/ha. Grapevines were cane pruned, vertically shoot positioned and hand pruned in winter. The Guyot training system canes were set at 0.9 m aboveground with two pairs of catch wires and a single catch wire providing trellising extending 0.9 m above the canes. The vineyard was rainfed and conducted under certified organic farming.

Pest and disease management programs were carried out according to local practices determined by field scouting, experience and weather conditions. During the two years of trial, usually at mid-June, shoots were mechanically trimmed when their growth exceeded the top wires.

The study was conducted on 48 vines organized into four randomized blocks with 12 vines each. Each block was divided in three plots of 4 vine each. Each plot consisted in of three consecutive interrow 135 m long and 9 wides, with two central experimental row. Plots were separated by a single border row. The following floor management systems were compared: the first plot consisted in a plurennial interrow grass-legume mixture of *Lolium perenne*, *Festuca rubra* (*Graminaceae), Trifolium repens* and *Lotus corniculatus* (*Legumonose)* with a mechanical weed control of the under row (T1), the second plot consisted in a annual interrow cover crop of *Trifolium alexandrinum* with a mechanical weed control of the under row (T2), the third plot consisted in an entire natural covering (T3).

Cover crops were seeded along T1 (grass-legume mixture) in October 2020 and T2 (legumes) in October 2020 and 2021 at rate of 30 kg ha^{-1}. Since the re-establishment of T1 in autumn 2021 was unsatisfactory, due adverse weather conditions, an over-sowing was performed in March 2022 at the rate of 30 kg ha^{-1} with the sod seeding technique.

No shoot thinning or bunch removal was performed.

During the 2-year period, the mean and maximum daily temperature and rainfall data were recorded at the Civil Protection site of Marche region (Meteo-Hydro-Pluviometric Regional Information System), which has a meteorological station 4,5 km from the vineyard. Growing degree-day (GDD, base 10 °C) accumulated from 1 April to harvest were calculated.

2.3.2 Cover crop assessment

In each cover crop plot, the following parameters were observed:

- herbage covering rate (%) and species composition (%) by visual estimation in three sampling areas of 100 x 100 cm in each plot before each mowing;

- dry matter yield (DMY) and its botanical composition in three sampling areas of 100 x 100 cm in each plot. Swards were mowed in according to local practises in order to control the cover crop vegetative growth and ensure a proper establishment and self-reseeding of annuals. Plant samples were oven-dried at 60°C to constant weight and then weighed to determine the above-ground dry matter yield.

2.3.3 Vine growth and canopy measurements

In each year the total leaf area (TLA) and canopy density (expressed as leaf layer number, LLN) were determined via Point Quadrat Analysis (Smart and Robinson 1991). On 1 September and 22 August, respectively in 2021 and 2022, the TLA and LLN were estimated using 100-120 insertions, according to the full height of the canopy, at 10 cm intervals with a thin metal rod following a sampling grid. The metal rod simulates the sunlight ray and, each contact with a canopy component, represents the sunlight interceptions.

Each year of the trial, the annual vine growth was assessed by counting and weighing the canes on all vines, and the Ravaz index (Ravaz, 1903), commonly used to evaluate the balance between vine growth and yield, was calculated as the ratio of yield to pruning mass.

2.3.4 Vine yield and grape composition

For each year of the study, after the veraison (indicatively starting from the last days of July), the total soluble solids [TSS (°Brix)], pH, titratable acidity (TA), tartaric and malic acid were weekly assessed on 100 berries, until the harvest. Vines were harvested on 13 September 2021 (DOY 256) and 23 August 2022 [Day of the year (DOY 235)] when the TSS began to level off, as measured in grapes sampled from representative positions in bunches.

Grapes were individually picked and the total number of bunches per vine was counted and weighed. Mean individual bunch weight was calculated as the ratio of total bunch weight per vine (yield) and the total number of bunches per vine.

At harvest, 100 berries per vine were collected and weighed to determine the berry fresh weight. The berries were crushed, and the juice was used to determine TSS, pH, titratable acidity (TA), tartaric and malic acid. The TSS were measured using a temperature-compensating Maselli LR-01 digital refractometer (Maselli Misure, Parma, Italy). Must pH was analysed with a Crison two decimal pH meter (Crison Instruments, Barcelona, Spain) by a glass electrode, TA with a Crison Titrator (Crison Instruments) using 0.25 N NaOH to a pH 7.00 endpoint, expressed as g/L of tartaric acid equivalent. The tartaric acid concentration was measured by the 'colorimetric method' based on the reaction between tartaric and vanadium acid with the produces an orange colour, measured by spectrophotometry at 500 nm; and the malic acid concentration with an enzymatic kit (Enzyplus-Raisio, Raisio, Finland). Over the two years of the study, the yeast assimilable nitrogen (YAN) concentration, including ammonium and α-amino acids, was estimated in each year following the Ogorodnik and Merkureua (1971) procedure reported in Gump et al. (2002). Bunch compactness was expressed and visually estimated using Organisation Internationale de la Vigne et du Vin (OIV) code 204 (Organisation Internationale de la Vigne et du Vin 1983), which uses a numbered scale to rank 'berries in grouped formation with many visible pedicels' as 1 and 'misshaped berries' as 9.

2.3.5 Statistical analysis

The results were tested with Statistica version 4.3 (StatSoft,Tulsa, OK, USA) for homogeneity of variance and subjected to ANOVA. The graphical representations were obtained using the Sigma Plot version 10 (SPSS, Chicago, IL, USA). In each year, data of TLA and LLN, PAR, grape composition at harvest and yield components were tested using means separation calculated by applying the Student–Newman–Keuls test at P ≤0.05.

In the figures, of the evolution of berry mass, TSS, must pH and TA are shown as mean values SE.

2.4 Results

2.4.1 Environmental conditions

Both years were characterized by drought in the period between bud break and harvest and high temperatures in the summer months, with unevenly distributed rainfall and torrential rain events, indeed on 15 September 2022 (DOY 258) fell 152.2 mm of rain in few hours (Data *from Protezione civile Regione Marche*).

The average temperature of summer was higher during the 2021 season than in 2022, respectively 24.93°C vs 24.52°C. The growing degree days (GDD, base 10°C) accumulated from April to harvest were 2121 in 2021 and 2287 in 2022 (Table7), besides for the Verdicchio variety, a requirement between 1400 and 1800 degree days is reported for full ripening.

The total rainfall from April to harvest was very low in 2021 (110.8 mm) and 2022 (130.4 mm). In the drier season 2021, only 110.8 mm of rainfall fell from bud break to harvest, the period encompassing fruit set, veraison and berry ripening. In 2022, even if the rainfall in spring was low, it rained about twice as much as in 2021(Table 7).

The summer 2021 recorded a higher number of days with temperatures above 35°C, equal to 23, compared to 2022, with 16 days. Besides, summer 2022 recorded a higher number of days with temperatures above 30°C, equal to 85, compared to 2021, with 65 days (Table7). Concluding both the 2021 and 2022 seasons were characterised by heat stress and severe summer drought.

Table 7. Weather variables and growing degree-days from April to October in Verdicchio vines.

year	Rain (mm/year)	Rain from budbreak-harvest (mm)	Rain in spring (mm)	Rain in summer (mm)	Average summer Temperature (°C)	Number of day T>30°C	Number of day T>35°C	GDD April-October
2021	754	111	61	108	24.93	65	23	2121
2022	702	130	129	285	24.52	85	16	2287

Daily average and maximum temperature and precipitation data were taken from the site of Protezione Civile Regione Marche. *GDD*, Growing degree-days (daily temperature base 10 °C). Data are from 1 April to 31 October in 2021 and in 2022.

2.4.2 Cover Crop

During the two years of the experiment, T3, the natural covering, ensured a higher soil covering rate compared to the other treatments in every sampling (Figure 10). Particularly, in the first year T3 showed a percentage of soil covering of 69%, 91% and 63% in the first, second and third mowing respectively. In the second year, the natural covering (T3), presented a soil covering near to 98% in both sampling (Figure 10). T2, the *Trifolium alexandrinum* cover crop, showed a higher soil covering compared to the grass-legume mixture (T1) only in the first two sampling of 2021, with a covering of 44% vs 29% and 69% vs 65% respectively. Then the legume covering (T2) presented the lowest percentage of soil cover among theses, and equal to 32% in the third mowing of 2021 and 52% and 42% in the first and second sampling of 2022 (Figure 10).

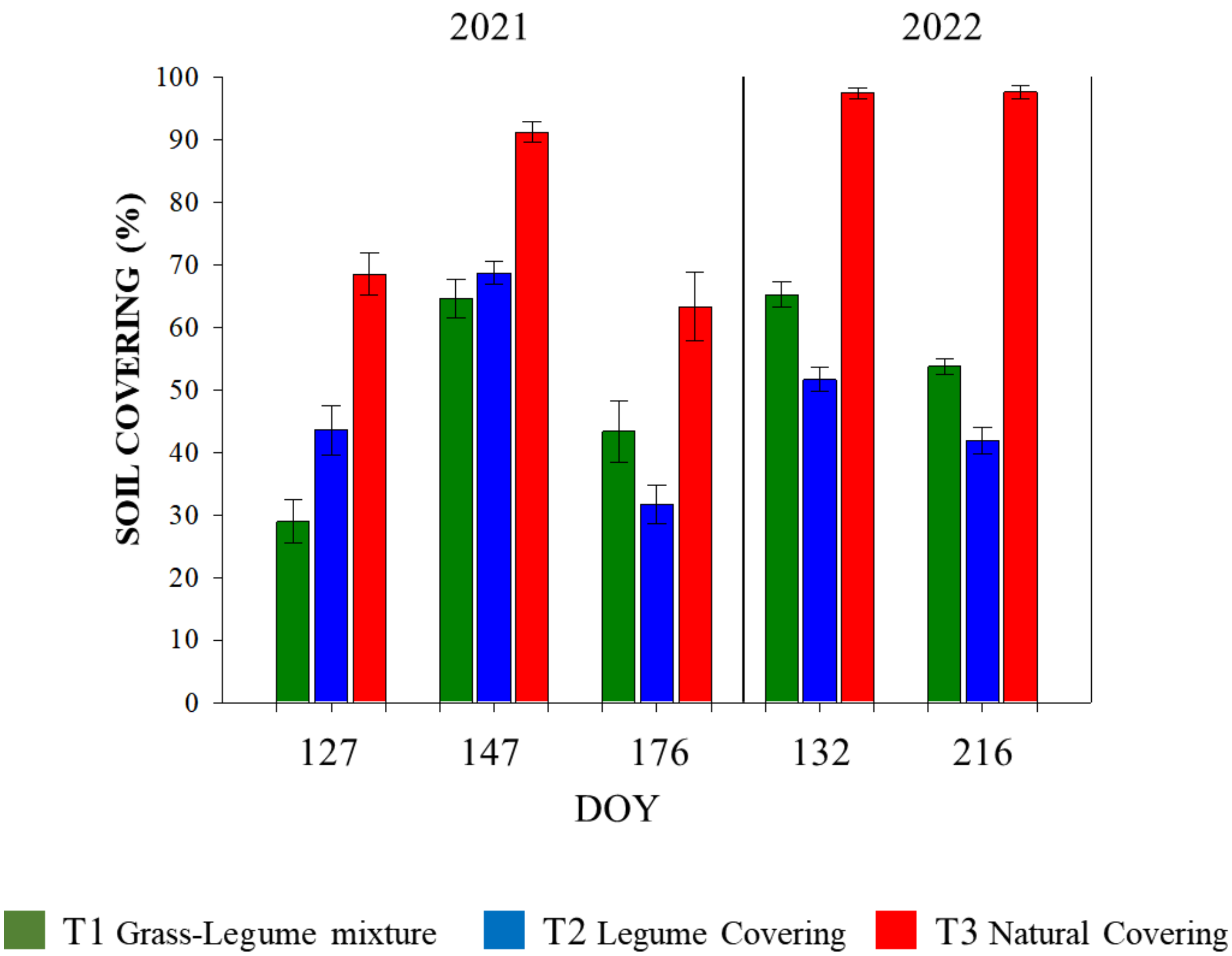

Figure 10. Percentage of soil cover during the survey.

Percentage of soil cover by different cover crops in 2021 and 2022. Data taken on the day of mowing, shown as DOY (day of the year).

The growth of cover crops and the mowing frequency, varied by year due to the climate conditions. Plots were mowed twice in 2021 and 2022, an exception regarded the natural covering, which grew spontaneously, requiring an extra cut in 2021 at the beginning of the growing season, when the other two theses had grown but did not reach a suitable height for cutting.

The production of dry matter yield differed significantly by mowing date and year (Figure 11 and 12). In the first chopping, when T1 and T2 had germinated but had not reached cutting height, T3 produced a quantity of dry matter of about 1300 kg per hectare. Generally, the natural covering (T3) produced the higher amount of dry matter yield in each mowing, except for the second chopping of 2021(Figure 11). T1 (grass-legume mixture) and T2 (*Trifolium alexandrinum*) showed similar values of dry matter yield in each mowing of 2021 and 2022 except the last date of sampling (DOY 216 of 2022), in which T2 presented lower values compared to T1 and T3.

For what concerns the composition of the soil covering, as the test was conducted under organic farming conditions, and therefore without the use of herbicides, the three cover crops showed an evolution of mixture components. The natural covering (T3) showed a progressive decrease in the presence of legumes, which disappeared in the last sampling of 2022 (Figure 11 and 12), while the amount of grasses resulted elevated and exceeded 50% in the second year of trial (Figure 12). Regarding the composition of T3, the grasses were represented by *Avena fatua* L. and *Poa pratensis* L., the legumes species by *Trifolium repens* L. and *Medicago arabica* L., and the other species by *Rumex acetosa* L., *Crepis foetida* L., *Plantago lanceolata* L., *Helminthotheca echioides* L., *Taraxacum officinale* F.H.Wigg., *Sonchus arvensis* L.

In T1 (grass-legume mixture) the presence of sown legumes species increased in the second year of trial, but it was registered also the presence of unsown species, in particular *Avena fatua* L. for grasses and *Rumex acetosa* L., *Cirsium Arvense* L., and Sonchus arvensis L. for the other species. In T2 (legume covering), the presence of legumes was higher that T1 and T3 in each sampling but it was also registered the presence of unsown species which are *Avena fatua* L. and *Poa pratensis* L. for grasses, and *Rumex acetosa* L., and *Cichorium intybus* L. for the other species.

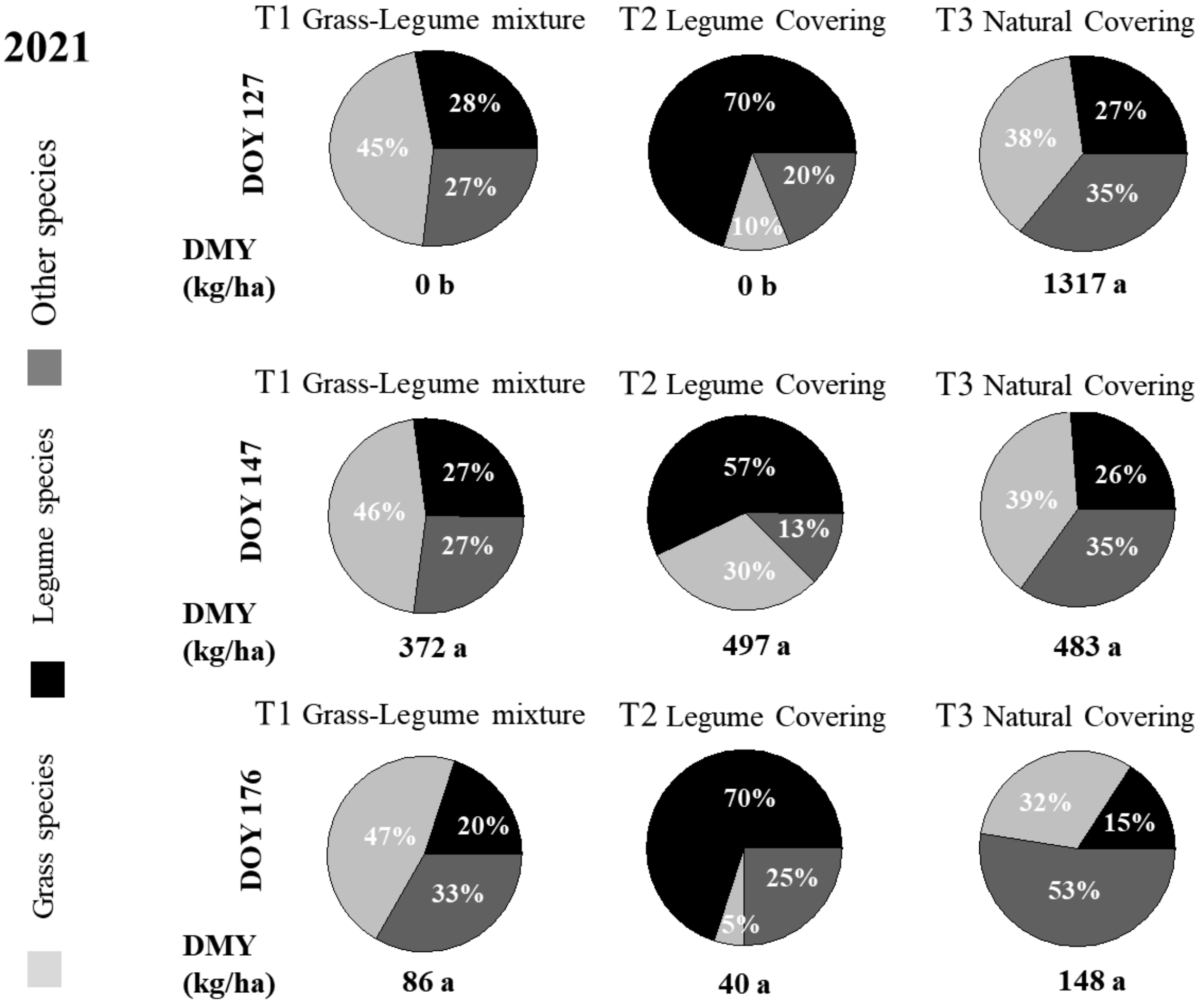

Figure 11. Dry matter yield and composition in 2021.

Dry matter yield (DMY) and percentages species contribution to dry matter production for each cut during the survey. Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters.

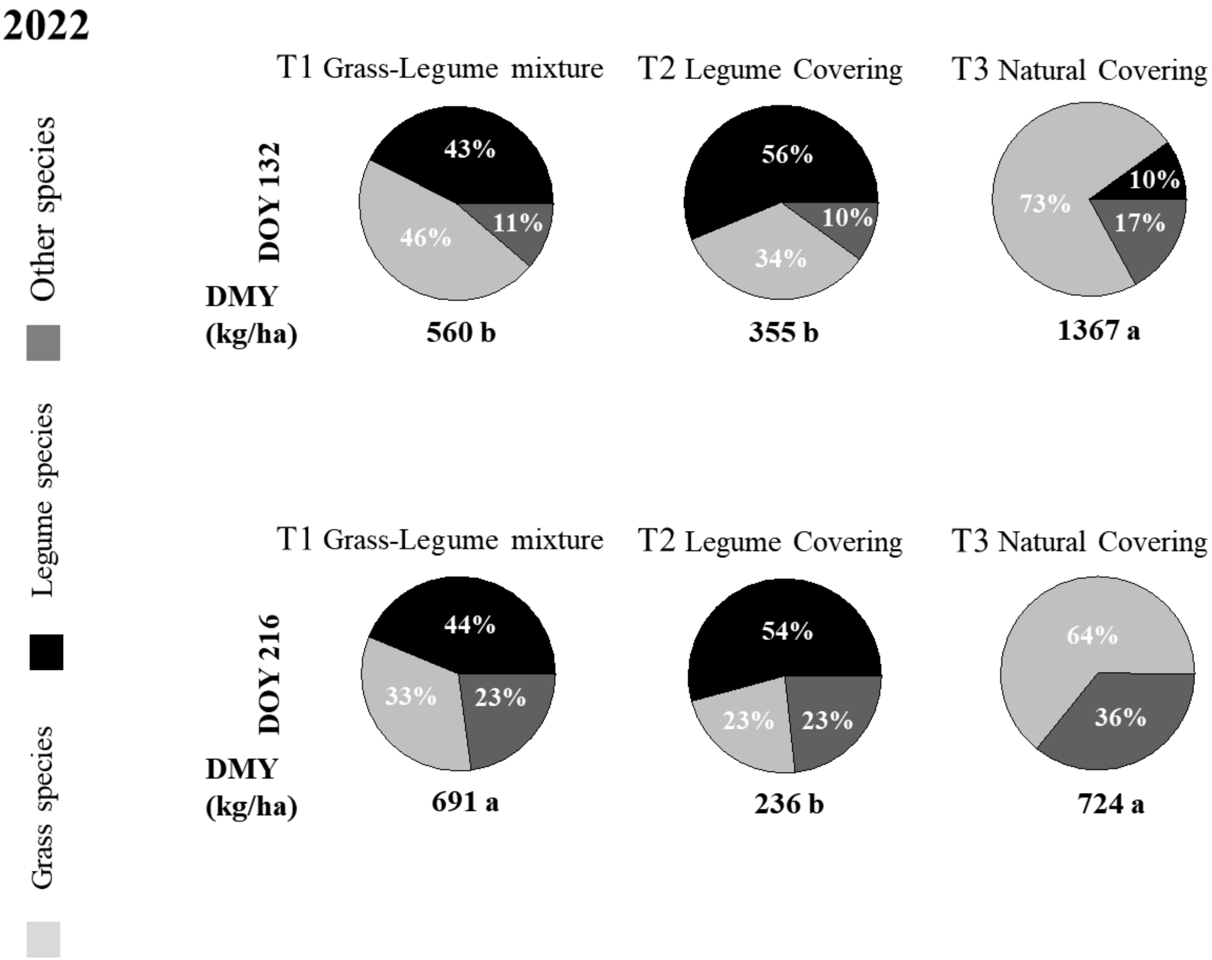

Figure 12. Dry matter yield and composition in 2022.

Dry matter yield (DMY) and percentages species contribution to dry matter production for each cut during the survey. Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters.

2.4.3 Canopy architecture and total leaf area

The vine canopy development was strongly influenced by the cover crop soil covering rate and its composition. Moreover, regardless the type of cover crop, in the 2021 growing season it was registered a reduced development of TLA, compared to the 2020 growing season.

In 2021 the number of shoots per vine was the same among the theses, equal to 16 shoots per vine, while in 2022 the natural covering (T3) showed a significantly lower number of shoots per vine, equal to 15, compared to the other theses that presented 20 shoots per vine (Table 8).

Regarding the TLA (total leaf area) per shoot, in 2021 the natural covering (T3) showed importantly lower values, equal to 0.23 m^2, in relation to the grass-legumes mixture (T1) and *Trifolium alexandrinum* cover-crop (T2) that presented similar values, of 0.32 m^2 and 0.30 m^2 for shoot respectively (Table 8). In 2022, we registered significative differences among the theses, the

Trifolium alexandrinum cover crop (T2) showed higher TLA for shoot, equal to 0.21 m^2, followed by the grass-legume mixture (T1), with intermediate values of 0.18 m^2, and the natural covering (T3) that showed significantly lower values, equal to 0.14 m^2 (Table 8).

Moreover, for what concerns TLA per vine, in 2021 T1 and T2 registered similar values, of 5.11 m^2 and 4.81 m^2 respectively, while T3 presented a decisive lower value, equal to 3.66 m^2. In 2022 the theses followed the same trend, with 3.56 and 4.21 m^2 per vine in T1 and T2 respectively, and significative lower values in T3, equal to 2.14 m^2 per vine.

Table 8. Total leaf area determined in T1, T2 and T3 vines.

year		Shoot/vine (n°)	TLA/shoot (m^2)	TLA/vine (m^2)
2021	T1	16 a	0.32 a	5.11 a
	T2	16 a	0.30 a	4.81 a
	T3	16 a	0.23 b	3.66 b
2022	T1	20 a	0.18 b	3.56 a
	T2	20 a	0.21 a	4.21 a
	T3	15 b	0.14 c	2.14 b

Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *TLA*, total leaf area. *T1*, grass-legume mixture, *T2, Trifolium alexandrinum*, T3, natural covering.

Regardless of the type of cover crop, in the 2021 growing season it was registered a reduced development of canopy height, compared to the 2020 growing season. In both years, the mean height of the canopy was significantly lower in the natural covering vines (T3), equal to 114 cm in 2021 and 99 cm in 2022, compared to the grass-legume mixture (T1) and the *Trifolium alexandrinum* cover crop (T2), that showed values of 124 cm and 123 cm in 2021 respectively and of 108 cm for both theses in 2022 (Table 9).

Furthermore, in 2021 the T3 showed decisive lower values of canopy thickness in all the sampled zone, coupled with importantly lower values of leaf layer number (LLN), compared to T1 and T2 that registered similar values (Table 9). Speaking in general, the natural covering vines presented a medium canopy thickness of 29 cm coupled with values of LLN equal to 2, while the T1 and T2 vines a medium canopy thickness of 40 cm coupled with values of LLN equal to 3 (Table 9).

Also, in 2022 the natural covering vines (T3) showed significantly lower values of canopy thickness in all the sampled zone, with values of 25 cm, 26 cm, and 29 cm in the distal, median and fruiting zone of the canopy respectively, coupled with lower values of LLN, equal to 2, 2 and 3 leaf in the distal, median and fruiting zone respectively. The *Trifolium alexandrinum* vines (T2) showed importantly higher values of canopy thickness in the distal zone of the canopy compared to grass-legumes mixture vines (T1), 45 cm vs 38 cm respectively, while these two theses registered similar values in the median and basal zones, equal to 47 cm in T2 for both zones and to 43 cm and 44 cm in T1 for the median and basal zone. Also, for the LLN, T2 showed significantly higher values in the distal zone of the canopy compared to T1, 4 vs 3 leaf respectively, while these two theses registered similar values of LLN in the median and basal zones, equal to 4 leaf (Table 9). Summarizing, in 2022, the natural covering vines presented the lower value of medium canopy thickness, equal to 29 cm, the *Trifolium alexandrinum* vines the higher value, equal to 50 cm, while the grass-legumes mixture an intermediate value of 43 cm (Table 9).

Table 9. Canopy architecture and leaf layer number in T1, T2 and T3 vines.

year		Canopy height (cm)	Medium canopy thickness (cm)	Thickness distal zone (cm)	Thickness median zone (cm)	Thickness fruiting zone (cm)	LLN distal zone (n°)	LLN median zone (n°)	LLN fruiting zone (n°)
2021	T1	124 a	40 a	37 a	40 a	38 a	3 a	3.1 a	3 a
	T2	123 a	40 a	39 a	39 a	37 a	3.3 a	3.4 a	3.3 a
	T3	114 b	29 b	24 b	24 b	26 b	2.1 b	2.2 b	2.3 b
2022	T1	108 a	43 b	38 b	43 a	44 a	3.3 b	3.5 a	3.8 a
	T2	108 a	50 a	45 a	47 a	47 a	4 a	3.9 a	3.9 a
	T3	99 b	29 c	25 c	26 b	31 b	2.3 c	2.5 b	2.6 b

Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. LLN, leaf layer number. T1, grass-legume mixture, T2, *Trifolium alexandrinum*, T3, natural covering.

2.4.4 Berry ripening and grape composition at harvest

The natural covering affected berry development only in the warmer season of 2021, with values significantly lower than the grass-legume mixture and the *Trifolium alexandrinum* cover crop (1.32g vs 1.58g and 1.64 g respectively for T3, T1 and T2). In the wetter seasons of 2022 (Table 11), the berry weight showed similar values between the thesis, values that were higher compared to the 2021, regardless of the soil covering (Figure 13 and Table 11).

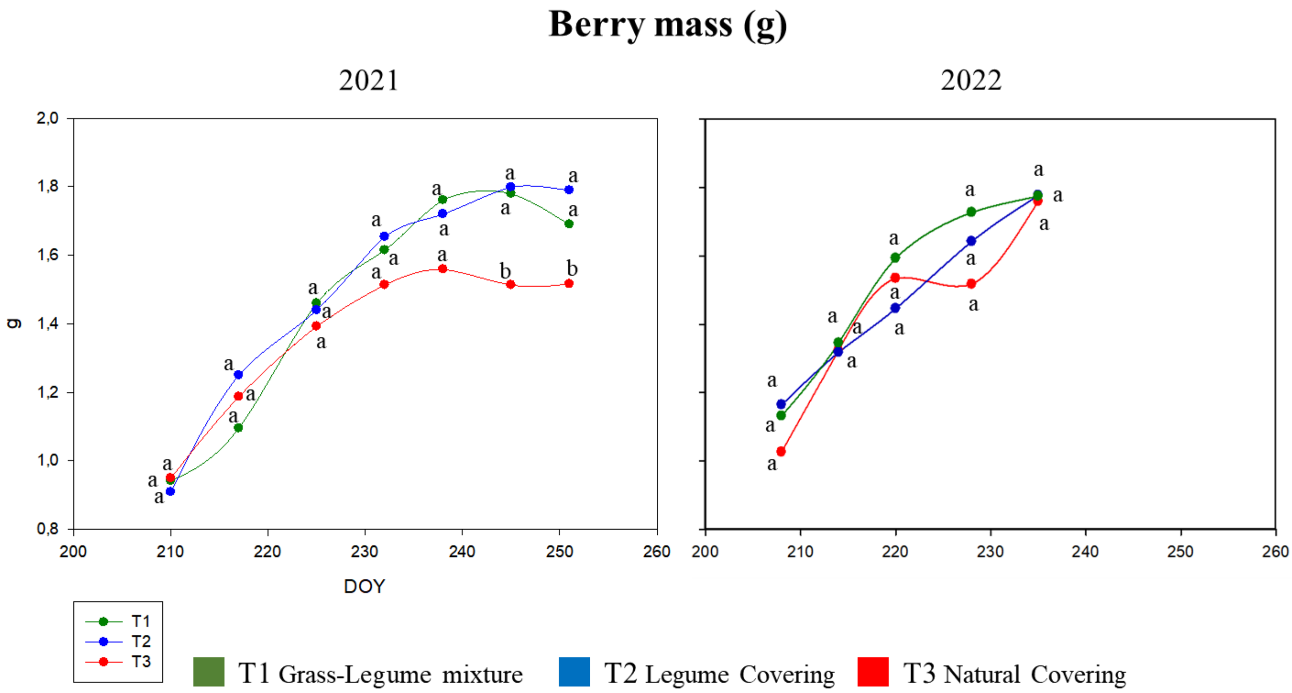

Figure 13. Evolution of berry mass in 2021 and 2022.

Mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. T1, grass-legume mixture, T2, *Trifolium alexandrinum*, T3, natural covering. (n = 100 berries per treatment, at harvest n = 100 berries per vine).

Over the two seasons, the grapes of T3 vines compared to those of T1 and T2 showed a delay in the sugar accumulation until the harvest, when the berries presented values statistically lower than T3 ones, respectively equal to 19.91 °Brix in T1 and 20.40 °Brix in T2 vs 23.76 °Brix in T3, in 2021, and 19.66 °Brix in T1 and 18.69 °Brix in T2 vs 23.78 °Brix in T3, in 2022 (Figure 14 and Table 10).

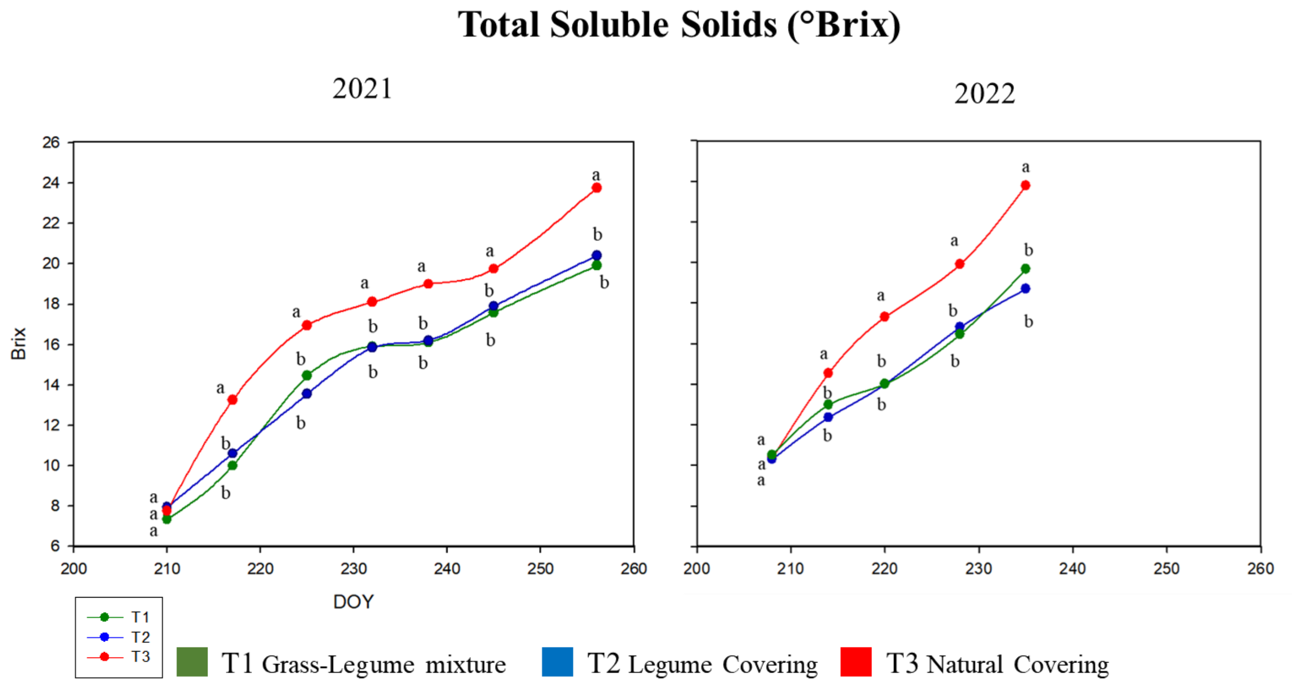

Figure 14. Evolution of Total Soluble Solid (TSS) in 2020, 2021 and 2022.

Mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. T1, grass-legume mixture, T2, *Trifolium alexandrinum*, T3, natural covering. (n = 100 berries per treatment, at harvest n = 100 berries per vine).

Similarly, the natural covering (T3) affected the berry pH, which was significantly higher starting from the second sampling to the harvest, when T3 showed pH values, in 2021, equal to 3.13 vs 3.01 and 3.05 of T1 and T2 respectively, and in 2022, equal to 3.08 while T1 and T2 registered both pH values of 2.97 (Figure 15 and Table 10).

pH

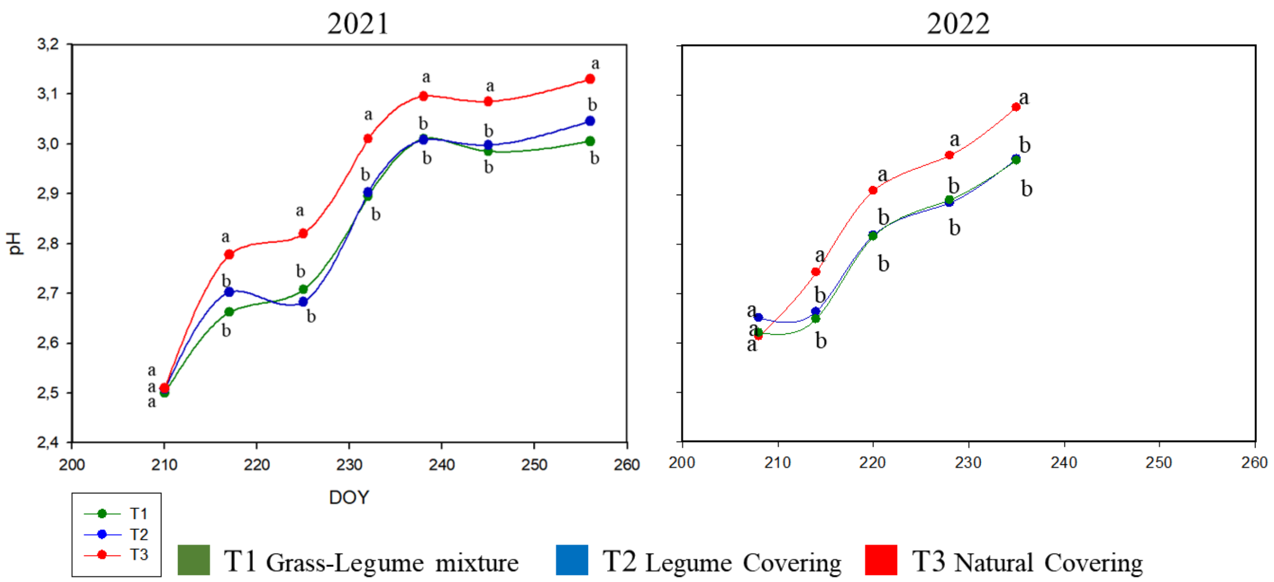

Figure 15. Evolution of pH in 2020, 2021 and 2022.

Mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. T1, grass-legume mixture, T2, *Trifolium alexandrinum*, T3, natural covering. (n = 100 berries per treatment, at harvest n = 100 berries per vine).

Furthermore, in both years the TA evolution in the grass-legume mixture (T1) and the legumes cover crop (T2) was delayed compared to T3 (Figure 16 and Table 10), with significantly higher values throughout the berry ripening phase. During the harvest of the 2021, T1 and T2 vines registered remarkable higher values compared to T3, of 7.68 g/l and 7.62 g/l vs 7.10 g/l respectively for T1, T2 and T3, while in the 2022 season only T2 showed significantly higher values compared to T3, with 7.65 g/L vs 7.05 g/L respectively for T2 and T3; T1 registered higher values compared to T3 (7,41 g/l vs 7,05 g/l respectively for T1 and T3) but without significant differences (Figure 16 and Table 10).

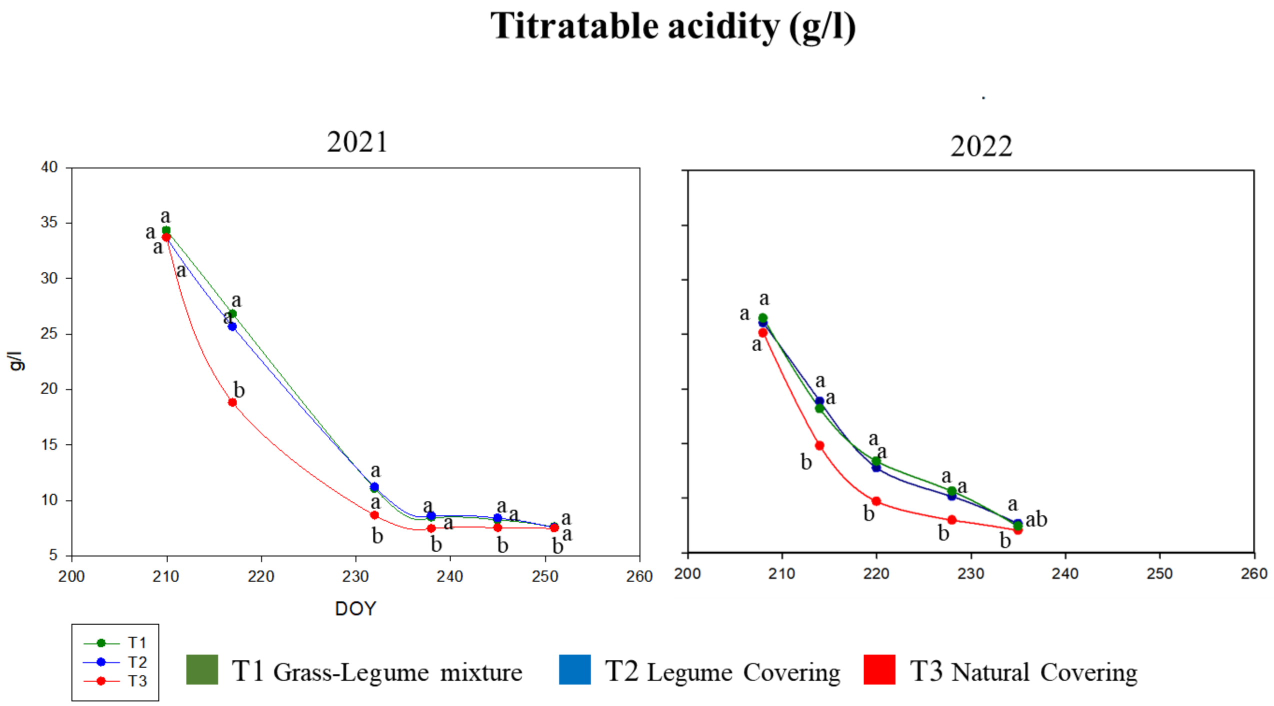

Figure 16. Evolution of Titratable Acidity (TA) in 2020, 2021 and 2022.

Mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. T1, grass-legume mixture, T2, *Trifolium alexandrinum*, T3, natural covering. (n = 100 berries per treatment, at harvest n = 100 berries per vine).

The same trend of TA was registered, in the harvest, in berry malic acid content (Table 10). The legume covering (T2) showed, in both years, significative higher values compared to natural covering (T3), with values of 0.74 g/l vs 0.44 g/l in 2021 and 1.43 g/l vs 0.90 g/l in 2022. The grass-legume mixture (T1) registered higher values of malic acid content compared to the natural covering (T3) but with important differences only in the first year of trial (0.74 g/L vs 0.44 g/L in 2021 and 1.13 g/L vs 0.90 g/L in 2022). Within the year, regardless of the thesis, the malic acid content resulted higher in the wetter season of 2022 (Table 10). The tartaric acid content showed significant differences only in 2021, with higher values in T3 rather than T1 and T2 (Table 10).

Finally, for what concerns the yeast assimilable nitrogen (YAN), during the harvest, the *Trifolium alexandrinum* vines (T2) showed, in both years, significantly higher values compared to natural covering vines (T3), equal to 86 mg/l vs 42 mg/l in 2021 and 126 mg/l vs 47 mg/l in 2022 (Table 4). Also grass-legume mixture vines (T1) registered, in both seasons, higher YAN content compared to natural covering vines (T3), with values of 84 g/l vs 42 g/l in 2021 and 83 g/l vs 47 g/l in 2022. In 2022 season it was registered significative differences of YAN among the three theses under analysis, with values of 126 mg/l in T2, 83 mg/l in T1, and 47 mg/l in T3 (Table 10).

Table 10. Must composition at harvest in T1, T2 and T3 vines.

	thesis	TSS (°Brix)	pH	TA (g/l)	Tartaric acid (g/l)	Malic acid (g/l)	YAN (mg/l)
2021	T1	19.91 b	3.01 b	7.68 a	9.30 b	0.74 a	84 a
	T2	20.40 b	3.05 b	7.62 a	9.19 b	0.74 a	86 a
	T3	23.76 a	3.13 a	7.10 b	10.18 a	0.44 b	42 b
2022	T1	19.66 b	2.97 b	7.41 ab	8.16 a	1.13 ab	83 b
	T2	18.69 b	2.97 b	7.65 a	7.55 a	1.43 a	126 a
	T3	23.78 a	3.08 a	7.05 b	8.21 a	0.90 b	47 c

Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. *TSS*, total soluble solids, TA, titratable acidity. T1, grass-legume mixture, T2, *Trifolium alexandrinum*, T3, natural covering.

2.4.5 Vine yield and vine size

The total yield per vine was higher, in both years, in grass-legumes mixture (T1) and in the legume covering (T2), with decisive differences in 2021 and 2022 compared to the natural covering (T3). T1 and T2 showed similar values in both years, equal to 6.44 and 6.81 kg/vine in 2021 and 5.15 and 5.80 kg/vine in 2022, while T3 registered significantly lower values, of 4.09 kg/vine in 2021 and 2.73 kg/vine in 2022 (Table 11).

In both years T1 and T2 vines showed a higher bunch number per vine than the T3 vines, 25 and 26 vs 19 in 2021, 21 and 21 vs 13 in the 2022 respectively (Table 11).

As regards to the bunch mass in 2021 grass-legume mixture (T1) registered a significantly higher value compared to natural covering (T3), with a mass of 268 g vs 221 g respectively, while T2 registered a higher value (254 g) than T3 even if without an important difference (Table 11). In 2022, T1 and T2 vines showed significantly higher values of bunch mass compared to T3 vines, equal to 370 g, 442 g and 218 g respectively (Table 11).

In conclusion, for what concerns the bunch compactness, the natural covering vines (T3) showed much fewer compact bunches compared to grass-legume mixture (T1) and *Trifolium alexandrinum* vines (T2) in both years (Table 11).

Table 11. Yield parameters at harvest in T1, T2 and T3 vines.

	thesis	Yield/vine (kg)	Bunches/vine (N)	Bunch mass (g)	Berry mass (g)	Bunch compactness (OIV)
2021	T1	6.84 a	25 a	268 a	1.58 a	7.9 a
	T2	6.41 a	26 a	254 ab	1.64 b	8.1 a
	T3	4.09 b	19 b	221 b	1.32 b	6.9 b
2022	T1	5.15 a	21 a	370 b	1.78 a	8.7 a
	T2	5.80 a	21 a	442 a	1.78 a	8.6 a
	T3	2.73 b	13 b	218 c	1.76 a	6.6 b

Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. T1, grass-legume mixture, T2, *Trifolium alexandrinum*, T3, natural covering.

In the first year of trial, the T3 vines showed a higher leaf area to yield ratio compared to T1 and T2 even if without significant differences (Table 12). Moreover, in the second year of trial, the T3 vines registered a higher leaf area to yield ratio compared to T1 and T2 with important differences, showing values equal to 0.75 m^2/kg, 0.76 m^2/kg, and 0.99 m^2/kg for T1, T2 and T3 respectively (Table 12).

In 2021, T1 and T2 registered significantly higher pruning mass per vine (1.02 kg and 1.01 kg respectively) compared to T3 (0.57 kg), while in 2022 the legumes (T2) vines showed the highest pruning wood per vine, equal to 1.16 kg, followed by grass-legumes mixture (T1) vines with 0.92 kg and by natural covering (T3) vines with 0.5 kg (Table 12).

The Ravaz index (yield-to-pruning mass) showed, in both years, higher values in T3, even if without significant differences (Table 12). The values of yield-to-pruning mass were, in 2021, 6.82 kg/kg, 6.70 kg/kg and 7.95 kg/kg for T1, T2 and T3 respectively and, in 2022, 5.70 kg/kg, 5.13 kg/kg and 5.90 kg/kg for T1, T2 and T3 respectively (Table 12).

Table 12. Vegetative and pruning characteristics recorded in T1, T2 and T3 vines.

	thesis	Leaf area/yield (m^2/kg)	Canes (N/vine)	Pruning wood (kg/vine)	Yield/pruning mass (kg/kg)
2021	T1	0.79 a	16 a	1.02 a	6.82 a
	T2	0.80 a	16 a	1.01 a	6.70 a
	T3	0.94 a	16 a	0.57 b	7.95 a
2022	T1	0.75 b	20 a	0.92 b	5.70 a
	T2	0.76 b	20 a	1.16 a	5.13 a
	T3	0.99 a	15 b	0.50 c	5.90 a

Within row mean separation performed with Student–Newman–Keuls test ($P < 0.05$) and shown by lowercase letters. T1, grass-legume mixture, T2, *Trifolium alexandrinum*, T3, natural covering.

2.5 Discussion

Both 2021 and 2022 were characterized by high temperatures during summer and drought in spring and summer. In 2022, if we do not consider the torrential rains that occurred on September 15, equal to 152.2 mm, which may have minimally infiltrated the soil in a sloping vineyard, the amount of rain fallen during the 2022 summer was slightly higher than the amount of 2021, equal to 133 mm and 108 mm respectively.

The use of cover crops in the vineyard, in addition to providing ecosystem services (Garcia et al., 2018), could be considered an adaptation strategy to climate change, as the cover crops moderate soil temperature, they improve water infiltration and soil fertility (Abad et al., 2021 a) and, for legume species, that are less competitive for water than grasses, they fix atmospheric nitrogen, improving the vine nitrogen content (Muscas et al., 2017) and the vine vigor. And as a result, this can lead to a delay in the ripening process. In line with our results for T1 and T2, Nauleau (1997) registered a reduction in soluble solid with a clover cover in France and Peng et al. (2022) found, in grapes growing with peanuts covering, lower total soluble solids (TSS) and higher titratable acidity (TA) in the berries.

As we observed in T1 and T2, the use of legumes species can increase the vine yield (Messiga et al., 2016), and it is known that a calibrated increase of yield, could reduce total soluble solid content in berries (Silvestroni et al., 2020). Conversely, a permanent cover crop, especially when composed ~~by~~ of grasses, as in the case of T3, it led to a decrease in grape yield (Palliotti et al., 2007, Mattii et al., 2005), that frequently is associated with an increase in the total soluble solid content (Abad et al., 2021 b). Regarding the canopy architecture, Tesic et al. (2007), found an increase of canopy openness, with few interior leaves, and a decrease in shoot length, with increasing percentage of soil covering by permanent cover crops, as in the case of T3.

Moreover, several studies found higher TSS content in vines grown on spontaneous vegetation (Pérez et al., 2018, Coniberti et al., 2018, Lopez et al., 2011) and higher TSS in vines grown on grasses than on legume mixture (Muscas et al., 2017). The decrease in TSS in T1 and T2 could be explained by different combined effects, the lower competition with vines of T1 and T2, the increase in the vigor of the vines due to N supply, and the consequent increase in the crop load (Silvestroni et al., 2020), that reduced the total leaf area-to-yield ratio (Kliewer and Dokoozlian, 2005) and caused a slowdown in ripening and a lower sugar concentration in the harvest.

In both seasons T3 showed lower values of titratable acidity (TA) compared to T1 and T2, in accordance with Lopez et al. (2011), who found that the presence of a spontaneous cover crop decreased TA in both of a two years study, and Fourie et al. (2007), who reported a decreasing acidity in the presence of a cover crop, that was more intense when the covering was maintained for a longer period of time during the season, as in our case for the natural covering.

Concerning the pH and malic acid content, we hypothesize that the lower pH and the higher content of malic acid in the cover crop vines containing legumes (T1 and T2) are due to the greater thickness and LLN of the canopy, which shade the bunches more than in the natural covering vines. Furthermore, Friedel et al. (2015) reported that a major intensity of shading in the fruiting zone can increase the total acidity and the malic acid content and decrease the pH in the musts.

For what concerns yeast assimilable nitrogen (YAN), in line with our findings in T1 and T2, Fourie et al. (2007) observed an increase in N in the must of Sauvignon blanc with the use of a legume cover crop, while several studies reported a decrease of YAN associated with the presence of a grass cover crop (Giese et al., 2015; Palliotti et al., 2007; Rodriguez-Lovelle et al., 2000), this is the case of T3.

Cover crops compete with vines for water and nutrients (Gomez, 2017), however in literature it was reported of increasing yields when legumes species, like *Trifolium* spp., were used (Messiga et al., 2016, Susaj et al., 2013, Ovalle et al., 2010). On the contrary, grasses permanent cover crops, like *F. arundinacea* (Palliotti et al., 2007, Mattii et al., 2005), lead to a decrease in the grape yield. Moreover, a decrease in the yield was observed when the plant cover took up the whole inter-row soil surface (Reeve et al., 2016), as in the case of T3, an entire natural covering of the vineyard with a high presence of grasses species.

In T3, the lower yield depended on a lower number of clusters per vine and on a lower bunch mass. Also, Muscas et al. (2017) observed a lower number of clusters per vine and a lower weight of cluster in grass-mixture vines compared to legume-mixture vines.

Focusing on the bunch compactness, Valdéz-Gomez et al. (2008), in a study on grapevines, that positively linked grey mould incidence with the vine vigor, it was observed that vigorous vines, showed a very dense canopy, more compact clusters and a delayed fruit maturity compared to less vigorous vines. Therefore, we could explain the lower bunch compactness in T3 vines with the reduced vigor, due to the higher competition for water and nutrients of the natural permanent covering, compared to T1 and T2, that showed lower competition plus supplied nitrogen.

Concluding, David et al. (2001), found a reduction in pruning weights in a tall fescue (*F. arundinacea*) cover crop, this is the case of T3 that reduced the number of shoots per vine and the pruning wood per vine, compared to T1 and T2.

2.6 Conclusions

The sown legume species (T1, T2), that provide less competition and fixed nitrogen, increased vine vigor, height and thickness of the canopy and leaf layer number compared to the permanent natural covering that on the contrary, decreased vigor. The supplied nitrogen and the lower competition for water and nutrients ensured a greater yield in T1 and T2; also, a reduction in the total leaf area-to-yield ratio produced a lowering in the amount of assimilates transmitted to the berries, compared to T3. The combined effects of the factors previously described led to a delay in the ripening process with a reduction in total soluble solid in the theses of the grass-legume mixture (T1) and the Trifolium *alexandrinum* cover crop (T2).

The delay in the grape ripening also affected the titratable acidity, the malic acid content, and the pH, thus T1 and T2 showed a higher TA and malic acid content and a lower pH compared to T3. The increased acidity was due also to the higher shading in the bunch zone compared to the natural covering vines which showed a less thick canopy with a lower LLN.

Finally, T1 and T2 registered a higher yeast assimilable nitrogen content, compared to T3, due to the larger presence of legumes. In the second year of trial, T2, that registered the higher presence of legumes, showed higher values of YAN in comparison also to T1, which recorded an intermediate presence of legumes between T2 and T3.

Concluding the use of cover crops containing legume species could, not only provide ecosystem services, but also be an appropriate technical strategy to mitigate the accumulation of sugars in the berries. Cover crops could also help to reduce the pH and to improve must microbiological stability, to increase the total acidity and malic acid content, both fundamental for the freshness of a white wine, and to increase yield. In addition, the presence of legumes allows the achievement of a higher YAN content in musts, a fundamental component for a regular fermentation process.

The use of legumes can therefore reduce, if not replace, nitrogen inputs over time, with economic savings and lower environmental risks, such as nitrogen leaks and the possibility of contamination of groundwater.

3. General conclusions

In this study we analysed the effects of two possible, non-flexible adaptation strategies to climate change on Verdicchio grapevine. The innovation of vine and pruning systems called High Cane, and the use of cover crop containing legumes (*Trifolium alexandrinum, Trifolium repens and Lotus corniculatus*) compared to a natural cover of the vineyard.

The innovation in vine training and pruning systems improved the number of buds per vine and per linear meter increasing the yield. The higher distance, in addition to possible effects on the microclimate of the canopy, reduced the canopy height. The combined effects of the abovementioned modifications reduced the total leaf area-to-yield ratio delaying the ripening process, and moreover, during the harvest musts showed lower sugar contents, lower pH values, and a higher total acidity.

The use of cover crops containing legume species, besides supplying nitrogen to the vines, and exerting lower competition for water and nutrients than the natural covering, improved the vine vigor and the vine yield and delayed the ripening process. During the harvest the must showed lower sugar content, lower pH values, and a higher total acidity and acid malic content. Moreover, the nitrogen supply in cover crops containing legumes, improved yeast assimilable nitrogen in the resulting musts, a fundamental component for the fermentation process.

In conclusion, both the innovation on vine training and pruning systems and the use of cover crops containing legumes, could be considerate possible adaptation strategies to climate change that allow a delay in the ripening process of the grape to obtain fresh wines with a moderate alcohol content that satisfy market needs.

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