



# Article Autochthonous Red Varieties in the Oltrepò Pavese Wine District: An Effective Tool for Adaptation to Climate Change

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Abstract: Global warming is challenging the performances of medium-to-late ripening red Vitis vinifera cultivars whose harvest dates might be consistently anticipated at the detriment of still insufficient phenolic maturity. A 3-year study (2021-2023) was devised to compare cv Croatina-the most grown red cultivar in the Oltrepò Pavese district—with the following five autochthonous genotypes: Croà, Moradella, Mornasca, Ughetta, and Uva Rara. Weather trends, main yield components, and grape composition parameters were recorded each year; in two out of the three trial seasons, ripening curves for total soluble solids (TSS) and titratable acidity (TA) were also derived. In terms of yield performance, all minor varieties showed a level of basal node fruitfulness (about 1.1 clusters/shoot) high enough to perform short pruning, which was not possible to achieve in Croatina. As per grape quality at harvest, Uva Rara behaved quite similarly to Croatina, whereas Ughetta, Moradella, and Croà were judged to be unsuitable due to poor berry coloration at harvest (less than 0.7 mg/kg). Conversely, Mornasca's performance was truly interesting and promising. In 2021 and 2023, Mornasca had delayed sugar accumulation, which was strongly uncoupled with total anthocyanins, which were not limited. In 2022, a hot and dry season, Mornasca outdid Croatina because the required TSS was assured, and the color significantly improved. The conclusion is that Mornasca is less susceptible than Croatina to imbalances in pigment formation or degradation, which typically occurs with berry overheating.

Keywords: Vitis vinifera; germplasm; yield; grape quality; climate change; ripening curves

# 1. Introduction

The extraordinary wealth of grape varieties is a distinctive feature of Italian viticulture. There are currently 635 registered wine grape varieties in Italy, including some long-forgotten ones that have been rediscovered, and each region boasts several of them [1]. Since 2010, 169 new varieties have been registered and authorized, 112 of which are classified as "autochthonous" (43% white and 57% red). Sometimes, they are also termed as "minor" or "local" to emphasize their very limited current diffusion, which, most of the time, does not exceed 200 hectares each.

As testified by several publications [2–8], retrieval, conservation, and assessment of local varieties are treasured in many grape-growing countries. An obvious reason for the revalorization of autochthonous genotypes is that they often lead to very distinct and peculiar wines that are strongly bound to the local, territorial features and thus emerge from the more homogeneous wine production pertinent to extensively grown international varieties [3]. A further push towards such kinds of germplasm is also linked to the challenges imposed by global warming [9], among which general phenology advancements for ripening to occur in a warmer-than-usual time window are very solid occurrences [10–13]. Under extremely high temperatures, grapevine functions may be impaired, leading to reduced metabolite accumulations, which may affect wine aroma and color [14]. Musts



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**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). with too high sugar concentrations might cause a stress response in yeast, which leads to increased formation of fermentation co-products, such as acetic acid. If not controlled by acid addition, the higher pH can lead to significant changes in the microbial ecology of musts and wines and increase the risk of spoilage and organoleptic degradation [15].

The rationale presented above has also stimulated analyses of the current and future climatic suitability of each variety in a given growing region [16,17]. An interesting application has been reported in Portugal, where spatial locations of 44 varieties were assessed using their growing degree day (GDD) requirements under a high-resolution dataset (<1 km) [16]. The results indicated that Portuguese varieties are highly adaptable because they are grown in a large range of thermal conditions. Another huge study performed on 465 cultivars assessed over four seasons in a hot Australian region [18], allowed the identification of cultivars grown under hot conditions with late budbursts to minimize frost risks, and had short growth periods, small canopies to improve water use efficiency, and early and late ripening to extend the season.

To preserve the specific and distinctive olfactory and tasting notes that link wines to their territory of origin in a warming scenario, the simplest solution is anticipating the harvest of grapes to limit wine alcohol levels as a viable alternative to the use of subtractive cellar technologies, which may cause compositional changes, compromising the aromatic quality of the product [19]. Then, an obvious question arises: To what extent can the harvest be anticipated while still providing an optimal balance between the different qualitative components of the berry, especially volatile and polyphenol concentrations and profiles? Literature is either scarce or contradictory about this issue [20,21]. When the ripening dynamics of Chardonnay grown in the premium sparkling wine district of Franciacorta (Italy) were followed for 3 years [22], it was impressive that in the memorably hot 2022 season, achieving average optimal must TA and pH levels (e.g., not less than 8 g/L and not higher than 3.00, respectively) would have required the harvest to be performed around 29 July, leaving unresolved doubts about adequate sugar content and aroma pool. Moving into reds grown in a warm environment, the most challenging factor is that while under optimal climatic conditions, balanced sugar-to-acid ratios, colors, and flavors will converge around a single chronological date; while under thermal stress, the ripening kinetics of different components tend to decouple with color and aroma considerably lagging behind technological maturity parameters [23,24].

If the general goal is, therefore, to push the harvest back for positioning in a hopefully cooler period, two main tools appear to be available. A few review papers have recently reported an array of viticultural practices that, in the short term, are viable to consistently delay ripening [25–27]. The most recent and extreme method is the "crop forcing" technique [28–30], where, even in a temperate climate, the vine is committed to crop twice due to the unlocking of the dormant buds during their first-year cycle. On the other hand, the solution could be to create or recur to late or very late ripening genotypes (scion, clone, or late ripening rootstocks) or genotypes able to produce high-quality wines under elevated temperatures [10,13]. Successful examples of how growing local autochthonous varieties might help to achieve or maintain desired grape qualities in a warming climate are already available. The main white varietal of the Colli Piacentini wine district (northern Italy), named Ortrugo, is best suited to sparkling winemaking and currently has very promising marketing appeal, yet it suffers from its inadequacy to maintain sufficient acidity at harvest mostly due to very low malic acid concentration. A 3-year comparison of Ortrugo against other local white varieties [31] has shown that especially those named Molinelli and Barbesino have a consistent ability to retain, at the same sugar concentration level, a higher TA (+2–3 g/L) than Ortrugo, mostly due to an increased malic acid pool pre-veraison and/or slower malic acid degradation rates post veraison.

The Oltrepò Pavese wine district is located across the borders of the Lombardy and Emilia Romagna Regions and currently boasts a vineyard acreage of about 13,300 hectares, with 3900 hectares grown with the leading red grape, Croatina. It tends to produce fruity, deeply colored wines that are mildly tannic and can benefit from bottle aging. With

global warming, ripening generally occurs around mid-September despite the varietal being categorized as a medium-to-late ripening genotype [32]. Another distinct feature of Croatina is its typically low fruitfulness of the basal nodes, which enforces a long cane pruning while also increasing year-to-year variability in the cropping levels.

An extraordinarily detailed climatic survey carried out for the Oltrepò wine district from 1961 to 2017 [33] provides a dramatic picture in terms of risk of meteorological drought in the area through the main following outcomes: (i) The mean annual average and maximum air temperature (T) have risen by 1.6 °C and 1.7 °C, respectively, when calculated for 1991–2017 vs. 1961–1990; (ii) the frequency of extreme T (i.e., >30 °C) on a monthly basis has increased from 25% in 1961–1990 to 75% in 1991–2017; (iii) over the whole observation time span, the period between 20 June and 20 August is categorized as "dry" with only 78 mm of rainfall; (iv) potential evapo-transpiration (ETP0) has increased from 948 mm/year in 1961–1990 to 1088 (+140 mm) in 2011–2017, with +59 mm in the Jun-Jul-Aug trimester. The above data provide full evidence for an increased risk of meteorological drought in the pilot area that, due to the main typology of winemaking (i.e., sparkling and spumante styles), is even more alarming.

The purpose of the present work is to provide a 3-year comparison of Croatina's yield performance and grape composition against those of five autochthonous red varieties, known as Moradella, Mornasca, Ughetta di Canneto, Croà, and Uva rara. The hypothesis is that some of the included local varieties could solve the issue of Croatina's scarce basal fruitfulness while postponing the harvest date into a likely cooler period.

## 2. Material and Methods

#### 2.1. Plant Material and Experimental Layout

The trial was conducted over three seasons (2021–2023) in a private, non-irrigated vineyard planted in 2017 at Azienda Tenute Scabini, Golferenzo, Italy, lat 44°57′43″20 N, long 09°18′25″20 E, 330 m a.s.l. The soil is a litho-stratigraphic formation named Val Luretta, which is about 150 cm deep until the bedrock is reached. The texture is about 10% sand, 40% clay, and 50% loam, which corresponds to a silt–loam soil type according to the USDA Soil Classification System. Soil hydrological constants calculated from [34] are 39.5% vol. (field capacity) and 24.1% vol. (wilting point) for a resulting 210 mm/mm of plant available water. Organic matter is around 1.5%, and active limestone is about 8%.

The vineyard is located on a moderate longitudinal slope (<5%), with north–south (NS) oriented rows and vines trained to a single-cane vertical shoot-positioned Guyot trellis at a spacing of 2.5 m  $\times$  1 m (inter- and intra-row) for a density of 4000 vines/ha. Each vine had a bud-load of about 9–10 plus a two-node spur maintained on the vine head. The fruiting cane was raised 90 cm from the ground, with three pairs of top catch wires for a canopy wall extending about 1.5 m above the main wire. Standard regional protocol for organic viticulture was applied in all the trial years. The canopy was mechanically trimmed once shoots outgrew the top foliage wire.

The vineyard used in this study also functions as a germplasm collection managed by the Lombardy Region. While extensively planted with the reference cultivar Croatina, it also features the following five autochthonous cultivars, each planted along a single 110 m long row: Uva rara, Moradella, Mornasca, Croà, and Ughetta. Although they are already registered in the national catalog of grape varieties, they are currently very sporadically grown. A representative cluster of each cultivar is shown in Figure 1.

During each season, daily minimum, mean, and maximum temperatures (°C) and rainfall (mm) were recorded by a weather station located within the vineyard. Every year, typically at budburst, inorganic fertilizers (ammonium nitrate, potassium chloride, and simple superphosphate) were distributed to provide equivalent amounts/ha of 50 kg N,  $30 P_2O_5$ , and 75 K<sub>2</sub>O. The primary disease threats of downy and powdery mildew were controlled on a calendar basis by spraying copper and sulfur within an average shoot length varying between about 10 and 100 cm; thereafter, Metalaxyl-M and Azoxystrobin were used until cluster closure according to the disease pressure. Cluster rot was absent in



2021 and 2002, whereas in 2023, a slightly more humid season, a visual assessment scored incidence rates lower than 5% in each cultivar.

Figure 1. Representative clusters of the six compared genotypes.

## 2.2. Yield Components and Grape Composition

A batch of 12 vines per cultivar was randomly selected and tagged after the 2021 winter pruning for subsequent more detailed determinations. Unless otherwise specified, the same vines were kept across seasons to better highlight any possible carryover effect. In 2022 and 2023 only, ripening curves tracking total soluble solids (TSS) and total acidity (TA) were included in the measuring protocol.

In each cultivar, ripening curves were drawn from the onset of veraison until ripening by taking, at 7- to 10-day intervals, three 50-berry samples from 8 of the 12-vine batch. The remaining four vines per cultivar were used only for harvest determination, therefore avoiding any interference that periodical berry sampling during the season might have caused to the dynamics of ripening. In 2022 only, before a quite early harvest, two post-harvest samples were taken at 7 and 14 days after harvest from the eight vine batches to determine the extent of ripening recovery in the more delayed cultivars.

Musts obtained from ripening curve samples were analyzed immediately for TSS using a temperature-compensated desk refractometer, whereas pH and titratable acidity (TA) were measured by titration with 0.1 N NaOH to a pH 8.2 endpoint and expressed as g/L of tartaric acid equivalents. To assess tartaric and malic acid concentrations, an aliquot of the must was diluted four times, then filtered through a 0.22 µm polypropylene syringe for high-performance liquid chromatography (HPLC) analysis and transferred to autosampler vials. All solvents were of HPLC grade. The chromatographic method was developed using an Agilent 1260 Infinity Quaternary LC (Agilent Technology, Santa Clara, CA, US) consisting of a G1311B/C quaternary pump with an inline degassing unit, a G1329B autosampler, a G1330B thermostat, a G1316B thermostatic column compartment, and a G4212B diode array detector (DAD) fitted with a 10 mm path and a 1  $\mu$ L volume Max-Light cartridge flow cell. An Allure Organic Acid column,  $300 \times 4.6$  mm and 5  $\mu$ m (Restek, Bellefonte, PA, USA) maintained at 30  $\pm$  0.1 °C, was used. Separation was performed in isocratic conditions using water, pH-adjusted to 2.5 using orthophosphoric acid, at a flow rate of 0.8 mL/min; 15  $\mu$ L of the sample was injected. The elution was monitored at 200–700 nm and detected by UV-vis absorption with a DAD at 210 nm. Organic acids were identified using authentic standards, and quantification was based on peak areas and

performed by external calibration with standards. Retention times (min) of citric, tartaric, and malic acid were 9.3, 10.0, and 11.1, respectively.

Each season, harvest was performed on the same date for all varieties (September 16, 13, and 20 in 2021, 2022, and 2023, respectively), having as a decision-making criterion that the reference Croatina had reached the minimum threshold of 22 Brix. At harvest, test vines were individually picked, the mass of clusters was weighed, and the total cluster number per vine was counted along with the shoot (cane) number. To better assess the impact of the varietal on basal node fruitfulness, in 2021 and 2022, yield per vine produced from the first count nodes on the fruiting cane was kept separate from the yield cropped at nodes >5.

Concurrently, three representative clusters per vine—usually inserted on basal, median, and apical cane portions—were taken to the laboratory for further processing. From each of the three clusters, a 50-berry subsample was taken by carefully cutting each berry at the pedicel with small sharp scissors and stored at -20 °C for phenolic determination according to [35]. In more detail, when still frozen, the berries were homogenized at 10,000 rpm with the Ultra-Turrax T25 (Rose Scientific, Edmonton, AB, Canada) homogenizer for 1 min; then, 2 g of the homogenate was transferred to a pre-tared centrifuge tube, enriched with 10 mL aqueous ethanol (50%, pH 5.0), capped and mixed periodically for 1 h before centrifugation at 959 g for 5 min. A portion of the extract (0.5 mL) was added to 10 mL of 1 mol/L HCl, mixed, and allowed to stand for 3 h; the absorbance was then measured at 520 and 280 nm on a Jasco V-530 UV spectrophotometer (Jasco Analytical Instruments, Easton, MD, USA). The concentration of total anthocyanins and phenolic substances was expressed as mg per g of berry fresh mass. The remainder of each cluster sample was crushed, and the resulting musts were analyzed for technological maturity parameters according to the methodology described above.

## 2.3. Chemicals and Reagents

The methanol, ethanol, acetonitrile, dichloromethane, sulphuric acid, hydrochloric acid, gallic acid, catechin, vanillin, Folin–Ciocalteu reagent, ethyl acetate, ethyl butyrate, ethyl isobutyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl isovalerate, ethyl lactate, ethyl pyruvate, ethyl succinate, ethyl propionate, butyl acetate, isoamyl acetate, methyl hexanoate, methyl octanoate, diethyl malate, diethyl succinate, 2-phenylethyl acetate, isoamyl lactate, diethyl malate, propyl acetate, *cis*-3-hexen-1-ol, 1-hexanol, 3-ethyl butanol, 1-pentanol, 2-octanol, isobutyl alcohol, isopentyl alcohol, hexyl alcohol, phenyl ethyl alcohol, methionol, benzyl alcohol, isohexyl alcohol, acetaldehyde, isobutyric acid, 2-methylbutanoic acid, butanoic acid, pentanoic acid, hexanoic acid, octanoic acid, and decanoic acid standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), and the chemicals were all at least of analytical grade. HPLC-grade water was obtained using a Milli-Q system (Millipore Filter Corp., Bedford, MA, USA).

#### 2.4. Data Analysis

Vine performance data were subjected to a two-way ANOVA using the SigmaStat 10.0 software package (Systat Software, San Jose, CA, USA). The homogeneity of error variances for the data taken on the same individuals over different years was assessed using Bartlett's test. The year was considered as a random variable, and the error term for the treatment factor was the year × treatment interaction mean square. Because variances were in all cases homogeneous, the year × treatment effects were tested using the pooled error mean square as an error term. Treatment comparison was performed using the Student–Neuman–Keuls test at  $p \leq 0.05$ . Year × treatment interaction was partitioned only when the F test was significant.

Repeated measures of the same parameters (TSS and TA) taken at different dates on the same individuals along the study seasons and days were analyzed with the repeated measures ANOVA routine embedded in the XLSTAT 2022.1 software package (Addinsoft, New York, NY, USA).

# 3. Results

The 2022 season stood out as an exceptionally hot and mostly dry season, with the GDD exceeding the 2400 °C threshold and total rainfall not reaching 400 mm (Figure 2). On the other hand, 2021 was cooler overall, with more abundant precipitation over the April–October period (596 mm), whereas 2022 behaved like an intermediate season. Notably, total rainfall recorded in 2021 and 2023 was still below the average referred to in the historical series from 1990 to 2020, setting at 720 mm.



**Figure 2.** Winkler Index given as cumulated growing degree days (GDD) from 1 April and 31 October, and cumulated monthly precipitation (P) recorded for each of the trial seasons by a weather station placed in close proximity to the vineyard.

While the correctness of the uniform baseline imposed at winter pruning in all varieties (about 9–10 nodes/vine) was confirmed by no significant variation in the total shoot number per vine (Table 1), Croatina did not fail to meet expectations of a mean node fruitfulness (0.81 clusters/node), which was always lower than values recorded in the remaining varietals (>1.33 clusters/node). When referring to the basal cane portion (nodes 1–5), Croatina had again the lowest cumulated fertility (2.5 clusters, to correspond to 0.5 shoots/node only), whereas the same calculation performed for the distal cane portion still led to lower fruitfulness in Croatina, which, however, was different when compared to Uva Rara and Croà only. Interestingly, in all autochthonous varieties, node fruitfulness was quite uniform over proximal and distal cane segments, and, when specifically referred to the first five count nodes, it varied from 1.1 clusters/node in Moradella to 1.5 clusters/node in Ughetta.

The overall picture just drawn for node fruitfulness and its components (proximal vs. distal) is not entirely mirrored by the yield response and the equivalent components. While Croatina still shows, on a 3-year basis, lower total yield per vine when compared to Moradella, Mornasca, and Croà, the same variable does not differ against Uva Rara and Ughetta (Table 1). The yield component allowing Croatina to compensate for its lower node fruitfulness was mean cluster weight, which exceeded 350 g versus about 200 g, which was measured only in Uva rara and Ughetta, whereas berry weight had no significant role.

**Table 1.** Effects of genotype and year on yield components over the three-year study (2021–2023). Data related to shoots/vine, clusters/vine-proximal, clusters/vine distal, yield/vine proximal, and yield/vine distal were not taken in 2023. This explains why, once added, the two-year-based clusters/vine-proximal and clusters/vine-distal do not exactly add to the three-year-based clusters/vine. The same explanation applies to yield per vine and its two components.

	Shoots/Vine	Clusters/ Vine	Clusters/Vine- Proximal	Clusters/Vine -Distal	Clusters/ Node	Cluster Weight (g)	Berry Weight (g)	Yield/Vine (kg)	Yield/Vine- Proximal (kg)	Yield/Vine- Distal (kg)
Genotype (G)										
Croatina	9.86	8.4 a	2.5 a	3.9 a	0.81 a	359 b	1.59 a	3.00 a	0.74 a	1.49 ab
Uva rara	8.86	14.0 b	6.6 b	7.4 b	1.49 b	207 a	2.03 b	2.89 a	1.39 ab	1.56 ab
Ughetta	10.29	14.4 b	7.7 b	6.7 ab	1.46 b	202 a	1.62 a	2.92 a	1.22 ab	1.16 a
Moradella	9.14	13.2 b	5.6 b	6.3 ab	1.41 b	317 b	2.28 b	4.17 b	1.57 ab	1.64 ab
Mornasca	8.86	13.3 b	5.7 b	6.7 ab	1.33 b	368 b	3.00 c	4.90 b	2.03 bc	2.26 b
Croà	10.43	16.9 b	7.0 b	8.3 b	1.64 b	343 b	2.17 b	5.79 c	2.36 c	2.40 b
F-prob	0.83 ns	8.19 **	6.88 **	4.10 *	13.01 **	11.82 **	20.36 **	16.80 **	8.29 **	5.19 **
Year (Y)										
2021	8.39 a	11.4 a	5.97	6.81	1.70 b	399 c	1.98	4.56 b	2.25 b	2.31 b
2022	10.46 b	11.9 a	5.58	6.38	1.16 a	198 a	2.26	2.36 a	1.03 a	1.32 a
2023	-	15.5 b	-	-	1.29 a	326 b	2.06	5.07 b	-	-
F-prob	9.89 **	34.20 **	0.48 ns	0.49 ns	20.06 **	37.21 **	2.98 ns	48.07 **	44.54 **	28.50 **
$\begin{array}{c} F\text{-}prop\\ G \times Y \text{ interaction} \end{array}$	0.67 ns	1.87 ns	0.29 ns	1.31 ns	1.95 ns	3.70 **	1.70 ns	3.22 **	4.71 **	2.09 ns

In the case of significance of the F test, within column mean separation indicated by lowercase letters was performed by SNK test. \* *p* < 0.05, \*\* *p* < 0.01, ns = not significant.

However, yield per vine and two of its components—cluster weight and yield referred to the proximal cane section—also showed a significant year × treatment interaction, which is partitioned in Figure 3. As per yield/vine (Figure 3A), the significant interaction essentially bursts from the overall low yield recorded at harvest in the hot and relatively dry 2022 and from a differential behavior observed in Croatina versus Uva Rara and Ughetta in each trial season. The same interaction analyzed for the two seasons available in terms of yield referring to the proximal cane portion (Figure 3B) disclosed that in the high-yielding 2021, differences in crop per vine between Croatina and others were amplified, whereas the same interaction was much smaller in magnitude in the low-yielding 2022. Cluster weight was the variable showing the highest variability across seasons, with 2021 scoring record cluster weights in Croatina and Mornasca (about 600 and 550 g, respectively), with a general drop in 2020 of around 150–220 g and a new resumption of cluster weight in 2023 (Figure 3C).



**Figure 3.** Partitioning of the significant year  $\times$  treatment interactions recorded for yield per vine (**A**), yield per vine referred to the proximal cane portion (nodes 1–5) (**B**), and cluster weight (**C**). Color codes are reported in one of the panel insets. Data are means for each year  $\times$  treatment combination (*n* = 4), and vertical bars are standard errors (SE). In (**B**), data are available only for 2022 and 2023.

Figure 4A–C shows the seasonal trend of berry fresh mass (A), TSS (B), and TA (C) recorded in 2022 over the post-veraison harvest period. With the sugaring process being greatly enhanced by the warm season (Figure 4B), harvest was performed on September 13 when Croatina touched the 24 Brix; yet it was followed for two more weeks (until DOY 270) to check recovery capacity by the more delayed cultivars (namely Moradella

and Mornasca). In terms of berry mass trends (Figure 4A), the smaller berries already recorded in Ughetta and Croatina upon first sampling (0.888 and 0.851 g, respectively) were maintained throughout the season, whereas Moradella, Mornasca, and Croà had an overall opposite behavior. Variability in berry size recorded over the two post-harvest sampling dates might also be due to some dehydration favored by climate conditions. Seasonal TSS evolution in 2022 marked significant TSS differences among cultivars at each sampling date. However, although Moradella and Mornasca started with the highest TSS at the onset of veraison (Figure 4B), their TSS at harvest was at least 2 Brix less than Croatina. Conversely, Croà and Uva rara stood out as quite effective sugar accumulator genotypes. Seasonal TA trend (Figure 4C) also showed large differences among cultivars; interestingly, although Moradella and Mornasca started with the lowest pool of acids (40.2 and 38.8 g/L, respectively), both retained, at harvest (DOY 256), higher TA than the reference Croatina. At the end of the two monitored extra weeks, Moradella still had 6.8 g/L, and Mornasca was set at 4.5 g/L.



**Figure 4.** Time trends of berry mass (**A**), total soluble solids (TSS as Brix) (**B**), and titratable acidity (TA as g/L) (**C**) recorded in 2022 on each cultivar from veraison until post-harvest. In each panel,

an arrow indicates the date of harvest. Data were subjected to Repeated Measure analysis with the XL-STAT package under the following outcomes: berry mass, TSS, and TA had highly significant between-subjects (cultivars) and time × cultivar effects with F = 74.38 (p < 0.0001 and F = 13.10 (p < 0.0001), respectively, for (**A**); F = 16.80 (p < 0.0001) and F = 12.41 (p < 0.0001), respectively, for (**B**); F = 169.44 (p < 0.0001) and F = 33.96 (p < 0.0001), respectively, for (**C**). Within each panel and date, vertical bars indicate standard error (SE), whereas \* implies significant differences among cultivars within a single date according to mean separation carried with the SNK test at p < 0.05. For clarity, lettering related to mean separation is reported for the harvest date only.

The wetter and cooler 2023 was conducive to a slower ripening, yet differences among cultivars were still very consistent (Figure 5A–C). Despite seasonal sampling starting with some delay versus the previous year (DOY 220 vs. DOY 199), fresh berry mass nicely confirmed that Ughetta and Croatina had initial lower berry size when compared to the other genotypes (Figure 5A), and the same relative differences were maintained until harvest (DOY 263). It seems especially relevant that the ranking of decreasing final berry size (i.e., Croà > Mornasca > Uva rara > Moradella > Croatina > Ughetta) was maintained over the two seasons, with the only exception being Croatina and Ughetta switching their positions.



**Figure 5.** Time trends of berry mass (**A**), TSS (as Brix) (**B**), and titratable acidity (TA as g/L) (**C**) were recorded in 2023 on each cultivar from post-veraison until harvest. In each panel, an arrow indicates the date of harvest. Data were subjected to Repeated Measure analysis with the XL-STAT package

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under the following outcomes: berry mass, TSS, and TA had highly significant between-subjects (cultivars) and time × cultivar effects with F = 67.70 (p < 0.0001) and F = 11.29 (p < 0.0001), respectively, for (**A**); F = 102.16 (p < 0.0001) and F = 17.24 (p < 0.0001), respectively, for (**B**); F = 50.27 (p < 0.0001) and F = 84.52 (p < 0.0001), respectively, for (**C**). Within each panel and date, vertical bars indicate standard error (SE), whereas \* implies significant differences among cultivars within a single date according to mean separation carried with the SNK test at p < 0.05. For clarity, lettering related to mean separation is reported for the harvest date only.

To mimic 2022 data, Mornasca and Moradella had significantly higher initial TSS than Croatina (around 10 Brix vs. 7.7 Brix in Croatina), whereas at harvest (DOY 263), Croatina slightly failed the ripening threshold of 22 Brix, likely due to the quite wet and cool season; Mornasca was confirmed to be a slow ripening genotype. As per TA trend (Figure 5C), 2023 started with Croatina retaining the highest pool (36.8 g/L) and Mornasca the lowest (23.9 g/L); nevertheless, Croatina progressively lost this advantage over the remainder of the season, and at harvest, these two cultivars did not differ.

Grape composition at harvest (Table 2) showed quite ample variability among cultivars for any measured variable, and, notably, a significant genotype × year interaction was found for TSS, TA, and tartaric and malic acid concentrations (Figure 6). TSS assessed as a main effect pooled over seasons showed two sub-groups—Croatina, Uva Rara, and Ughetta averaging around the 22 Brix target—and Moradella, Mornasca, and Croà showed a consistent delay in sugar accumulation. However, the same two-group pattern was overall reflected by only malic acid concentration, whereas all remaining variables resulted in a more heterogeneous response among cultivars. Indeed, the total anthocyanin concentration measured at harvest had high discriminant values, placing Croatina and Mornasca as the best ranking and Croà as a truly poorly colored variety (Table 2).

**Table 2.** Effects of genotype and year on must composition recorded at harvest in the three-year study (2021–2023).

	TSS (°Brix)	TA (g/L)	pН	Tartaric Acid (g/L)	Malic Acid (g/L)	Total Anthocyanins (mg/g)	Total Phenols (mg/g)
Genotype (G)							
Croatina	22.64 b	6.95 a	3.37 b	8.94 b	1.63 a	1.147 cd	2.931 b
Uva rara	22.77 b	7.17 a	3.41 b	9.14 b	1.80 ab	0.953 c	2.400 b
Ughetta	21.94 b	9.69 c	3.26 a	9.94 c	2.34 cd	0.640 b	1.940 a
Moradella	18.72 a	8.74 b	3.33 bc	8.76 b	2.70 d	0.538 b	1.603 a
Mornasca	18.68 a	6.78 a	3.34 bc	7.18 a	2.12 bc	1.257 d	2.821 b
Croà	19.75 a	8.97 b	3.24 a	8.48 b	2.71 d	0.334 a	2.931 b
F-prob	15.62 **	30.39 **	6.93 **	19.08 **	10.47 **	25.90 **	38.77 **
Year (Y)							
2021	19.06 a	8.85 c	3.30 b	10.05 c	2.71 c	0.913 b	2.407
2022	23.65 b	7.16 a	3.49 c	7.76 a	2.42 b	0.791 ab	2.128
2023	19.11 a	8.34 b	3.18 a	8.73 b	1.63 a	0.698 a	1.786
F-prob	61.97 **	28.63 **	99.87 **	51.90 **	35.42 **	3.39 *	9.47 **
<i>F-prob</i> $G \times Y$ interaction	3.72 **	8.93 **	1.58 ns	4.83 **	6.89 **	1.56 ns	2.47 ns

In the case of significance of the F test, within column mean separation indicated by lowercase letters was performed by SNK test. \* = p < 0.05, \*\* p < 0.01, ns = not significant.

Partitioning of the genotype × year interaction for TSS (Figure 6A) unraveled that the nature of the interaction was originated by a differential behavior of each cultivar across quite different seasons, with 2022, namely, being fairly hot and dry. In fact, inter-cultivar variability for TSS diminished in 2022 and amplified in 2021 and 2023. The most responsive cultivar was Croà, whose ripening speed greatly varied over the years (i.e., in 2022, Croà had a slightly higher TSS than the reference Croatina), whereas the sugaring process of this variety was significantly delayed in the other seasons. A similar ripening pattern across the

years was also shown by Mornasca, whereas Croatina, Uva Rara, and Ughetta were the most stable over time, always setting above 20 Brix.

TA interaction (Figure 6B) showed an overall mild reactivity of final TA levels of Croatina and Mornasca to the remarkably different yearly climate trends. Croà was the only cultivar to retain high TA (9.7 g/L) in the hot 2022, whereas Moradella and Ughetta proved to be extraordinarily responsive to the year effect. Tartaric acid concentration at harvest prompted that while Mornasca was almost a no responder in terms of year-to-year variability, all remaining varieties drastically reduced tartaric acid concentration in the hot 2022, especially compared to the cooler and wetter 2021 (Figure 6C). Finally, absolute values of malic acid retained at harvest in each cultivar varied less than expected with the different climate years (Figure 6D). In fact, even in 2022, malic acid content at harvest was more than adequate for the envisaged winemaking; Croatina only, in 2023, lowered malic acid concentration below the 1 g/L threshold.



**Figure 6.** Partitioning of the significant year x treatment interactions recorded at harvest for TSS (**A**), TA (**B**), tartaric acid (**C**), and malic acid (**D**). Color codes are reported in one of the panel insets. Data are means for each year x treatment combination (n = 4), and vertical bars are standard errors (SE). In (**B**), data are available only for 2022 and 2023.

# 4. Discussion

Yearly weather trends recorded over the 3 years of the trial were somewhat ideal for assessing the adaptability of the compared genotypes. In fact, the hot and dry 2022 alternated with the cooler and wetter 2021, whereas 2023 was overall warm yet with more abundant rainfall than 2022 over the growing period.

The first item requiring discussion relates to the yield potential of the tested genotypes. The reference and most grown Croatina cultivar is well known for its low fruitfulness of the basal nodes, which was confirmed timely in our study, as only 0.74 kg of grapes were obtained over the first five basal nodes (Table 1). This behavior explains why Croatina is usually cane pruned, which, besides increasing winter pruning costs and hindering other mechanical operations [36], sometimes also leads to a marked biennial bearing pattern [37]. Although older work has shown that Croatina can successfully adapt to a short mechanical pruning followed by a certain degree of hand finishing, which is able to compensate for the low fruitfulness with an increased shoot number [38], the technique has not been very successful mostly for the distrust of growers versus a non-selective pruning approach.

Another physiologically viable solution could be to prolong the cane length of a given vine until the apical cane portion overlaps with the basal cane portion of the subsequent vine, thus solving the issue of low basal productivity. Drawbacks of this technique are that it is not always easy to find a mature cane of suitable length and, above all, the basal cane portion "de facto" has two overlapping canes, increasing the requirement for shoot thinning, which, moreover, is bound to carefully select the fruitful ones while removing the others.

The significant year  $\times$  genotype interaction found for total yield/vine also points out that Croatina is quite prone to a biennial bearing pattern, which, rather than depending on a significant variation in the basal cane fruitfulness (Figure 3B), is the result of large changes in the cluster weight component (Figure 3C).

In all varieties other than Croatina, mean node fruitfulness was significantly increased primarily because clusters/vines recorded in the proximal cane portions also increased. Overall, within a wide range of yield per vine (2.89–5.59 kg), each tested autochthonous cultivar is suited to a spur pruning, which, however, should not exceed the three count nodes/spur to avoid undeveloped basal buds [39].

If a range of minor cultivars warrants good productivity under short pruning, then the final grape quality becomes the main discriminant in finding a possible alternative to Croatina. Croatina is described as a medium-to-late ripening variety with a seasonal heat requirement comprising 1800–2000 GDD [37]. The final grape composition should target an alcohol content of 12–13° vol., pH = 3.2–3.4, and total acids of 5.0–7.0 g/L. Croatina is quite often used in blends, the most known being the Gutturnio wine, where color and relatively low acidity brought by Croatina mix nicely with the usually lightly colored and acidic Barbera grapes. If the above optimal intervals are taken into account, Croatina's harvest performed on September 16, 13, and 20 in 2021, 2022, and 2023, respectively, meet the expected standards. However, when cumulated GDD were calculated until these harvest dates, outcomes were of 1882, 2145, and 1941 for 2021, 2022, and 2023, respectively. Therefore, the dry 2022 season was conducive to a somewhat quite early harvest (10 September), and, nevertheless, the GDD that accumulated until that specific day was largely in excess of the upper optimal limit of 2000 GDD.

However, the core of the discussion is on (i) how Croatina behaves in terms of technological and phenolic maturity over the years, greatly differing in weather trends, and (ii) whether there is any viable alternative within the range of the observed local cultivars. Although total anthocyanins at harvest did not display a significant year  $\times$  genotypes interaction (Table 2), partitioning of the interaction (Figure S1) and comparison with Figure 6A leads to an interesting and enlightening outcome. When the two "opposite" years, 2021 (cool and wet) and 2022 (hot and dry), are compared, Croatina had adequate TSS and outstanding color in 2021, whereas in 2022, it was exactly the opposite with high TSS and overall, insufficient color (slightly <1 mg/g) if the final grape destination is considered. While 2023 was somewhat intermediate under this respect, it is rather confirmed that, in Croatina, decoupling between sugar and color accumulation is aggravated in hot years with a great acceleration in sugar accumulation and a delay in color formation. The nature and mechanism of such enhanced decoupling under changing temperatures have been the object of several publications [40–45]. A finding shared by all the studies cited above is that positive thermal anomalies (e.g., a period that is, on average, warmer than a previous historical series) have contributed, with or without a concurrent water deficit, to widening the decoupling between technological and phenolic maturity. Another factor that contributes to increased asynchrony between sugar and color accumulation in grapevine berries is that while a temperature rise might have rather controversial effects on sugar accumulation (fostering or inhibiting the process under the always possible interference of an artefactual sugar concentration due to unmet berry water loss), the impact of high temperatures on anthocyanin synthesis and accumulation is generally negative [46-48]. Another puzzle associated with ripening dynamics is whether the frequently observed higher sugar accumulation rates—which, in turn, lead to early harvests—are due to an advancement in the

onset of veraison or rather due to a faster solute accumulation without any significant shift in the date of veraison. A study conducted in Australia [49] provided evidence that the former hypothesis holds, claiming that under global warming, the speed of ripening (e.g., Brix/day) did not significantly increase. Although a number of modified cultural practices have been devised and tested to de-synchronize sugar and color (i.e., slowing down the former while not hindering the latter) [25,26,50], another possible solution is a varietal change or, if allowed, using a given fraction of grapes to blend with the standard cultivar.

Based on the above and within the array of cultivars tested in our three-year study, attention must be paid to genotypes able to reunite a slow sugaring process and, concurrently, an impaired color accumulation process. Looking at the 3-year means reported in Table 2 for final grape composition, while Uva Rara is already known for having quite similar behavior to Croatina in terms of final grape composition, cultivars Ughetta, Moradella, and Croà show a quite clear inefficiency in terms of color accumulation, rendering unrealistic a hypothesis for Croatina replacement.

On the other hand, Mornasca has a very interesting and promising outlook. While showing acceptable node fruitfulness regardless of position along the spur or cane (Table 1), its features of high productivity (4.9 kg/vine correspond to a remarkable 19.6 t/ha) and large berries, in theory, might not be the best combination to warrant good berry pigmentation. Instead, Mornasca was able to associate a slow sugar accumulation while total anthocyanins concentration at harvest was even higher (1.257 mg/g), albeit not significantly higher than the Croatina amount (Table 2). Looking at the ripening curves available for years 2022 and 2023, it is also remarkable that the lower TSS recorded at harvest in Mornasca does not link with an already delayed sugar pool at the beginning of sampling (Figures 4 and 5). As a matter of fact, at the beginning of sampling, Mornasca had a fairly high TSS value, and clearly, the sugar limitation manifested later in the season. Also, in 2022, when an early and dry season allowed the performance of post-harvest sampling to assess recovery performance, Mornasca had a consistent TSS increase, which, instead, was not registered in Moradella.

The peculiar behavior of Mornasca emerges if, as already shown for Croatina, its TSS and color values at harvest are compared for the quite different 2021 and 2022 seasons. In the fairly cool 2021, with Croatina perfectly targeting both TSS and color thresholds, Mornasca had a somewhat decent color (1.07 mg/g), but grapes were just unripe (TSS =  $16.3 \degree$ Brix). However, since the limiting factor was sugar and the growing seasons have significantly increased duration due to global warming, a delayed Mornasca harvest could have been, indeed, a possibility. In 2022, when the dry season forced Croatina to be harvested early in the season (10 September) at high TSS (24.1 Brix) and overall modest anthocyanins concentration (0.98 mg/g), Mornasca showed outstanding behavior, obtaining ideal values of both parameters. Mornasca's behavior in 2023 overall partially mirrored that observed in 2021, although the too-low TSS at harvest (17.2) was paralleled by a more than adequate color content (1.3 mg/g) to reconfirm that the option of further harvest postponement is feasible and solid.

# 5. Conclusions

The hypothesis made in this three-year study was that germplasm available for local red cultivars, which might represent an alternative to the widely grown Croatina genotype, might represent a tool to either increase vineyard efficiency and profitability while concurrently offering solutions to adapt to climate change challenges. Data were consistent enough to hold that, as far as GDD accumulated from budburst and harvest are falling within the 1800–2000 °C range, the final grape composition of Croatina grapes meets the targets. Conversely, despite an anticipated harvest, in the hot 2022, Croatina showed a too-high TSS/color ratio (24.6), with color accumulation consistently lagging behind sugar accumulation.

Within the above scenario, Mornasca has proved to be a viable alternative to Croatina for at least three orders of reasons: (i) good basal node fruitfulness, with new perspectives

in terms of a shift toward spur pruning; (ii) in cool or average seasons (2021 and 2023, respectively), Mornasca confirms a slow pace in sugar accumulation, which however, seems to be strongly decoupled from the total anthocyanins accumulation which is anything but limited. Consequently, harvest could be consistently postponed by exploiting a normally long growing season to reach the minimum TSS target; (iii) in the hot and dry 2022, Mornasca had an ideal behavior at ripening as TSS was adequate (yet not too high) and color was definitely abundant. It can, therefore, be concluded that Mornasca, despite its high-yielding attitude and large berries, is able to regulate sugar and maintain more than adequate berry coloration in a hot season when Croatina is showing apparent limitations.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10060658/s1, Figure S1: Means of year  $\times$  treatment combinations recorded at harvest for total anthocyanins. Color codes are reported in one of the panel insets. Data are means for each year  $\times$  treatment combination (n = 4), and vertical bars are standard errors (SE).

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