THE ROLE OF 'M SERIES' ROOTSTOCKS IN THE ADAPTATION OF SPARKLING WINE VITICULTURE IN FRANCIACORTA WINE REGION TO CLIMATE CHANGE (NORTH ITALY)

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"Trees can be likened to men turned upside down,
with their heads stuck in the ground and their feet in the air".

Democritus, Abdera: 460-370 B.C.

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It was slightly changed to adapt its structure to the one requested for a dissertation, trying to avoid the loss of the main aim.

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Abstract

New adaptation strategies and techniques are needed to increase resilience of vineyards to the effects of climate change. Currently, most of the vineyards are grafted onto few century-old rootstocks, selected from a relatively small gene pool. The development of new biotypes can represent a turning point for modern viticulture. In fact, the rootstock choice from an enriched pool of genotypes, could represent a winning adaptive solution, positively influencing the behaviour of the scion by increasing its performance, considering the specific environmental context and oenological target.

The M series rootstocks, developed by the University of Milan in the eighties, when the first signs of climate change effects appeared and officially registered in 2014, were tested in different environments and with different grafting combinations.

This work focuses on the evaluation of rootstocks performances in combination with Chardonnay in the specific context of sparkling wine production in Franciacorta (Lombardy, Italy). The four M rootstocks (M1, M2, M3, M4) and the widely adopted SO4 and 1103P were analysed considering drought stress tolerance, vegetative growth (vigour), yield and berry quality. Microvinifications of grapes from each scion-rootstock combination were made in 2022 and 2023 and analysed and evaluated by a tasting panel.

The rootstock 1103P showed good tolerance to water stress condition and was characterized by late ripening and a balanced sugar and acidity profile. However, it did not perform well in terms of sensorial analysis of wine. SO4 demonstrated a very good tolerance to water stress and maintained a stable but average sugar/acidity ratio along the four seasons. The M4 revealed to be the most interesting of the M-series showing a good water stress tolerance, a high acidity-sugar ratio, concurrent with an earlier ripening that allows for a more balanced ripening in terms of aromas, as confirmed by the tasting panel scores.

Keywords: climate change, novel rootstock, M-series, viticulture, sustainability.

Resumo

Para aumentar a resiliência das vinhas face aos efeitos das alterações climáticas que ameaçam o setor vitícola, são necessárias novas estratégias e ferramentas de adaptação. Atualmente, a maioria das vinhas são enxertadas em porta-enxertos centenários, selecionados a partir de uma pequena "pool" genética. O desenvolvimento de novos biótipos pode representar um ponto de viragem para a viticultura moderna. De facto, a escolha de porta-enxertos a partir de um conjunto enriquecido de genótipos, pode representar uma estratégia de adaptação vencedora, influenciando positivamente o comportamento da variedade, aumentando o seu desempenho, considerando o contexto ambiental específico e os objectivos enológicos.

Os porta-enxertos da série M, desenvolvidos pela Universidade de Milão nos anos oitenta, aquando dos primeiros sinais dos efeitos das alterações climáticas, foram oficalmente registados em 2014, e foram testados em diferentes ambientes e com diferentes combinações de enxertia.

Neste trabalho é avaliado o desempenho de porta-enxertos em combinação com a variedade *Vitis vinifera* Chardonnay no contexto específico da produção de vinho espumante em Franciacorta (Lombardia, Itália). Os quatro porta-enxertos (M1, M2, M3, M4) e os amplamente adotados (SO4, 1103P) foram comparados considerando a tolerância à secura (stress hídrico), crescimento vegetativo (vigor) e produção. Foram também feitas microvinificações de cada combinação cv. Chardonnay-porta-enxerto em 2022 e 2023 que foram analisadas e avaliadas por um painel de provadores.

O porta enxerto 1103P apresentou boa tolerância ao stresse hídrico e caracterizou-se por maturação tardia e perfil equilibrado de açúcar e acidez. Todavia não apresentou bom desempenho em termos de análise sensorial do vinho. O SO4 demonstrou uma tolerância muito boa ao stress hídrico e manteve uma relação açúcar/acidez estável, mas média, ao longo das quatro estações. O M4 revelou-se o mais interessante da série M apresentando uma boa tolerância ao stress hídrico, e resultando numa elevada relação de acidez-açúcar, resultante de um amadurecimento mais precoce o qual permite uma maior equilíbrio aromático, como confirmado pelas pontuações do painel de degustação.

Palavras-chave: alterações climáticas, novos porta-enxertos, série M, viticultura, sustentabilidade.

Extended Summary (Portuguese)

Para aumentar a resiliência das vinhas aos efeitos das alterações climáticas, que ameaçam o equilíbrio do setor vitivinícola, são necessárias novas estratégias de adaptação a aplicar na vinha. O aumento das temperaturas (do ar e do solo) determinam uma antecipação das fases fenológicas da vinha com particular atenção ao abrolhamento e maturação com as respetivas consequências de maior risco de danos causados por geadas tardias e modificação da composição dos bagos. A maior produção de açúcares, a diminuição da acidez e o rápido aumento do pH, conjugados com a redução da complexidade aromática provocada por um fenómeno de dissociação entre maturação tecnológica e aromática, colocam em risco a quantidade e a qualidade das principais zonas vitícolas. Além disso, a maior frequência de ondas de calor e períodos de seca mais longos determinam fenómenos de stress hídrico moderado e severo com consequentes problemas de desidratação dos cachos, diminuição da taxa fotossintética e reduções na produção, bem como a longevidade das vinhas e que representam o maior desafio deste século. Numerosos estudos demonstraram formas de se intervir no terreno para neutralizar eficazmente estes danos. Estas técnicas de adaptação dividem-se em estratégias a curto prazo (por ex, gestão da sebe , rega , uso de protetores), a médio prazo (por ex. sistema de condução , poda mínima, uso de redes de sombreamento) e estratégias a longo prazo (por ex. orientação das linhas, escolha do porta-enxertos, alteração da exposição e altitude). Uma nova abordagem à escolha dos porta-enxertos poderia ser uma solução vencedora para o futuro da viticultura. De fato, o uso de porta enxertos representa um exemplo efetivo de controle biológico contra a filoxera e também uma importante ferramenta agronómica, capaz de influenciar fortemente o comportamento das variededades que acontece, de forma consolidada, em outros setores da fruticultura. Atualmente, a maioria das vinhas são enxertadas em porta-enxertos estabelecidos há mais de um século a partir de um conjunto genético relativamente pequeno. Dos porta-enxertos disponíveis cerca de dez conseguem cobrir a maior parte da área de vinha cultivada a nível global. São exemplos os genótipos 779/1103P, SO4, K5BB, 110R, 420A e 140R. O desenvolvimento de novos biótipos, combinado com descobertas recentes no campo da genética, pode representar um ponto de viragem, uma vez que a viticultura moderna, enquanto tal, não pode depender de material genético antigo. Os porta-enxertos desenvolvidos pela Universidade de Milão (série M) na década de 80, aquando dos primeiros sinais de mudança climática, foram registrados oficialmente em 2014, e estão ainda em observação para se avaliar o seu desempenho em diferentes ambientes e com diferentes combinações de enxertos. Em particular, o seu uso e desempenho em combinação com a casta Chardonnay está a ser avaliado na região vinícola lombarda de Franciacorta (BS) para a produção de espumantes de base. Os testes são realizados numa vinha experimental e onde as respostas fisiológicas, agronómicas e enológicas da casta Chardonnay são analisadas em combinação com os porta-enxertos da série M: M1, M2, M3 e M4 e em comparação com videiras enxertadas em porta-enxertos comerciais mais utilizados na área: SO4 e 1103P. Durante o período de quatro anos em análise, houve anos (2022 e 2023) caracterizados por temperaturas

médias do ar acima da média e, no primeiro, uma forte componente de stress ligada à presença de inúmeras ondas de calor.

Os dados, recolhidos desde 2020, demonstram a competitividade dos novos porta-enxertos M, em termos de resistência às status de stress hídrico, equilíbrio vegetativo-produtivo, qualidade e desempenho produtivo, com mostos que apresentam bons níveis de acidez acompanhados de valores de açúcar e azoto suficientes para fins enológicos, com uma boa produção de uvas por videira e por sarmento. Estes porta-enxertos também são capazes de produzir vinhos interessantes do ponto de vista sensorial. Mesmo em condições de stress abiótico, os porta-enxertos da série M demonstraram a capacidade de adaptar, de forma diferenciada, o cultivo da vinha aos efeitos das alterações climáticas e promover a sustentabilidade da viticultura moderna com uma redução significativa da pegada hídrica. Exemplos são M3 e M4 que conseguiram manter uma temperatura foliar da sebe abaixo de plantas enxertadas no 1103P em condições de elevado stress hídrico. Além disso, o M4 foi considerado o melhor em termos de linearidade da tendência acidez-açúcar, garantindo altos níveis de acidez, embora não atrase a maturação como o 1103P. Embora nenhum porta-enxerto M tenha mostrado sinais de superioridade absoluta, com exceção do M4, que se revelou comparável ao 1103P e SO4, é evidente que os estudos e a recolha de informação tem de continuar nesta área, bem como em outras regiões vitícolas ameaçadas pelas alterações climáticas.

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1. Literature review

1.1. The main rootstock's roles in viticulture

For over a century, viticulture was characterized by the practice of grafting and the use of rootstocks which are the basis of the vitality of every production area except for small oases in which phylloxera (*Daktulosphaira vitifoliae*, Fitch 1856) did not proliferate. Grafting onto American based genotypes was the response of European viticulture at the end of the 1800s to this small aphid, coming from North America, which has almost ended centuries of history and winemaking tradition (Granett *et al*., 2001). In fact, the European vine *Vitis vinifera sativa* has a marked sensitivity to the insect that causes it to dieback in a short time due to severe root damage. The solution was found overseas, exploiting the resistance inherent in American vines against the insect given the co-evolutionary process through which they were subjected.

The main rootstocks produced were derived from a hybridization process between three main American species: *Vitis riparia*, *Vitis rupestris* and *Vitis berlandieri* (Figure 1). These three species show not only high resistance to phylloxera but also a marked intra and interspecific hybridization compatibility (Levadoux, 1956; Figure 4). Despite that, these species, if used in purity, are not able to fully satisfy to the multiple needs of European viticulture like high $CaCO_3$ tolerance, the crosses with *Vitis vinifera* proved capable to respond to the peculiarities and specific needs of the different viticultural areas (Palliotti *et al*., 2021). Indeed, about 90% of *Vitis vinifera* plants are currently grafted on a combination of the three American species (Keller *et al*., 2015; Serra *et al*., 2013) but other species were also used such as *Vitis vinifera*, *Vitis candicans* and *Vitis cordifolia* (Palliotti *et al*., 2021). It is also important to point out that very few varieties of those species are used (Scienza, 2021).

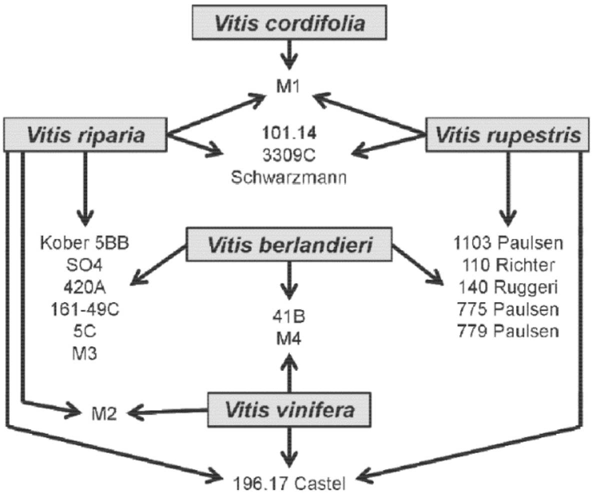


Figure 1: Most used grapevine rootstocks and their pedigree (Corso and Bonghi, 2014).

The first rootstocks were obtained in the late XIX and early XX centuries, including 1103P (1895), K5BB (1896), 101-14 and 41B (1882) or 420A (1887) (Maul *et al*., 2024). This first generation was followed by a second one, which, however, only minimally improved on the previous achievements. The high efficiency of the first rootstocks was decisive in the duration of their use as they showed to be highly protective against phylloxera but also able to tolerate the calcareous soils which is typical of the main European wine-growing areas. The success achieved meant that viticulture was able to reassert itself but, at the same time, research in this sector crystallized (Figure 2). In fact, it should be considered that generally a genetic improvement program for selection of a new rootstock hardly requires less than 20-25 years, with a consequent expense in economic and material terms (Falginella *et al.*, 2022).

Nowadays, the choices available to the viticulturists are limited to rootstocks that are over a hundred years old, which are beginning to show signs of limitations given the new challenges and problems that modern viticulture must go through. Old rootstocks are showing phenotypical instability leading to important yield variation between the years in function of climate. This limits a correct vineyard management, yield prediction and profits.

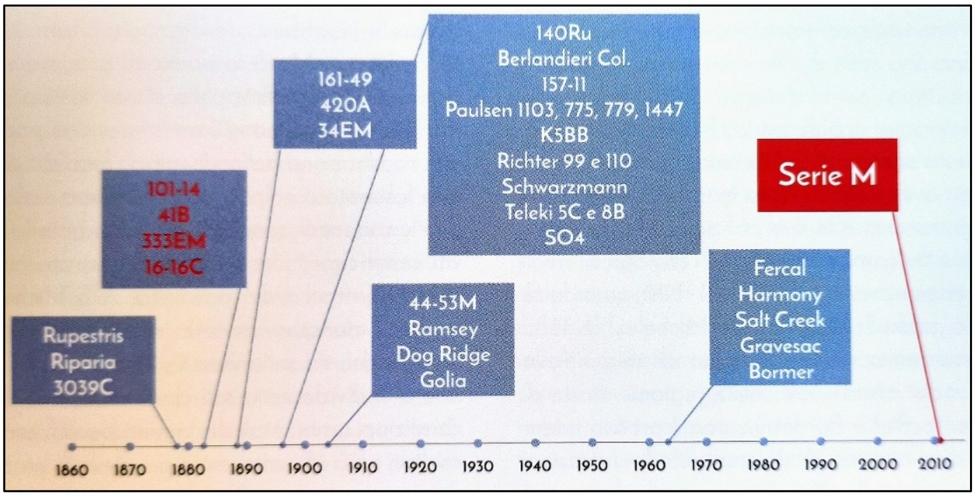


Figure 2: Chronology of the constitution of the main vine rootstocks from XIX century to the present day (Scienza, 2019).

Climate change is one of the major concerns as it determines intense variations in rainfall distributions accompanied by a rise in temperature, making water deficit phenomena increasingly common, especially in Mediterranean areas, influencing the normal physiological processes of the plant and the composition of the grapes (Costa *et al.*, 2023, 2016; Fraga *et al.*, 2012; Jones, 2006; Moutinho-Pereira *et al.*, 2004; Paranychianakis *et al.*, 2004; Hardie and Martin, 2000; Austin and Bondari, 1988; Hardie and Considine, 1976). These environmental conditions, coupled with the presence of calcareous or saline soils, are emphasized, leading to strong limitations for vine's development due to the difficulty of necessary nutrients absorption (Falginella *et al.*, 2022). This follows the role of the rootstock as a mediator between soil and vine, regulating and influencing scion's physiology in different ways. However, the choice of rootstock must always consider that there is not one perfect biotype which can be perfectly suited with terroirs specificities and the chosen grape variety.

Therefore, knowledge of the cultivated environment, the compatibility between the two bionts, the oenological objectives, the tolerance to biotic and abiotic stresses are fundamental parameters to be considered when planting a new vineyard. Among them, it is possible to recognize the importance of climate as one of the main drivers of rootstock behaviour. Climate change further emphasizes the importance of this factor in the decision (Leeuwen *et al.*, 2019).

The difficulties of forecasting future scenarios, added to the usual longevity of an average vineyard, accentuate the need for careful research in this area. As mentioned above, the rootstock can meet different needs. First, tolerance to root parasites such as phylloxera, rootworms and nematodes. The former, present in almost all vineyard areas, is easily controlled by the most common American rootstocks, although the presence of *Vitis vinifera* genes could make them particularly susceptible to aphid attack over time, such as the cases of 26G or AXR#1 (Palliotti *et al.*, 2021; Granett *et al.*, 1983). Nematodes are more common in sandy soils but are also widespread in other textures (Renčo *et al.*, 2020). Among the various damages that can be caused to the root system there is the transmission possibility of GFLV by *Xiphinema index* or TBRV by *Longidorus* spp. Rootstocks such as Teleki 5C, Riparia Gloire and Borner shown to be tolerant to *X. index* but unable to block the transmission of the related virus (Rahemi *et al.*, 2022; Palliotti *et al.*, 2021; Van Zyl *et al.*, 2012).

Rootstocks must adapt to the different environmental conditions of the different ranges to be fully functional in its role as a biological mediator. Not only it must adapt to the present soil and climate conditions, but it must also guarantee high quantitative-qualitative standards and production stability from year to year. The main stability indexes considered during rootstock characterization are "b", related to results stability across vintages (results goes from 0 to 2, b=1 represents a medium stability level), "Wi^2", which indicates stability though the environments, and the "coefficient of variation" (CV) that is related to measures dispersion. The lower those indexes are the higher would be the stability level (rootstock performance in this case) (Brancadoro and Failla, 2019).

The main soil characteristics considered when choosing a rootstock are the presence of active and total limestone, water shortage or stagnation, and the availability of mineral elements. Prior to the introduction of phylloxera, European vines, were cultivated on own-roots and were not affected by the presence of carbonates in the soil due to the marked resistance of *Vitis vinifera* to iron chlorosis (Palliotti *et al.*, 2021; Bavaresco *et al.*, 2003). American species, and therefore also their hybrids, sometimes without this character, induce symptoms of iron chlorosis (Figure 4), especially in the early phenological stages. Symptoms are recognizable by internerval discoloration of the apical leaves coupled by reduced growth and yields due to low photosynthetic levels (Brancadoro *et al.*, 1995, Marshner *et al.*, 1995; Bavaresco *et al.*, 1992). *Vitis vinifera* is in fact able to maintain a good iron supply even in the presence of limestone because it has a well-developed root system and leaf systems that are efficient in regulating metabolism. The absorption capacity of European grapevine is proportional to the emission of root exudates responsible for the acidification of the surrounding soil with the consequent reduction of iron and its absorption (Palliotti *et al.*, 2021; Marastoni *et al.*, 2020; Keller, 2015; Kobayashi *et al.*, 2012;). The active limestone content in the soil is therefore one of the main drivers of rootstock choice. Levels below 6% are not shown to be limiting, values around 16-20%

require medium-tolerant rootstocks such as M2, M3, M4, 110 Richter, Kober 5BB. Active limestone values above 20% require tolerant rootstocks such as M1, Fercal and 41B (Palliotti *et al.*, 2021; Falginella *et al.*, 2022). Another crucial factor to consider when choosing a rootstock is soil salinity (Figure 4). Salinity negatively affects the development of the vine as it affects its physiology. In particular, the presence of Cl^- and Na^+ induce negative changes in stomatal conductance, electron transport chain rate, leaf water potential, chlorophyll content, fluorescence, osmotic potential, and ionic concentration (Cramer *et al.*, 2007). Moreover, salinity promote synthesis of ROS, degradation of cell membranes, formation of toxic metabolites, difficulty in uptake of nutrients from the soil and activation of genes related to the production of plant hormones such as auxins and jasmonate (Ismail *et al.*, 2012; Cramer *et al.*, 2007). Different vine species respond differently to soil salinity depending on their ability to exclude, at the root level, Cl^-. In ascending order of salinity tolerance: *V. berlandieri*, *V. champini*, *V. cinerea* and *V. rupestris* (Corso and Bonghi, 2014). The rootstock has a strong influence on salinity tolerance and particular combinations can prove to be advantageous such as 1103 P, 101.14, Kober 5BB, 140 Ruggeri and M4 (Palliotti *et al.*, 2021; Meggio *et al.*, 2014; Walker *et al.*, 2002).

The Mediterranean viticultural regions have low and irregular rainfall patterns in the summer months and climate change is intensifying this trend, leading to the observation of extremely rainy and dry years (Costa et al., 2023; Essa *et al.*, 2023). In areas subject to high levels of water in the soil due to the local climatic trend or soil texture, rootstocks with *V. berlandieri* as parental are not recommended given the low tolerance to anoxia but also to attacks by root parasites such as *Armillaria mellea* (Palliotti *et al.*, 2021). In most Mediterranean areas, problems related to sever water deficit situations are causing problems to the vitality of vineyards. Despite the ability of the vine to tolerate situations of water scarcity through mechanisms such as the control of stomatal aperture, aquaporin activity and recovery of embolisms (Zufferey *et al.*, 2011; Lovisolo *et al.*, 2010, 2002), situations of prolonged severe water stress combined with high temperatures can cause potential damage to vineyards. Previous research shows that deficiency leads to a reduction of stomatal conductance (Iacono *et al.*, 1998), decrease in photosynthesis level (Dias and Brüggemann, 2010), lower canopy growth/expansion (Lovisolo *et al.* 2010, Cramer *et al.*, 2007), xylem cavitations episodes (Hochberg *et al.*, 2017; Lovisolo *et al.*, 2008; Alsina *et al.*, 2007) and yield reduction (Wenter *et al.*, 2018; Chaves and Oliveira, 2004).

Vine's evolutionary path contributed to the development of traits of resistance to water stress in a more marked way for some varieties, called anisohydric (slow stomata closure rate), rather than for others called isohydric (fast stomate closure rate).The consequences can be observed by the different physiological response in the presence of lack of water (Dal Santo *et al.*, 2016; Gerzon *et al.*, 2015; Chaves *et al.*, 2010; Vandeleur *et al.*, 2009). The rootstock is able to play a fundamental role in drought resistance/tolerance as it directly influences water absorption. Its activity is therefore influenced by two main factors: water availability and grafting compatibility (Palliotti *et al.*, 2021).

Although each rootstock has its own root morpho-anatomical and physiological characteristics such as geotropic angle (Figure 3), growth capacity, root pressure, suberification, microbiota, etc., the graft is able to regulate its development and expression (Vinks *et al*., 2021; Ferlito *et al*., 2020; Ibacache *et al*., 2020; Clingeleffer *et al*., 2019; Tandonnet *et al*., 2010). Geotropic angle was widely used in the past to characterize root-system drought tolerance and water uptake capacity. Little values are typical of *Vitis rupestris* as its great capacity to explore deep soil layers, the opposite for *Vitis riparia* (Palliotti *et al*., 2021). Rootstocks, as hybrids are influenced by pedigree traits (Zambon, 2022).

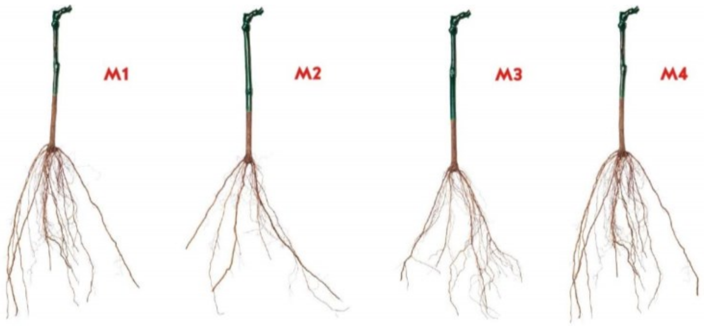


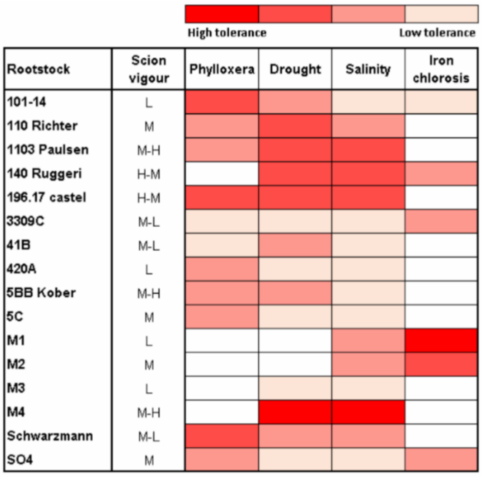
Figure 3: M rootstocks geotropic angle (Zambon, 2022).

In a context of climate change, with the consequent increase in duration and intensity of drought periods, the choice of rootstock could prove to be one of the most advantageous and economical to ensure a correct supply of water to the typical vines of the different terroirs (Figure 4). Among the rootstocks typically chosen for dry summer environments there are several examples: 110 Richter, 140 Ruggeri, 779/1103 Paulsen, M2 and M4 (Palliotti *et al*., 2021). Mineral nutrition is another crucial point in the choice of rootstock. The different ability to absorb and translocate nutrients such as K, Ca and Mg is typically evaluated wisely. In hot and dry environments, a disproportionate absorption of the above-mentioned nutrients in favour of potassium is observed, influencing berry composition (Kodur *et al*., 2011, 2011; Brancadoro *et al*., 1994;).

Rootstocks such as SO4 and 101.14 prefer K absorption over Ca and Mg. Rootstocks such as 1103P, 420A, 110 Richter have a medium-low absorption capacity, while M2 or Kober 5BB are able to absorb K in a balanced way (Palliotti *et al*., 2021). Regarding magnesium absorption, Livigni *et al*., (2019) observed that 1103P is able to tolerate Mg deficiency better than SO4. Just as the scion has an influence on the rootstock, the opposite statement is also true.

The rootstock is responsible for the absorption of water and minerals but also for the production of hormones which influence plant metabolism (Keller, 2020; Richard, 1983). The diversity of the environmental conditions in which the vine is grown forces the rootstock to adapt in such a way as to maintain the highest possible efficiency degree (Eshel and Beeckman, 2013; Smart *et al*., 2007; Nikolaou *et al*., 2000; Brancadoro *et al*., 1995). The degree of vigour induced on the scion by the rootstock (Figure 4) depends on the ability of the roots to provide the necessary resources (e.g. water, nutrients) for the metabolic processes of the scion, which responds by adjusting the growth rate and production load (Pou *et al.*, 2022; Kidman *et al.*, 2013; Cookson *et al.*, 2012; Tandonnet *et al.*, 2010, 2008; Paranychianakis *et al.*, 2004; Grant and Matthews, 1996; Tardáguila *et al.*, 1993). Previous studies showed a direct correlation between rootstock vigour and productive load as they occur, as analysed by Kidman *et al.*, (2013), a higher induced vigour results in a higher bud fertility. According to a general classification reported by Palliotti *et al.*, (2021), VCR (2023), Yin *et al.*, (2023) and Scienza (2019), rootstocks can be classified into three macro-classes of vigour. Vigorous rootstocks include Kober 5BB, 1103 Paulsen, 140 Ruggeri, M2; among those of medium vigour Fercal, 110 Richter, 779 Paulsen, M4; low vigour ones can be SO4, 101.14, 420 A, M1, M3. Some rootstocks, classified as anisohydric, are able to induce this trait in the grafted vine, thus making the plant less sensitive and more tolerant to drought (Prinsi *et al.*, 2021; Tramontini *et al.*, 2013).

In addition, the rootstock can also influence grapes maturation. An experiment conducted by Corso *et al.*, (2016) studies the different behaviour of Cabernet Sauvignon grafted onto 1103P and M4. The results show that the rootstock, in this case 1103P, is able to delay ripening, as confirmed by other studies (Gambetta *et al.*, 2012; Koundouras *et al.*, 2008). The hypothesis that rootstock also plays an important role in grape composition was confirmed by several authors (Li *et al.*, 2019; Nèmeth *et al.*, 2017; Nassur *et al.*, 2014; Pulko *et al.*, 2012) which underline how, in particular the phenolic profile, it can be strongly influenced by the chosen combination with direct repercussions on oenological quality, nutraceuticals and appreciation of the wine. As a matter of facts grapevine vigour, induced by rootstock activity, tends to delay ripening phase, veraison in particular, with a consequent stronger effect on secondary metabolites whose production is influenced more than the primary metabolites one. Possible explanations can be found in an indirect reason where it is assumed that, in a vigorous vine, clusters are more shaded, and a direct one that involves the role of auxins which, if produced and transferred in a lower amount, can delay verasion and ripening rate (Brancadoro and Failla, 2019; Corso *et al.*, 2016). Another important grape component influenced by rootstock is the YAN (Yeast Assimilable Nitrogen) content from which oenological quality depends on and which content is related to vine vigour (Brancadoro and Failla, 2019; Lee and Steenwerth, 2011).



█ ▓ ▒ ░

High tolerance … Low tolerance

Rootstock	Scion vigour	Phylloxera	Drought	Salinity	Iron chlorosis
101-14	L	▓	▒	░	░
110 Richter	M	▒	▓	▒	
1103 Paulsen	M-H	▒	▓	▓	
140 Ruggeri	H-M		▓	▓	▒
196.17 castel	H-M	▓	▓	▓	
3309C	M-L	░	░	░	▒
41B	M-L	░	▒	░	
420A	L	▒	░	░	
5BB Kober	M-H	▒	▒	░	
5C	M	▒	░	░	
M1	L			▒	█
M2	M			▒	▓
M3	L		░	░	
M4	M-H		█	█	
Schwarzmann	M-L	▓	▒	▒	
SO4	M	▒	░	░	▒

Figure 4: Grapevine rootstocks and their response to abiotic stresses. Low (L), medium (M) and high (H) rootstock vigour is reported. Degree of tolerance to phylloxera, drought, salinity, and iron chlorosis is present (Corso and Bonghi, 2014).

1.2. The M rootstock series

Since 1985 an intense breeding program was carried out at the University of Milan (DISAA) to develop new rootstocks from parents belonging to American species and, sometimes, *Vitis vinifera* or *Vitis cordifolia* (Figure 1). Simple (two parents) or complex rootstocks (more than two parents) were created. The main parental specie used was *Vitis berlandieri* whose drought and limestone tolerance genes were introduced with the backcross technique considering its rooting difficulties (Brancadoro, 2021). In 2014, starting from about 8000 seedlings, four rootstocks were officially registered: M1, M2, M3 and M4 (G.U. N° 127 4/06/14).

The varied characteristics of these four new rootstocks are particularly useful in the current context of climate change. In fact, they can be used in different terroirs and in different grafting combinations. In the last 5 years, different cultivar and M rootstocks combinations were studied along the Italian peninsula characterized by different environmental conditions. Results showed that rootstock explain variability for around 50% of grapevine vigour and production (effect of rootstock, rootstock*year, rootstock*variety) (Brancadoro, 2022). A study conducted by Bianchi *et al* in 2020 analysed the physiological and genetic response of M1, M3 and M4 in the context of increasing water deficit. The results seem to be encouraging in terms of resilience to climate change, as the behaviour of the M was comparable to the tolerant rootstocks 1103P and 110R widely used in viticulture nowadays. It was observed that at severe water deficit levels, rootstocks induced a reduction in stomatal conductance, following an increased transcription of genes related to ABA biosynthesis, and a decrease in stem water potential without showing significant decrease in photosynthetic activity. In the case of M4 it was observed that the reduction of stomatal conductance is not due to an up-regulation of genes related to ABA but to a down-regulation of those related to its catabolism (Corso *et al*., 2015). These results suggest an excellent predisposition of M rootstocks to tolerate moderate to severe water stress situations. In the work of Frioni *et al*., 2020 M4 was compared with 1103P in combination with Grechetto Gentile and it proved to be well superior in terms of tolerance to water stress. In a third work (Bianchi, 2021), the effects of M4 and 1103P in combination with Pinot Blanc were compared and it was observed that under conditions of moderate water deficit no significant difference appeared. Under severe water stress conditions, 1103P induced faster stomatal closure, higher ABA transcription and lower photosynthesis than M4, which maintained a good level of water use efficiency (WUE) and photosystem II functionality. SO4 was compared to M4 and this last one induced, under water stress conditions, higher WUE withing a higher content of sugar and anthocyanins despite a similar yield reduction (Merli *et al*., 2016). The comparison between M4 and 101.14 was the subject of another study in which the behaviour of Cabernet Sauvignon grafted onto the two rootstocks and autografted was studied (Prinsi *et al*., 2021). The results showed that M4 has a great ability to maintain higher levels of WUE and lower levels of stem water potential compared to other combinations. The adaptability of M4 to water deficit situations was also observed by the higher capacity to absorb water from the soil and the lower reduction in root's growth rates. In the study, there was less ABA concentration in leaves and in roots than in the other combinations, coupled by reduced transcription of the relevant genes. A proteomic analysis was carried out on M4 which shown the ability to regulate osmolarity and keep root's cell structures intact. In addition, an interesting enzymatic activity linked to the degradation of starch and sucrose synthesis was observed in order to compensate for the reduction of photosynthetic activity (Prinsi *et al.*, 2020, 2018; Regier *et al.*, 2009). M4 was also shown to be effective in the photosynthetic activity recovery following a period of water stress, this indicates its adaptation capacity to different water availability situation typical of a climate change context (Meggio *et al.*, 2014). In addition to drought resistance, M4 was showed to tolerate high salinity soils level due to the lower concentration of salts in the leaves, unlike what was observed, in the case study, in 101.14 (Meggio *et al.*, 2014). The comparison between M4 and 101.14 also shown that M4 is able, in situations of water deficit, to express more markedly genes related to the production of stilbenes, resveratrol in particular, which play a fundamental counteracting action against ROS. This behaviour allows a greater development of the lateral roots with a consequent greater uptake of water (Corso *et al.*, 2015). Stilbenes produced, translocated in grapes, in stressful situations also play an important nutraceutical role for human health (Pastor *et al.*, 2019; Navarro *et al.*, 2018; Georgiev *et al.*, 2014).

From a study by Bavaresco *et al.*, (2015) integrated with Caramarico (2020), the following information can be obtained: M1 and M3 are characterized by a medium-low vigour and a medium-high productivity induced. As far as K and Mg absorption is concerned, M3 was shown to be more efficient than M2, especially regarding Mg absorption, but this last one allows a more equilibrate uptake between the two elements. Mg absorption improves the phenolic quality of the grapes as it favours a good production of anthocyanins and polyphenols associated with a good sugar content. However, M1 proven to be effective in inducing excellent phenolic accumulation in grapes. The sugar production of M2, M3 and M4 is excellent, and the acidity conferred by M3 is interesting especially if associated with climate changes effects on grapes (Van Leeuwen and Destrac-Irvine, 2017). M2 is able to confer good drought resistance. M1 and M3 are compared to 101.14 and 1103P in an experiment (Zamboni *et al.*, 2016) where the increase in the dose of available N showed different behaviours. M1 was characterized by lower vigour, similar to 1103P, and greater apical dominance than commercial rootstocks, M3 showed no sign of declining vigour. Both M1 and M3 increased WUE as the nitrogen dose increased as well as 101.14 compared to 1103P. This was due to a maintenance of leaf assimilation accompanied by a reduction in transpiration. All rootstocks showed a lower leaf concentration of K, P, Mg and B. In conclusion, M1 proved to be more valid than M3 as it combined more desirable effects while maintaining a more balanced level of foliar nutrition. A nutritional evaluation work conducted by Porro and colleagues (2012) shown the high Ca and B uptake capability demonstrated by M1 within a weak tendency to absorb Fe that can lead to reduce chlorosis symptoms. M3 had good results in Mn uptake but a lower one for K. Opposite was M2 that is able to take Mn and K in an equilibrate way. M4, as M3, showed K uptake limitations. The authors suggested that M1 and M3 would be suitable in limiting irrigation environments. As with all rootstocks, M's are also able to influence the behaviour of traditional vines more than the so-called international French ones (Brancadoro and Failla, 2019).

At the nursery level, the highest combination efficiency rates are observed for M2 and M3 (77-78%) and lower for M1 and M4 (73-74%), values similar to those of SO4 and 1103P; It is important to underline that the graft combination plays a fundamental role (Falginella *et al.*, 2022).

The oenological quality of berries (red and white) derived from M rootstocks was tested by Biasi (2021) in different Italian wine regions. Results show the tendency to keep the same yield but with low cluster weight as compared to commercial rootstocks used. The same study shows the tendency of M rootstocks to induce higher acidity levels but with low malic acid content coupled with a lower sugar content. However, there are wine regions where results are not confirmed like in Prosecco wine region with Glera variety. M rootstocks general influence on oenological properties were confirmed by Storchi and Bogoni (2021) especially cluster weight reduction, tartaric acid content increase and sugar content reduction tendency. Storchi (2021), on more than ten years test on the varieties Sangiovese and Cabernet Sauvignon grown in Tuscany, observed also that M1 and M2 were able to increase anthocyanins. M4 increased their content as well and it guaranteed higher water stress tolerance which was connected with a higher wine equilibrium.

In terms of sustainability and more in particular regarding the Water Footprint, it was observed that M2 and M4 reduced water consumption by about 25-30% in the entire grapevine annual cycle (Lunelli, 2019). In general, M rootstocks show higher phenotypical stability levels through the years and between the different testing areas making them more reliable in a future prospective. Their stability is evident in difficult years characterized by drought, heat waves or high pluviometry (Brancadoro, 2021, Scienza, 2021).

PAPER

2. Paper

THE POTENTIAL ROLE OF NEW AND COMMERCIAL ROOTSTOCKS IN THE ADAPTATION OF SPARKLING WINE VITICULTURE TO CLIMATE CHANGE: THE CASE OF FRANCIACORTA (NORTH ITALY)

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2.1. Abstract

New adaptation techniques are needed to increase resilience of vineyards to the effects of climate change. Currently, most of the vineyards are grafted onto few century-old rootstocks, selected from a relatively small gene pool. The development of new biotypes can represent a turning point for modern viticulture. In fact, the rootstock choice from an enriched pool of genotypes, could represent a winning adaptive solution, influencing the behaviour of the scion by increasing its performance, considering the specific environmental context and oenological target.

The M series rootstocks, developed by the University of Milan in the eighties, when the first signs of climate change effects appeared and officially registered in 2014, were tested in different environments and with different grafting combinations.

This work focuses on the evaluation of Chardonnay cv., grafted onto different rootstocks, performances in the specific context of sparkling wine production in Franciacorta (Lombardy, Italy). The four M rootstocks (M1, M2, M3, M4) and the widely adopted SO4 and 1103P were compared considering drought stress tolerance, vegetative growth (vigour), yield and berry quality. Microvinifications of grapes from each scion-rootstock combination were made in 2022 and 2023 and analysed and evaluated by a tasting panel.

The rootstock 1103P showed good tolerance to water stress condition and was characterized by late ripening and a balanced sugar and acidity profile. However, it did not perform well in terms of sensorial analysis of wine. SO4 demonstrated a very good tolerance to water stress and maintained a stable but average sugar/acidity ratio along the four seasons. The M4 revealed to be the most interesting of the M-series showing a good water stress tolerance, a high acidity-sugar ratio, concurrent with an earlier ripening that allows for a more balanced ripening in terms of aromas, as confirmed by the tasting panel scores.

Keywords: climate change, novel rootstock, M-series, viticulture, sustainability.

2.2. Introduction

Grapevine is a plant well known for high adaptability to different environmental condition and resistance to abiotic stresses. During the process of diffusion and evolution, grapevine was favoured by its high degree of resilience and adaptability to weather and soil conditions (Grassi and De Lorenzis, 2021, Hardie, 2000). This allowed, especially in Europe, the establishment of many cultivation areas, characterized by significant pedoclimatic differences (Khan *et al.*, 2020).

Today, climate change is challenging Mediterranean viticulture. In fact, the current climate phase is characterized by a severe increase of temperature both at global and local level that in most viticultural areas translates in stressing conditions during summer months (Rogiers *et al.*, 2022; Venios *et al.*, 2020). With reference to precipitation, while it is hard to identify clear changes in the precipitation levels, some areas are currently characterized by modification in the seasonal pattern and by an increase in the length of periods without precipitation events (Essa *et al.*, 2023, Massano *et al.*, 2023; Cook *et al.*, 2018; Trenberth, 2011). Additionally, the increase of temperature translates into a higher atmospheric evapotranspiration demand, determining the increase of drought conditions and connected effects on vine behaviour (van Leeuwen and Destrac-Irvine, 2017). Furthermore, the increase of spring thermal resources determines the advance of phenological timing, with early sprouting that leads to a shift of ripening during the warmest times of the season, when the frequency of heat waves is higher, with relevant effects on the ripening dynamics, affecting the berry composition and threating the production of specific and traditional wines. This causes economical losses coupled many times to vine's death caused by severe drought (Jones *et al.*, 2022).

In recent years the sector responses mainly aimed to reduce the impact of environmental factors on vine physiology and, consequently, production parameters (Tombesi *et al.*, 2022; Andreoli *et al.*, 2019; Santos *et al.*, 2019; Costa *et al.*, 2016; Fraga *et al.*, 2012; Olesen *et al.*, 2011; Jones, 2006). Research had therefore first studied and then developed several adaptation strategies, which can be divided into short-term, medium-term and long-term (Bucur and Dejeu, 2022). The first two represent the simplest and most flexible choices that can be done by producers. in fact, short-term solutions can be applied immediately during a vine growing season to face and contrast the negative effects of the threats occurring along the season. Canopy management, irrigation, sunscreen applications, pests' control and soil management are examples of possible actions to perform (Santos *et al.*, 2021 A). Medium term strategies imply and investment for the future growing seasons, without losing the possibility to change and adjust them in function of changing environmental conditions. Training systems changes, minimal or delayed pruning and shading nets are the most used solution (Zheng *et al.*, 2017; Frioni *et al.*, 2016; Novello and De Palma, 2013; Valenti *et al.* 2012). On the other hand, long-term techniques imply long lasting investments and given the time perspective and the cost of implementation, in front of future scenarios unpredictability, are often put in the background (Santos *et al.*, 2021 B) both in terms of research and operative application.

The adoption of high-performance rootstocks represents a powerful long-term tool to modify the behaviour of vines and therefore make them less exposed to the effects of climate change, improving the adaptation to adverse environmental conditions (Gonzaga Santesteban *et al.* 2023; Marín *et al.*, 2021). Rootstock represents a pivotal agronomical tool. Without rootstocks *Vitis vinifera* could not be cultivated in most European terroirs due to phylloxera (*Daktulosphaira vitifoliae*, Fitch 1856) pressure since second part of XIX century (Granett *et al.*, 2001). The union between the two scions allows important interactions in both directions, even if the influence of rootstock on grapevine variety grafted on it has a higher importance. In detail, rootstock influences nematodes tolerance (Palliotti *et al.*, 2021; Van Zyl *et al.*, 2012), limestone and iron chlorosis tolerance (Bavaresco *et al.*, 2003, 1992; Brancadoro *et al.*, 1995), salinity tolerance (Ismail *et al.*, 2012, Cramer *et al.*, 2007), drought tolerance (Bianchi *et al.*, 2021, 2020; Palliotti *et al.* 2021; Prinsi *et al.*, 2021; Tramontini *et al.*, 2013) and mineral nutrition (Kodur *et al.*, 2011, 2011; Brancadoro *et al.*, 1994). Additionally, rootstocks are also able to influence scion vigour and phenology and berry composition (Yin *et al.*, 2023; Pou *et al.*, 2022; Brancadoro and Failla, 2019; Li *et al.*, 2019; Scienza, 2019; Nèmeth *et al.*, 2017; Corso *et al.*, 2016).

Nowadays, in a complex environmental, economic and regulatory context, the sector is dealing with different and new viticultural and vinicultural models. For this reason, the introduction of new rootstocks could help to guarantee grape quality and yield by optimizing the scion-rootstock interaction according to current and future environmental pressures, also considering the specific oenological goals of different vinicultural models. This could be beneficial for environmental and economic sustainability, because a more efficient use of resources leads to a decrease in the input request and a stabilization of yield and quality (Darriaut *et al.*, 2022; van Leeuwen *et al.*, 2019). Moreover, this strategy could play a relevant role in the context of organic viticulture, helping a sustainable management of the organic vineyards as in increase of vineyard resilience to climate change negative effects could be crucial for this production type.

In this regard, the University of Milan (DISAA) recently invested in the selection of new rootstock biotypes, called the “M series”. The breeding selection, started in 1985, focused on the main American grapevine species and more specifically on *V. berlandieri, V. vinifera* and *V. cordifolia*. The main driver of the breeding program was of the creation of new genotypes able to increase vine performances facing climate change effects (Maul *et al.*, 2024). The M series rootstocks, released in 2014, are proving to be effective with respect to the above-described goals, being tested in different viticultural contexts. The data collected in recent years (Falginella *et al.*, 2022; Bianchi *et al.*, 2021, 2020; Prinsi *et al.*, 2021, 2020, 2018; Camarico, 2020; Frioni *et al.*, 2020; Brancadoro and Failla, 2019; Lunelli, 2019; Merli *et al.*, 2016; Zamboni *et al.*, 2016; Bavaresco *et al.*, 2015; Corso *et al.*, 2015; Meggio *et al.*, 2014; Porro *et al.*, 2012) show the competitiveness of the M series as compared to conventional rootstocks.

Climate change particularly threatens sparkling wine production (Van Leeuwen and Darriet, 2016; De Orduna, 2010; Jones *et al.*, 2005), as the quality of wines is strongly related to maturity balance with regards to grape acidity (Nesbitt *et al.*, 2022; Briche *et al.*, 2014). The production of sparkling wine in Italy is very important and the demarcated wine region (DOCG) of Franciacorta (Lombardy, North Italy) is one of the most relevant areas for this kind of production, being Chardonnay the most adopted variety. In Franciacorta, the clearest effects of the recent warming are:

- phenological rhythm shift, with an advanced harvest that take place during a warmer period of summer.
- ripening stage length shortening.
- generally increased drought conditions because of higher evapotranspiration demand by a warmer atmosphere, concurrently with variable precipitation level.

All those effects can determine a decrease in the quantity and quality of the harvested grapes, threatening the production of this important wine area. The role of viticulture tools could limit the negative effects of climate change, making also sparkling wine regions able to counteract them. In detail, this work focuses on one of the most effective long-term strategies, namely the choice of plant material, and particularly the rootstock.

This study tests the performances of the M-series rootstocks (M1, M2, M3, M4) in comparison with the widely adopted SO4 and 1103P ones. The main aim of the work is the evaluation of the effects of the rootstocks on the water stress status of vine (cv. Chardonnay), with reference to quality and quantity of both grape and wine production, in the specific context of Franciacorta sparkling wine production. The research was conducted over 4 years (2020-2023), allowing to test the phenotypic stability of the different rootstock scion combinations under different environmental conditions.

2.3. Materials and methods

2.3.1. Experimental site and design

This work was carried out in Franciacorta DOCG demarcated wine region, located in the Province of Brescia (Lombardy, North Italy), along the period 2020-2023. The experimental vineyard under study (Figure 5) is part of Vezzoli farm (45,61° N, 9,95° E) within the municipality of Adro. The vineyard is VSP trained and Guyot pruned with a NE-SW orientation of the rows, a spacing of 2 m along the row and 0.9 m between the rows, for a plant density of 5556 plants/ha and planted in 2014. The inter row is characterized by spontaneous grass cover that is generally mulched three times per year. The soil is loamy clay, with coarse loam grain size and shallow skeleton. The vineyard is divided into six plots on single row with fifty plants each (figure 5). Chardonnay (*Vitis vinifera* L.) was grafted, in 2014, onto rootstocks 1103P, M1, M2, M3, M4 and SO4. The main features of the six rootstocks are listed in Table 1.

Figure 5 - Experimental site with the corresponding rootstock genotypes used in the trial. Satellite image processed with QGIS.

Table 1 - Pedigree and main characteristics of the rootstock series used in the trial (1103P, M1, M2, M3, M4, SO4).

ROOTSTOCK	PEDIGREE	MAIN CHARACTERISTICS
1103P	*V. berlandieri* X *V. rupestris*	Vigorous, maturation delayer, enhances grape acidity. Good drought, salinity and asphyxia tolerance.
M1	106/8 [*V. riparia* x (*V. cordifolia* x *V. rupestris.*)] X Resseguier N°4 (*V. berlandieri*)	Low vigour, high resistance to ferric chlorosis. Medium resistance to drought and salinity.
M2	8B Teleki (*V. berlandieri* x *V. riparia*) X 333 E.M. *(V. vinifera x V. berlandieri)*	Vigorous, good resistance to drought, salinity and iron chlorosis. It favours high productions.
M3	R27 (*V. berlandieri* x *V. riparia*) X 5C Teleki (*V. berlandieri* x *V. riparia*)	Low vigour, efficient in potassium absorption, medium drought and salinity resistance.
M4	41B (*V. vinifera* x *V. berlandieri*) X Resseguier N°4 (*V. berlandieri*)	Medium-high vigour, high resistance to drought and salinity. Good tolerance to limestone.
SO4	*V. berlandieri* X *V. riparia*	Medium vigour, medium drought and salinity tolerance. Good limestone tolerance.

2.3.2. Agrometeorology characterization

To understand the rootstock behaviour along the four years of the trial, agrometeorological characterization of the four season was based on data from a weather station of the Agrometeorological Network of the Brescia Province, located in Corte Franca (BS), close to the experimental vineyard (5 km). The four years were analysed and compared with the historical time-series 1997-2023, considering average yearly temperature, the yearly number of hot days (maximum temperature above 32°C – Modina *et al*., 2023, Zhu *et al*., 2020), the yearly total precipitation and the precipitation of the April-August period, representative of the growing season of Chardonnay in the investigated area.

To provide a synthetic characterization of the four years in terms of grapevine water stress, a simplified single layer reservoir water balance model with a daily time step was performed for the variety Chardonnay, based on the daily weather data of the Corte Franca weather station, and which considered an average soil with a Potential Available Water of 130 mm/m of depth (Cola *et al*., 2014). Spontaneous grass cover was considered as calculation model reference, to take into account water competition (Allen *et al*., 1998). The sum of daily grapevine maximum (ETM) and real (ETR) evapotranspiration were calculated for the period April 2nd to August 20th, representative of the average growing season of Chardonnay in Franciacorta, from phenological stage BBCH 8 (bud burst) to BBCH 89 (harvest maturity). The ratio ETR/ETM was adopted to estimate the grapevine water deficit along the growing season (Liu *et al*., 2017) since the maximum evapotranspiration represents the ideal condition of the crop without any stress and the real one, the real ability of the soil-plant-atmosphere system to respond to the potential evapotranspiration demand. This report on the seasonal total provides a measure of the water stress that the plant suffered during the season.

To describe the effects of environmental conditions on grapevine growth and development and properly describe the four years in terms of thermal conditions for grape growth and development (Tonietto and Carbonneau, 2014; Irimia *et al*., 2013; Jones *et al*., 2010), the following bioclimatic indexes were calculated:

- Winkler index (Amerine and Winkler, 1944)

$$Winkler\ Index = \sum_{1^{st}\ Apr.}^{31^{st}\ Oct.} \left(T_{average} - 10°C\right) \quad T > 10°C$$

- Huglin Index (Huglin, 1978)

$$Huglin\ Index = \sum_{1^{st}\ Apr.}^{30^{th}\ Sep.} \frac{\left(T_{average} - 10°C\right) + \left(T_{max} - 10°C\right)}{2} k \quad k = 1{,}04$$

- Cool Night Index (Tonietto and Carbonneau, 2004)

$$Cool\ Night\ Index = \sum T_{min}\ 30\ days\ before\ harvest$$

2.3.3. Grapevine analysis: water status, temperature and Ravaz Index

The surveys performed in the experimental vineyard focused on physiology during the growing season and production parameters at harvest. For each plot six plants, equally distributed along the row, were sampled. Regarding physiology, the water status of grape was assessed, considering one leaf per plant, placed in the model position of a primary shoot and measuring the leaf water potential ($\psi_{pre\text{-}dawn}$) at pre-dawn (3 a.m. - 5:30 a.m.) with a pressure chamber (Scholander *et al*., 1965), produced by PMS Instrument Company, Corvallis, Oregon (USA). At this time of the day, the water potential of the leaf is in equilibrium with the soil one (Améglio *et al*., 1999) and the measurement provides an accurate estimation of the soil water content. Deloire *et al* (2020) and Carbonneau (1988) proposed the following stress classes: no-stressed (0 to -0.3 MPa), mild (-0.3 to -0.5 MPa), moderate (-0.5 to -0.8 MPa) and severe (< -0.8 MPa).

Following the methodology described by Bellvert *et al*. (2014), during the season, the temperature of the vines canopy of each plot was measured with an infrared thermal camera (Thermo Gear Model G100EX/G120EX (Detector Uncooled focal plane array; number of pixels 320 (H) × 240 (V); spectral range 8–14 µm; dynamic resolution at 14 bit), produced by InfReC, NEC Avio Infrared Technologies CO) between

10 a.m. and 2 p.m. Each thermal picture, taken in the shaded side, frames the vine canopy and two control elements, consisting of two cardboard tags, one dry and one wet, fundamental to properly tune the temperature range of the thermal picture. The photographs were processed using the "InfReC Analyzer NS9500 Lite" software (DAQLOG Systems Ltd, UK). The temperature of three dry leaves, one representative for the basal, median and apical portion of the vine canopy wall were extracted considering two points per leaf (Bianchi *et al.*, 2018). The data obtained were then used to calculate the CWSI (Crop Water Stress Index) (Jones, 2002; Idso *et al.*, 1981).

$$CWSI = \frac{T_{point} - T_{wet}}{T_{dry} - T_{wet}}$$

The CWSI is in fact indicated to evaluate the level of water stress of the plant based on the temperature of the plant canopy wall, which is related to the level of instantaneous stomatal transpiration. The index takes values ranging from 0 to 1; where zero indicates no stress and maximum respiration rate, while one indicates maximum levels of water stress and total absence of transpiration due to maximum stomatal closure.

At harvest, for each vine, the parameters measured were the number of bunches produced, the number of shoots and the yield per plant. In every season, all the plots were harvested at the same time. During winter pruning, the removed wood was weighted in order to calculate Ravaz Index as the ratio between yield per vine (kg) and pruning weight (kg) (Ravaz, 1911). The obtained value gives information related to vegetative-productive habitus balance. In general terms, a well-balanced vine is characterized by a Ravaz Index value between 5 and 8. Lower or higher values indicate an unbalanced vine status with respectively an under cropped or an overcropped condition respect to plant vigour or the opposite (Palliotti *et al.*, 2021; Almanza-Merchán, 2014; Aliquó *et al.*, 2010).

2.3.4. Berry must analysis

At harvest, for each plot must was obtained from four bunches per plant, per six plants per plots. Bunches were sampled from the middle position of the median shoots and manually crushed (about 100 mL). Must was added with 0.2% sodium azide (NaN_3) to prevent fermentation, to perform compositional analysis. pH was measured by pH-meter. Titratable acidity, expressed in g/L of titratable acid, was measured by an automatic titrator (Flash Automatic Titrator, Steroglass, Perugia, Italy). Sugar amount, expressed as Brix degrees (°Bx), was determined by using a digital refractometer (geass DBR35, Geasse, Turin, Italy), measuring total soluble solids (TSS). Yeast Assimilable Nitrogen (YAN) content was measured following Sörensen method (Formol number method – Casalta *et al.*, 2013). K^+ content was measured by using the automated AMS Alliance SmartChem® 450 Discrete Analyzer (AMS-Alliance, France).

2.3.5. Wine analysis

For each plot, 100 kg of grapes were randomly collected along each plot to perform microvinifications, following a standard protocol. The microvinification protocol is characterized by crushing the morning after the day of harvesting, addition of 2 g/L of pectolytic enzymes, 5 g/hL SO_2 and 10 g/hL PVPP to the must. The must is then decanted and then 20 g/hL of selected yeasts and 20 g/hL of specific activator are added. At the end of fermentation, racking is done with the addition of 4 g/hL of SO_2. Decanting is carried out after 3-15-30 days and 1 g/hL of SO_2 is added for each of them. At the time of bottling, 2 g/hL of SO_2 are added. Microvinification were performed only in 2022 and 2023. Alcoholic strength, residual sugars, dry matter, titratable acidity (g/L tartaric acid), pH and volatile acidity (g/L acetic acid) were measured by using the wine analyser WineScan™ (FOSS, Padova, Italy).

Sensorial analysis of the wines was performed with a blind test by a selected panel of tasters (n°9 in 2022 and n°12 in 2023). Normalized results of the evaluation were adopted to characterize wines by means of spider charts.

2.3.6 Experimental design and statistics

In order to assess and represent the effect of rootstock, year and their interaction, production parameters, water status and sensorial perceptions was processed with statistical software as Microsoft® Excel® for Microsoft 365 MSO (Version 2405 Build 16.0.17628.20006) at 64 bit and SPSS statistical environment (IBM SPSS Statistics 29.0.1.0, USA). After assumptions assessment, one-way ANOVA was performed taking into consideration significance levels of $p \leq 0.001$ (***), $0.001 < p \leq 0.01$ (**), and $0.01 \leq p \leq 0.05$ (*); $p > 0.05$ (n.s.). Duncan post-hoc test was performed where a statistical difference was observed.

The minimization of vintage variability is for sure one of the most important goals of vineyard management. To assess phenotypic stability induced by rootstock genotypes on vines and grapes production parameters Wricke's ecovalence Wi^2 (Wricke, 1962) was calculated. Obtained value results from the interaction with the vintage, squared and summed across four years. Low values mean low variability and a relatively higher stability. To assess thermal, pluviometry and evapotraspiration level effects on vegetative (wood weight), productive (yield and yield/shoot), and qualitative (sugars, acidity, pH, YAN and K^+) parameters a regression was performed by ordering vintages in a chronological order. Sugars – Acidity relation was studied by a regression analysis by using all available data spitted per rootstock genotype.

2.4. Results and discussion

2.4.1. Agrometeorological results

To characterize the climate of the area under study, figure 6-a shows the total precipitation considering the whole year while figure 6-b focuses on the precipitation of the growing season semester (March-August) for Chardonnay in Franciacorta. Average yearly air temperatures and number of days with maximum temperature above 32°C are presented in figure 6-c and figure 6-d respectively.

To quantify the water stress of each studied season, a single layer reservoir water balance was performed, considering a standard soil with a potential available water of 130 mm for 1 m depth (Cola *et al.*, 2014).

Figure 6-e shows the total maximum grapevine evapotranspiration estimated from 2nd April to August 20th, the average date of bud break (8 BBCH) and harvest (89 BBCH) in the Franciacorta area.

With reference to precipitation, the average yearly cumulation level is 1140 mm, while the average cumulation of the vine growing period (March-August) is 608 mm, with a relevant variability the years. With reference to the four experimental years, 2020, 2021 and 2023 they were characterized by slightly above-average rainfall values (+2%; +6%; +9%), while 2022 was particularly dry (-32%). With reference to the growing season semester (March – August), 2020 was particularly high in precipitation rate (+50%), 2021 was average (+7%), 2022 confirmed as a dry season with -43% when compared to the average and 2023 showed a +35% increase. The analysis of yearly average temperature (Figure 6-c) shows that there are important variations from one year to the next. In the vintages studied, 2020 and 2021 were in the medium range, while the 2022 and 2023 vintages were found to be warmer. Analysing the number of hot days, 2022 was characterized by a high value (4° in total classification); 2020, 2021 and 2023 remained under the mean value. By observing, in the years considered, the maximum and minimum thermal averages from the beginning of veraison to harvest, 2020 and 2023 were within the average while 2021 was colder and 2022 warmer. Analysing in detail the ripening period of the grapes it can be observed that 2022, compared to other years, was characterized by the absence of rainfall and temperatures (maximum and minimum) higher than the other vintages. 2022 was also the year with the highest frequency of temperatures above the threshold of 32°C. 2020, 2021 and 2022 had a higher frequency of lower temperatures.

In terms of evapotranspiration (ET) (Figure 6-e), the difference among vintages is clear. 2022, compared to the others, was characterized by an estimated reduced grapevine transpiration compared to the maximum one. This could be explained by stomata closure under conditions of moderate and severe water stress effect (Parolin, 2022).

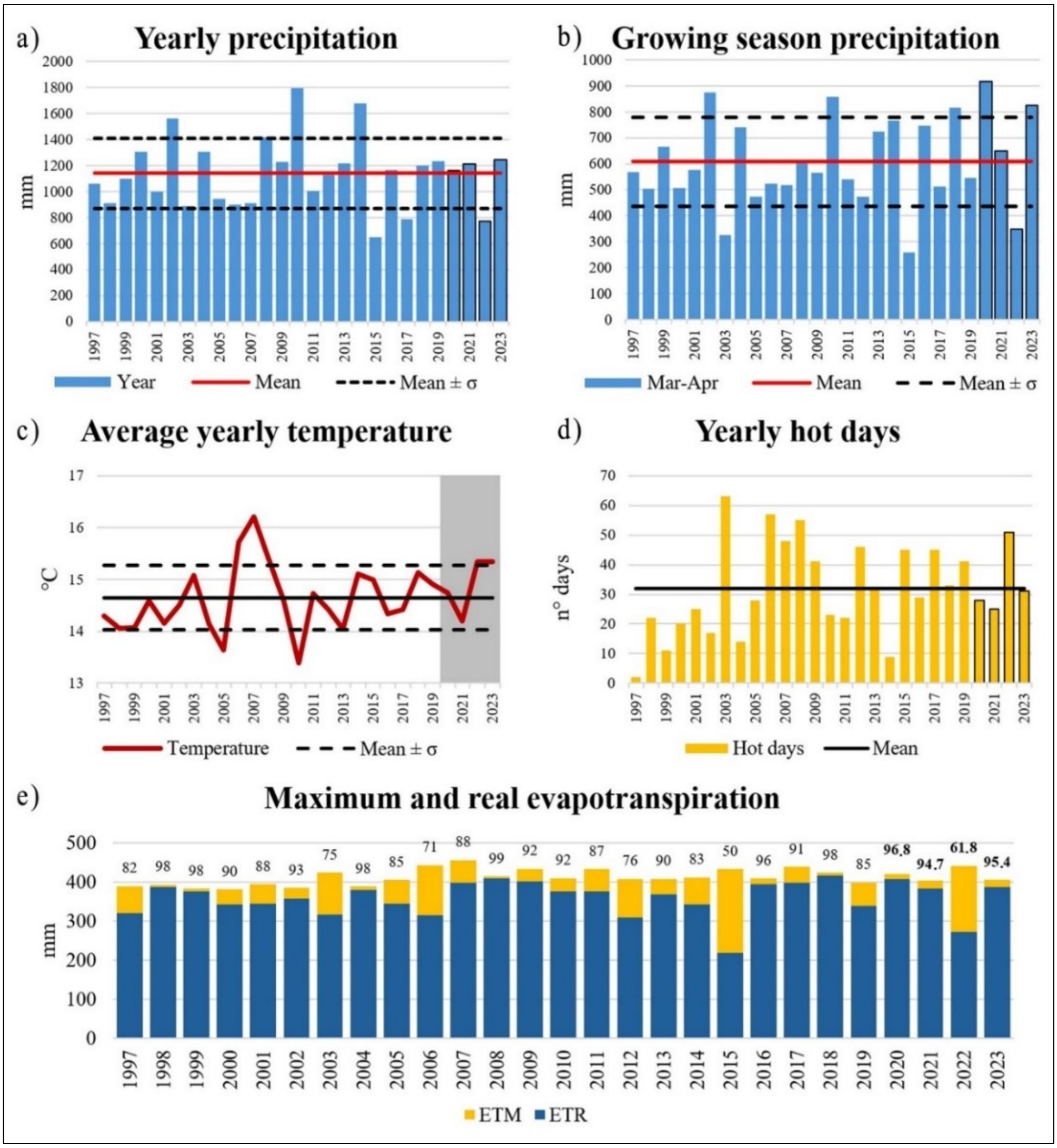

Figure 6 - Agroclimatology characterization of the four years. Yearly precipitation ***(a),*** *growing season (Mar-Aug) precipitation* ***(b),*** *average yearly temperature* ***(c),*** *yearly hot days (T > 32 °C)* ***(d)*** *and yearly maximum/real evapotranspiration* ***(e).***

From the analysis of Table 2, it can be observed that no marked differences are present, in terms of phenology and ripening period lengths, between 2020, 2021 and 2023 due to the low stress level perceived. Oppositely in 2022, despite a regular beginning of veraison time, harvest was anticipated inducing a ripening period shortening.

Table 2 - Beginning of veraison and harvest days within maturation period length during the studied vintages.

Year	85 BBCH – Beginning of veraison (DOY)	Harvest (DOY)	Length (n° days)
2020	194	232	38
2021	202	235	33
2022	195	223	28
2023	198	233	35

The effects of environmental conditions on grapevine were assessed by means of bioclimatic indices Winkler Index, Huglin Index and Cool Night Index as shown in Table 3. The values of the Winkler Index were classified following Jones *et al*., (2010). Class IV corresponds to a warm-temperate climate and class V to a warm climate. 2020 and 2021 resulted as class IV while 2022 and 2023 as class V. According to Irimia *et al*., (2013) the Huglin heliothermic Index, which is the same for all four vintages, indicates the presence of a warm climate (IH_{+2}). According to Tonietto and Carbonneau (2004), the Cool Night Index defines the thirty nights prior to ripening as warm.

Table 3 – Values for the bioclimatic indices (Cool Night, Winkler and Huglin) in the studied years, compared with historical statistics for 1997-2023. For all the three indexes, classification of yearly values is reported and for minimum and maximum values relative year is placed in brackets (Jones et al., 2010).

Bioclimatic Index	Minimum (1997-2023)	Average (1997-2023)	Maximum (1997-2023)	2020	2021	2022	2023
Winkler	1967.8 (2010)	2199.6	2536.7 (2006)	2126.7	2022.1	2415.2	2292.5
	IV	IV	V	IV	IV	V	V
Huglin	2423.2 (2002)	2646.3	3007.7 (2007)	2646.9	2530.9	2827.3	2675.4
	IH_{+1}	IH_{+2}	IH_{+3}	IH_{+2}	IH_{+2}	IH_{+2}	IH_{+2}
Cool Night	16.3 (2010)	19.5	22.9 (2003)	19.9	18.8	20.8	18.1
	CI-1	CI-2	CI-2	CI-2	CI-2	CI-2	CI-2

Season 2020 was characterized by average annual and during the growing cycle abundant precipitation. With reference to temperature, the year was close to normal values, with a reduced number of hot days during summer. This translated in the second lowest level of Winkler and Huglin indexes. Regarding the Cool Night Index 2020 it was the second highest among the four years. The ETM/ETR ratio was the highest, suggesting no relevant water stress along the season.

Season 2021 showed normal precipitation both regarding the whole year and the growing season, normal yearly temperatures and the lowest level of hot days among the four years. This was the lowest year of the four regarding all Winkler and Huglin indexes, and second lowest for the Cool Night Index. The ETM/ETR was very high, with no relevant water stress for grapevine.

Season 2022 showed abnormal low precipitation at yearly and growing season level. Yearly temperature overcame the normal value for more than one standard deviation interval, and the number of hot days was the highest of the four and the fourth highest of the whole 1997-2023 timeseries. This translated into the highest values of the thermal indexes and in the lowest ETM/ETR ratio with consequent severe drought for grapevine as also confirmed by predawn water potential (ψ_{PD}) data.

Season 2023, precipitation wise, it was like 2020, with normal yearly precipitation and abundant growing cycle precipitation. Yearly temperature overcame the normal value for more than one standard deviation interval, but the number of hot days (T > 32 °C) was in accordance to the average level. 2023 was second highest for Winkler and Huglin indexes, but the lowest for the Cool Night Index. Similarly to 2020 and 2021 the ETM/ETR ratio was very high.

2.4.2. Ecophysiology: Water status and CWSI

Predawn water potential (ψ_{PD}) was measured for the four vintages under analysis in order to monitor plant water status and indirectly assess soil water content. On the same day, thermal surveys of the plant wall were carried out to calculate CWSI. The water potential varied between 0 and -0.3 MPa confirming the absence of water stress in 2020 and 2023. Figure 7 shows the CSWI values for each rootstock combination. In 2021 only mild stress conditions were observed, while in 2022 the water stress increased during the season, moving from moderate to severe.

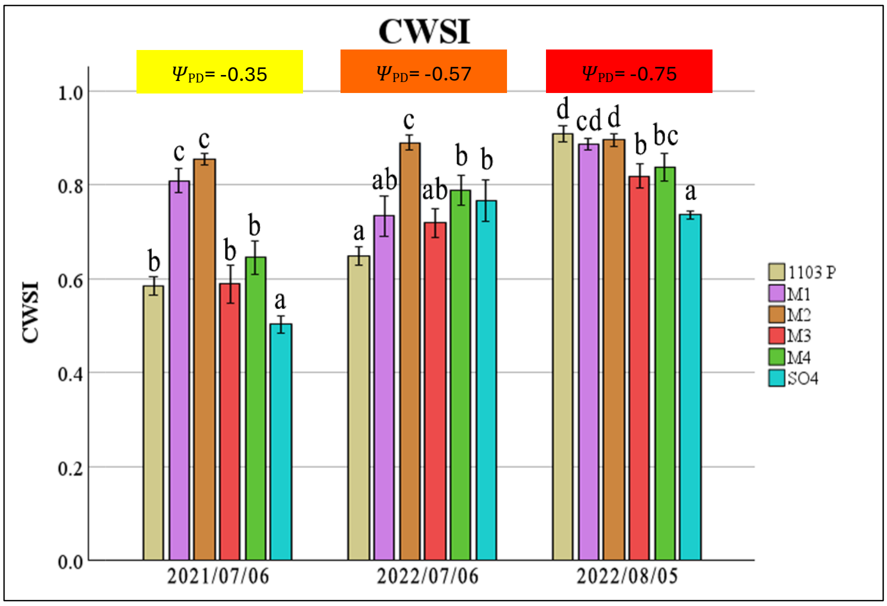

Figure 7 - CWSI level of each rootstock plot during water stress conditions. For each date the average pre-dawn water potential (ψ_{PD}) is shown, mild stress is represented in yellow, moderate in orange and severe in red. ANOVA and post hoc test were performed taking into consideration a significance level of $p \leq 0.001$ (***). Data from 2020 and 2023 are not shown, since the two seasons were not affected by water stress.

Compared to the other rootstocks, SO4 showed low CSWI values (0.50; 0.77; 0.74 respectively) in all the three dates, while 1103P moved from low values in the mild and moderate stress conditions to the highest in the third event. M1 showed high values except for the second date, while M2 constantly showed high values, M3, and M4 showed similar behaviour to SO4, with slightly high values in all the dates. Considering the severe stress condition of the third date, 1103P, M1 and M2 showed high CWSI levels, SO4 the lowest and M3, M4 an intermediate behaviour, meaning different way to respond to water stress (Bianchi *et al.*, 2021 and 2020).

2.4.3. Agronomic and yield parameters

The influence of rootstock on vines *V. vinifera* Chardonnay behaviour was investigated considering productive parameters such as grape production per plant and grape production per shoot, vegetative parameters like wood weight and Ravaz Index, qualitative parameters such as sugars, titratable acidity, pH, YAN and K^+ content. Multivariate ANOVA was performed after assumptions assessment on all the above-listed parameters, considering as factors the four years (2020, 2021, 2022 and 2023), the six rootstocks (1103P, M1, M2, M3, M4, SO4) and the year per rootstock interaction. Significance levels of $p \leq 0.001$ (***), $0.001 < p \leq 0.01$ (**), $0.01 \leq p \leq 0.05$ (*) were considered; $p>0.05$ (n.s.). Table 4 shows the significance level of all the combination analysed.

Table 4 - After assumptions assessment, multivariate ANOVA was performed considering the significance levels of $p \leq 0.001$ (***), $0.001 \leq p \leq 0.01$ (**), and $0.01 \leq p \leq 0.05$ (*).

	Production (kg/vine)	Production/ Shoot (kg/shoot)	Wood Weight (g)	Ravaz Index	Sugars (°Bx)	Titratable Acidity (g/L tartaric acid)	pH	YAN (mg/L)	K^+ (mg/L)
Rootstock	**	*	**	***	**	***	***	n.s.	n.s.
Year	***	***	***	***	***	***	***	***	***
Rootstock X Year	n.s.	***	n.s.	n.s.	*	n.s.	**	*	n.s.

Figure 8 shows the average parameters for Chardonnay vines grafted on the different rootstocks along the four years of the trial. For each parameter, the Duncan post-hoc test was performed when a statistical difference was observed. The average production per plant (kg/vine) of 1103P was higher (3.5 kg) than the average of the other five (2.6 kg), while in the case of production per shoot (kg of grapes/number of developed shoots), 1103P obtained 0.21 kg/shoot versus the lowest productions of M1 (0.17 kg/shoot) and M4 (0.16 kg/shoot). Only M2 showed a significant lower wood weight (g), with 533 g versus an average value of 768 g for the other five rootstocks. Consequently, M2 scored the highest Ravaz index (5.6), followed by M3 (4.3) while M4 reached an average value of 3.3.

In terms of berry composition, the 1103P showed the lowest average sugar accumulation (19.3 °Brix), followed by SO4 (19.8 °Brix). The M series rootstocks reached higher average values, with M4 reaching 20.5 °Brix. Regarding titratable acidity, 1103P reached 6.8 g/L, followed by SO4 and M4 with 6.2

g/L both. Regarding the pH, only 1103P showed a significant lower average (3.3) values compared to the other five rootstocks (3.4). YAN and K^+ content did not show significant differences among the rootstocks.

Rootstocks statistical differences on agronomic parameters

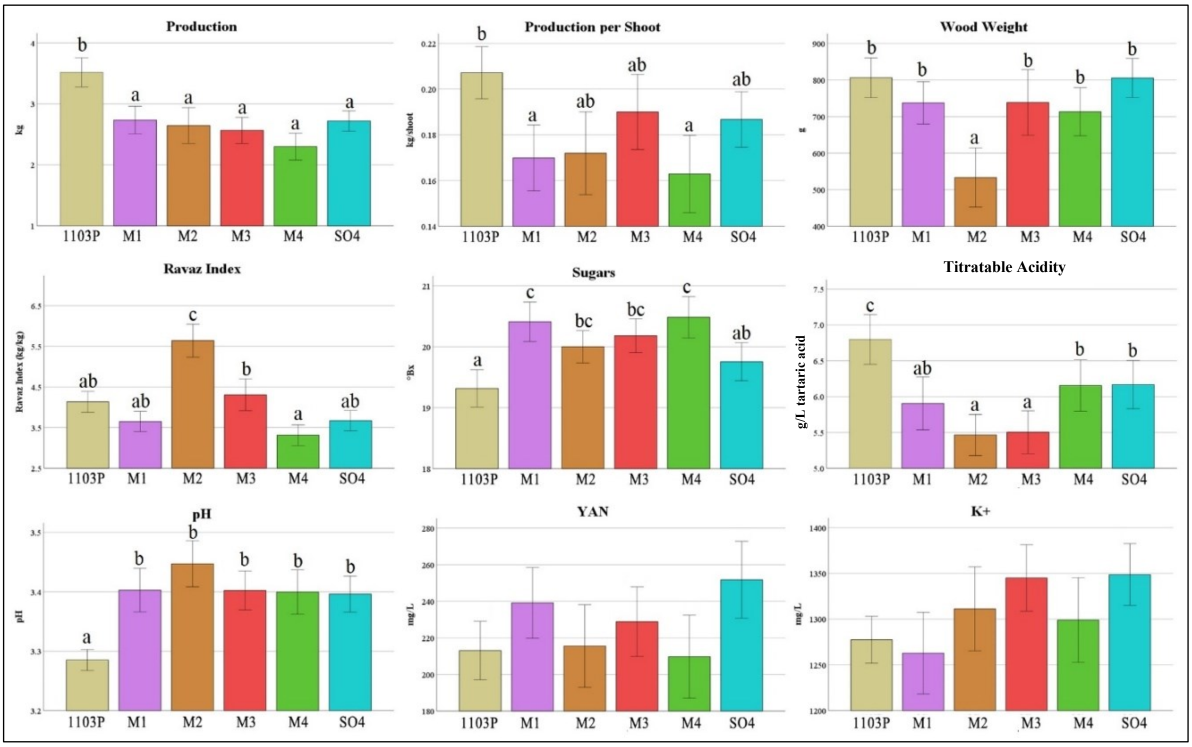

Figure 8 – Effect of the different rootstock on the vine and berry parameters (V. vinifera Chardonnay). ANOVA was performed considering significance levels of $p \leq 0.001$ (***), $0.001 < p \leq 0.01$ (**), and $0.01 \leq p \leq 0.05$ (*); $p>0.05$ (n.s.).

To investigate how Chardonnay, grafted on different rootstocks, responded differently depending on meteorological conditions, a linear regression between the productive (production and production/shoot), the vegetative (wood weight) and qualitative parameters (sugars, acidity, pH, YAN and K^+) and the environmental indices was performed for each rootstock (Table 2S and Figure 7S).

The environmental indices considered were yearly temperatures (YT), yearly hot days (HD), Winkler Index (WNK), Huglin Index (HUGH), yearly precipitation (P_Y), growing season precipitation (P_MA) and the ratio between maximum and real evapotranspiration (ETM/ETR). In the case of temperature-related indices (TY, HD, WNK, HUGH), most of the grafting combinations showed high R^2 (> 0.5) for production/shoot, pH and K^+ content. In M3 and M4, production/shoot was well related (the highest the index value, the highest the production) to all the temperature-related index, while in 1103P only to TY and WNK. The results showed a direct relation between HD and pH. All grafting combinations had $R^2 > 0.5$, but in the case of the rootstock 1103P, due its lowest slope coefficient (B), happens to be less sensitive to HD. K^+ content showed a direct relation with HD, WINK and HUGH. Also in these cases, 1103P showed the lowest B, confirming to be the less sensitive to air temperature in terms of the K^+ accumulation.

About the rain-related indices, P_MA influenced more the above reported parameters than the P_Y. Generally, the increase of P_MA is related to the decrease of sugar content and pH combined with the increase of acidity and wood weight. For all those parameters, the regressions with P_MA showed $R^2 > 0.5$ for most of the grafting combinations. M1 and M2 got the lowest R^2 and B values for sugars, acidity and, only for M2, wood weight, that seems less influenced by the precipitations during the season. Those are the only two rootstocks that got $R^2 > 0.5$ for yield and yield/shoot versus P_MA.

In the case of evapotranspiration, most of the grafting combinations showed high R^2 (> 0.5) for yield/shoot, pH, pruning wood weight and K^+ content versus the ETM/ETR ratio. The results achieved showed a strong relation between ETM/ETR and pH, but 1103P and SO4 happened to be less influences by water stress, as showed by the lowest B value. 1103P, M1 and M2 got the lowest R^2 for yield/shoot. Finally, there was a negative relation between ETM/ETR and K^+ content.

As acidity-sugar amount is crucial to define the harvesting date and for must/wine quality. Six cv-rootstocks related linear models were produced using all available data in the four vintages (Figure 9). Every year grapes were collected in the same day. This regression approach highlights some relevant points:

- R^2, even its significance level was not checked, shows the goodness fit of the linear model. M4 and 1103P shown the highest values while SO4, M1 and M3 medium ones, M2 the lowest one. This could be interpreted in terms of a higher stability in the sugar-acidity relationship for M4 and 1103P, no matter how the environmental and management conditions are.
- the points cloud of 1103P and SO4 are positioned more on the left side of the chart in comparison with other rootstocks, meaning a lower sugar accumulation which can be translated into a delay in technological maturity. This suggest that the M-series rootstocks need specific advanced harvesting times to ensure the best berry composition or a fresher climate conditions. In the specific context of Franciacorta, this could imply a further anticipated harvest, which consequence deserve future analysis.
- Franciacorta Consortium defines 19° Bx as the best sugar content level for harvesting Chardonnay in the area. Considering this threshold, and applying the linear models above described the prediction of titratable acidity for 1103P, M1, M2, M3, M4 and SO4 is 7.1, 6.9, 6, 6.5, 7.4 and 6.7 g/L respectively. 1103P and M4 could grant higher titratable acidity, a well-desired trait for the specific oenological goals of the region.
- the points cloud of SO4 shows a fast decrease of acidity when reaching 20°Brix. This could be a relevant problem when dealing to the territorial management of harvest, with possible delays between the best theoretical harvesting time and the operative one.
- the slope of the regression model could suggest a better capability to keep acidity, concurrently with sugar accumulation. However, the less leaned slope is obtained for plants of Chardonnay grafted on M2, which had the lowest level of acidity among the six treatments, considering the same sugar content. Considering the rootstock with highest levels of calculated acidity at 19 °Brix the higher

capacity to keep higher acidity level during sugar accumulation is given by 1103P, M4 and SO4 in ascending order.

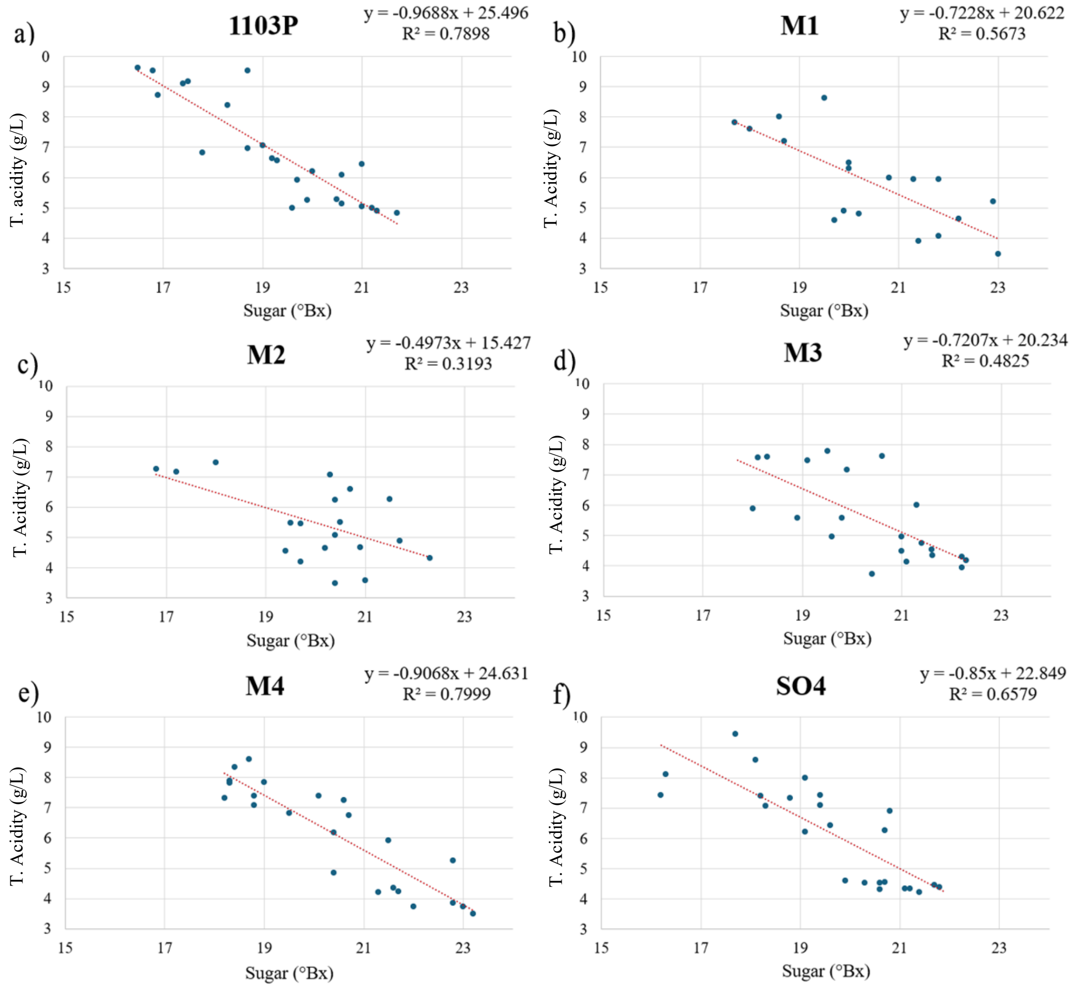

Figure 9 – Titratable acidity expressed as g/L of tartaric acid vs Sugars regression for each rootstock: a) 1103P, b) M1, c) M2, d) M3 e) M4, f) SO4. Regressions are based on the four years repetition data. Points indicate averages (n =6). Regression equation and R^2 score are reported.

Table 5 shows the results of the Wricke's ecovalence test, performed to assess the effect of rootstocks on the stability of vine performances along the four years of experimentation. M2 and M3 presented the higher number of stable parameters in comparison to the other combinations. Plants grafted on the SO4 and M1 rootstocks had intermediate stability while M4 and 1103P were the less stable. As can be seen from results, the differences among the rootstocks are generally small more data should be picked to reinforce those affirmation.

It is important to highlight that stability is not a positive feature by itself and it is strongly dependent on the average value of each parameter analysed. For example, it was found a higher stability for the Ravaz Index in the case of M3 but obtained absolute values of the index highlight that it was the most unbalanced rootstock (5.64 as Ravaz Index value). On the other hand, SO4 is stable in terms of grape production per vine, also ensuring medium-high production.

Table 5 - Wricke's ecovalence and regression coefficient values. The most stable value is reported in bold.

W_i^2	Production	Production/ Shoot	Wood Weight	Ravaz Index	Sugars	Titratable Acidity	Sugar/ T. Acidity	pH	YAN	K^+
1103P	124.7	0.5	8384531.6	281.2	6390.5	596.4	202.3	184.6	828590.8	27152818.0
M1	119.3	0.6	**8368263.6**	267.7	6423.6	590.4	205.3	183.5	844759.4	27728400.0
M2	122.3	0.5	8617112.6	264.9	6433.6	566.1	206.8	183.9	**803919.2**	**26918360.0**
M3	121.3	**0.5**	8412681.8	**65.1**	**6367.9**	**554.4**	205.9	**183.3**	813459.7	27507825.4
M4	121.6	0.5	8398838.8	80.7	6412.8	593.4	203.5	183.4	827678.5	27422637.4
SO4	**118.6**	0.5	8574833.5	69.5	6399.7	598.2	**190.9**	183.9	834847.2	27553211.2

2.4.4. Wines composition and sensorial results

To gain a complete knowledge of the effects of rootstock on Chardonnay vines and berries, the resulting wines were analysed for the vintage 2022 and 2023 as the only vintages when microvinifications were performed (Table 6). Significance was not checked. Alcoholic strength of wine is correlated to the sugar content in grapes. Grafting on the 1103P and M4 rootstocks resulted in the most alcoholic wines in 2022, while the M1 and M2 gave the highest value in 2023. Furthermore, M1 (+11,5%) M2 (+7.5%) and SO4 (+2.5%) increasing their alcoholic strength from 2022 to 2023, while 1103P (-2.8%) and M3 (-2.6%) decreasing their value. It seems that the first group had a lower sugar accumulation in 2022, probably due to water stress as also supported by physiological data. Indeed, M1 and M2 had high values of CWSI.

Titratable acidity (g /L Tartaric acid) varied from 5.18 (M4) and 5.70 (SO4) in 2022 and between 6.30 (M2) and 7.30 (M3) in 2023.

For all rootstocks, values increased from 2022 to 2023 which should be related to the dominant meteorological conditions experienced by plants and berries in those years. Indeed, 2023 had higher precipitations (+ 477 mm) during the growing seasons that increased soil water contents and promotes a higher titratable acidity level (Modina *et al.*, 2023). pH values were related to titratable acidity and in both years, there were very low difference between rootstocks. SO4 guaranteed higher malic acid level in 2022 as it was able, even in severe water stress status, to keep lower leaf wall temperature (due to higher transpiration) in comparison to the other combinations, as shown by CWSI values. In the same year, SO4 was also able to reduce volatile acidity content. M4 presented its lowest value in 2023 when 1103P, followed by M3, presented high malic acid level.

Table 6 - Grape must parameters differences induced by rootstock genotype and vintage.

Rootstock	**Alcoholic strength (v/v)**		**Reducing Sugars (g/L)**		**pH**		**Titratable Acidity (g/L)**		**Malic Acid (g/L)**		**Volatile Acidity (g/L acetic acid)**	
	2022	**2023**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
1103P	12.05	11.71	1.91	0.10	3.34	3.25	5.48	7.10	1.18	3.29	0.21	0.57
M1	11.35	12.66	2.07	0.60	3.34	3.27	5.43	6.50	1.13	2.20	0.21	0.49
M2	11.79	12.67	1.89	0.40	3.31	3.29	5.64	6.30	1.15	2.30	0.21	0.47
M3	11.99	11.68	1.85	0.00	3.39	3.27	5.30	7.30	1.17	3.00	0.21	0.54
M4	12.03	12.05	1.89	0.00	3.40	3.26	5.18	7.00	1.15	2.80	0.21	0.45
SO4	11.75	12.04	2.75	0.10	3.30	3.29	5.70	6.90	1.22	2.70	0.19	0.58

Sensorial wine evaluation was performed for seasons 2022 and 2023 that, as previously discussed, represent two opposite climatic situations (Figure 10 a/b). Significance was not checked. Generally, it was possible to discriminate both the season and the rootstock effect. In 2023, SO4 was characterized by the highest olfactory intensity, with a prevalent aroma of white fruits. It was also the most persistent and balanced and, definitively, the favourite by the tasting panel. It seems that under average meteorological conditions for this wine growing area, such as the ones occurring in 2023, the SO4 wines got the best performance. This is in accordance with the fact that the SO4 is the prevalent rootstock adopted in Franciacorta, and it is well adapted to humid soils. However, it is interesting to analyse the wines of a dry season, such as 2022. In these conditions, M4 obtained good performances, having the highest sapidity and structure, a good acidity and spicy and white-fruit prevalent aroma. In light of this characteristics, M4 was the favourite wine of the tasting panel. Finally, despite the good performances of 1103P reported in the previous chapter, in both years it had the lowest overall score.

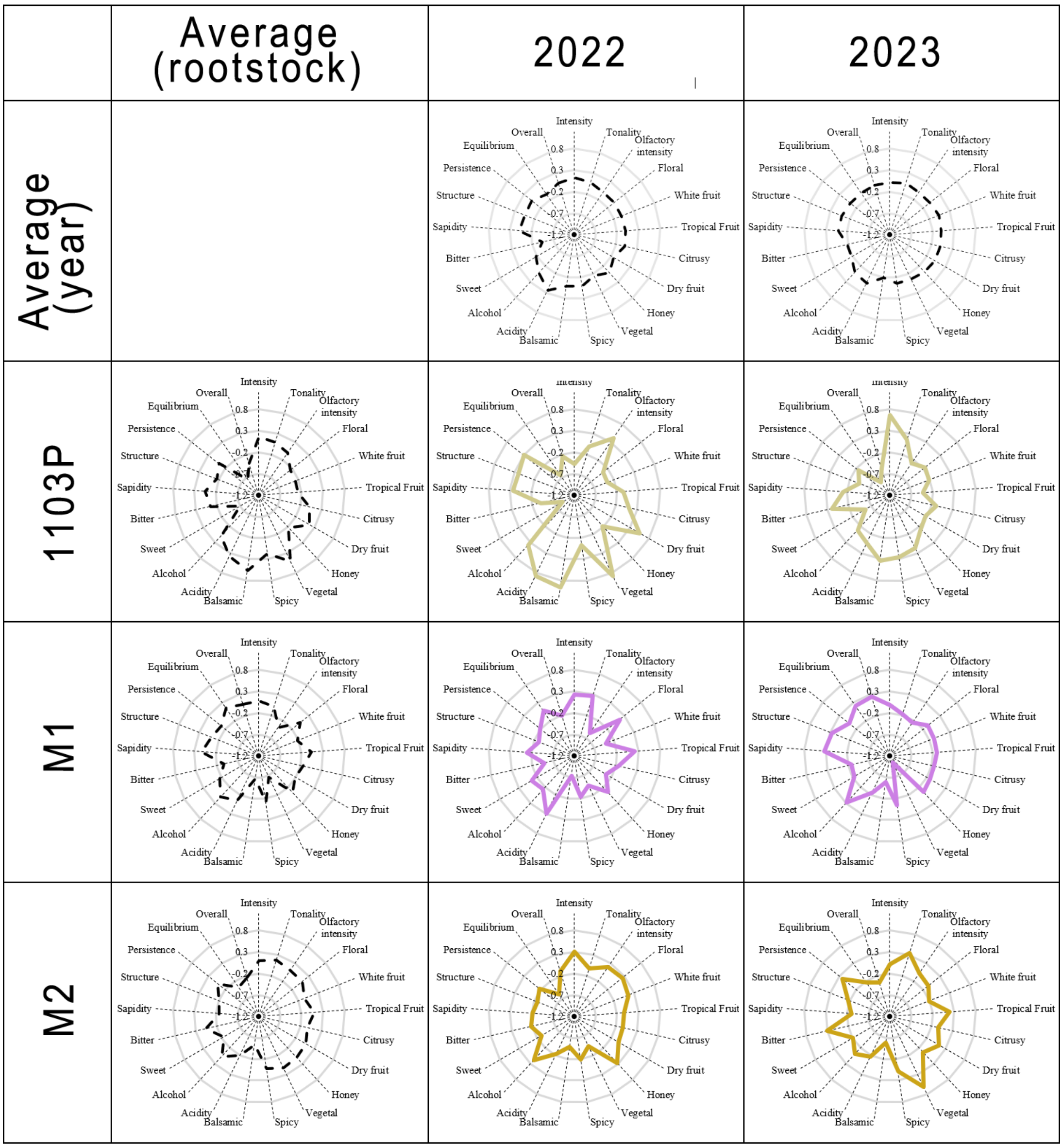

Figure 10A - Results of the sensorial analysis of the microvinifications from grapes harvested from Chardonnay vines grafted on 1103P, M1 and M2 experimental plots. Yearly average and rootstocks average diagrams are also reported.

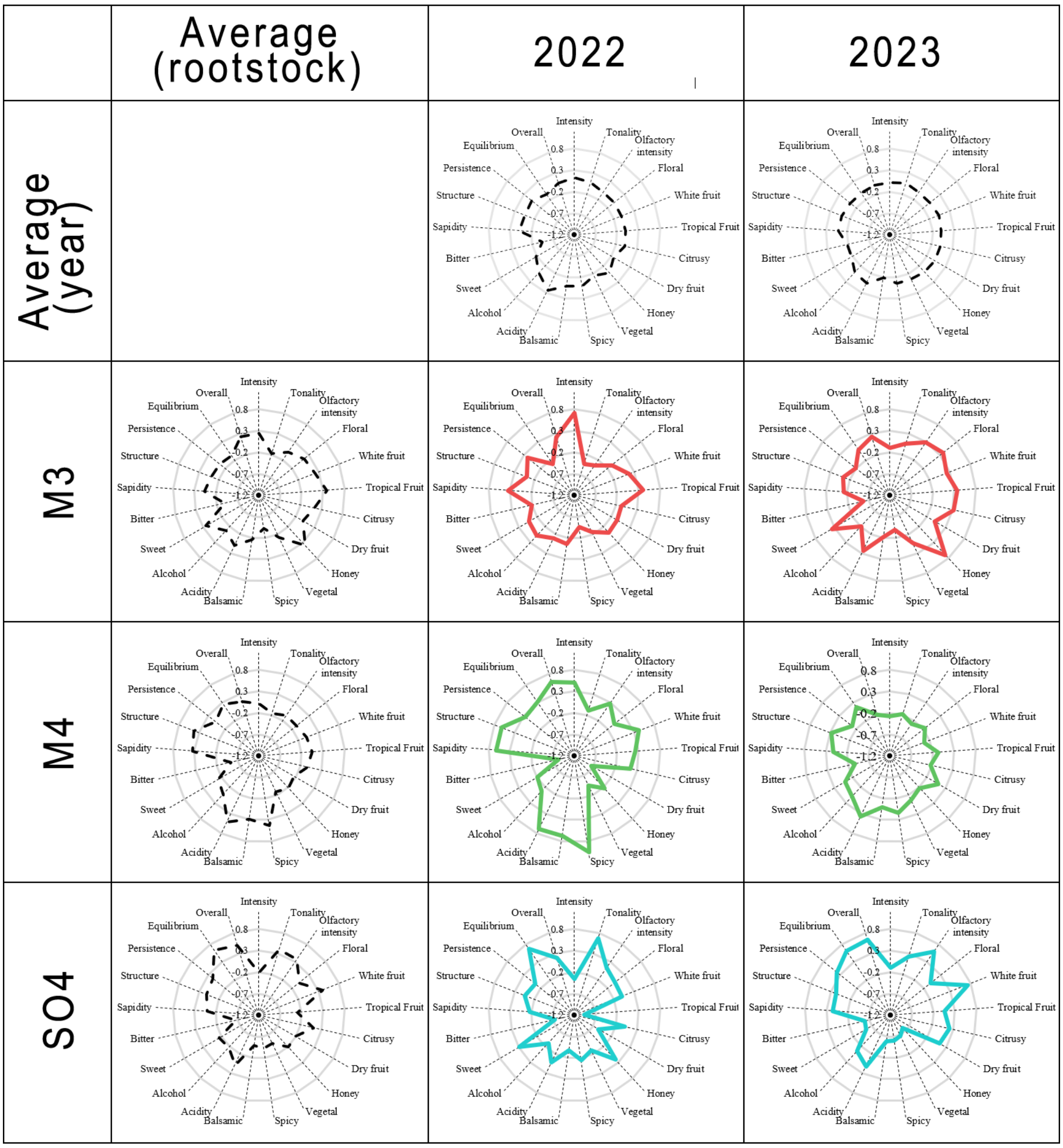

Figure 10B - Results of the sensorial analysis of the microvinifications from the M3, M4 and SO4 experimental plots. Yearly average and rootstocks average diagrams are also reported.

2.5. Conclusions

This work was devoted to the evaluation of the effects of different rootstocks on the eco-physiology of grapevine (*Vitis vinifera* cv. Chardonnay), with reference to berry quality and yield and wine quality, in the specific context of Franciacorta sparkling wine production, assessing the phenotypic stability of the different rootstock scion combinations under different environmental conditions.

With the exception of YAN and K^+, all the vegetative, productive and qualitative parameters of grapevine were affected by the scion-rootstock combination. Considering the environmental effects on scion-rootstock combination, precipitation-based indices (P_MA, P_Y and ET/ETR) showed higher R^2 values with field parameters (yield and yield/shoot). The relationships were particularly strong for pH, K^+ content, Wood Weight and Ravaz Index. Good R^2 values were also shown for pH, Ravaz Index and K^+ by HD, representative of summer heat stress. Generally,1103P scored the lowest slopes indicating the smallest variation of the investigated parameters as a response to the variation of environmental driving conditions.

In general terms, 1103P showed good general performances in terms of field parameters. It showed good tolerance to mild/moderate water stress condition, and it was characterized by late ripening and a balanced sugar and acidity profile, ideal for sparkling wine production. However, the tasting panel did not appreciate the wines derived from plants grafted on this rootstock. SO4, the main diffused rootstock in the area, demonstrated a very good tolerance to water stress, an average but stable sugar/acidity ratio along the four seasons and a good panel appreciation. Among the M rootstocks, M4 proved to be most interesting for the adoption in the specific context of Franciacorta viticulture. In fact, it was characterized by a good water stress tolerance, a high acidity-sugar ratio, concurrent with an earlier ripening that allows for a more balanced ripening in terms of aromas, as confirmed by the tasting panel scores.

3. Prospects and Future Improvements

In future, therefore, and with reference to the topic dealt with in this work, it will be necessary to study the effect of the new M rootstocks in different Italian areas and abroad, carefully analysing the effects induced on multiple thousands of cultivars existing only in Europe or at least in the most cultivated (Tempranillo tinto, Merlot noir, Garnacha Tinta, Airen, Trebbiano toscano and Chardonnay – Eurostat, 2024). The comparison with other rootstocks, and the development of new biotypes, would represent a significant achievement for modern viticulture. In particular, it would be interesting, and probably useful to analyse the American species internal variability as only few varieties of *V. riparia/berlandieri/rupestris* are used. There are promising resources in areas like Missouri River, Jack Fork River, Fredericksburg and Arizona with e.g. *Vitis arizonica* that is highly salinity tolerant or *Vitis rufotumentosa* which tolerates *Xiphinema* spp. presence (Scienza, 2021). The use of rootstocks, old or new, as agronomic tool in combination with other agronomic techniques (eg. canopy or soil management) undoubtedly represents a further perspective of study. As a matter of fact, best results will probably be found by combining different agronomical approaches to reach a winning strategy in order to resist against future and current climate change effects.

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5. Appendix

The following figures and tables are part of the statistical elaboration that were produced but, at the end, was not inserted inside this work. However, was decided to show the most interesting results.

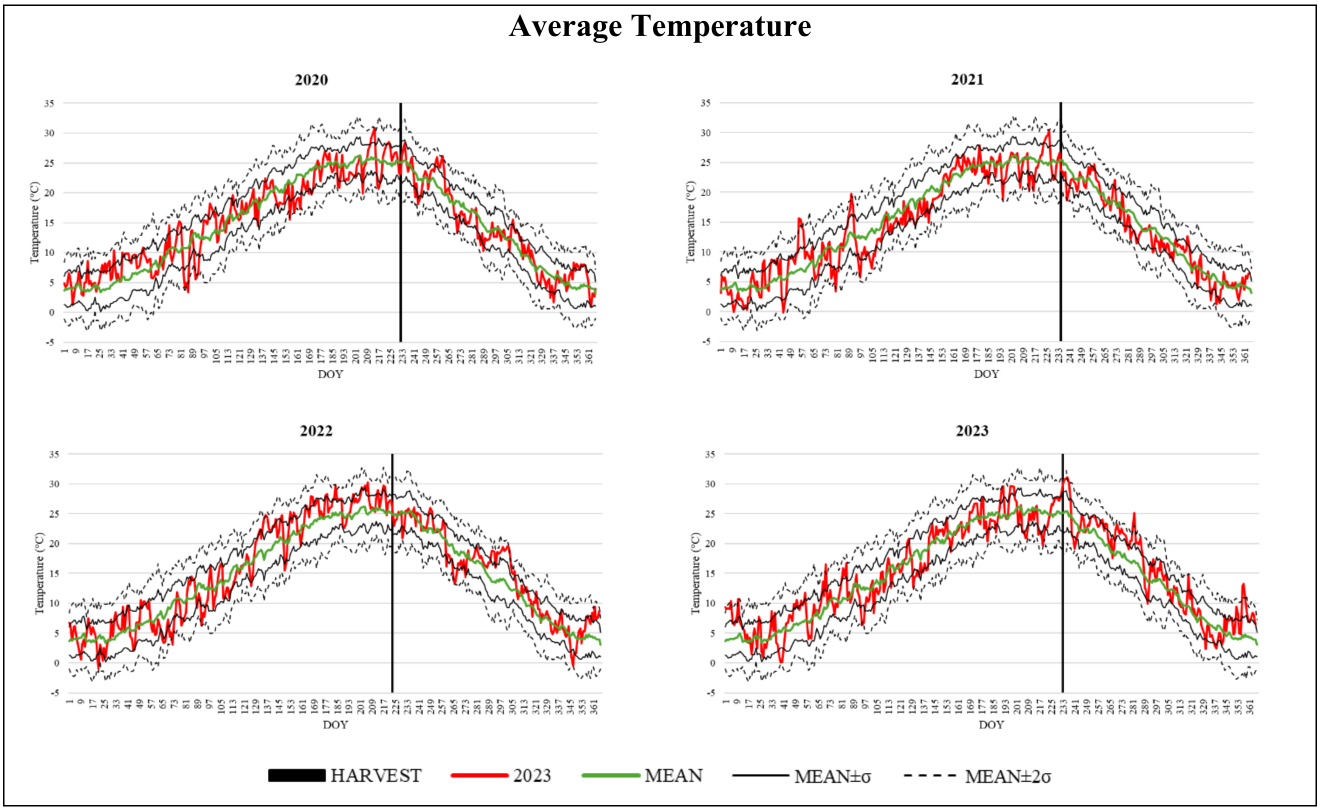


Figure 1S – Average air temperature of the four seasons compared with 1997-2023 mean.

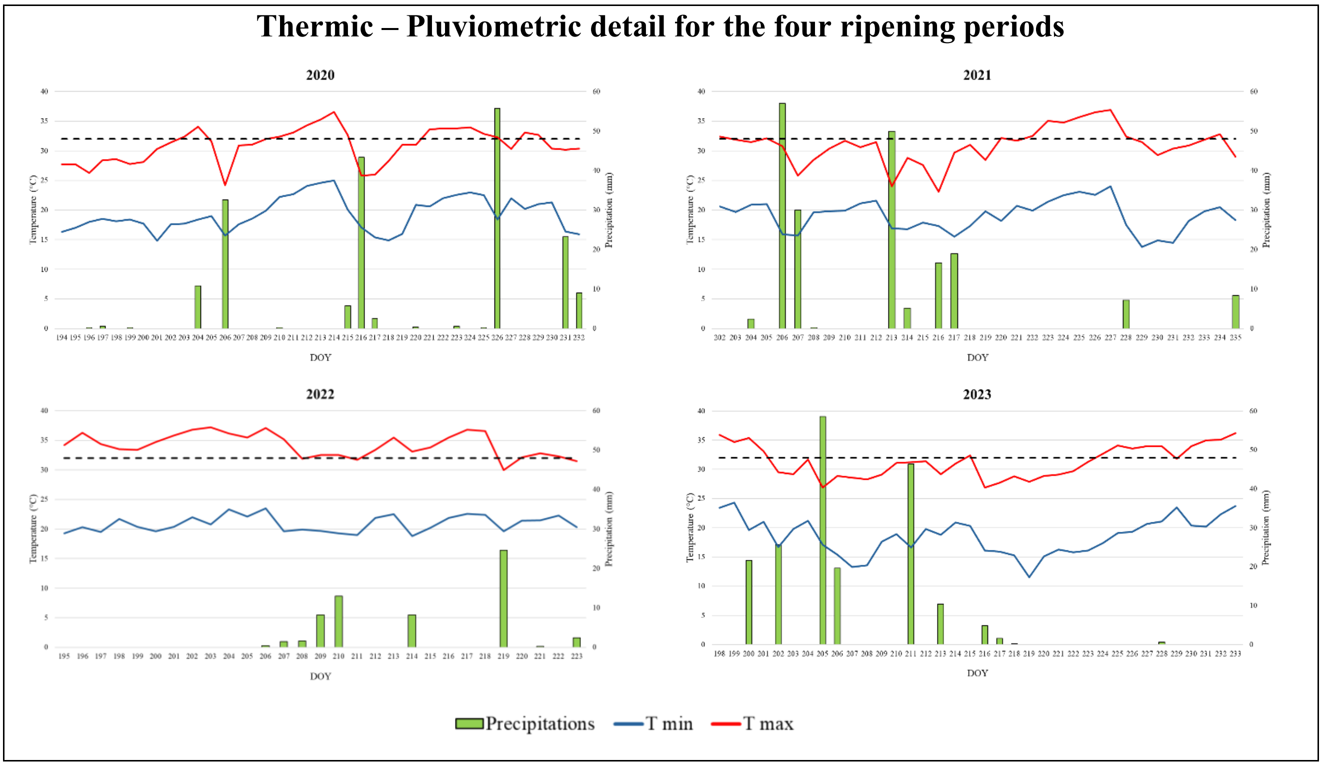


Figure 2S – Temperature and rains behaviour during the 2020, 2021, 2022 and 2023 ripening periods. Maximum temperature (red line), minimum temperature (blue line) and rains (green columns). Dotted line represents 32°C threshold.

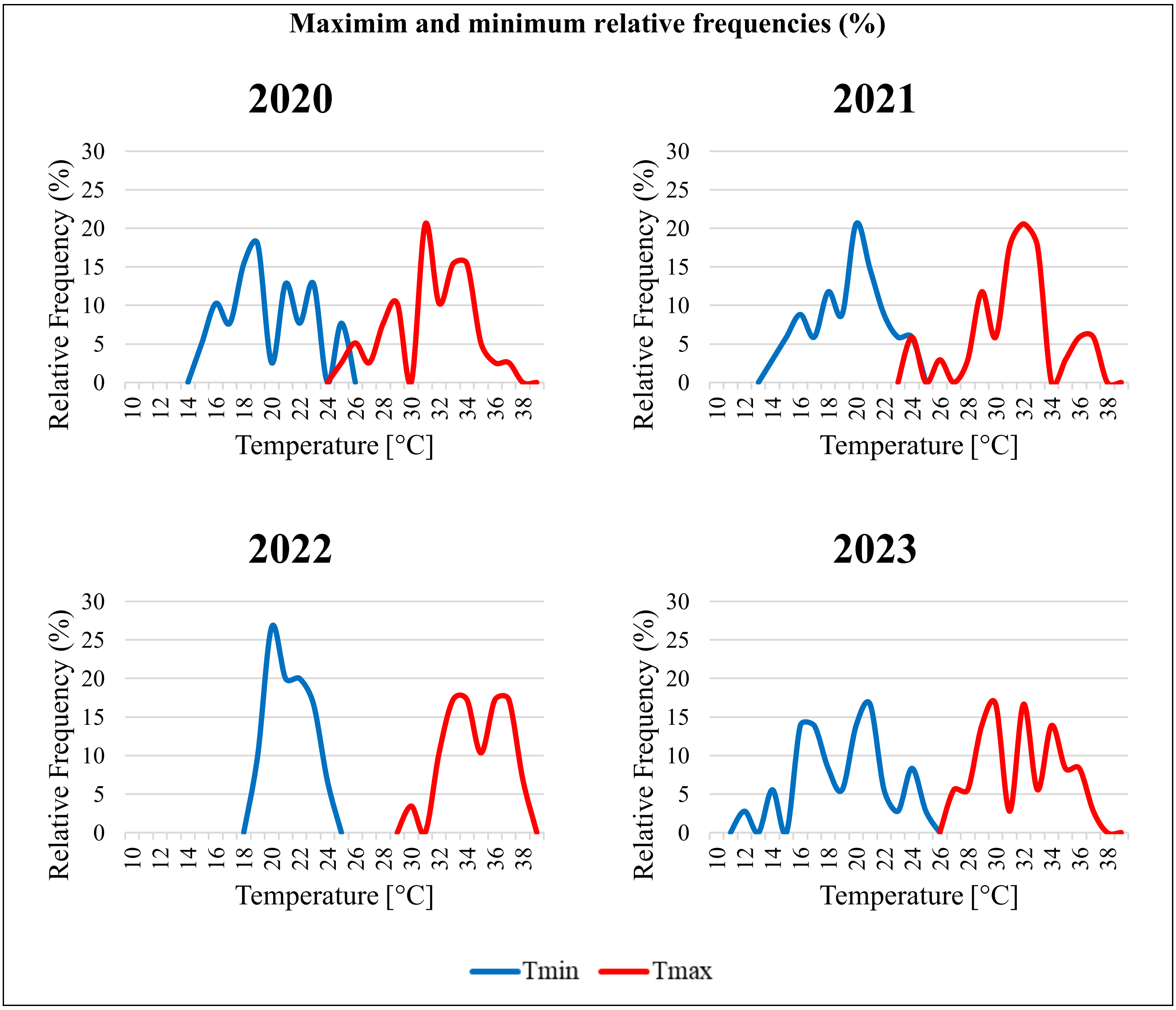


Figure 3S – Maximum and minimum temperatures relative frequencies (%) during the 2020, 2021, 2022 and 2023 ripening periods. Maximum temperature (red line) and minimum temperature (blue line).

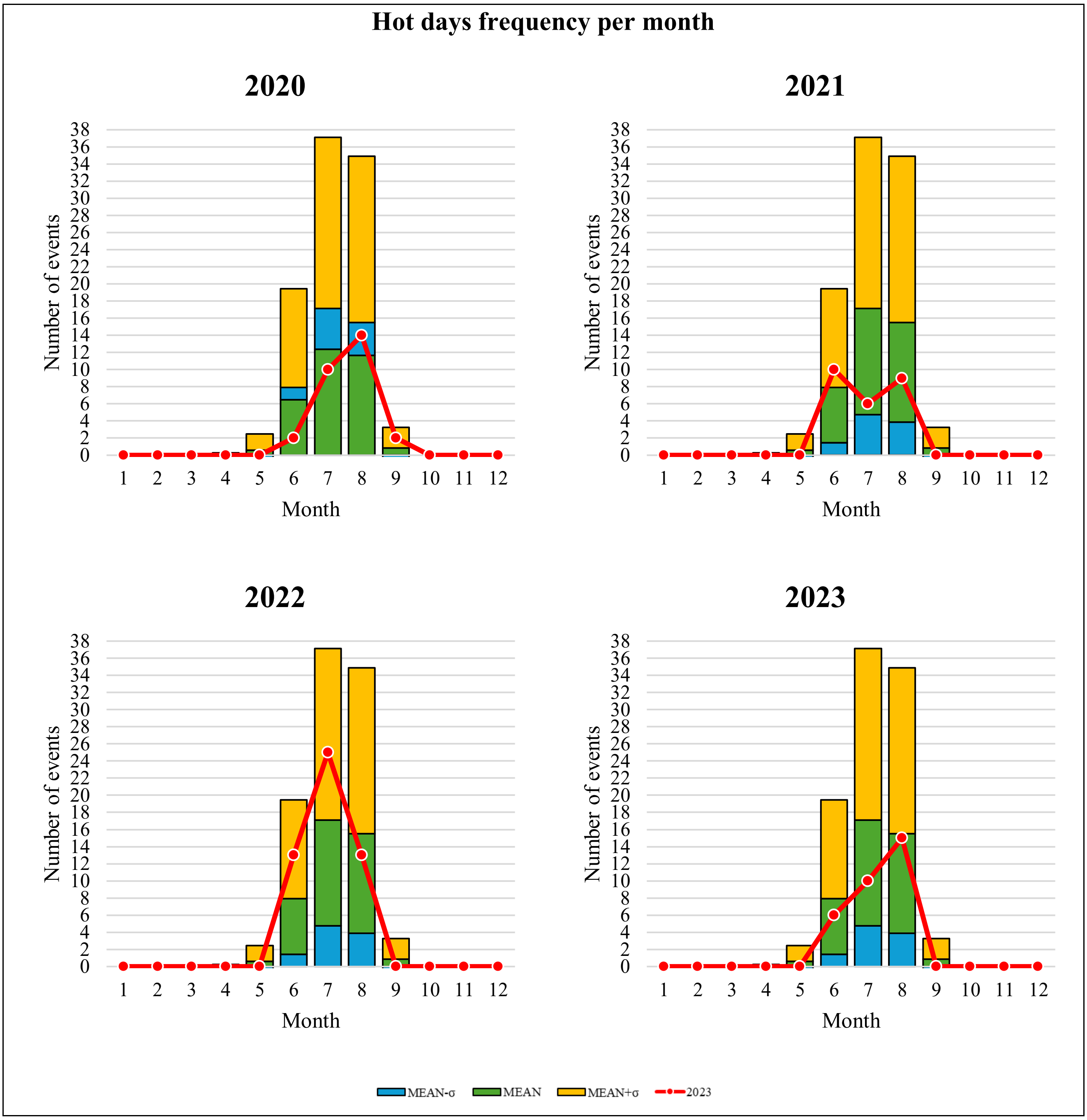
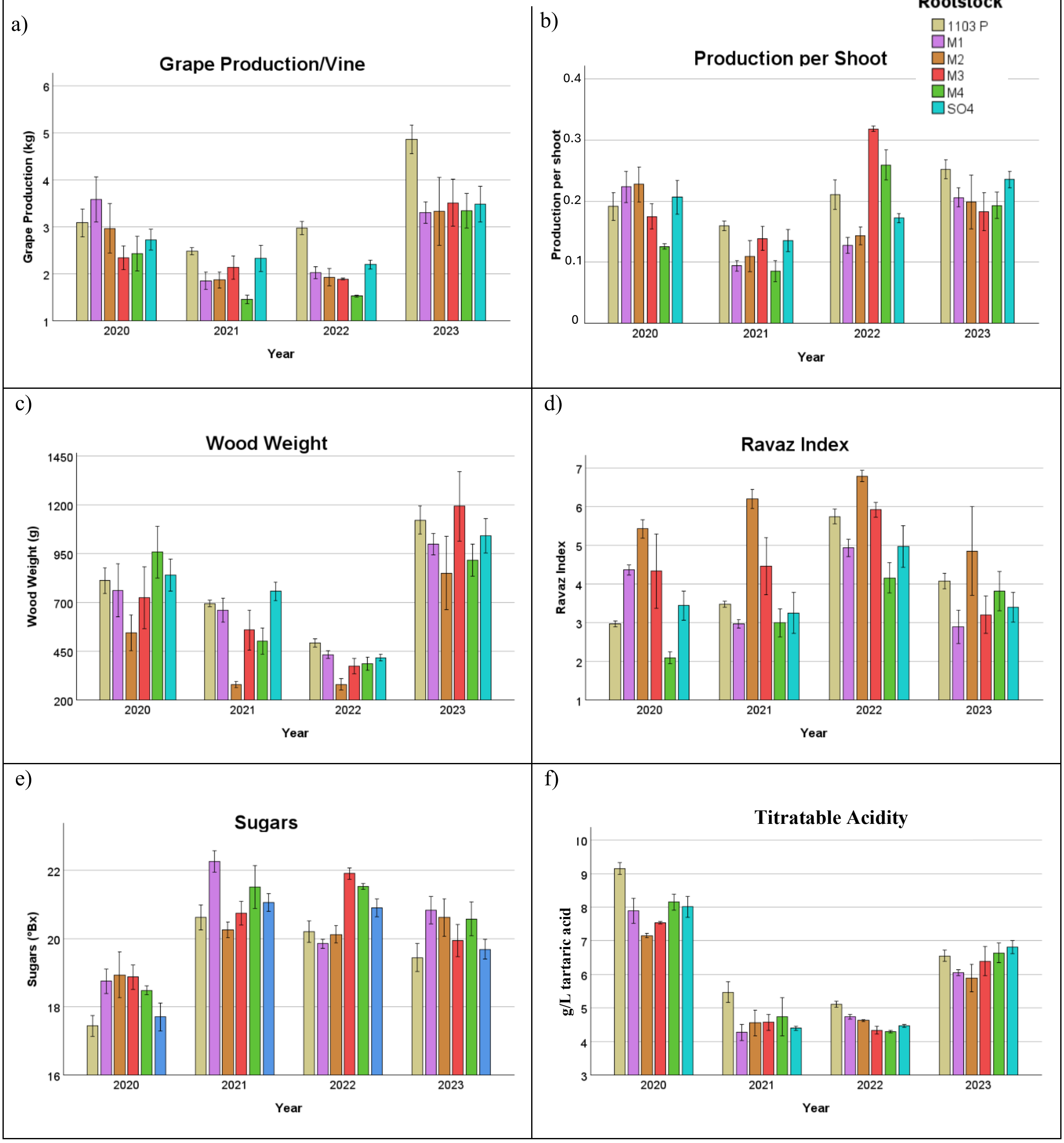


Figure 4S – Hot days (T>32°C) frequencies per month (red line) compared with mean (green) obtained from 1997-2023 data; light blue and yellow correspond to negative or positive anomalies respectively.

Vine and grape parameters in function of rootstock genotype and vintage effect



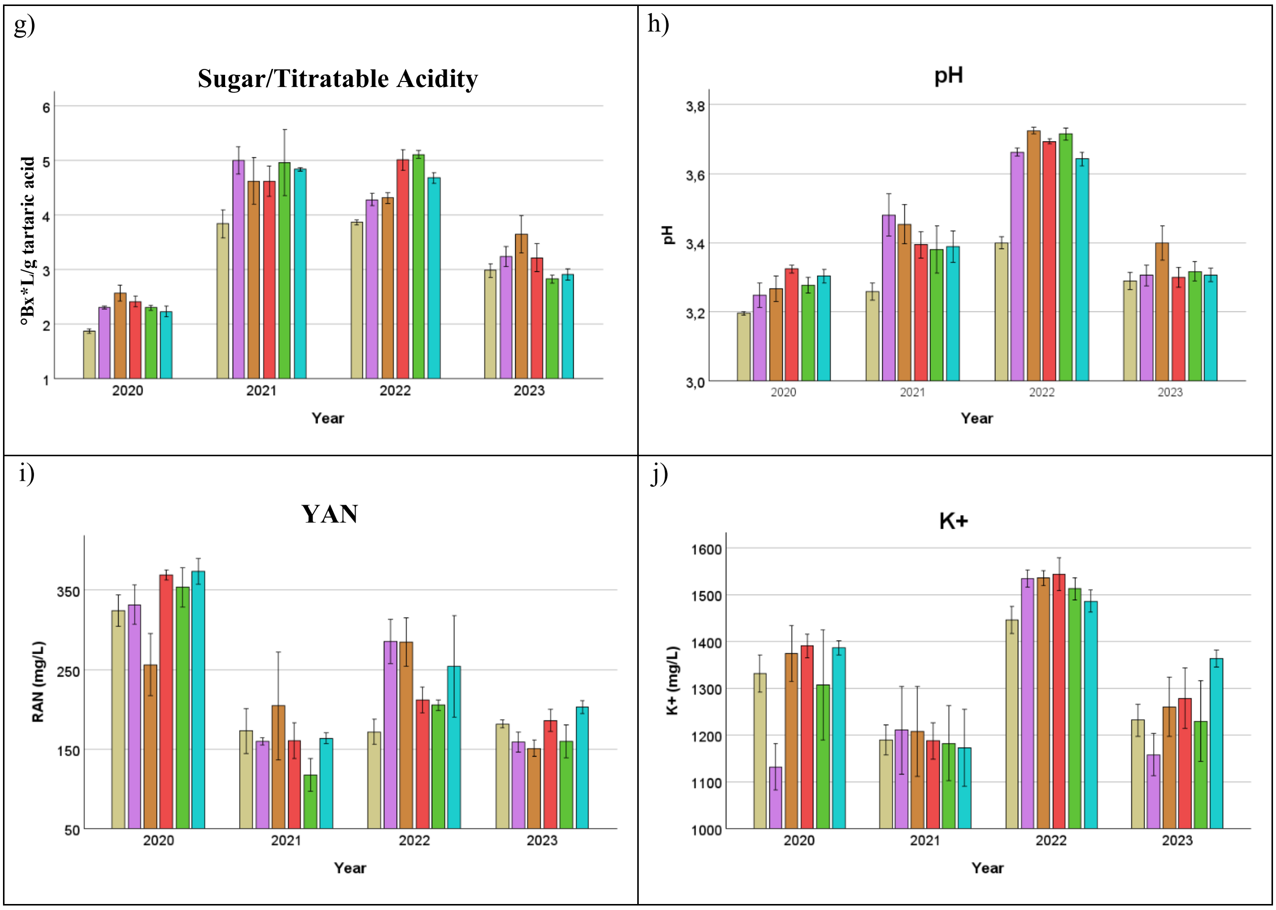
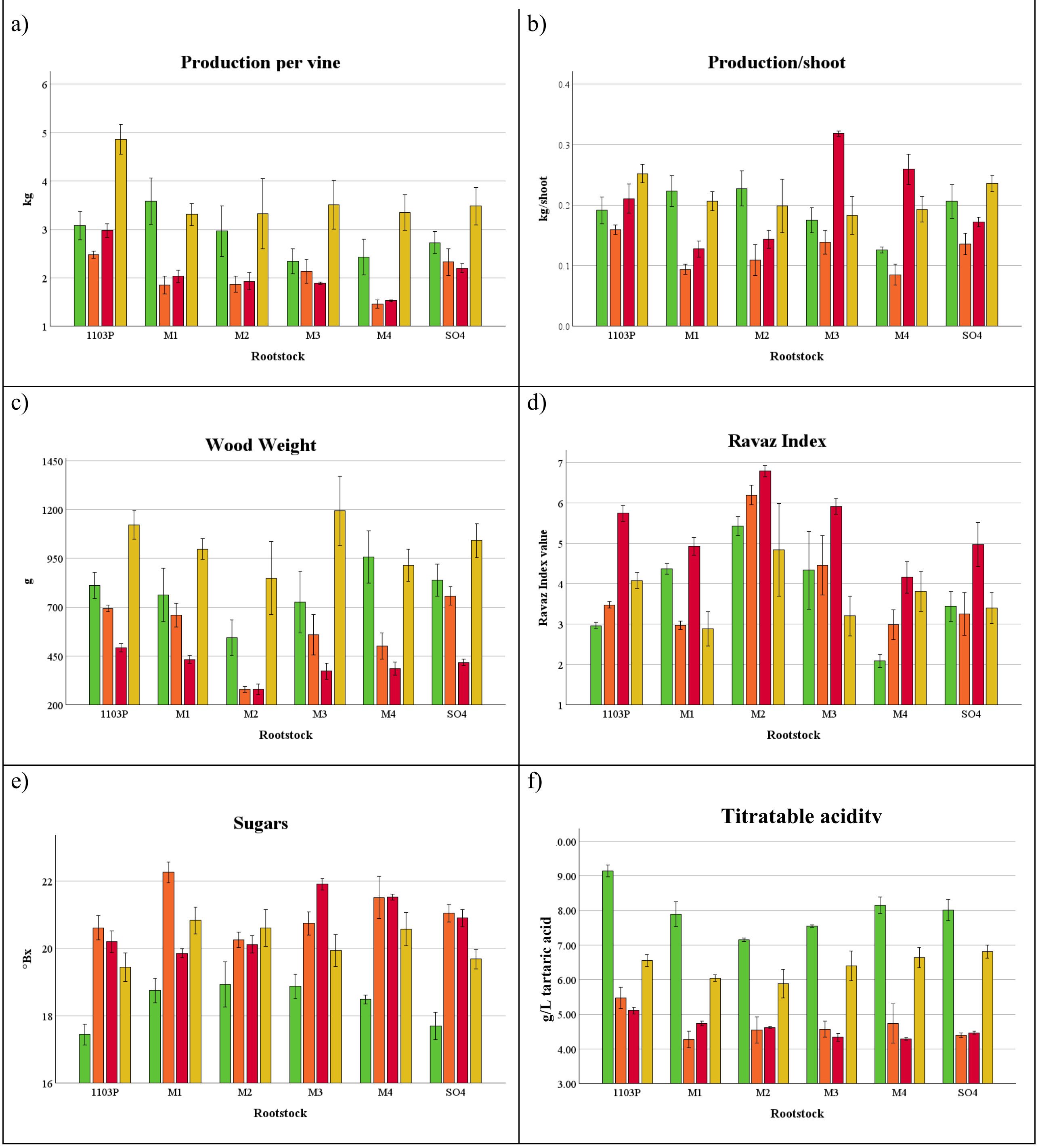


Figure 5S - Vine and grape parameters statistical differences induced by rootstock genotype and vintage. ANOVA was performed taking into consideration significance levels of p ≤ 0.001 (***), 0.001 ≤ p ≤ 0.01 (**), and 0.01 ≤ p ≤ 0.05 (*); p>0.05 (n.s.).

Table 1S - Post-hoc test (Duncan) on CWSI values. ANOVA was performed taking into consideration significance levels of $p \leq 0.001$ (***), $0.001 \leq p \leq 0.01$ (**), and $0.01 \leq p \leq 0.05$ (*); not significative (n.s.).

2020										
Rootstock	**Grape Production (kg)**	**Production/Shoot (kg/shoot)**	**Wood Weight (g)**	**Ravaz Index**	**Sugars (°Bx)**	**Titratable Acidity (g/L tartaric acid)**	**Sugars/T.Acidity (°Bx*L/g tartaric acid)**	**pH**	**YAN (mg/L)**	**K^+ (mg/L)**
Significance	n.s.	n.s.	n.s.	**	*	**	***	n.s.	*	*
1103P	-	-	-	ab	a	b	a	-	ab	b
M1	-	-	-	bc	b	a	bc	-	b	a
M2	-	-	-	c	b	a	c	-	a	b
M3	-	-	-	bc	b	a	bc	-	b	b
M4	-	-	-	a	ab	a	bc	-	b	b
SO4	-	-	-	ab	ab	a	b	-	b	b
2021										
Rootstock	**Grape Production (kg)**	**Production/Shoot (kg/shoot)**	**Wood Weight (g)**	**Ravaz Index**	**Sugars (°Bx)**	**Titratable Acidity (g/L tartaric acid)**	**Sugars/T.Acidity (°Bx*L/g tartaric acid)**	**pH**	**YAN (mg/L)**	**K^+ (mg/L)**
Significance	*	n.s.	***	**	*	n.s.	n.s.	n.s.	n.s.	n.s.
1103P	b	-	bc	a	a	-	-	-	-	-
M1	ab	-	bc	a	b	-	-	-	-	-
M2	ab	-	a	b	a	-	-	-	-	-
M3	ab	-	bc	a	a	-	-	-	-	-
M4	a	-	b	a	ab	-	-	-	-	-
SO4	b	-	c	a	ab	-	-	-	-	-
2022										
Rootstock	**Grape Production (kg)**	**Production/Shoot (kg/shoot)**	**Wood Weight (g)**	**Ravaz Index**	**Sugars (°Bx)**	**Titratable Acidity (g/L tartaric acid)**	**Sugars/T.Acidity (°Bx*L/g tartaric acid)**	**pH**	**YAN (mg/L)**	**K^+ (mg/L)**
Significance	***	***	**	***	***	***	***	***	n.s.	n.s.
1103P	c	bc	c	bc	a	d	a	a	-	-
M1	b	a	bc	ab	a	c	b	bc	-	-
M2	b	a	a	c	a	bc	bc	d	-	-
M3	ab	d	b	bc	c	a	de	cd	-	-
M4	a	c	b	a	bc	a	e	d	-	-
SO4	b	ab	bc	ab	b	ab	cd	b	-	-
2023										
Rootstock	**Grape Production (kg)**	**Production/Shoot (kg/shoot)**	**Wood Weight (g)**	**Ravaz Index**	**Sugars (°Bx)**	**Titratable Acidity (g/L tartaric acid)**	**Sugars/T.Acidity (°Bx*L/g tartaric acid)**	**pH**	**YAN (mg/L)**	**K^+ (mg/L)**
Significance	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
1103P	-	-	-	-	-	-	-	-	-	-
M1	-	-	-	-	-	-	-	-	-	-
M2	-	-	-	-	-	-	-	-	-	-
M3	-	-	-	-	-	-	-	-	-	-
M4	-	-	-	-	-	-	-	-	-	-
SO4	-	-	-	-	-	-	-	-	-	-

Agronomic parameters grouped per rootstock



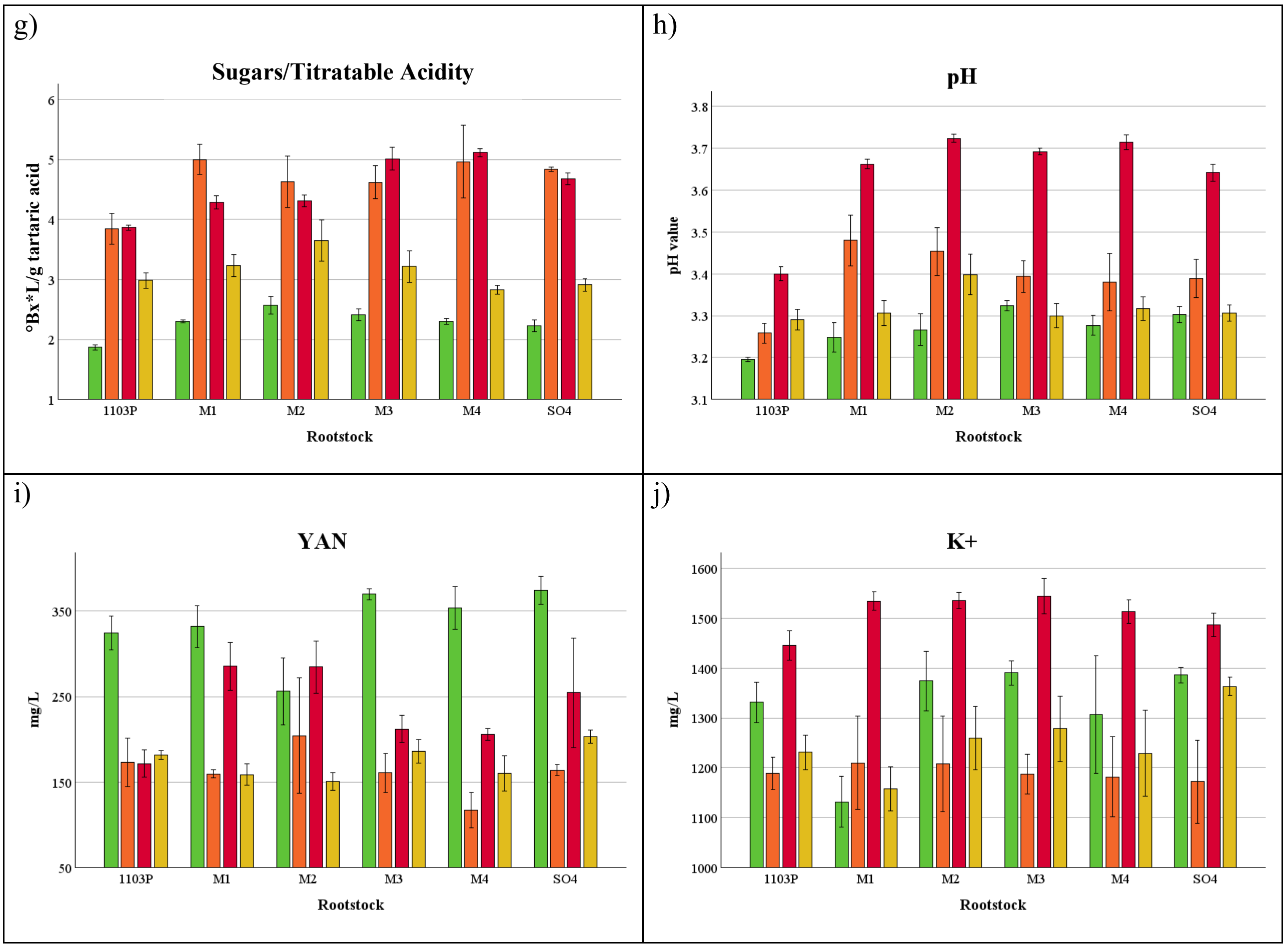


Figure 6S - Vine and grape parameters statistical differences induced by rootstock genotype and vintage. ANOVA was performed taking into consideration significance levels of $p \leq 0.001$ (***), $0.001 \leq p \leq 0.01$ (**), and $0.01 \leq p \leq 0.05$ (*).

Table 2S - R^2 and regression coefficient B of the regressions calculated on productive, vegetative and qualitative parameters for different agrometeorological factors (TY, HD. WNK, HUGH, P_Y, P_MA, ETM/ETR).

TY	**Yield (kg)**		**Yield / Shoot (kg/shoot)**		**Sugars (°Bx)**		**pH**		**Titratable Acidity (g/L tartaric acid)**		**Wood weight (g)**		**Ravaz index**		**K^+ (mg/L)**		**YAN (mg/L)**	
	R2	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**
1103P	0.440	1.270	**0.804**	0.063	0.006	-0.192	0.372	0.095	0.009	-0.319	0.031	84.960	0.450	1.478	0.326	118.779	0.030	-23.714
M1	0.080	0.470	0.171	0.470	0.202	-1.221	0.019	0.047	0.021	0.423	0.006	33.220	0.126	0.660	0.185	146.087	0.034	29.583
M2	0.150	0.520	0.152	0.380	0.069	0.347	0.156	0.138	0.008	0.203	0.206	224.228	0.020	-0.221	0.339	153.936	0.001	4.021
M3	0.470	0.500	**0.510**	0.101	0.049	0.517	0.115	0.112	0.006	0.222	0.082	1830.065	0.004	0.128	0.413	179.886	0.000	-0.617
M4	0.220	0.760	**0.850**	0.129	0.001	0.990	0.171	0.150	0.001	0.075	0.013	60.130	0.443	1.117	0.363	160.219	0.012	20.398
SO4	0.160	0.420	0.450	0.053	0.002	-0.128	0.117	0.099	0.027	0.540	0.008	-43.423	0.351	0.871	**0.733**	204.458	0.022	24.918
HD	**Yield (kg)**		**Yield / Shoot (kg/shoot)**		**Sugars (°Bx)**		**pH**		**Titratable Acidity (g/L tartaric acid)**		**Wood weight (g)**		**Ravaz index**		**K^+ (mg/L)**		**YAN (mg/L)**	
	R2	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**
1103P	0.002	-0.003	0.106	0.001	0.083	0.034	**0.812**	0.007	0.222	-0.073	0.330	-12.870	**0.899**	0.097	**0.751**	8.416	0.121	-2.210
M1	0.123	-0.260	0.047	-0.001	0.110	-0.042	**0.535**	0.012	0.095	-0.043	0.432	-13.118	**0.523**	0.063	**0.883**	14.901	0.144	2.847
M2	0.128	-0.220	0.033	-0.001	0.028	0.010	**0.755**	0.014	0.161	-0.042	0.104	-7.449	0.371	0.044	**0.790**	10.977	0.350	2.968
M3	**132.000**	-0.220	**0.992**	0.007	**0.534**	0.080	**0.827**	0.014	0.236	-0.063	0.227	-14.231	**0.557**	0.071	**0.763**	11.417	0.013	-0.927
M4	0.094	-0.230	**0.838**	0.006	0.160	0.049	**0.863**	0.016	0.269	-0.079	0.319	-13.892	**0.503**	0.056	**0.889**	11.701	0.000	0.105
SO4	0.143	-0.019	0.001	0.001	0.139	0.049	**0.819**	0.012	0.172	-0.063	**0.601**	-17.140	**0.978**	0.068	**0.626**	8.816	0.006	0.594
WNK	**Yield (kg)**		**Yield / Shoot (kg/shoot)**		**Sugars (°Bx)**		**pH**		**Titratable Acidity (g/L tartaric acid)**		**Wood weight (g)**		**Ravaz index**		**K^+ (mg/L)**		**YAN (mg/L)**	
	R2	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**
1103P	0.184	0.003	**0.531**	0.000	0.019	0.001	**0.665**	0.000	0.105	-0.003	0.016	-0.192	**0.753**	0.006	**0.505**	0.466	0.108	-0.141
M1	0.000	0.000	0.017	0.000	0.139	-0.003	0.196	0.000	0.010	-0.001	0.052	-0.309	0.248	0.003	0.485	0.745	0.049	0.112
M2	0.006	0.000	0.016	0.000	0.095	0.001	0.437	0.001	0.029	-0.001	0.023	0.235	0.032	0.001	**0.534**	0.609	0.058	0.081
M3	0.011	0.000	**0.781**	0.000	0.250	0.004	0.385	0.001	0.042	-0.002	0.000	-0.024	0.125	0.002	**0.579**	0.671	0.015	-0.065
M4	0.027	0.000	**0.995**	0.000	0.067	0.002	0.468	0.001	0.066	-0.003	0.041	-0.336	**0.614**	0.004	**0.603**	0.650	0.000	0.004
SO4	0.010	0.000	0.159	0.000	0.036	0.002	0.390	0.001	0.014	-0.001	0.153	-0.583	**0.654**	0.004	**0.750**	0.651	0.004	0.032
HUGH	**Yield (kg)**		**Yield / Shoot (kg/shoot)**		**Sugars (°Bx)**		**pH**		**Titratable Acidity (g/L tartaric acid)**		**Wood weight (g)**		**Ravaz index**		**K^+ (mg/L)**		**YAN (mg/L)**	
	R2	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**
1103P	0.040	0.002	0.301	0.000	0.000	0.000	**0.584**	0.001	0.042	-0.003	0.112	-0.723	**0.705**	0.008	**0.793**	0.834	0.018	-0.082
M1	0.000	0.000	0.016	0.000	0.312	-0.007	0.234	0.001	0.000	0.000	0.179	-0.813	**0.544**	0.006	**0.591**	1.175	0.238	0.343
M2	0.001	0.000	0.025	0.000	0.002	0.000	0.438	0.001	0.009	-0.001	0.001	-0.071	0.110	0.002	**0.814**	1.074	0.265	0.249
M3	0.019	-0.001	**0.917**	0.001	0.224	0.050	**0.525**	0.001	0.030	-0.002	0.053	-0.665	0.306	0.005	**0.849**	1.161	0.009	0.075
M4	0.000	0.000	**0.928**	0.001	0.016	0.002	**0.560**	0.001	0.046	-0.003	0.063	-0.596	0.382	0.005	**0.852**	1.104	0.052	0.192
SO4	0.012	-0.001	0.086	0.000	0.007	0.001	**0.504**	0.001	0.008	-0.001	0.320	-1.205	**0.815**	0.006	**0.894**	1.015	0.082	0.215
P_Y	**Yield (kg)**		**Yield / Shoot (kg/shoot)**		**Sugars (°Bx)**		**pH**		**Titratable Acidity (g/L tartaric acid)**		**Wood weight (g)**		**Ravaz index**		**K^+ (mg/L)**		**YAN (mg/L)**	
	R2	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**
1103P	0.114	0.002	0.001	0.000	0.066	-0.002	**0.646**	-0,0003	0.179	0.003	**0.623**	0.940	**0.728**	-0.005	**0.821**	-0.468	0.052	0.077
M1	0.213	0.002	0.116	0.000	0.127	0.002	**0.657**	-0.001	0.101	0.002	**0.715**	0.899	**0.700**	-0.040	**0.922**	-0.810	0.270	-0.208
M2	0.302	0.002	0.076	0.000	0.001	0.000	**0.725**	-0.001	0.174	0.002	0.324	0.698	**0.619**	-0.003	**0.853**	-0.607	**0.628**	-0.211
M3	0.397	0.002	**0.911**	0.000	**0.530**	-0.004	**0.937**	-0.001	0.282	0.004	**0.512**	1.136	**0.833**	-0.005	**0.784**	-3616.000	0.000	0.001
M4	0.300	0.002	**0.565**	0.000	0.125	-0.002	**0.899**	-0.001	0.300	0.004	0.452	0.880	0.266	-0.002	**0.927**	-0.636	0.012	-0.052
SO4	0.395	0.002	0.069	0.000	0.127	-0.002	**0.905**	-0.001	0.228	0.004	**0.851**	1.085	**0.973**	-0.004	0.485	-0.413	0.030	-0.072

P_MA	**Yield (kg)**		**Yield / Shoot (kg/shoot)**		**Sugars (°Bx)**		**pH**		**Titratable Acidity (g/L tartaric acid)**		**Wood weight (g)**		**Ravaz index**		**K^{+} (mg/L)**		**YAN (mg/L)**	
	R2	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**
1103P	0.152	0.002	0.011	0.000	**0.515**	-0.004	**0.811**	0.000	**0.674**	0.006	0.424	0.824	**0.775**	-0.004	0.309	-0.253	0.429	0.195
M1	**0.681**	0.003	**0.544**	0.000	0.032	-0.001	**0.988**	-0.001	**0.583**	0.005	**0.553**	0.785	0.207	-0.002	**0.923**	-0.715	0.002	-0.014
M2	**0.628**	0.002	**0.551**	0.000	0.148	-0.001	**0.969**	-0.001	**0.685**	0.004	0.220	0.749	**0.781**	-0.003	0.350	-0.343	0.257	-0.119
M3	0.360	0.002	**0.604**	0.000	**0.949**	-0.005	**0.914**	-0.001	**0.794**	0.005	0.277	1.009	**0.690**	-0.004	0.274	-0.321	0.240	0.184
M4	0.489	0.002	0.352	0.000	**0.607**	-0.004	**0.942**	-0.001	**0.813**	0.006	**0.806**	1.078	**0.496**	-0.003	0.479	-0.403	0.161	0.164
SO4	0.472	0.002	0.309	0.000	**0.620**	-0.005	**0.947**	-0.001	**0.733**	0.006	**0.680**	0.920	**0.721**	-0.003	0.090	-0.157	0.119	0.126

ETM/ ETR	**Yield (kg)**		**Yield / Shoot (kg/shoot)**		**Sugars (°Bx)**		**pH**		**Titratable Acidity (g/L tartaric acid)**		**Wood weight (g)**		**Ravaz index**		**K^{+} (mg/L)**		**YAN (mg/L)**	
	R2	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**	**R2**	**B**
1103P	0.057	0.015	0.014	0.000	0.172	-0.034	**0.823**	-0.005	0.333	0.062	**0.531**	11.310	**0.879**	-0.067	**0.680**	-5.557	0.171	1.814
M1	0.290	0.028	0.173	0.002	0.042	0.018	**0.761**	-0.010	0.208	0.044	**0.634**	11.041	**0.508**	-0.043	**0.983**	-10.900	0.117	-1.779
M2	0.314	0.024	0.139	0.001	0.030	-0.007	**0.871**	-0.011	0.299	0.040	0.274	8.357	**0.596**	-0.039	**0.725**	-7.284	0.432	-2.283
M3	0.285	0.023	**0.924**	-0.004	**0.690**	-0.063	**0.961**	-0.010	0.407	0.057	0.415	13.329	**0.734**	-0.056	**0.661**	-7.361	0.037	1.066
M4	0.261	0.027	**0.652**	-0.004	0.262	-0.043	**0.973**	-0.012	0.437	0.069	**0.533**	12.444	0.456	-0.037	**0.839**	-7.880	0.006	0.455
SO4	0.318	0.019	**0.680**	0.001	0.253	-0.046	**0.956**	-0.009	0.336	0.061	**0.788**	13.598	**0.979**	-0.047	0.425	-5.031	0.000	0.059

Figure 7S: Regression charts can be consulted online: https://unimi2013-my.sharepoint.com/:f:/g/personal/gabriele_cola_unimi_it/EqyKmEjEkZlCrUIvYVChxQMBp88idxTXmhf8kfMyITRnPg?e=n36pko