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Adaptation of the regional agronomic diagnosis for grapevine yield analysis

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ABSTRACT

Yield issues are crucial for winegrowers. In France, yield is often lower than winegrowers' objectives, and there is a need to understand the causes and magnitude of yield variability in vineyard systems. The yield formation process for grapevine (*Vitis vinifera*) takes place over two consecutive years. Grapevine is also characterised by a limited control of phenology, a winter dormancy period and the existence of storage organs. We adapted the regional agronomic diagnosis approach as defined by Doré *et al.* (1997) to quantify grapevine yield variability in two complementary case studies. The regional agronomic diagnosis proved useful in acquiring knowledge on grapevine functioning, reflecting on the data collection strategy, and leveraging interventions to better control yield by integrating multiple factors, particularly in the context of transitioning viticulture systems. Furthermore, the successful application of a regional agronomic diagnosis to grapevine crops can be considered as a major contribution to increasing the understanding of yield formation. It helps winegrowers manage yield variability when addressing planned or unplanned changing conditions, such as an agroecological transition or climate change.

KEYWORDS: grapevine yield, pest pressure, berry quality, water status, nitrogen status, limiting factors

INTRODUCTION

In modern agriculture, maintaining sustainable yield levels involves reducing agricultural threats to the environment and dealing with climatic change, among other challenges.

For perennial crops like grapevine (*Vitis vinifera*, Figure 1), replanting is slow and highly constrained by regulations. The best option for winegrowers is often to implement new vineyard management strategies. In fact, some innovative crop management practices can help ensure greater sustainability and mitigate the negative effects of climate change or pest pressure on grapevine production in terms of quantity and quality. Numerous studies have investigated the management and technological options, decision support tools or innovative agricultural methods to manage grapevine yields, regulate pests and support optimal use of soil water and nitrogen resources in changing contexts (Davy *et al.,* 2010; Léger *et al.*, 2010; Pertot *et al*., 2017; Ripoche *et al*., 2011; Victorino *et al.,* 2020). These studies often examine whether production remained stable by comparing the yields of a reference system and an assessed system with a modified management strategy over a multi-year period. This method is especially relevant when a vineyard management system is undergoing an agroecological transition or adapting to climate change. However, interannual variability is not explicitly considered in these studies, although multi-year productivity must be maintained when adjusting vineyard management strategies.

In agriculture, a crop's potential yield corresponds to the yield that would be possible if water and nutrients were not limited and biotic stress was low (van Ittersum and Rabbinge, 1997). Annual and interannual factors such as solar radiation, temperature and atmospheric CO_2 strongly impact grapevine fruitfulness and overall yield potential (Zhu *et al.*, 2020). Indeed Zhu *et al.* (2020) showed that temperature, radiation and rainfall explained 60 % to 85 % of interannual variations in cluster number, berry number, berry and cluster mass, and yield per vine in four separate vineyards located in one region and employing two vertical shoot position training systems. In practice, winegrowers do not have a clear idea of potential yield because their main concern is to maintain stable yields from year to year to achieve consistent fruit quality, supply and income. One difficulty when trying to study grapevine yields is that yields differ greatly from one plot to another depending on soil, variety, microclimate and production objectives. Grapevine yields are also highly subject to interannual variations, which usually exceed 15 % and often 35 % (Clingeleffer, 2010), which is higher than those observed in other crops. As a perennial crop, the grapevine features an indeterminate development pattern (Lebon *et al*., 2004) and has a lifespan of up to several decades. The vegetative and reproductive phases usually occur simultaneously and over several years. More specifically, grapevine yield formation extends over two consecutive years that will be hereafter referred to as year *n-1* and year *n*, with year *n* being the year of harvest. Although grapevines are grown in very diverse soil conditions, they are always managed according to yield potential, through the choice of architecture and pruning or certified origin product standards. Finally, the harvest date does not correspond to agricultural or physiological maturity but rather to oenological maturity (Bonada and Oscar Sadras, 2015). Thus, "maturity" is a complex trade-off between grape quantity, berry health, sugar, colour, acidity and aromatic components. In fact, winegrowers do not generally try to reach the potential yield but instead strike a balance between the quantity and quality of the grapes at harvest. Grapevine yield formation is therefore a complex issue with multiple variables.

Consequently, methods and tools are required to structure the grapevine yield analysis (Laurent *et al.*, 2021). This is particularly true when winegrowers are transitioning to agroecological practices such as organic viticulture, soil cover cropping, lower pesticide use and increasing biocontrol use, all of which may affect yield variability.

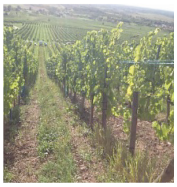
	Case study	ALSACE	PACA
Alsace 	Vineyard area	Alsace	Côtes du Rhône
	Climate	Semi-continental	Mediterranean
	Viticulture systems	Certified origin products; integrated, organic or biodynamic certification	Certified origin products; organic certification
	Wine production	Vinification by the winegrower	Cooperative and vinification by the winegrower
PACA 	Number of fields	7 fields	44 fields
	Grape varieties	Riesling; Pinot noir	Grenache
	Explanatory factors	Water, nitrogen, Botrytis	Water, nitrogen, pests and diseases
	References	Thiollet-Scholtus *et al.*, 2016	Merot and Smits, 2018; Merot *et al.*, 2020

FIGURE 1. Main characteristics of the two case studies described in this paper: network size, grape varieties and potential explanatory factors for yield variability. The regional agronomic diagnosis was applied to these complementary case studies, which cover a range of economic and climatic contexts.

Doré *et al.* (1997) proposed a methodological framework for analysing cropping system drivers, namely the regional agronomic diagnosis. Some studies on perennial crops have demonstrated the relevance of regional agronomic diagnosis for identifying and ranking limiting factors for crop yield at a regional scale (Jagoret *et al.*, 2017; Woittiez *et al.*, 2017).

In this paper, we go further by adapting and applying regional agronomic diagnosis to grapevine to help winegrowers quantify yield variability and uncertainty. We hypothesised that the grapevine agronomic yield diagnosis in organic and low input systems can be used to identify and rank the factors which limit yields and yield components in changing environments. To demonstrate this, we applied the regional agronomic diagnosis separately to two complementary datasets obtained from two wine regions in France over several years. We followed the six-step methodology and discuss the limiting factors of yield. After presenting these individual results, we then discuss the added value of this regional agronomic diagnosis method and put the two case studies into perspective together.

MATERIALS AND METHODS

1. Yield formation and related processes

Regardless of the production system or region, grapevine yield can be analysed by breaking it down into three main yield components (Guilpart *et al*., 2014; Zhu *et al*., 2020; Merot and Smits, 2020). These yield components are determined successively during the production cycle (Guilpart *et al.*, 2014): first, the number of clusters per vine, then the number of berries per cluster, and finally the average mature berry mass (Eq. 1). According to the literature, the number of clusters per vine and the number of berries per cluster account for about 60 % and 30 % of yield variation, respectively, whereas the berry mass accounts for about 10 % of yield variation (Clingeleffer, 2010; Dry, 2000; Guilpart *et al.*, 2014).

Eq. 1. Yield (kg per plant) = *number of clusters* (per plant) x *number of berries per cluster x average berry mass (kg)*

The formation of grapevine yield and its components (Figure 2) starts in year *n-1* at flowering and finishes at harvest in year *n*. Inflorescence initialisation and differentiation take place in year *n-1* from March to August, and their development occurs in year *n* after bud break (Keller *et al.*, 2010). The number of mature berries per cluster depends on the number of flowers per inflorescence, that is, the number of flower buds that actually produce a flower. The number of flowers per inflorescence is established in year *n* from April to May in the northern hemisphere (Srinivasan and Mullins, 1981). The percentage of fruit set reflects the number of flowers that turn into green berries after flowering. The green berries grow from fruit set stage to bunch closure stage, at which point the ripening phase begins; this constitutes the third yield component: mature berry mass.

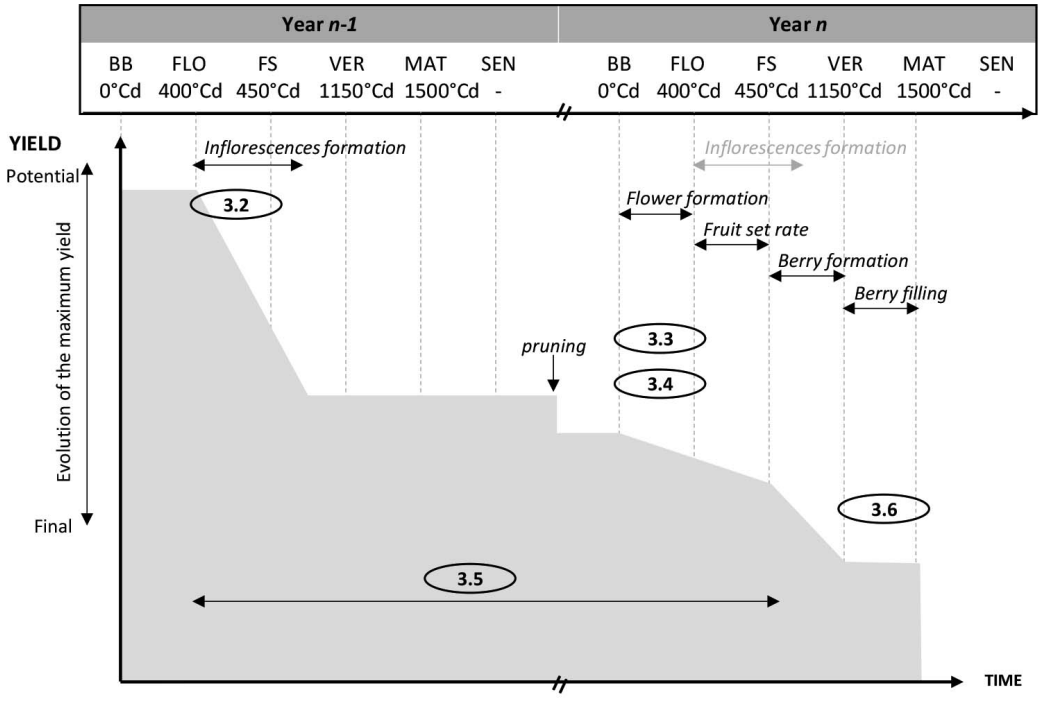


FIGURE 2. Stages of grapevine yield formation over the two-year development process (adapted from Guilpart *et al.*, 2014) expressed as a function of the potential yield. Phenological stages and total thermal time from budburst (in degree-days); BB: Bud break; FLO: Flowering; FS: Fruit set; VER: Veraison; MAT: Maturity (harvest); SEN: Leaf senescence. The numbers in ovals refer to the paragraph of Section 3 where this part of the yield formation process is studied.

Harvest then takes place in September or October in France. Berries can be damaged or destroyed from their formation until harvest, resulting in yield loss.

The evolution from potential to actual yield over the two-year formation process is presented in Figure 2. The potential yield of a plot depends on the climate and soil type (van Ittersum *et al.*, 2013) but also in vineyard systems on planting density, training system, the number of buds retained per plant and grape variety. Limiting factors at each stage of yield development may reduce each yield component, and consequently yield to a maximum achievable value at harvest. Information about all limiting factors at each stage and their quantified effects (e.g., the effect of water stress at the flowering of year *n-1* on inflorescence formation, Guilpart *et al.*, 2014) can be used to produce indicators of expected yield. Comparing these indicators with the reference yield can help winegrowers adapt their practices to achieve the reference yield. Figure 3 shows an example of a plot where the inflorescence formation is the main yield-limiting stage (in a Mediterranean climate in our field experiment).

Radiation, temperature, water status and mineral nutrition are reported to affect the formation of inflorescences during year *n-1* (Vasconcelos *et al.*, 2009). Vasconcelos *et al.* (2009) proposed quantitative relationships between temperature and bud fertility and found a range of temperatures from 20 °C to 35 °C were suitable for the formation of inflorescence primordia. Low radiation on latent buds reduces inflorescence formation, most likely because of degraded carbon status and assimilate supply to the buds (Dry, 2000; Keller, 2010; Lebon *et al.*, 2006). Water deficit reduces bud fertility regardless of the bud position on the cane (Guilpart *et al.*, 2014). Nitrogen (N) deficiency reduces inflorescence formation because an optimal supply of N is needed to reach the maximum formation of inflorescence primordia and differentiation of flowers (Keller, 2010; Vasconcelos *et al.*, 2009).

Apart from these abiotic stresses, pests and diseases also play a decisive role in yield formation and reduction. For example, powdery mildew (*Uncinula necator*) is the most widespread and destructive grapevine disease worldwide

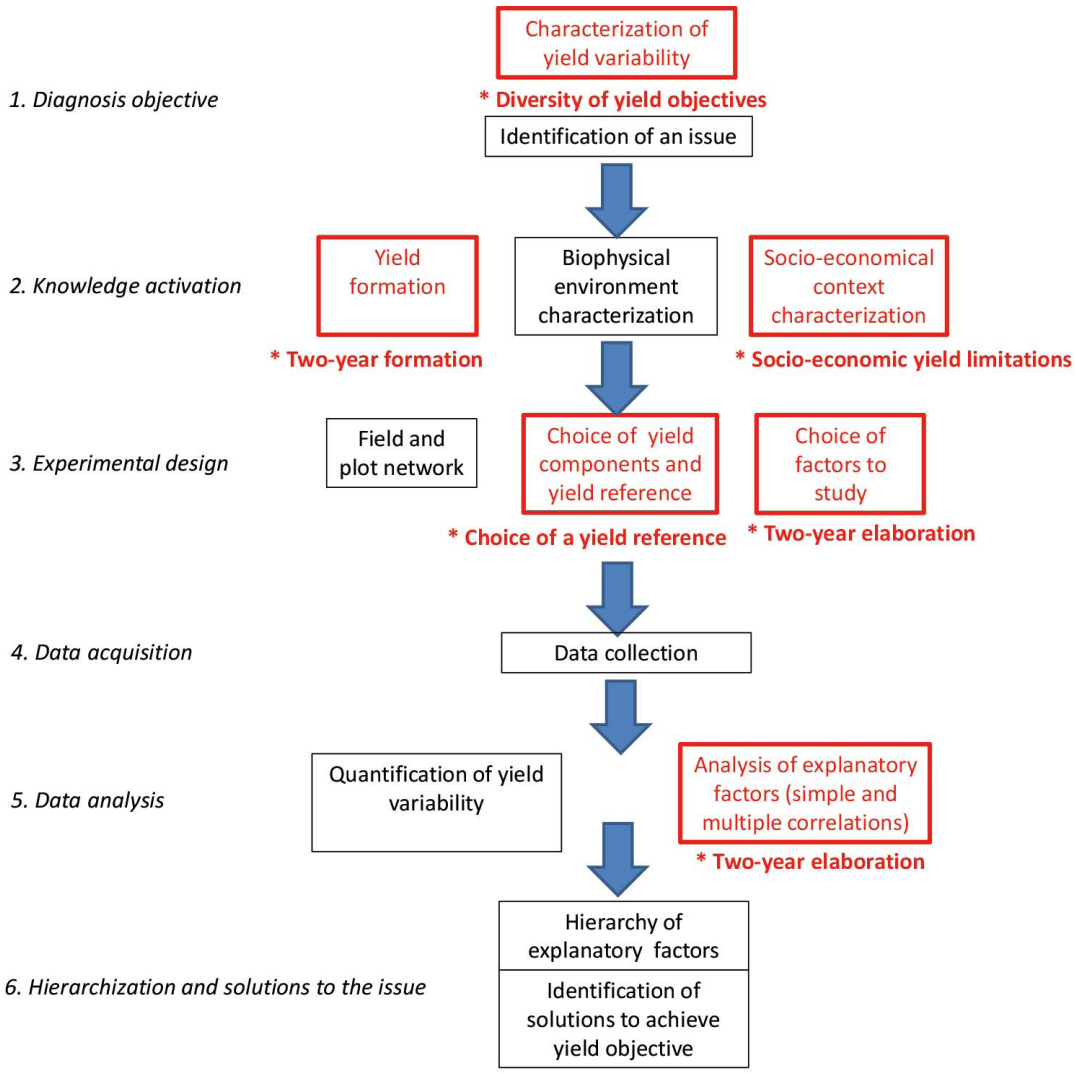


FIGURE 3. Steps of the regional agronomic diagnosis method as adapted to grapevine (adapted from Doré *et al.*, 1997). The red boxes highlight the specificities of grapevine yield.

and is the main target of fungicides used on *V. vinifera*. Integrated pest management strategies include better-targeted treatments through epidemiological and phenological surveys. They may be economically viable and are less environmentally destructive than systematic pesticide sprayings. As a result, they are increasingly popular, but the trade-off is that winegrowers have to tolerate higher levels of disease pressure. A clear understanding of the links between disease and yield as well as disease and wine quality is therefore crucial (Calonnec *et al*., 2004).

2. Regional agronomic diagnosis method

The diagnosis approach presented here was adapted from Doré *et al.* (1997), where it was mainly applied to annual crops. We used the same approach to analyse issues related to grapevine yields, integrating the key features of grapevine yield and formation. By "issues", we mean yield loss as well as interannual and spatial yield variability specific to perennial crops, for which yield formation takes place over two consecutive years. The diagnosis approach follows a six-step process (Figure 3).

Step 1 defines the problem to solve and outlines the studied system in spatial and temporal terms at a scale relevant for the analysis. Yield variability must be characterised by drawing on multiple sources of information to get a clear overview of the magnitude of yield variation. In this study, we chose to source our experimental data from a network of vineyards.

Step 2 corresponds to the activation of knowledge regarding the socio-economic context, the biological processes and the environment (pedoclimatic and biotic) to identify the question and formulate hypotheses to explain yield variability within the network more precisely. This step mobilises various sources of scientific and non-scientific knowledge. Doré *et al*. (1997) suggested paying particular attention to biophysical environment characterisation. Additionally, to adapt to perennial crops, we will consider the factors that are known to put limits on grapevine yield, i.e., certified origin product specifications and sales channels (Figures 1 and 2).

Step 3 is the design of the experimental protocol. To test the intra-regional variability of the regional agronomic diagnosis, we propose using plots in a large range of production contexts. The networks are therefore made up of various plots according to the following criteria, which are identified in the literature as influencing grapevine growth (Figures 1 and 2): (i) climate variation in terms of rainfall and temperature; (ii) socio-economic context variations in terms of wine quality specifications and sales channels; (iii) vineyard system management in terms of environmental specifications, and (iv) grapevine variety. To adapt the diagnosis to perennial crops, we include experimental protocol data from two consecutive years for each yield factor. The chosen yield components are quantitative and qualitative. The quantitative yield components are the number of clusters per vine, cluster mass (g), berry mass (g), number of berries per cluster. We used the yield components to get the calculated yield per vine (kg.plant^{-1}) and the yield achievement ratio (no unit), obtained by dividing the calculated yield by the winegrower's target yield (kg.plant^{-1}). The qualitative yield components we monitored were juice sugar in harvested berries and total acidity in the harvested berry juice. This third step also includes the choice of yield reference for each plot in each network as a reference to compare with the grapevine yields collected in step 1.

Step 4 is the data acquisition phase. We collected the data on paper forms directly in the plots, then transferred the data to storage files.

Step 5 consists of yield data analysis. First, intra-network variability is statistically characterised for each yield component. Second, we use regression to investigate the hypothesis of relationships between the assessed factors (Section 2.1) and yield components. The two-year yield formation process is also taken into account by investigating the hypothesis of a relationship between year *n* and year *n-1* assessed factors and yield components.

Step 6 first involves creating a hierarchy of the assessed factors impacting yield components. This hierarchy then allows us to identify possible points of leverage to reach the yield objective.

3. Case studies

The regional agronomic diagnosis was applied to two case studies in France: one in the Provence-Alpes-Côte d'Azur (PACA) region and one in the Alsace region (Figure 1) to determine how yields vary when implementing new practices to reduce pesticides. These two case studies cover a range of situations in terms of the number of variables available to perform the regional agronomic diagnosis related to the hypothesis raised. In the PACA case study, we studied organic farming as a way to eliminate synthetic herbicides, synthetic fungicides and synthetic insecticides. The Alsace case study focused on reducing all types of inputs used in vineyard management, including chemicals and machinery use (fossil fuels). These two French regions are identified to be in the B-4 climatic scenario zone (Blenkinsop *et al.*, 2008). They show a range of temperate/maritime-influenced climates, summarised as warm with moderate precipitation. The assessed factors include several soil and climate conditions, production systems, grape varieties, red and white wines, wine production and marketing contexts for several vineyards, located at experimental sites or in commercial vineyards (Figure 1), that have been monitored for at least two consecutive years.

The PACA case study is a vineyard plot network in the Côtes du Rhone wine region (Merot *et al*., 2020). A total of 44 plots were monitored from 2013 to 2016. The PACA network diversity takes into account the following assessed factors (i) geographical areas including differences in microclimate of the plot, (ii) microclimate influence on pests and diseases severity, (iii) management conditions (i.e., different conventional and organic specifications), and (iv) yield target expected by sales channels (i.e., cooperative wines versus wines sold directly by the winegrower). Plots were planted with the Grenache cultivar, which is typical of the region and grown under a wide range of organic

management conditions. The year of planting ranged from 1980 to 2004 and the density was homogenous across the network (4000 vines per hectare). All plots were fully in production. The nearest weather station is Carpentras-Serres. All PACA plots were fully in production and had a low vine planting density (averaging 4000 vines per hectare) that was homogeneous across the network.

The Alsace case study is a vineyard plot network in the Alsace wine region. The data were collected from seven plots located near Colmar (8°4' N, 7 21' E) and monitored from 2013 to 2018 (Thiollet-Scholtus *et al.*, 2020). The Alsace network diversity takes into account the following factors (i) pedoclimatic geographical areas, (ii) pests and diseases, and (iii) management conditions (i.e., conventional, organic and biodynamic specifications). The Alsace plots are planted with the Riesling variety (Châtenois, Ingersheim, and Ribeauvillé plots) and with the Pinot noir variety (Rouffach plot). The nearest weather station is Colmar. The Alsace plots were fully in production (11 to 42 years old) and had a low vine planting density that was homogeneous across the network (averaging 4300 vines per hectare). All of the Alsace plots benefit from Origin Products certification.

Calculated yield and cluster mass are available for each year for both case studies. We also have detailed data on the number of flowers per inflorescence, the number of berries per cluster, and average berry mass for the PACA case study, and details on the number of clusters per plant, sugar and total acidity of harvested berries for the Alsace case study. These output yield variables were analysed in relation to input explanatory variables such as 12C/13C ratio (δ13C) and available nitrogen in must for Alsace, and downy mildew, grey mould, powdery mildew and grape moth severities for PACA.

The δ13C ratio assesses vine water uptake conditions between veraison and harvest, in other words: during the ripening period. The δ13C is measured on must sugars in mature berries. The δ13C is the ratio between 12C and 13C. The δ13C ratio is expressed under zero, and the slightly negative values of the δ13C ratio indicate water stress (van Leeuwen *et al*., 2001).

RESULTS

1. Regional agronomic diagnosis in organic farming (PACA case study)

Step 1: Winegrowers and technical institutes often report high grapevine yield variability in organic farming. For example, the organic extension service in south-western France (SVBA) observed large variations in actual yield in organic vineyards, ranging from 17 to 54 hl.ha^{-1}. A survey reported that the average yield of 50 to 60 hl.ha^{-1} decreased from 5 to 30 hl.ha^{-1} when the management of the vine was changing in organic farming (Source: GDDV41 *Groupement Départemental Des Viticulteurs Loir-et-Cher, 2004*). Lower yields than the regional reference, are also reported in organic farming in the scientific literature (Seufert *et al.*, 2012; Meissner *et al.*, 2019). As such, the agronomic diagnosis for the PACA case study aimed to explain the yield variability when grapevines are grown according to organic standards.

Step 2: The PACA case study plots are located along the Rhone Valley, an area which has a Mediterranean climate with the following features: minimum average day temperature of 8.1 °C, maximum average day temperature of 20.6 °C, rainfall 648 mm and 65.7 rainy days a year (source Météo-France, data from Carpentras-Serres, 1981–2010). This case study presents various landscape and soil conditions, such as hills, terraces, stony slopes and plains.

PACA is the second-largest wine region in France after the Bordeaux region in terms of production land area (89.500 ha in 2020). The main cultivars grown and studied are Shiraz, Grenache and Mourvèdre. Various types of wines are produced and the majority of grapevines are grown according to certified Origin Products standards with yield limitations (max 51 hl.ha^{-1}). Although some "famous" appellations (e.g., Châteauneuf du Pape, Beaumes de Venise) enjoy a comfortable economic situation, about 63 % of the overall production volume in 2020 was sold to cooperatives, which pay lower prices to winegrowers. To compensate for the lower margins, some winegrowers reduce field interventions such as fertilisation. The PACA region is also characterised by a high rate of organic viticulture: nearly 17.8 % of the PACA vineyard area is organic.

Step 3: The experimental design used to assess grapevine yield variability uses the following points: (i) field network, (ii) yield reference, (iii) yield component description and (iv) characterisation of assessed factors.

Field network. This study is based on the PACA network presented in the Material and Method part.

Yield reference. To be able to compare yields between plots with different yield objectives, we had to define a yield reference. The plot network covered an important variability in the terroirs. For each plot, we considered different reference options, such as average or maximum yield observed in the field over the last 10 years, the regional yield for Grenache variety etc., but the various growing conditions made it impossible to select a single regional reference. Moreover, since winegrowers do not keep track of yields over long periods, and because interannual variability is considerable, average or maximum values were not available for each field. We, therefore, chose the yield objective declared by each grower as a reference (Table 1). The range of values for yield objectives defined by growers is high (from 0.39 to 3.41 kg.plant^{-1}). We calculated the yield achievement ratio as the ratio between the actual yield harvested in the plot on a given year and the yield objective. Thus, when the grower reached the yield objective, the yield achievement ratio is equal to or greater than 1.

Yield components. The transition to organic farming leads to changes in many different practices. Therefore, we did not focus on a single yield component or a single potential limiting or reducing factor in this PACA case study.

TABLE 1. Variability of main yield indicators in the PACA case study: number of observations; mean, maximum and minimum values; and standard deviation.

Yield indicators	Number of observations	Unit	Mean value	Maximum observed	Minimum observed	Standard deviation
Calculated yield per plant	757	kg.plant^{-1}	2.32	3.19	0	3.10
Yield objective	44	kg.plant^{-1}	1.64	2.31	0.39	0.59
Yield achievement ratio	42	no unit	1.02	2.55	0.17	0.50

Instead, we examined all the processes affecting yield throughout the yield formation process to understand the yield variations caused by changes in practices to transition from conventional to organic farming (Merot *et al*., 2019).

Assessed factors. We assumed that a large part of the yield variability could be explained by the main abiotic and biotic factors: nitrogen status, water status and pest and disease damage, after year and location influences had been taken into account.

Step 4: We focused on the characterisations of two particularly important grapevine stages: flowering and harvest. Harvest is the final stage, while flowering is a key stage not only for assessing water and nitrogen stress (Guilpart *et al*., 2014) but also for assessing early damage from pests and disease (Fermaud *et al*., 2016). The number of clusters per plant, the number of flowers per inflorescence, the number of berries per cluster and the berry mass were measured on 30 plants per field. The calculated yield per plant was obtained by multiplying the number of clusters per plant by the number of berries per cluster and by the berry mass.

We estimated water and nitrogen status and pest and disease damage at flowering and harvest (Merot and Smits, 2020). Since we were working on a network of commercial vineyards, we chose easy-to-measure indicators. One shoot per plant on the 30 plants was monitored, and we measured the nitrogen status on the selected shoot at flowering with an N-tester SPAD unit on the eighth to tenth leaves (Cerovic *et al*., 2015). Early water status was estimated at flowering using the apex index measured on this shoot (Trambouze *et al*., 2009). On each of the 30 plants, one cluster per plant was monitored to follow pest and disease severity. We observed the percentage of these clusters presenting grey mould, downy mildew and powdery mildew damage at flowering and harvest. The number of berry moth larvae per cluster was estimated using the "brine method" (Stockel *et al*., 1994) at harvest.

Step 5: Data analysis dealt with (i) yield variability description and (ii) explanatory factor analysis. We observed a large variability in the calculated yield in the PACA case study, from 0 to 2.32 kg.plant^{-1} (Table 1). This range, therefore, had to be considered relative to the range in yield objectives, which varies between vineyards: yield objectives ranged from 0.39 to 2.31 kg.plant^{-1} (Table 1). The yield achievement ratio ranged from 0.17 to 2.55 with an average of 1.02. Among the 42 plots, 14 winegrowers did not achieve their yield objectives (i.e., yield ratio $\leq$ 0.9), 18 exceeded their yield objectives (i.e., yield ratio $\geq$ 1.1) and 10 reached their yield objectives (i.e., 0.9 < yield ratio < 1.1) (Table 1).

Explanatory factors for yield variability were analysed using a correlation matrix (Figure 4a). The correlations between measured variables (Figure 4a) and yield ratio highlight the limiting and reducing role of diseases at harvest (–0.49 and –0.26, respectively, for powdery and downy mildews) and water stress at flowering (–0.16) on the yield achievement ratio in year *n*.

Nitrogen content for years *n-1* and *n*, δ13C for year *n* and water stress at flowering for year *n-1* were positively correlated to the yield ratio (Table 2). The higher the δ13C, the more the vine suffered from water stress. In the PACA region, water stress is a major concern for growers. Water stress at flowering for year *n* limited the yield ratio. We observed non-significant correlations between yield components and grape moth's severity, and yield components and powdery mildew (Table 2). Downy mildew at harvest was the most important factor correlated to the yield ratio in the PACA case study because it directly impacted the number of berries per cluster observed at harvest. More specifically, downy mildew negatively impacted the number of flowers per inflorescence at flowering (P = 0.031), the number of berries per cluster (P = 0.011) and cluster mass (P = 0.009).

In the Mediterranean climate, spring and early summer rainfall may be limited depending on the year, and temperatures increase rapidly during this period. This means that early water stress can occur during flowering and fruit sets and can limit the mineralisation process (Celette *et al.*, 2009), thus reducing the soil nitrogen availability as well. These conditions can impact the formation of inflorescences in year *n-1* and therefore the number of clusters per plant harvested the following year. Under organic farming conditions, nitrogen and water stress during flowering and fruit sets can be aggravated by inappropriate weed and intercropping management between rows and under the row (Steenwerth and Belina, 2008).

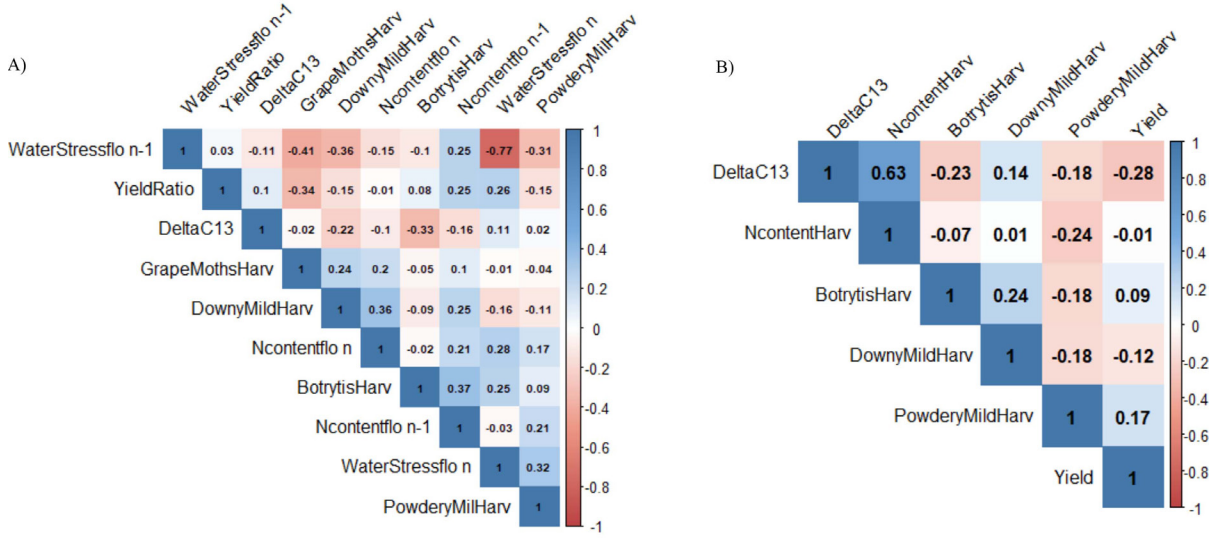


FIGURE 4. Relationships between yield, yield indicators and main potential explaining factors: A) Correlations among variables measured on the 48 plots of the PACA network; B) Correlations among variables measured on the seven plots of the Alsace network. BotrytisHarv, DownyMildHarv, PowderyMildHarv: frequency of grey mould (*Botrytis cinerea*), downy mildew and powdery mildew attacks on bunches at harvest, respectively.

TABLE 2. Significance of linear regressions (p-value with 0.05 threshold) performed for the PACA case study between yield components (in columns) and explanatory factors (in rows).

	Number of flowers per inflorescence	Cluster mass (g)	Calculated yield (kg.plant^{-1})	Number of berries per cluster	Average berry mass (g)
Downy mildew severity (%)	0.031	0.009	0.069	0.011	0.336
Grey mould severity (%)	0.649	0.012	0.024	0.056	0.058
Powdery mildew severity (%)	0.284	0.211	0.022	0.322	0.503
Grape moths' severity (number of larvae)	0.240	0.896	0.749	0.905	0.628

Labour is a major constraint in organic farming systems, especially during spring and early summer, when pesticide spraying, bud pruning, soil management and trellising are needed. Soil management may be performed too late, creating excessive competition for resources (nitrogen and water) between the grapevines and weeds. Finally, approved organic pesticides are non-systemic and mostly leachable, so winegrowers must apply them more frequently and must plan applications more carefully around rainfall. Pest and disease severities can rise quickly and cause yield loss if spraying is delayed (Fermaud *et al*., 2016).

Step 6: The ranking of factors and solutions to improve the regional agronomic diagnosis was performed using correlation analysis and principal component analysis. Correlation analysis on the PACA case study highlighted seven yield limitation factors (Figure 4a), which we ranked using the correlation coefficients for the yield ratio. These seven factors are, in decreasing order of correlation to yield ratio: powdery mildew at harvest (correlation: 0.49), δ13C (correlation: 0.4), water stress at flowering year *n-1* (correlation: 0.28), nitrogen at flowering year *n-1* (correlation: 0.27), downy mildew at harvest (correlation: -0.26), nitrogen at flowering year *n* (correlation: 0.26), and water stress at flowering year *n* (correlation: 0.16). The correlation includes information on both the frequency and intensity of impact on yield.

Although controlling powdery mildew and grey mould are both parts of a winegrower's plant protection strategy, the practices implemented to achieve yield objectives differ. In southern France, fungicides against downy mildew are very frequently applied while pesticides against grey mould are not used as much. Preventive measures are recommended to limit grey mould, which develops in humid microclimates. They are also recommended against

powdery and downy mildew, along with better scheduling of fungicide applications, especially copper-based ones. Winegrowers need to adjust the doses and frequency of application while also taking into account weather forecasts. As with the application of pesticides, water stress is also highly dependent on climatic conditions. Rainfall cannot be managed, so aside from irrigation, one way to limit water stress is to avoid water loss from the soil during spring and summer and increase water storage during winter and autumn through good management of natural and sown intercropping. Finally, nitrogen stress is associated with two main practices: fertilisation and intercropping. Choices made regarding intercropping duration and species are critical.

2. Regional agronomic diagnosis on a variety of low input vine systems (Alsace case study)

Step 1: The Alsace case study sought to assess the impact of innovative management techniques aimed at significantly reducing input use. This approach was studied through a limited number of well-understood, controlled and easy-to-analyse plots (Figure 1). The interest of the Alsace network in our study is the combination of acceptable yield objectives with high grape quality for PDO wines at harvest (Thiollet-Scholtus *et al*., 2021). The main assumption in the Alsace case study is that the main limiting factor of yield in north-eastern France in the summer water deficit.

Step 2: With regard to the pedoclimatic context, the Alsace region has a semi-continental climate with a mean annual rainfall of 575 mm and average temperatures ranging from –11 °C to +8 °C in winter and from 10 °C to 35 °C in summer. Viticulture in Alsace is oriented towards highly aromatic white wines obtained mainly from three varieties that are sensitive to grey mould. Grapevines are grown under PDO standards with yield limitations (75 $hl.ha^{-1}$) that are higher than in other French regions. Organic farming is also widespread and data traceability is high, which facilitates data collection. With regard to grapevine management, intercropping and high trellising (planting density is 4500 $vines.ha^{-1}$) are much more common compared to the PACA case study. Grey mould and water constraints were essential factors for the study in Alsace. Fungal diseases such as grey mould (Botrytis) are very problematic due to the few fungicides permitted for use by organic farming standards.

Step 3: The network was chosen to present contrasting situations between plots based on soil type and grapevine management practices. The data collected in Alsace are from plots that all have PDO status. The soil is very different from one plot to another. The data were collected in seven well-documented plots located near Colmar (8° 4’ N, 7° 21’ E) in four locations: Châtenois, Ingersheim, Ribeauvillé and Rouffach. The nearest weather station is the Colmar station. The network is representative of the regional diversity according to PDO and environmental specifications (Renaud-Gentié *et al.*, 2019). As with the PACA case study, the yield objective for each plot in the Alsace network was used as a reference to analyse a level of yield achievement ratio (Table 3). For the Alsace case study only, the yield reference was always analysed considering grape quality at harvest (i.e., sugar and total acidity in harvested berries). The network was designed to understand the impact of more sustainable agricultural practices on yield. The Alsace case study takes into account all the components of yield and focuses on abiotic limiting factors (water and nitrogen) and one biotic limiting factor (grey mould linked to grape quality at harvest) in relation to climate.

Step 4: We collected the data over five growing seasons (2013–2017) in each plot. The yield components (e.g., number of clusters per plant and cluster mass in g) were measured at harvest in each plot. The plot yield per hectare was then calculated using the actual planting density recorded for that plot and year. The δ13C was measured on must sugars in harvested berries. Available nitrogen in must, sugar and total acidity in harvested berries were measured

TABLE 3. Variability of yield indicators in the Alsace case study: number of observations; mean, maximum and minimum values, and standard deviation.

Yield components	Number of observations	Unit	Mean value	Maximum observed	Minimum observed	Standard deviation
Number of clusters per plant	35	no unit	27.97	41.55	14.46	7.23
Cluster mass	35	g	90	140	50	20
Calculated yield	35	$kg.plant^{-1}$	2.42	4.02	1.31	0.65
Yield objective	35	$kg.plant^{-1}$	2.27	2.80	1.76	0.46
Yield achievement ratio	35	no unit	1.01	2.14	0.44	0.34
Sugar in harvested berries	35	$g.L^{-1}$	194.91	219.34	150.48	20.14
Total acidity of harvested berries	35	$g.L^{-1}$	6.46	11.58	4.1	1.70

TABLE 4. Significance of linear regressions (p-value with 0.05 threshold) performed for the Alsace case study between yield components in columns and explanatory factors in rows.

	Number of clusters per plant	Cluster mass (g)	Calculated yield ($kg.plant^{-1}$)	Sugar in harvested berries ($g.L^{-1}$)	Total acidity of harvested berries ($g.L^{-1}$)
12 C/13 C (no unit)	0.548	0.347	0.284	ND	ND
Available nitrogen in the must ($mg.L^{-1}$)	0.026	0.217	0.861	ND	ND
Grey mould severity (%)	ND	ND	ND	0.017	0.013

ND: no data.

in the juice of the same 10 vine groups at harvest. There are clearly huge differences in maturity in the Alsace network, which result in very different impacts of water availability and botrytis risk, which is explained by grey mould severity. Grey mould severity was reported on paper forms directly in the plots at each harvest date. Experts from local extension services assessed damage to clusters due to grey mould at harvest by visual observation.

Step 5: Data analysis deals with (i) yield variability description and (ii) explanatory factor analysis in the same way as for the PACA case study.

Yield variability: Calculated yield is slightly higher than in the PACA case study: 2.42 **versus** 2.32 $kg.plant^{-1}$ (Table 3). The yield and quality objectives for the Alsace network were achieved each year, with an average calculated yield objective ratio of 1.01 (Table 3). This means that on average the Alsace winegrowers can (i) achieve their yield objectives and (ii) sell their wines under the Alsace PDO certification.

Explanatory factors for yield variability are analysed in Table 4 and the correlation matrix in Figure 4b. In accordance with the hypothesis put forward, we observed a negative correlation between yield parameters and "water stress during berry maturation" measured with the **δ**13C indicator (correlation: –0.28; Figure 4b). The Alsace data demonstrate a positive correlation (correlation: 0.63) between "nitrogen available in the must at harvest" and the **δ**13C parameter (Figure 4b). This is in line with the results of van Leeuwen *et al*. (2001): the **δ**13C parameter is relevant to characterise the water constraint and thus the ease of accumulation of berry sugars, then measured at harvest time. We also observed a linear relationship between the "grey mould severity" parameter and the two harvest quality indicators: "sugar rate in must at harvest" (correlation: 0.017) and "total acidity in must at harvest" (correlation: 0.013) (Table 4). This confirms the links between grey mould attacks and grape juice quality found by Barbeau *et al.* (1999) following the Alsace case study. Finally, Figure 4b shows that grey mould (botrytis) reduces yield (correlation: 0.09) and highlights an existing negative role of downy mildew at harvest (correlation: –0.12) on calculated yield (Figure 4b).

Step 6: Ranking of the assessed factors and solutions to improve the regional agronomic diagnosis was performed using the Alsace correlation matrix (Figure b). The factors ranked were: δ13C, powdery mildew at harvest for year *n* and downy mildew at harvest for year *n*. As in the PACA case study, Alsace biotic factors such as powdery mildew were associated with the plant protection strategy.

DISCUSSION

This study used two varied and complementary datasets from two wine regions in France to show how to adapt the regional agronomic diagnosis to grapevine yield variability analysis. The aim of adapting this method is to help growers manage yield variability and uncertainty when transitioning to a more sustainable wine-growing system. In this study, we proposed applying the regional agronomic diagnosis using a six-step process. This process starts by defining the problem and includes recommendations on using a range of information on processes, field networks, characteristics and various multi-year data.

The regional agronomic diagnosis applied to grapevine yield variability was relevant in understanding the processes responsible for yield formation and yield variability analysis with a systemic approach. This will be very useful for fine-tuning the adaptation of vine management to climate or other changing conditions. One way in which the method provides added value is in its systemic approach. Doré *et al*. (1997) emphasised the importance of understanding processes that link, limit or reduce factors, and therefore yield variability. Fully understanding grapevine yield variability entails having solid knowledge of many complex processes (e.g., storage processes or competition between vegetative and reproductive processes) involved in yield formation – knowledge which is often lacking. In fact, the regional agronomic diagnosis for both case studies highlighted variations of yield and yield components that could not be explained by one major factor

only. A combination of factors can have a differing degree of influence on yield depending on the year. Thus, when studying only one factor, the quality of linear regression is low even if a significant effect is observed. Identifying hypotheses about the explanatory factors or yield diversity was an essential step to ensure that data collection was based on the right variables. Thus, identifying hypotheses can help limit the number of observations made in the network, or give greater importance to some measurements compared to others. The implementation of an agronomic diagnosis was required to study both the biophysical and socio-economic diversity.

Secondly, the regional agronomic diagnosis required choosing integrated indicators consistent with yield formation processes, further highlighting the need to understand these processes. Indicators chosen for the diagnosis must be relevant to (i) link a constraint or limiting factor to a given yield component and (ii) when possible, associate limitation and reduction on yield with practices, in relation to phenological stages. Having a detailed understanding of these indicators will be very useful to more easily adapt vine management during an agroecological transition or to adapt to climate change. Accordingly, it was preferable to choose simple and sometimes less precise indicators. Numerous studies detail indicators and their meaning or how to choose indicators and how to sample (Fermaud *et al*., 2016). Two main principles must be retained: adaptation to the case study and parsimony. The agronomic diagnosis can quickly become cumbersome to perform as yield components, factors to assess and factors of diversity in the region or the chosen vineyard management system are added. This is why, in the PACA case study, no soil measurements (e.g., water content, nitrogen content, carbon storage, biological processes) were performed. Furthermore, some yield components are more burdensome to assess than others. For example, the measurement of the number of flowers per inflorescence is tedious and time-consuming over a very short period (flowering), even if it indicates the potential number of berries considering only abiotic factors of yield limitation. Often, assessing the number of berries per cluster at harvest will suffice. This measurement was made in the PACA case study, but not in the Alsace case study. Two yield components measurements are essential: the number of clusters per plant and cluster mass. The number of clusters per plant is important because this figure explains much of yield variability (Martinez-Casasnovas *et al*., 2012) and because it provides information about what happened in year *n-1*. Cluster mass includes both the number of berries per cluster and the average berry mass, so it mostly reflects what happened in year 2. In fact, cluster mass, sugar rate and acidity are much easier to assess than the number of berries per cluster or the average berry mass. The diagnosis for the two case studies also highlights the importance of the multi-year approach. It seems important to work with multi-year databases and to increase the number of plots analysed the same year, taking into account the specificity of the year.

Thirdly, the last step of the regional agronomic diagnosis was used to characterise promising possibilities for reducing pesticides use. The regional agronomic diagnosis helped identify the period during the yield formation process that needs to be managed differently. Although we observed relationships within one particular year, such as the year of harvest (e.g., in the PACA case study, water stress in year 2 affected the yield in year 2), the two case studies highlighted the significant role of the first year of yield formation. In the critical period identified, the identification and ranking of processes responsible for yield variability shed light on the possible management options to address the issue. Thus, in Alsace, grey mould pressure around harvest was noted, and so one proposal could be to modify the protection strategy against grey mould. Whereas in the PACA case study, the downy mildew strategy was a bigger issue. The regional agronomic diagnosis of the Alsace case study complemented the PACA case study to provide a greater variety of options for sustainable vineyard management. Since the two case studies are specific to wine regions, they offer highly diversified information to support the agroecological transition of viticulture.

Finally, regional agronomic diagnosis can be an effective way to support the design of sustainable grapevine systems. In both case studies, new information on the factors that limit and reduce yield was collected directly within the context of increasing organic and low input vineyard systems. In the PACA case study, pests and disease, as well as mineral (water and nitrogen) status, were highlighted as they explained yield reduction. In Alsace, grey mould and water status were shown to be explanatory factors of yield variability. These results should encourage researchers to focus on these factors in further developing a design process. Thus, the regional agronomic diagnosis can support knowledge acquisition, formation of representation and identification of the crucial issues to concentrate on and required in the design process (Toffolini *et al*., 2020).

CONCLUSION

There is a need to understand the causes and magnitude of yield variability in grapevines, particularly when winegrowers transition to a more agroecological system or adapt to climate or other changes. Yield is a key factor in any future changes in vine management. We propose adapting the regional agronomic diagnosis method, initially intended for annual crops, to the specific characteristics of grapevine crops as viticulture becomes more likely to change in the context of climate change and agroecological transition. The specific characteristics that must be considered are the multi-year yield development, the choice of the reference yield, the limited control of phenology, and the winter dormancy period. The regional agronomic diagnosis applied to viticulture is useful for acquiring knowledge on vine functioning, reflecting on the data collection strategy in viticulture experimentation, and proposing options to better control yield in the design process by integrating multiple factors, particularly in a context of changing winegrowing systems.

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