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### ► To cite this version:

Anne Merot, Guillaume Coulouma, Nathalie Smits, Elsa Robelot, Christian Gary, et al.. A systemic approach to grapevine decline diagnosed using three key indicators: plant mortality, yield loss and vigour decrease. *OENO One*, 2023, 57 (1), 10.20870/oeno-one.2023.57.1.5575 . hal-03960649

**HAL Id: hal-03960649**

**<https://hal.inrae.fr/hal-03960649>**

Submitted on 27 Jan 2023

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ORIGINAL RESEARCH ARTICLE

# A systemic approach to grapevine decline diagnosed using three key indicators: plant mortality, yield loss and vigour decrease

Anne Merot<sup>1</sup>, Guillaume Coulouma<sup>2</sup>, Nathalie Smits<sup>1</sup>, Elsa Robelot<sup>1</sup>, Christian Gary<sup>1</sup>, Lucia Guerin Dubrana<sup>3</sup>, Jouanel Poulmarch<sup>4</sup>, Xavier Burgun<sup>5</sup>, Anne Pellegrino<sup>6</sup> and Marc Fermaud<sup>3</sup>

<sup>1</sup> ABSys, INRAE, CIHEAM-IAMM, CIRAD, Institut Agro, Univ Montpellier, Montpellier, France

<sup>2</sup> LISAH, INRAE, IRD, Institut Agro, Univ Montpellier, Montpellier, France

<sup>3</sup> INRAE, UMR SAVE, Bordeaux Science Agro, ISVV, F-33882, Villenave d'Ornon, France

<sup>4</sup> Chambre Agriculture Hérault – Montblanc, France

<sup>5</sup> IFV Institut Français de la Vigne, Cognac, France

<sup>6</sup> LEPSE, Institut Agro, INRAE, Univ Montpellier, Montpellier, France



\*correspondence:  
anne.merot@inrae.fr

Associate editor:  
Alonso Pérez-Donoso



Received:  
2 June 2022

Accepted:  
16 January 2023

Published:  
27 January 2023



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## ABSTRACT

Grapevine decline, a major global viticulture issue, is defined as a multi-year decrease in vine productivity and/or increase in vine mortality. Although grapevine trunk diseases are one of the most studied causes, the decline is multifactorial and associated with more than 70 factors, including abiotic and biotic hazards. With so many factors to consider, the phenomenon is difficult to understand. Our study aims to make it easier to determine and assess grapevine decline by focusing on three key indicators: yield, mortality and vegetative vigour. We investigated the relationships between these indicators from both a temporal and spatial perspective to propose a set of diagnostic indicators. Thus, we conducted a winegrower survey, a historical analysis of grapevine decline and field measurements of the abovementioned indicators on plot networks in three major French winegrowing regions (see graphical abstract): Bordeaux, Cognac and Languedoc. We found that winegrowers' perceptions of decline were consistent with an objective characterisation based on field measurements of the indicators. Although vine mortality progressively spread over the years, neither the survey nor the historical analysis showed a direct link between decline and yield loss. Rather, large yearly fluctuations in yield, which did not systematically decrease over time, account for this finding. As a result, the mortality rate and the normalised difference vegetation index (NDVI) indicators were shown to be earlier indicators of grapevine decline than yield loss (addressed from the yield achievement ratio, YAR). We performed a multifactorial analysis of the overall data set from the three regions to deepen our understanding of the diversity of declining situations and the underlying environmental and management factors contributing to decline. Finally, two ground-based NDVI indicators and an image-analysis methodology using aerial photographs were proposed as easy-to-obtain indicators of grapevine decline. NDVI indicators were linearly correlated with both the YAR and mortality rate. This study provides a better understanding and promising tools for the early diagnosis of grapevine decline.

**KEYWORDS:** decline, dieback, yield loss, vine mortality, vigour, diagnosis, indicators

## INTRODUCTION

Over the last several decades, the phenomenon of decline has affected numerous woody species (Bettenfeld *et al.*, 2020). The term decline, first used in forestry science (Sinclair, 1965; Manion, 1991), was originally associated with reduced growth and mortality in trees. The causes of decline are difficult to pinpoint because multiple abiotic and biotic stresses are involved (Walters and McCarthy, 1997; Haavik *et al.*, 2015). Similar to the forestry industry, the winegrowing sector has begun to experience a grapevine decline phenomenon in the last twenty years, so that decline is considered an increasingly widespread problem in many vineyards across the world (De la Fuente *et al.*, 2016). Hofstetter *et al.* (2012) even estimated that replacing dead vines could cost approximately US\$1.5 bn per year globally, which represents a considerable economic loss for the wine industry (Gramaje *et al.*, 2011). The incidence of this phenomenon can vary from one country to another, but most wine-producing countries are affected: Australia, the United States, New Zealand, Italy (Laura Mugnai, personal communication), Spain, Portugal and France (De la Fuente *et al.*, 2016).

In France, a National Plan for Grapevine Decline (PNDV) was launched in 2016 to study the possible causes of grapevine decline and support French winegrowers in limiting the agronomic and economic impacts of decline (Riou *et al.*, 2016). The PNDV defines grapevine decline as ‘a multi-annual decrease in grapevine yield or a premature, brutal or progressive death of vines, which afflicts viticulture’ (Riou *et al.*, 2016). According to the wine industry, grapevine decline is a serious concern for the certified French-origin viticulture product sector because it encompasses approximately 10 % of the entire vineyard area (BIPE, 2016). At the national scale, Schaumberger *et al.* (2018) observed a yield decrease of 0.3 hl ha<sup>-1</sup> year<sup>-1</sup> since the 1980s, regardless of wine type.

The spread of grapevine decline may be due to numerous factors related to cropping practices and the grapevine environment, including biotic and/or abiotic hazards. Global warming and climatic hazards (frost, heat, severe drought) have a direct impact on phenology and some yield components inducing yield loss (Van Leuwen and Darriet, 2016; Becard *et al.*, 2022) or an indirect impact by increasing susceptibility to pathogens and pests (Bois *et al.*, 2017; Reinke and Thiéry, 2016). Specific causes were sometimes identified; notably, 50 % of low-yield productive vineyards in France were associated with rootstock 161-49C and the Syrah variety (Beuve *et al.*, 2013). Furthermore, the loss of biodiversity and functionality in soils was recently demonstrated to play a role in grapevine decline (Darriaut *et al.*, 2021). Grapevines are frequently exposed to biotic hazards, including insects, viruses, fungi, bacteria and phytoplasma (Wilcox *et al.*, 2015). Specifically, viruses and phytoplasma involved in the infectious degeneration of grapevine may cause grapevine decline and mortality, despite sanitary measures aimed at preventing their

propagation (Foissac and Wilson, 2010; Maliogka *et al.*, 2015). Since the 1980s, grapevine decline related specifically to grapevine trunk diseases (widely known as GTDs) has been the most studied around the world, with a focus on the following fungal agents: *Eutypa*, Esca complex, *Phomopsis* and *Botryosphaeriaceae* (Carter, 1991; Mugnai *et al.*, 1999, Larignon, 2012; Lecomte *et al.*, 2012; Gramaje *et al.*, 2018). These complex diseases, associated with the woody development of different fungal species, are assumed to be major threats to vineyard longevity (Bertsch *et al.*, 2013, Guérin-Dubrana *et al.*, 2019; De la Fuente *et al.*, 2016), and Esca impacting both directly and indirectly (Mugnai *et al.*, 1999; Fontaine *et al.*, 2016; Calzarano *et al.*, 2004).

Given the complexity of the factors involved and the strong context-dependent nature of the decline phenomenon, different scales should be considered, including the plant, plot, vineyard and region (Walters and McCarthy, 1997). Studies have been carried out at all scales to describe a considerable range of symptoms and damage related to decline. At the plant scale, grapevine decline is characterised by a number of non-specific symptoms, including delayed budburst, reduced shoot growth, shoot dieback and shoot or arm death (Emmett *et al.*, 1992). Before declining, some warning signs may be observed, such as the fluctuation of individual vine vigour and yield from year to year. At the plot scale, yield and mortality are related because dead vines do not produce bunches. Thus, at plot and vineyard scales, the incidence of mortality and/or yield loss over time may account for the decline (Emmett *et al.*, 1992). In southwestern France, the mortality rate varies between vineyards, and the mortality risk was shown to be higher for vines expressing Esca and/or *Eutypa* dieback than for asymptomatic vines (Guérin-Dubrana *et al.*, 2013). Vines expressing *Eutypa* dieback show a significant yield reduction explained by fewer bunches (Munkvold *et al.*, 1994). Finally, in ‘declining’ vineyards, the progressive loss of productivity was associated with an increase in mortality rate, which reached up to 60 % over two decades (Emmett *et al.*, 1992; Fuentes *et al.*, 2016; Kaplan *et al.*, 2016).

As grapevine decline may be the result of the exposure level (time and severity) of grapevines to some environmental hazards combined with genetics and management factors conditioning vineyard susceptibility, decline studies should adopt global and systemic approaches (Bréda and Peiffer, 2014; Claverie *et al.*, 2020). The present study aims to characterise the temporal dynamics of grapevine decline by focusing on three key indicators: yield, mortality and vegetative vigour. The major objectives are i) to define and assess grapevine decline in a simple way for three French winegrowing regions, ii) to characterize the spatial and temporal variability of the relationships between yield, mortality and vine vigour, and iii) to propose a set of diagnostic indicators to help characterise grapevine decline at the plot scale. Surveys and field measurements were simultaneously conducted to shed light on the relationship

between farmers' perceptions of decline and its objective characterisation based on measured indicators.

## MATERIALS AND METHODS

This work was based on three datasets obtained from a network of grapevine plots: data from winegrowers' interviews, yield and mortality dynamics data at the plot scale, and plant indicators observed in 2019 at both the plot and subplot scales.

### 1. Vineyard network

The studied network included 16 vineyard plots that winegrowers considered as exposed to decline across three French winegrowing regions. Eight plots, subjected to an oceanic climate, were located in southwestern France, in the Bordeaux and Cognac regions. Eight other plots were in the Languedoc region in southern France with a Mediterranean climate. The main plot characteristics are presented in Table 1. This network of commercial plots allowed us to take into account a large range of conditions, including target yield, vineyard age, age of decline, cultivar and rootstock, vineyard management (density, pruning system, irrigation, cover crop), disease pressure, and some key abiotic constraints, such as climatic water balance, soil depth and water holding capacity (Table 1).

In 2019, three subplots of 30 consecutive vine locations (with or without vines) within one row were delimited in each vineyard. The three subplots were scattered in the plot to account for their heterogeneity in terms of soil properties and grapevine decline based on expert knowledge.

## 2. Qualitative analysis of winegrowers' perceptions of decline based on a survey dataset

To understand winegrowers' perceptions of decline, we surveyed the 16 winegrowers associated with the vineyard network. The survey consisted of a mix of closed-ended and open-ended multiple-choice questions divided into four sections:

- plot characteristics (size, certification status, irrigation, soil features, target yield),
- perception of grapevine decline (definition, rate), and potential explanatory factors,
- crop management sequence,
- exposure to abiotic and biotic factors.

We studied the frequency of answers given by winegrowers. Since their answers varied widely, especially regarding descriptions of practices, we presented only the answers that were cited several times by the winegrowers. We analysed the answers in light of the decline framework proposed by Brunier *et al.* (2020) through three pillars: vulnerability, issues and exposure.

### 3. Variables describing vine productive status

#### 3.1. Categories of vine productivity

In all plots studied, we identified various states of productivity for individual vines (Table 2). We considered all vines that contributed to the annual yield as productive. Non-productive vines were dead (D), absent (A) or newly planted and

**TABLE 1.** Vineyard network and major features of the plots studied.

Planting density (per ha)	Plot size (ha)	Pruning system	Age in 2019 (y)	Age beginning decline (AgeD, y)	Mortality	Target yield (TY; hl. ha <sup>-1</sup> )	Soil depth (soildeep)	Within field soil variability	Irrigation	Inter-row cover crop (Cover%)	Esca intensity	Eutypa intensity	Court-noué intensity
3640	0.61	Royat	38	16	high	37	medium	low	no		medium	0	medium
4000	0.46	Royat	20	16	high	37	low	low	no	0	medium	0	medium
4440	1.29	Royat	21	8	medium	90	medium	high	yes	50	high	low	low
4000	1.88	Guyot	24	12	medium	75	NA	NA	no	NA	NA	NA	NA
4440	2.13	Lépine	27	12	high	75	medium	high	yes	50	high	low	high
4440	0.79	Guyot	19	NA	medium	90	medium	low	no	0	0	0	0
4000	0.53	Guyot	22	23	high	75	medium	low	no	25	low	0	0
2500	2	Double Guyot	30	18	medium	110	high	NA	no	50	medium	low	0
2500		Double Guyot		NA	NA	NA	high	NA	no	NA	NA	NA	NA
2500		Double Guyot	30	NA	NA	NA	high	NA	no	NA	NA	NA	NA
2500	0.78	Double Guyot	20	10	medium	140	high	NA	no	100	medium	0	0
2777		Double Guyot	35	10	medium	130	high	low	no	50	low	0	0
8264	0.79	Double Guyot	20	8	high	45	medium	low	no	0	high	0	0
8264	1.44	Double Guyot	20	12	medium	45	low	low	no	0	low	0	low
8264	1.44	Double Guyot	20	12	medium	45	low	high	no	0	low	0	low
8564	1.14	Double Guyot	20	12	medium	45	medium	low	no	0	low	0	low

The region and the grapevine variety are included in the plot names: Cab = Cabernet-Sauvignon; Chard = Chardonnay; Syr = Syrah; Ugni = Ugni blanc.

**TABLE 2.** Categories of vines and use in different normalised difference vegetation index (NDVI) field measurements and yield assessment.

Vine group	Vine category	NDVI_tot	NDVI_AI	NDVI_N	YIELD <sub>plot</sub>	YIELD <sub>subplot</sub>	YIELD per vine	YIELD per N-vine
Scale of measurement	/	Subplot	Subplot	Subplot	Plot	Subplot	Plot	Subplot
Non-productive	A = Absent	yes	no	no	no	no	no	no
	D = Dead	yes	no	no	no	no	no	no
	NP = Newly Planted	yes	yes	no	yes	no	yes	no
Productive	S = Symptomatic (chronic diseases)	yes	yes	no	yes	yes	yes	no
	OA = One-Arm vine	yes	yes	no	yes	yes	yes	no
	N = Normal	yes	yes	yes	yes	yes	yes	yes

not yet producing (NP). Absent vines corresponded to previously dead vines that had been removed from the plot. Among the productive vines, we identified three sub-categories in the 2019 experiment. Vines affected by significant levels of chronic disease were referred to as ‘symptomatic’ (S). Symptomatic vines included vines with typical foliar symptoms of Esca (Es) (in this study, Esca and black dead arm were not differentiated according to Lecomte *et al.* (2012)), *Eutypa* (EU) and virus-like diseases, namely, court-noué caused by the grapevine fanleaf virus (GFLV). Vines expressing symptoms of annual epidemics only, such as downy and powdery mildews or Botrytis bunch rot, were not included in the ‘symptomatic’ (S) category. They were considered normally productive vines, similar to those that looked healthy. Vines having lost one of their two main branches (OA for one-armed vines) constituted a second category of productive vines. Finally, other productive vines that looked healthy were classified into a third category named normally producing (N).

### 3.2. Mortality estimates

We estimated mortality using two different methods. At the plot scale, all vine locations within each plot were visually classified in September 2019 according to the above-defined categories (Table 2). The mortality at the plot scale was estimated by the proportion of A and D vines (AD%) in the plot. Moreover, the specific 30 vines from each subplot were also *de facto* classified, and the mortality rate in each subplot was calculated.

The 2019 field estimation was completed by a historical approach for each plot in Languedoc in 2012 and 2015. For this purpose, we used free airborne orthoimages covering the plots collected from the French National Institute of Geographic and Forest Information (IGN). Orthoimages were chosen according to the following criteria: i) images taken from May to July when the canopy development was sufficient but with reduced overlapping between plants, ii) image resolution lower than 0.25 m to detect the canopy of each vine and iii) images taken if possible when the

sun reaches the zenith to limit the shadow of the row. For each plot, the locations of each initially planted vine were predicted from the planting density, and the direction of the rows was automatically recognised from the image. The lack of vegetation at some predicted locations was depicted using a GIS software program (ArcGis). These empty places were qualified as ‘AD’ vines without distinction between the two categories (Figure 1), and a mortality rate was calculated. The mortality levels for each year from 2012 to 2019 were interpolated from the three analysed years with the assumption of a linear progression of mortality between each analysed year.

### 3.3. Yield estimates

#### 3.3.1. Yield estimates at the plot scale

At the plot scale, we collected from the winegrowers the actual harvested yield measured at the time of entry into the cellar (yield expressed in hectolitres per hectare, hl ha<sup>-1</sup>) over the 2007–2020 period. To compare yields obtained in plots with different yield regulations, we then calculated the yield achievement ratio at the plot scale, referred to as YAR<sub>plot</sub> in Fermaud *et al.* (2016). YAR<sub>plot</sub> is defined as the ratio between the actual harvested yield and the target yield of the plot (TY<sub>plot</sub>). The target yield, obtained from the winegrowers (Table 1), represents the quantitative production planned at winter pruning, allowing the grower to cover production costs and ensure the targeted economic margin (Equation 1) (Fermaud *et al.*, 2016).

The cumulative yield loss over the period (2007–2020) was assessed through the integrated value of the YAR<sub>plot</sub> below the optimum threshold (YAR<sub>plot</sub> = 100 %). This integrated value was later divided by the number of years (y) of measurements of harvested yield to normalise the integrated YAR<sub>plot</sub> between the plots (YAR<sub>int</sub>) (Equation 2).

Finally, the harvested yield per vine was calculated at the plot scale as the ratio between the harvested yield of the plot and the density at the time of planting (Table 1, Table 2). This density was corrected each year ( $\gamma$ ) by the proportion of AD vines (Equation 3).



**FIGURE 1.** Detection of absent and dead (A/D) vines from old orthoimages (IGN June 2012) in the Languedoc\_7\_ Chard plot. The yellow circles indicate the A or D vines, without distinction; the white lines correspond to the studied subplots.

### 3.3.2. Yield estimates at the subplot scale

In 2019, at the subplot scale and on a per-vine basis, the harvested yield, the number of bunches, and the number of shoots were recorded separately for symptomatic (S) vines and normally producing vines (five N-vines per subplot). The harvested yield per N-vine at the subplot scale is expected to be higher than the harvested yield per vine at the plot scale because the S-vine yield was reduced by viruses and grapevine trunk diseases. The average bunch mass (for N-vines and S-vines) was calculated as the ratio between the harvested yield per vine and the number of bunches per vine. The harvested yield for each subplot ( $Yield_{subplot}$ ) was determined using the frequency of vines and the average yield per vine in each productive category (S, OA, N) (Table 2). The productivity was considered null for all non-productive categories (A, D, NP). Finally, the harvested yield at the

subplot scale ( $Yield_{subplot}$ ) was calculated in Equation 4, where  $i$  is the vine category (N, S, OA).

Based on the measurements made on N-vines only, we calculated the potential yield per subplot ( $PY_{subplot}$ ) in Equation 5.

Finally, the yield achievement ratio at the subplot scale ( $YAR_{subplot}$ ) was calculated as the ratio between the yield and the potential yield per subplot (Equation 6).

## 4. Plant growth and pathological indicators

### 4.1. Vigour indicators

Vegetative development was evaluated in all plots in 2019 by using the normalised difference vegetation index (NDVI) indicator. We measured the NDVI using a GreenSeeker® sensor (Trimble Navigation Ltd., Sunnyvale, CA, USA) at three phenological stages: flowering, veraison and

► **Equation 1:**  $YAR_{plot} (\%) = (Harvested\ yield / TY_{plot}) \times 100$

► **Equation 2:**  $YAR_{int} (\%) = \frac{\sum_{y=2007}^{y=2020} (100 - YAR_{plot,y})}{number\ of\ y}$

► **Equation 3:** Harvested yield per vine<sub>y</sub> (plot scale) = Harvested yield<sub>y</sub> / [(1 - AD%)<sub>y</sub> × density].

► **Equation 4:**  $Yield_{subplot} (kg) = 30\ vines \times \sum_{i=1}^{i=3} frequency_i \times bunch\ mass\ per\ vine\ i (kg)$

► **Equation 5:**  $PY_{subplot} (kg) = 30\ vines \times (Number\ of\ shoots\ per\ vine \times number\ of\ bunches\ per\ stem \times average\ bunch\ mass (kg))$  in N-vines

► **Equation 6:**  $YAR_{subplot} (\%) = (Yield_{subplot} / PY_{subplot}) \times 100$

harvest. The NDVI was measured from one row side on the 30 consecutive vine locations comprising each subplot. The GreenSeeker® sensor was oriented horizontally towards the middle of the canopy height at approximately 1 m away. We placed a white screen with a very low NDVI value ( $< \sim 0.05$ ) behind the canopy on the other side of the row to differentiate the canopy from background interferences.

On each day of measurement, we measured NDVI in three different ways according to the visual state of every vine in the subplot: NDVI\_tot, NDVI\_AI and NDVI\_N (Table 2). We first measured the NDVI of the total 30-vine subplot area (NDVI\_tot). In the second measurement (NDVI\_AI), we considered only the vines that were alive (N, OA, S, NP), thus excluding the absent and dead vines (A, D). Finally, only the N-vines were included in the third NDVI measurement (NDVI\_N). In the three winegrowing regions, the NDVI\_tot values at veraison and at harvest were averaged because they were very similar. The same calculation was performed for the NDVI\_N value of productive vines.

At veraison, we calculated the NDVI difference between the N-vines and the live vines (NDVI\_N-l) as well as the NDVI difference between the N-vines and the entire subplot (NDVI\_N-tot). These new NDVI indicators allowed us to characterise the impact of dead and absent vines, as well as that of vigour loss due to pests and chronic diseases over the season.

#### 4.2. Pathological indicators

In the 2019 experiment, we assessed the incidence (%) of vines showing foliar symptoms of different chronic diseases regardless of their severity level, notably grapevine trunk diseases, in all vineyards. Key epidemiological stages were selected for subplot monitoring as follows. At flowering, we assessed *Eutypa* dieback incidence by observing the canopy of every vine to discriminate between apparently healthy and symptomatic vines showing typical *Eutypa* leaf and shoot symptoms (Munkvold *et al.*, 1994). At veraison, grapevines showing virus-like disease were recorded on the basis of typical foliar discolouration (yellowing, chlorotic or bright yellow mottling), stunting and reduced cane and foliar growth mostly related to grapevine court-noué disease according to experts. At the veraison stage, we also monitored Esca symptoms that were confirmed by further observations at harvest, according to Lecomte *et al.* (2012). At harvest, we also recorded the vines showing one live arm and one dead arm (OA vines, Table 2). Data from the three subplots sampled in the vineyard plot were averaged.

### 5. Weather data

The cumulative rainfall (R), cumulative Penman reference evapotranspiration (ET0) and average temperature (TEMP) were collected at a monthly frequency over the 2007–2020 period from three weather stations located at the sites of Merignac (near Bordeaux), Cognac and Roujan (Languedoc region). The weather stations were in the same town as the vineyards for Bordeaux but 5 to 37 km away from the vineyards in the Cognac and Languedoc regions. Two regional weather indicators were calculated each year.

The first indicator was the climatic water balance, defined as the difference between cumulative rainfall and cumulative reference evapotranspiration over the year (R-ET0). This indicator aimed to evaluate the balance between water supply and potential water demand in a simplified way. The climatic water balance was chosen over the soil water balance because input data and parameters required to run a soil water balance in our network of vineyards, which included different crop and soil management, were not available or not accurate enough (Lebon *et al.*, 2003, Celette *et al.*, 2010). The second climatic indicator was the average temperature during the spring period from April to June (TEMPaj). The values of each indicator (R-ET0; TEMPaj) were averaged over two successive years ( $y$  and  $y-1$ ) to cover the two-year period of the yield elaboration process.

## 6. Statistical analyses

### 6.1 Means comparison and linear mixed-effects modelling

The analyses were carried out with R statistical software version 4.1.1 (R Core Team, 2021).

To investigate the temporal change in the studied variables, we used the linear mixed-effects model. We assumed that the studied variable  $Y$  (in this study,  $Y = AD\%$ , harvested yield per plot, harvested yield per vine or  $YAR_{plot}$ ) varied for each plot (Plot) over time (YEAR) depending on the winegrowing region. Several models with increasing structural complexity were tested for each variable. These models were built with a fixed YEAR effect, and the Region and/or Plot were integrated as random effects according to the following equations:

$$\text{mod1} = \text{lme}(X \sim \text{YEAR}, \text{random} = \sim 1 | \text{Plot})$$

$$\text{mod2} = \text{lme}(X \sim \text{YEAR}, \text{random} = \sim 1 | \text{Region/Plot})$$

In the case of heteroscedasticity, a correction with a variance structure component was integrated into the model. Two corrections were tested, one with Plot (cor1) and one with Region and Plot (cor2).

$$\text{mod3} = \text{lme}(X \sim \text{YEAR}, \text{random} = \sim 1 | \text{Plot} + \text{cor1})$$

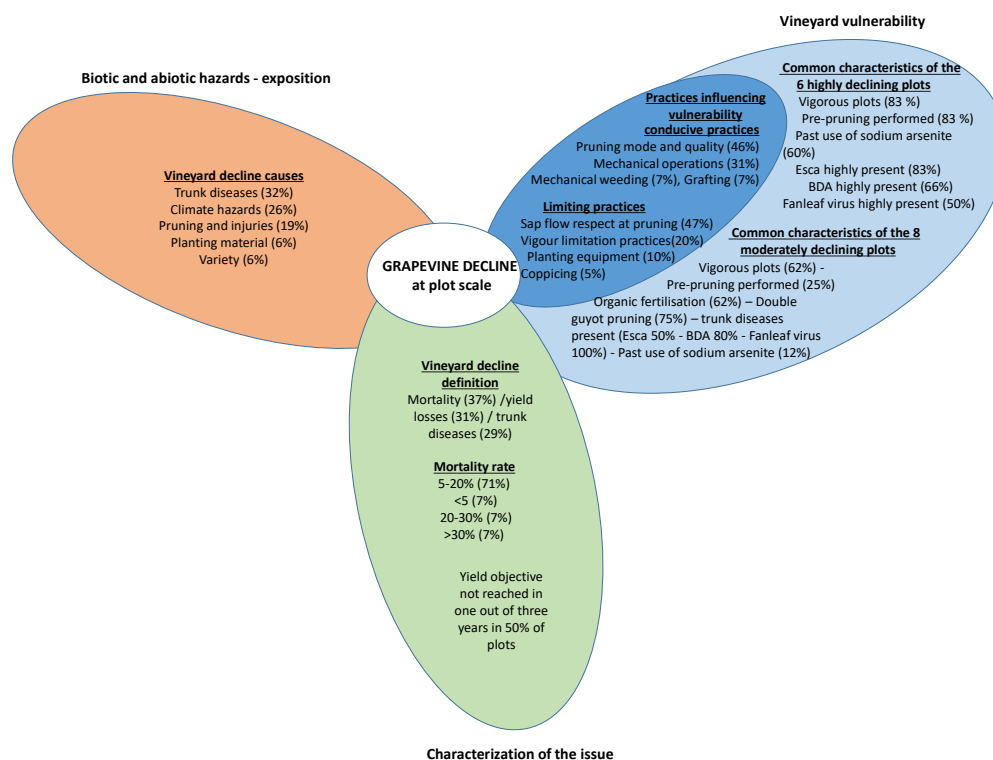
$$\text{mod4} = \text{lme}(X \sim \text{YEAR}, \text{random} = \sim 1 | \text{Plot} + \text{cor2})$$

$$\text{mod5} = \text{lme}(X \sim \text{YEAR}, \text{random} = \sim 1 | \text{region/Plot} + \text{cor1})$$

$$\text{mod6} = \text{lme}(X \sim \text{YEAR}, \text{random} = \sim 1 | \text{region/Plot} + \text{cor2})$$

For each variable, we used the Akaike information criterion (AIC) to select the best model. Then, we tested the YEAR effect for each variable by performing an ANOVA with the best model. If the residuals did not follow a Gaussian distribution, a permutation test was performed. When the model showed a significant effect of the year, we performed multiple comparisons of means to better understand this effect. We considered significance at the classic level of 0.05.

In the particular case of vigour measures, an analysis of variance was performed to compare the NDVI\_N to the NDVI\_tot for each region during the veraison-harvest period



**FIGURE 2.** Grapevine decline perception among the interviewed winegrowers. Answers to the survey organised following the three pillars of crisis management (Brunier *et al.*, 2020). Green petal: characterisation of the issue; orange petal: exposure to hazards; blue petal: vineyard sensitivity (dark blue: practices that are conducive to or limit vulnerability; light blue: common characteristics of highly vulnerable plots). The frequency of answers are given in brackets. BDA = black dead arm.

and the NDVI\_N-AI at flowering to the NDVI\_N-AI during the veraison-harvest period.

## 6.2. Principal component analysis

The major relationships among all the variables recorded were identified by principal component analysis (PCA) using the StatBox software program (Version 6.6, Grimmer Logiciels, Paris, France). A total of 17 variables were included in the PCA, including 14 active variables participating in the axis definition. The four other variables (cover%, AsNa, VIG\_R, soilDeep) were considered supplementary variables because they were qualitative and more subject to winegrowers' perceptions (see Table 1 for abbreviations and values of these variables).

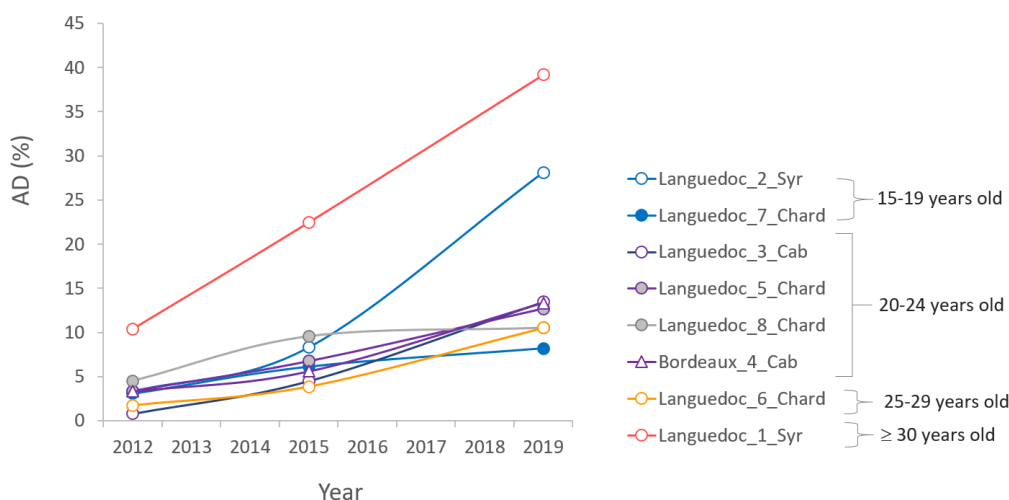
# RESULTS

## 1. Characterisation of grapevine decline based on interviews with winegrowers

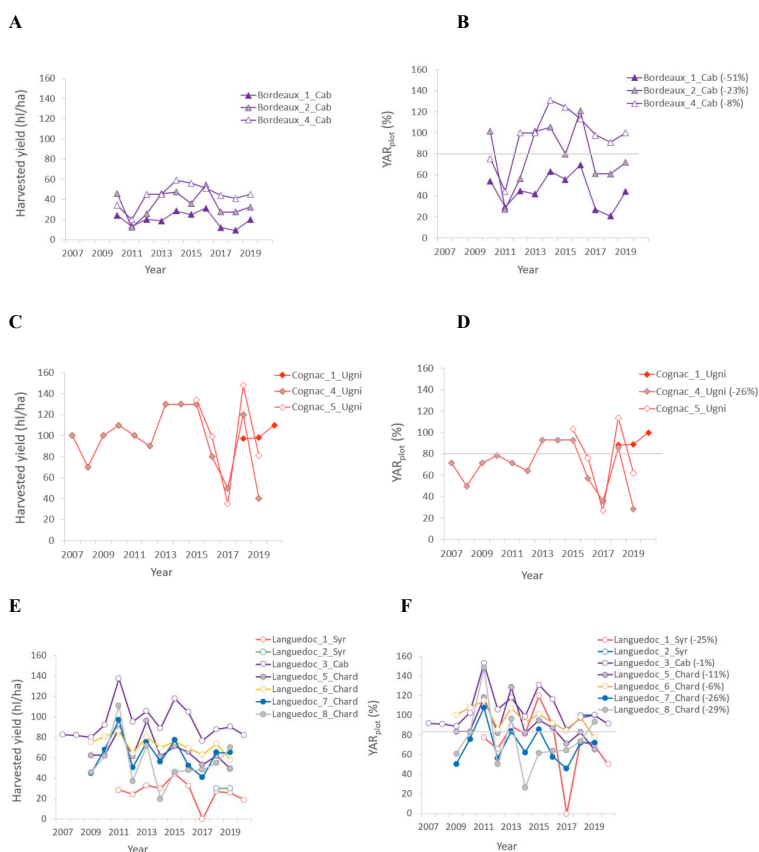
All the interviewed winegrowers identified grapevine decline in their plots and their decline definition was often very similar to that proposed by the French National Plan for Grapevine Decline, especially in terms of vineyard longevity (Figure 2). Mortality (37 %) and yield loss (31 %) were considered synonyms of decline, and trunk diseases were also mentioned

in 32 % of the interviews. When focusing on the plots studied, winegrowers declared a mortality rate ranging between 5 % and 20 % for 71 % of the studied plots in 2019 (Figure 2) and higher than 30 % in one plot. The target yield assigned to the plot was reached only one year out of three in 50 % of the plots. The decline was mainly identified in plots that were more than 10 years old, mainly when plots were between 11 and 15 years old. The factors that are conducive to grapevine decline in the plots studied according to the winegrowers were trunk diseases (32 % of answers), climate hazards (26 %) and injuries related to pruning and mechanical operations (19 %) (Figure 2—exposure to biotic and abiotic hazards). Thus, winegrowers considered that imprecise and insufficiently meticulous pruning increased decline, whereas pruning that did not interfere with sap flow (47 %) and vigour limitation practices (20 %) may limit decline occurrence (Figure 2—vineyard vulnerability, practices influencing vulnerability). Regarding plot vulnerability to decline (as defined in Brunier *et al.*, 2020), higher vulnerability was mainly associated with vigorous plots subjected to pre-pruning and with higher trunk diseases and virus pressure levels (Figure 2). Furthermore, the winegrowers also identified limiting and conducive technical factors involved in vineyard sensitivity to decline, including mechanical operations (31 %), notably mechanical weeding (7 %) and planting equipment (10 %) (Figure 2—conductive and limiting practices). Curative practices such as cutting





**FIGURE 3.** Rates of absent (A) and dead (D) vines (AD%, see Table 2) in 2012, 2015 and 2019 according to the age class of the plots.



**FIGURE 4.** Harvested yield (A, C, E) and yield achievement ratio (YARplot; B, D, F) from 2007 to 2020 in Bordeaux (A, B), Cognac (C, D) and Languedoc (E, F).

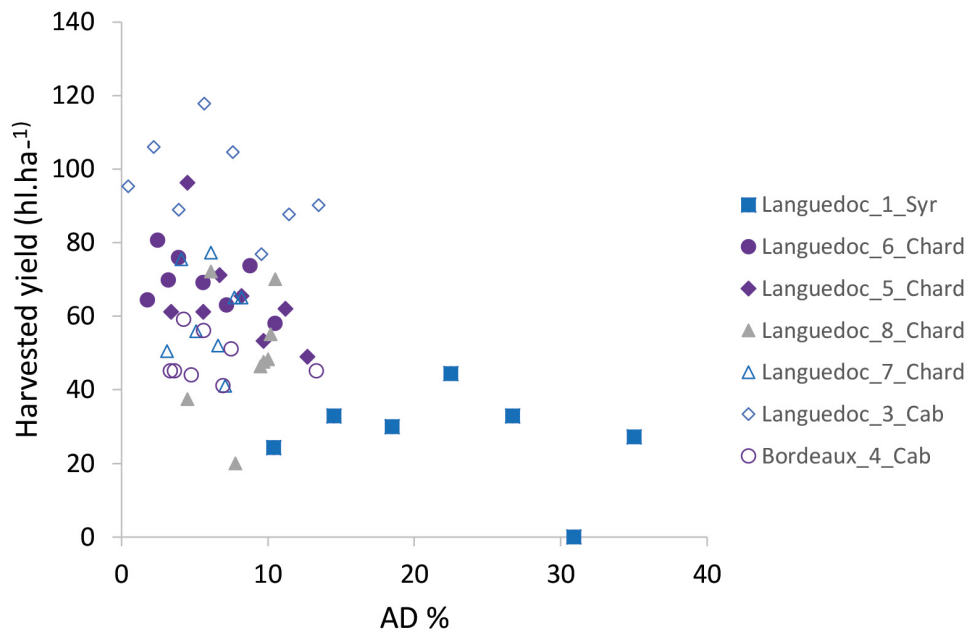
The values in brackets represent the normalised integrative values of the YARplot (YARint). The grey horizontal lines represent the YARplot threshold of 80 %.

back and trunk renewal were also cited as ways to limit decline at the plot scale, whereas over-grafting tended to increase decline. Finally, in the practice description part of the survey, co-planting, allowing live vine shoots to grow over an adjacent empty space, and limitation of large wounds during pruning were the only three practices winegrowers in the network actually used to address decline (Figure 2), representing 57 %, 38 % and 100 % of answers, respectively.

## 2. Dynamics of vine mortality and yield in the three regions

### 2.1. Dynamics of mortality at the plot scale

The mortality indicator (AD%) at the plot scale increased significantly from 2012 to 2019 ( $\text{mod}4-p < 0.001$ , obtained with a permutation test) in all the plots (Figure 3). The increase in AD% was higher from 2015 to 2019 than



**FIGURE 5.** Harvested yield as a function of mortality at plot scale (AD%, see Table 2) over the 2012–2019 period in two of the three studied regions (Bordeaux and Languedoc).

from 2012 to 2015. In fact, mortality dramatically increased for Languedoc\_1\_Syr (the oldest plot in the network), Languedoc\_2\_Syr (the youngest plot in the network) and Languedoc\_3\_Cab.

## 2.2. Yield dynamics at the plot scale

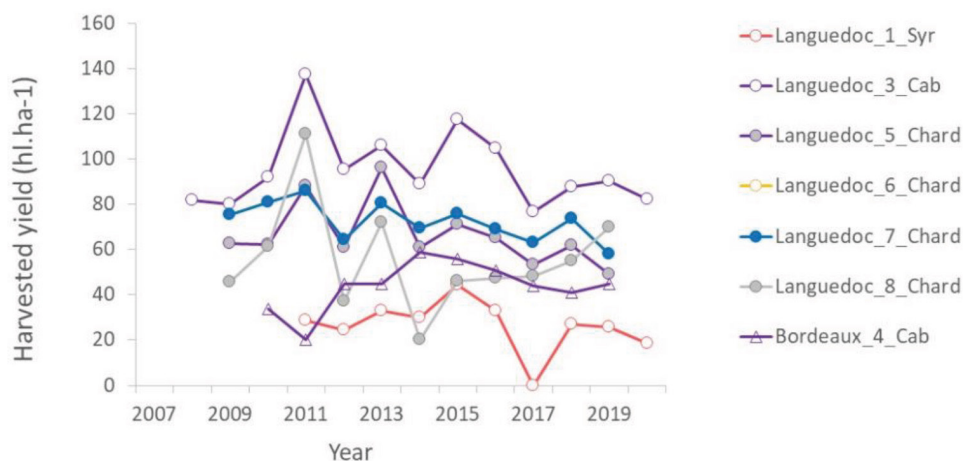
The results for harvested yield and yield achievement ratio ( $YAR_{plot}$ ) calculated over the 2007–2020 period are presented in Figure 4. The linear mixed-model-based analysis showed a significant effect of time on the harvest yield for the whole network ( $mod5, p < 0.001$ ). The time effect was mostly related to inter-annual variability rather than a regular decrease in harvested yield, even if the yield tended to decrease in more than 75 % of the plots over the studied period. As expected, the harvested yield varied among regions, with the highest overall values in Cognac, the lowest values in Bordeaux and

intermediate values in Languedoc. Within each region, high yield variations were observed among vineyards and years. When analysed individually, a significant effect of time (year) was observed on Lang\_6\_Chard (Figure 4).

The minimum  $YAR_{plot}$  was between 0 % and 30 % in all regions, and the normalised integrative value of the YAR below the optimum ( $YAR_{int}$ ) varied between -51 % (Bordeaux\_1\_Cab) and -1 % (Languedoc\_3\_Cab). From statistical analysis, no consistent trend of yield loss over time was observed across the whole network, apart from a significant vintage effect ( $mod3, p < 0.0001$ ).

## 2.3. Relation between yield and mortality at the plot scale

Overall, the harvested yield decreased significantly ( $p$ -value  $< 0.001$ ) as the AD% increased (Figure 5). However, when considering individual vineyards, the harvested yield



**FIGURE 6.** Harvested yield per productive vine from 2012 to 2019 in Bordeaux and Languedoc plots.

decrease was either clear (e.g., Languedoc\_6\_Chard), or, in contrast, poorly discernible (e.g., Languedoc\_1\_Syr or Bordeaux\_4\_Cab). The expected decrease in harvested yield with an AD% increase may be disturbed by compensation from the productive vines. As a verification, the dynamics of yield per productive vine over the period 2007-2019 were assessed on available data (Figure 6). No significant trend was found (mod4—p value = 0.16) in the whole network mixing plots with low and medium AD% values. The higher AD% values were observed only on Languedoc\_1\_Syr with a low yield over this period, mainly due to soil characteristics and low target yield.

#### 2.4. Relationship between decline and climatic indicators

Relationships between regional climatic water balance (R-ET0) or the mean temperature in spring (TEMPaj) with the dynamics of harvested yield and yield achievement ratio ( $YAR_{plot}$ ) are presented in Figure 7 and Supplementary data S1. No relationship between the harvested yield or  $YAR_{plot}$  and the climatic water balance was observed for the three regions (Figure 7). Similarly, no clear trend was observed between the harvested yield or  $YAR_{plot}$  and the spring temperature (Supplementary data S1).

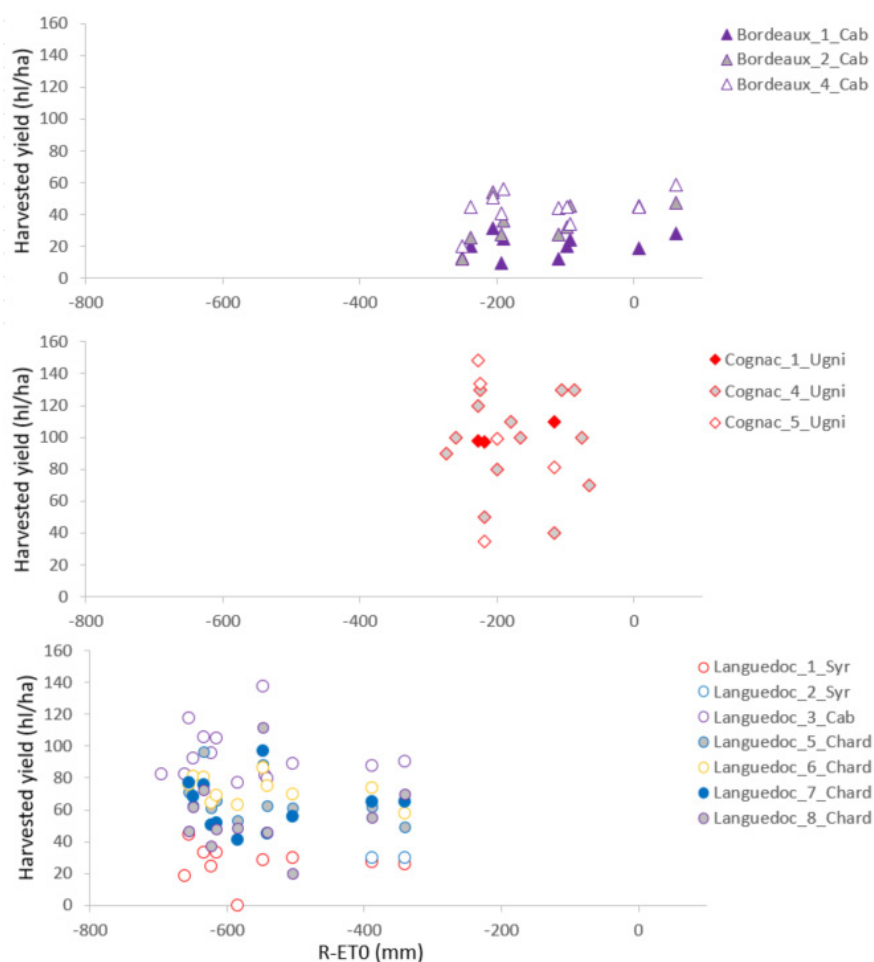
### 3. Decline analysis in 2019 at the plot and subplot scales

#### 3.1. Non-productive vine frequency and yield

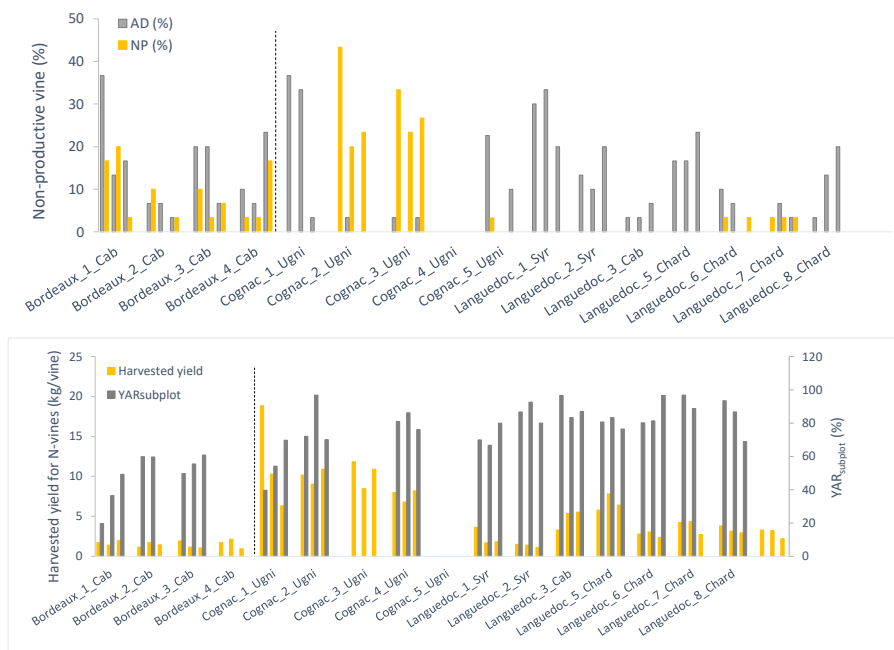
The average frequency of AD vines was similar among the regions, with a high degree of variability among plots and within each plot (Figure 8 and Supplementary Data S2). Conversely, newly planted vines (NP) represented a significant proportion of non-productive vines in Bordeaux (8 %) and Cognac (16 %), whereas replanting was not a common practice in Languedoc. The harvested yield per vine for N-vines showed high interregional differences, with the highest values in Cognac and the lowest subplot yields harvested in Bordeaux, partly due to the high vine density. The yield per vine for N-vines measured at the subplot scale was consistent with the yield per vine calculated at the plot scale (Figure 6). The yield achievement ratio calculated at the subplot scale ( $YAR_{subplot}$ ) also presented substantial variability among regions and plots. When compared to the  $YAR$  calculated at the plot scale in 2019, the  $YAR_{subplot}$  was higher in 40 % of the plots and lowered in the other 60 %.

#### 3.2. Vegetative vigour

Considering the various NDVI indices, the values of NDVI<sub>N</sub> and NDVI<sub>tot</sub> from veraison to harvest were lower than



**FIGURE 7.** Harvested yield as a function of the climatic water balance (R-ET0) averaged over two successive years over the 2007–2020 period in the three regions (Bordeaux, Cognac and Languedoc).



**FIGURE 8.** Rates of non-productive vines (AD% and NP%) and productivity (harvested yield and yield achievement ratio in each subplot YARsubplot) of all vineyards in Bordeaux, Cognac and Languedoc at the subplot scale. Each bars corresponds to one subplot.

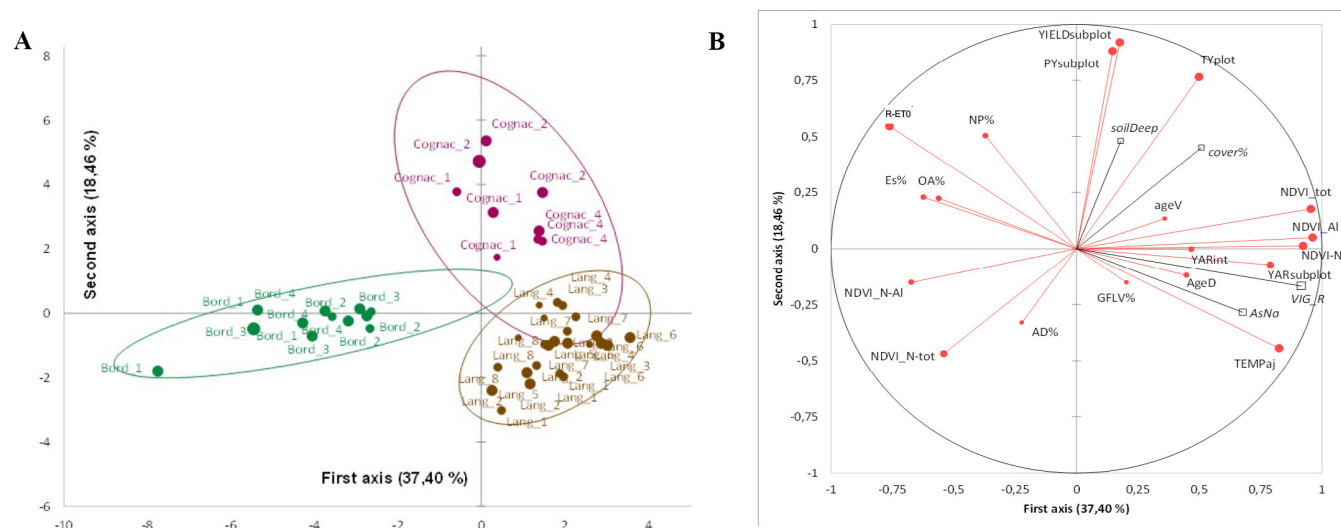


**FIGURE 9.** Normalised difference vegetation index (NDVI) indicators measured at flowering (Flo) or during the veraison-harvest period (ver-har) in each subplot of all vineyards in Bordeaux, Cognac and Languedoc.

A) NDVI<sub>N</sub> (measured on normal vines only) and NDVI<sub>tot</sub> (measured on all vines) over the veraison-harvest period. B) Difference of NDVI between the normal vines and alive vines (NDVI<sub>N-AI</sub>) at flowering and for the veraison-harvest period. Each bar corresponds to one subplot.3.3. Multi-factorial analysis of decline in 2019 in the three regions

0.51 in Bordeaux, while they were higher than 0.76 in both Cognac and Languedoc (Figure 9A). The average NDVI<sub>N</sub> (ver-har) values were significantly higher than the NDVI<sub>tot</sub> (ver-har) values ( $p < 0.05$ ) in Bordeaux and Languedoc, while these two indicators were similar in Cognac ( $p > 0.05$ )

(Figure 9A). The NDVI<sub>N-AI</sub> values varied strongly among plots and regions (Figure 9B). The coefficient of variation of NDVI<sub>N-AI</sub> (ver-har) among the subplots was, on average, 68 % in Bordeaux, 84 % in Languedoc and 134 % in Cognac.



**FIGURE 10.** The first two axes of the principal component analysis of the studied grapevine plots in three regions (and subplots): Bordeaux (“Bord” in green), near Cognac (in purple) and Languedoc in south-eastern France (“Lang” in brown). A) scattering of the plots (and subplots); B) variable projection.

i) Active variables (red dots) characterising grapevine decline and incidence of chronic diseases (AD%, Es%, OA%, GFLV%, AgeD), yield indicators (Yield<sub>subplot</sub>, PY<sub>subplot</sub>, TY<sub>plot</sub>, YAR<sub>int</sub>, YAR<sub>subplot</sub>), vine age and vegetative vigour (ageV, NP%, NDVI<sub>tot</sub>, NDVI<sub>N</sub>, NDVI<sub>N-tot</sub>, NDVI<sub>N-AI</sub>, NDVI<sub>AI</sub>), regional climatic features (R-ETO, TEMP<sub>aj</sub>), and ii) supplementary variables (black squares, italics) related to soil depth and management (soilDeep, cover%), vine vigour (VIG<sub>R</sub>) and past use of sodium arsenite (AsNa).

Moreover, it significantly decreased from flowering to veraison in Languedoc (from 0.04 to 0.02) ( $p < 0.05$ ).

In the principal component analysis (PCA), the first two main axes accounted for more than half of the total variance, i.e., 55.8 % (Figures 10a,b) and 67.1 % by including the third PCA axis. As a first important result, the three regions were clearly differentiated (Figure 10a), which was expected because of very different production targets and associated cropping techniques. This was best exemplified by clear-cut differences in i) key production parameters, notably growers’ target yields (TY<sub>plot</sub>), vine density, which is twice as high near Bordeaux (> 8000 vines/ha) than in Languedoc (approx. 4000 vines/ha) and in ii) contrasting climatic conditions, i.e., oceanic versus Mediterranean (Figure 10b, Figure 7, Supplementary data S1).

Figure 10b shows the inter-relations among various production system variables and three key grapevine decline indicators, i.e., AD%, Es%, and YAR. The first composite axis was mainly representative (respective contribution in brackets) of the yield achievement ratio in 2019 (YAR<sub>subplot</sub>? 8.8 %) and three high-vigour NDVI variables, i.e., the total NDVI (NDVI<sub>tot</sub>, 12.8 %), the NDVI of live vines (NDVI<sub>AI</sub>, 13.0 %) and the NDVI of normal productive vines (NDVI<sub>N</sub>, 12.0 %). Thus, the right-hand pattern on Axis 1, including the supplementary variable (VIG<sub>R</sub>) indicating increased vigour due to the rootstock, corresponded to high vine vigour and reduced yield loss. The key decline variable, Esca vine incidence (Es%, 5.5 %), showed a clear contrast on Axis 1 with regard to the previous variables, most particularly with the YAR<sub>subplot</sub> (coefficient  $R = -0.56$  (at  $p = 0.01$ )). This may further substantiate a causal link between yield loss (2019) and such a key decline variable.

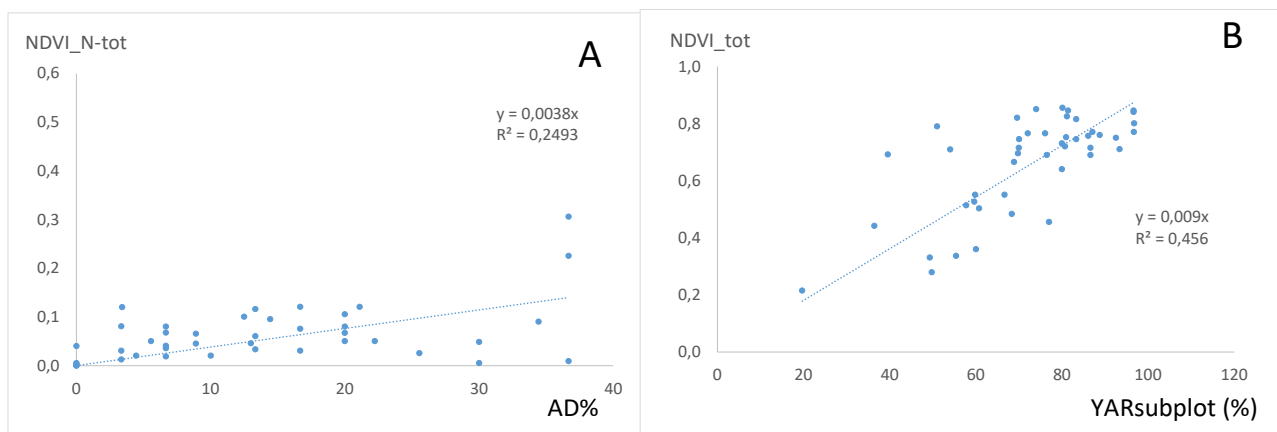
Very interestingly, the differential NDVI index (NDVI<sub>N-AI</sub>) also contributed markedly to Axis 1 (6.4 %). Since the NDVI decrease (NDVI<sub>N-AI</sub>) was significantly ( $p = 0.01$ ) and positively correlated with Esca symptoms ( $R = 0.51$ ) and negatively correlated with the YAR in 2019 ( $R = -0.51$ ), we propose it as a very promising indicator for diagnosing decline. Interestingly, the supplementary survey variable *AsNa*, indicating past use of sodium arsenite, was positively and significantly correlated with the plot age when decline first appeared ‘AgeD’ ( $R = 0.47$ ,  $p = 0.01$ ).

The second main PCA axis mostly represented (on the positive side) three yield-related variables: the subplot yield (Yield<sub>subplot</sub>? 24.1 %), the potential yield at the subplot level (PY<sub>subplot</sub>? 22.2 %) and the target yield (TY<sub>plot</sub>? 16.7 %). Furthermore, the third PCA axis (not shown) highlighted two crucial decline variables, i.e., AD% and the vineyard age when decline first appeared (AgeD), as correlated positively with the plot age ageV (significant  $R$  coefficients of 0.32 and 0.69, respectively).

Finally, the PCA showed a significant correlation between the decline indicator AD% and the NDVI indicator NDVI<sub>N-tot</sub> (Pearson  $R = 0.54$ ,  $dF = 43$ ,  $p < 0.001$ ) (Figure 11). In the same way, another significant linear relationship was established between the yield achievement ratio (YAR<sub>subplot</sub>) and the overall NDVI indicator based on all vines, NDVI<sub>tot</sub> (Pearson  $R = 0.71$ ,  $dF = 43$ ,  $p < 0.0001$ ) (Figure 11).

## DISCUSSION

This study proposed an analysis of grapevine decline, at both the plot and subplot scales, based on winegrowers’ answers



**FIGURE 11.** Relation between NDVI indicators, mortality and yield.

A) Significant linear regression between the decline indicator AD% (dead and absent vines) and the NDVI indicator NDVI\_N-tot, i.e., the difference between the normal-vine NDVI and the total NDVI. B) Significant linear regression between the yield achievement ratio indicator (YAR) and the NDVI indicator NDVI\_tot, assessed by including every vine in the vineyard subplot monitored: each point represents each studied subplot.

to a survey as well as experimental measurements in different vineyards.

### 1. Main features of winegrowers' perception of decline

The winegrowers' survey showed that decline was a major concern in the three French regions studied. Winegrowers' perceptions of decline were based on vine mortality rather than on yield decrease. The yield issue was considered more in terms of inter-annual variability than of a regular decrease over time. Winegrowers related decline to grapevine trunk diseases (GTDs). GTDs were cited in the three petals of the vulnerability flower plot (Breda and Pfeiffer, 2014), showing the difficulty in differentiating the definition, the processes and the explanatory factors of grapevine decline. This may be because, at least partly, it is easier to visually detect trunk diseases than other disorders, such as those related to vine hydraulic conductivity or soil issues (Lecomte *et al.*, 2012). This finding highlights the current lack of a set of validated and operational indicators of decline. As a direct consequence, we were not able to draw conclusions about the interest in some practices to lower grapevine decline.

### 2. The particular role of trunk diseases and cultivar effect

The focus on trunk diseases found in the surveys was consistent with previously published results. Several studies observed a strong relationship between vine mortality and Esca-*Eutypa* dieback prevalence or incidence (Fussler *et al.*, 2008; Guérin-Dubrana *et al.*, 2013). Our study highlighted the range of declining situations and the variability in the expression of GTDs between the three winegrowing regions. This may be explained by various factors, such as the climate, the different varietal susceptibilities to trunk diseases and possible interactions with regional soils and/or practices. The higher level of GTDs in the Bordeaux and Cognac regions may also relate to climatic conditions that

are more conducive since chronic symptoms of Esca appear more frequently during cool and rainy springs or summers (Surico *et al.*, 2000, Marchi *et al.*, 2006). Moreover, the typical Cabernet-Sauvignon cultivar in Bordeaux is known to be highly susceptible to trunk disease pathogens (Bruez *et al.*, 2013, Murolo and Romanazzi, 2014). Conversely, the lower prevalence of GTDs in Languedoc vineyards, mostly planted with Syrah, corroborated the observations from the French National Grapevine Wood Diseases Survey, reporting Syrah as one of the varieties that expressed symptoms of trunk diseases the least (Grosman and Doublet, 2012). However, the plots showing high mortality rates in Languedoc were planted with this cultivar, which is associated with a particular type of decline known as "Syrah decline", which is well documented in California and France (Battany *et al.*, 2004; Beuve *et al.*, 2013). Similar to Syrah, the Chardonnay cultivar generally expresses fewer Esca symptoms (Grosman and Doublet, 2012, Bruez *et al.*, 2014) but expresses more court-noué symptoms (Yobrégat *et al.*, 2020).

In addition to mortality, yield losses on live vines have been reported. For *Eutypa* dieback, a reduction in both the bunch number and the yield, from 30 % to 60 %, has been demonstrated according to the disease severity (Munkvold *et al.*, 1994). For Esca, canes of symptomatic plants exhibited reduced carbohydrate reserves during the winter rest period, which may account for a decrease in plant vigour and yield loss (Petit *et al.*, 2006).

### 3. Comparing the winegrowers survey to field measurements of decline

The historical analysis of the plot network confirmed the winegrowers' perceptions of grapevine decline as being mainly driven by increasing proportions of non-productive vines. Thus, according to the airborne orthoimage analyses, the rate of non-productive vines increased from 2012 to 2019 in every plot. The link between grapevine decline and yield loss was not straightforward from either the survey or the

historical dynamics of yield. Interestingly, the winegrowers' perception of decline was supported by a trend of yield decrease in the historical analysis (2007–2019) for more than 75 % of the plots studied and even a significant yield decrease for Languedoc\_6\_Chard. Generally, the  $YAR_{plot}$  was high (> 80 %) for most of the vineyards studied in the three regions over the entire period considered, with the exception of a few particular years (2017 with severe spring frost and 2019). Thus, yields generally met the targets set by winegrowers over the studied period. Nevertheless, the high inter-annual variability in yield, reported by the winegrowers as an indicator of grapevine decline, was consistent with the year-to-year variations in the harvested yield measured at the field scale. Moreover, it is probable that the strong inter-annual variability in yields over time masked the yield decrease due to the decline. In addition, the increasing yield per vine observed for some plots was consistent with the increase in space available for live vines adjacent to absent and dead vines and with the compensatory management practices adopted by winegrowers to limit yield loss at the plot level.

#### 4. Specific abiotic issues of decline, depending on the region

The survey highlighted the diversity of abiotic production contexts, which echoed the results of our quantitative analyses. The regional features were very apparent in the PCA multi-factorial analysis of the 2019 data. The Languedoc vineyards, with a high level of water deficit, warm springs, intermediate yields and virus pressure, were clearly distinguishable from the Bordeaux and Cognac vineyards, which were characterised by lower water deficits and cooler springs. The high soil depth in Cognac, the large degree of variability in soil properties and depth in Languedoc, and the use of irrigation in some plots could affect and explain, at least partly, the poor link between the yield and water deficit in those regions. The differences in target yields between the plots (varying by more than 60 hl ha<sup>-1</sup> in the Languedoc dataset) may also affect the analysis of the effect of climate on yield. Finally, although warmer temperatures are expected to favour vine fruitfulness (Sanchez and Dokoozlian, 2005), no relationship was found between spring temperatures and harvested yields in any region, suggesting that many other factors (e.g., specific microclimate, soil characteristics and crop management at the field scale) underlying yield elaboration may have overcome the impact of regional temperature.

#### 5. Temporal and spatial progression of decline

This study allowed us to identify the relationships between yield, mortality, diseases and vine vigour from both temporal and spatial perspectives. The historical analysis confirmed that decline is a long-term phenomenon. We showed that the mortality rate in a given year was an earlier indicator of grapevine decline than yield or YAR. Mortality progressively spread over the studied plots year after year, but a large degree of variability within each vineyard was observed, with non-productive vines distributed irregularly within each

vineyard. The subplot data were probably not sufficient to study this spatial variability. A geostatistical approach was not possible with this dataset, and a specific, larger dataset may be suitable to analyse the pattern of non-productive vines. Conversely, the yield studied at the subplot scale was a more efficient way to understand the potential compensatory effect of non-productive vines. Similarly, the subplot scale was suitable for studying the variables related to vigour.

Although no clear yield loss was observed in any individual vineyard plot at the time of the study, yield tended to be negatively correlated with the rate of non-productive vines within our whole network. Additionally, the dynamics of productivity indicators, such as yield or YAR, were less precise grapevine decline markers than the mortality rate because of their higher inter-annual variability. Since yield is an integrative variable of all practices and abiotic/biotic stresses during the two years of yield elaboration, such inter-annual yield fluctuations are not surprising (Merot and Smits, 2020; Merot *et al.*, 2022; Clingeleffer, 2010).

The results based on Figure 5 show a slightly decreasing trend in yield according to the non-productive vines in some plots, with a large amount of data below a threshold of 10 % of mortality (AD). Based on a mean increase rate of 1 % of AD per year (from the results of the historical analysis, see Figure 3), the AD (25 %) after 25 years likely corresponds to an economical and legal threshold (only for Protected Designation of Origin (PDO) products), which determines the decision to pull up the vineyard.

From a historical point of view in France, it is important to note that our study took place after 2010. This is significant because, according to Schauburger *et al.* (2018), viticulture yields decreased at a national level from approximately 1990 to 2010 and then plateaued afterwards. Thus, the decline phenomenon in French vineyards was not as strong over the last decade as it was in the previous thirty years (1980–2010). Ultimately, the decline phenomenon should be addressed over a longer period of time.

#### 6. Indicators applied to diagnose decline

This study aimed to identify indicators that may help winegrowers and technicians detect and diagnose a decline situation at the plot or subplot level. Winegrowers often wait to implement practices to limit the effects of decline. Such management practices can be particularly expensive and time-consuming, which slows their implementation every year on all plots (Kaplan *et al.*, 2016). Furthermore, the uncertainty and long time lags in profit losses may delay the adoption of such practices and reduce profitable vineyard lifespans, even if the benefits of early adoption are considerable (reducing profit losses by up to almost 50 % in some cases) (Kamplan *et al.*, 2016). Thus, early decline diagnostic indicators are of vital importance to help and promote early adoption of practices limiting the effects of decline.

A new methodological approach was developed to quantify the dead or absent vines at the plot scale. We used

orthoimages available at no cost from the French National Institute of Geographic and Forest Information (IGN) covering the plots from the past 10 years, depending on their availability. Various criteria (3.2) were applied to choose the appropriate images. The areas not covered by canopy within the rows of the vineyard were manually depicted through GIS software to obtain the dead or absent vines. An automated process is currently in development based on various published studies (DeLenne *et al.*, 2010; Padua *et al.*, 2018) to produce specifications from free IGN orthoimages for future applications.

The other technological approach to identify and develop decline indicators was the use of ground-based NDVI assessments resulting from field measurements at precise phenological stages during berry maturation, i.e., at veraison and harvest (Figure 10). The NDVI\_tot indicator, including all vines, allowed us to propose a new, easy-to-obtain field indicator of grapevine decline according to the promising significant linear relationship established with the yield achievement ratio (YAR). This relationship must be confirmed under other experimental and/or regional vineyard conditions. At least two major factors can explain the reduced NDVI\_tot values. First, the presence of missing, dead or newly planted vines may have contributed to a lower NDVI value. Second, it may also be due to marked foliar disease symptoms, notably the typical GTD tiger-stripe symptoms (Lecomte *et al.*, 2012). NDVI monitoring can actually reveal shifts originating from pathological infections on plants (Thomas *et al.*, 2018), such as Esca symptoms and viral leaf symptoms, such as red blotch or grapevine leafroll-associated viruses (Bendel *et al.*, 2020). The sensitivity of hyperspectral vegetation indices was also recently applied to disease severity grading in the canopy, in cotton (Martins *et al.*, 2018) and in grapevine (Manganiello *et al.*, 2021). Accordingly, we also consider another key NDVI-based indicator, NDVI\_N-AI, as a very promising diagnostic indicator of decline, particularly related to the intensity of Esca foliar symptoms. The NDVI\_N-AI indicator was especially helpful, in complement to NDVI\_tot, for distinguishing Esca foliar symptoms from a more general vigour reduction. Thus, both NDVI indicators measured at the ground level and the aerial photograph methodology are very promising tools to better diagnose grapevine decline situations. A further step will be to determine the threshold value(s) for interpreting these indicators in various production contexts. Beyond the choice of the new indicators, diagnosing a declining situation is also a matter of where and when these indicators are collected.

In this study, the plot level has been shown to be an interesting scale for investigating changes in decline and dynamics over several years. However, the subplot scale (here, 30 consecutive vine locations) was also particularly relevant in detecting decline using mortality and NDVI indicators. In most of our plots, the decline did not actually spread over the entire plot surface but was mostly located in certain zones clearly identified by winegrowers. Although the subplot data were probably not sufficient to study infra-plot variability, this result may lead to a recommendation to regularly

monitor the decline indicators in one to three subplot(s) in the plot instead of conducting a one-time check over a wider area. This may allow technicians and growers to increase the number of plots studied, which may be helpful considering the large diversity of decline situations. Moreover, because the mortality dynamics in the network did not show any abrupt changes, a yearly check of the decline phenomenon in a particular plot does not seem necessary. A 2- to 3-year time step seems more appropriate to limit data acquisition time and explain decline with a minimum number of variables.

## CONCLUSION

In this study, we investigated the relationships between vine mortality and yield decrease, which are associated with vine decline by winegrowers, and plant development indicators (NDVI), considering both a temporal and spatial perspective. The objective was to propose a set of indicators based on their sensitivity to vine decline. Vine mortality, yield and NDVI-based indicators can all contribute to the diagnosis of decline but not at the same time over the plot lifetime. Thus, mortality and NDVI indicators permitted the early detection of decline. This study revealed that the degree of implementation practices varied markedly between plots and winegrowing regions. In that respect, although the production variables, i.e., yield and YAR, were shown to be late indicators of vine decline, they are helpful in quantifying the potential economic losses for the winegrowers. The subplot analysis was complementary to regional and plot-scale analyses. It allowed us to point out the yield compensatory practices implemented by the winegrowers as the rate of non-productive vine increased and to build new sensitive and easy-to-measure indicators of vine decline based on key NDVI assessments, including alternatively all plants, live plants or normal productive plants. Further study is necessary to set specific thresholds of those NDVI indicators related to vine decline in various winegrowing regions.

## ACKNOWLEDGEMENTS

This research was supported by FranceAgrimer/CNIV as part of the programme ‘Plan National Dépérissement du Vignoble’ within the TRADEVI project. The authors are grateful to the winegrowers of the studied plots for their help. The authors acknowledge the Bordeaux INRAE–BSA–IFV Joint Technology Unit UMT SEVEN for its support, as this study was carried out partly within the UMT SEVEN framework. The authors would also like to thank Teri Jones-Villeneuve and the Springer Service to Authors service for their English language editing and reviews. The authors declare no conflicts of interest.

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