



# Vineyard age-specific cohorts display similar climate x esca relationships but suggest hidden drivers in younger vineyards

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## ABSTRACT

Esca is a grapevine disease with a unique visual impact. Plants exhibit leaf necrosis with a tiger-striped pattern, sometimes dramatically apoplectic. Together with the variability in leaf symptom expression from one season to the next, esca is conspicuously visible to vine growers, amplifying their perception of its propagation. A global increase in esca incidence has been observed in the first 10–15 years of this century, making esca one of the major causes of vine mortality and vineyard decline. Research has improved the understanding of this complex disease with symptoms caused by a combination of distinct pathogens in association or succession, as well as by structural and physiological changes. The multifactorial complexity of esca has made understanding the disease challenging. Among the factors environment plays a critical role. Climate change could be contributing to the observed growth in esca incidence, and if true, could continue to exacerbate the disease in the future. This study builds upon an extensive surveying effort carried out by annually monitoring ~57,000 vines across 12 estates in the Bordeaux region for 9 years. We integrated this extensive regional esca incidence database with localised daily weather data to quantify the impact of climatic and pedoclimatic factors on esca incidence. The study confirmed that factors previously explored such as the amount of precipitation early in the season and temperature dynamics later in the season have a clear relationship to disease incidence, but also highlights an important change in disease behaviour in plots planted in the last 10–15 years. Isolating the effect of age of the plots uncovered disease patterns that suggest hidden drivers unrelated to climate which could possibly include changes in propagated vines and/or management techniques.

**KEYWORDS:** dieback, climate effect, grapevine, wood disease, pedoclimate, wine

## INTRODUCTION

Cultivated over 7.3M ha worldwide, grapevine (*Vitis vinifera* L. spp.) is the most important perennial fruit crop economically, with a high commercial value for wines and juices, fresh table grapes, and dried fruit (OIV, 2023). Grapevines are also known for their wide range of pathogens, especially fungal pathogens that are responsible for highly destructive diseases resulting in yield loss and/or vine mortality. Some fungal diseases negatively affect fruit yield and/or quality in the current season but rarely result in carry-over effects or vine death that threaten the long-term sustainability of the vineyard. Other fungal diseases, such as esca, lead to vine mortality and are responsible for vineyard decline. Esca incidence has been increasing globally over the last 20 to 25 years, resulting in important economic losses (De la Fuente *et al.*, 2016; Gramaje *et al.*, 2018).

Despite its long documented history, esca is still poorly understood, the pathogens responsible for esca are not clearly defined and esca remains a complex syndrome (Graniti *et al.*, 2006d; Dewasme *et al.*, 2022). Until the end of the 1990s, it was considered a disease affecting old vines, where pruning wounds of the trunk susceptible to rot, particularly white rot, accumulated over the years (Fischer, 2002; Mondello *et al.*, 2018). Esca is a complex disease involving a series of fungal pathogens, mainly *Phaeoacremonium chlamydospora*, *Phaeoacremonium minimum*, *Fomitiporia mediterranea*, *Eutypa lata*, and *Botryosphaeria* spp., with the entirety of all the microorganisms involved still to be defined (Mugnai *et al.*, 1999; Bruez *et al.*, 2015; Gramaje *et al.*, 2018). Esca symptoms can range from vascular necrosis in perennial wood, which can be reproduced under controlled conditions by inoculation of these fungi, to leaf chlorosis and interveinal necrosis whose causes and erratic appearance in vineyards are still not understood (Laveau *et al.*, 2009; Lecomte *et al.*, 2012; Gramaje *et al.*, 2018).

A major source of contamination in vineyards is annual pruning wounds which serve as entry points for spores, while other studies have concluded that significant contamination also occurs during the plant propagation process resulting in vines that are already infected at planting (Eskalen *et al.*, 2007; Gramaje and Armengol, 2011; Gramaje *et al.*, 2018). Management of the disease is challenging, as complete eradication is unattainable due to the uncertainty of the target (i.e., the pathogen to be controlled), the difficulty of reaching the pathogens once they have entered the wood, and the prohibition of chemical treatments. Currently, the most successful strategy of vine growers is prophylaxis and costly corrective surgery such as curettage or re-grafting (Creaser and Wicks, 2004; Mondello *et al.*, 2018; Dewasme *et al.*, 2022).

Several factors are likely contributing to a rise in observed esca incidence, including the global increase in demand for new plantings in the 1990s, the adoption of new plant propagation techniques, the increasing use of selective fungicide, the substitution of more adapted indigenous grape varieties with international varieties, the introduction

of new rootstocks, changes in cultural practices, such as training systems, and an increased use of mechanical pruning (Surico *et al.*, 2004; Surico *et al.*, 2006; Lecomte *et al.*, 2012; Gramaje *et al.*, 2018; Guérin-Dubrana *et al.*, 2019; Claverie *et al.*, 2020). Climate change is also evoked as a factor contributing to the rise in esca incidence.

The relationship between esca expression and climate patterns has been analysed as part of numerous studies. In Italy, Surico *et al.* (2000) showed that cool and rainy summers favour the slow form of esca while hot and dry summers are favourable to the apoplectic form. Murolo and Romanazzi (2014) showed that increased precipitation from June to September along with moderate temperatures that did not exceed 35 °C were particularly conducive for esca. Andreini *et al.* (2014) also found a positive correlation between rainfall from June to August and esca incidence. Calzarano *et al.* (2018) followed two vineyards in central Italy for incidence and severity of esca foliar symptoms for 21 consecutive years (1994 to 2014), recording rainfall and temperature, showing a high correlation of July rainfall and temperature with the incidence and severity of leaf symptoms. Gramaje *et al.* (2018) reported that the seasonal variation of esca foliar symptoms observed in France, the USA, and Australia can be connected to winter rainfall and the temperature in spring, where higher temperatures showed lower esca incidence in general. It also consolidated the conclusions of Sosnowski *et al.* (2011b) on Eutypa dieback, where extreme air temperatures and soil moisture conditions in potted plants translated to higher foliar expression, suggesting they may play a role as either predisposing or inciting factors. Examining relationships between water deficit and esca has yielded different conclusions—Martín *et al.* (2019) suggested that moderate water deficits amplified the physiological disturbance from esca leaf symptoms (e.g. decreases in assimilation), while Bortolami *et al.* (2021) demonstrated that severe drought reduced the incidence of foliar expression of esca overall. Larignon (2009), exploring 6 years of Botryosphaeria dieback expression against monthly precipitation and temperature, highlighted a good correlation between the annual percentage of Botryosphaeria dieback and the rainfall in May. Later, Larignon (2020) generalised that observation for esca with longer-term data from the beginning of the past century.

Nevertheless, the complex setup of climate studies usually limits conclusions. Most studies that are conducted over shorter periods can be used to highlight cultural or physiological factors but fail to reveal relations with climate variables that demand longer time series. The main limitations of the current body of knowledge include the paucity of longer-term data following the same plots, the same vines, with the same methods. Longer time frames are often consolidations of observations made with different methods and even over multiple locations, while some of the more precise observations with consistent methods and locations are not long-term.

The present study aimed at integrating a robust, long-term observational study with reliable climate and pedoclimate

data, exploring the explanatory power of climate variables to explain esca incidence. To do so, the study is based on the exploitation of one of the largest and most robust databases of esca incidence to date, conducted for 9 years (following the same 57,000 vines) in the Bordeaux winegrowing region.

## MATERIALS AND METHODS

### 1. Esca incidence data

Our starting database of esca incidence was the Dewasme *et al.* (2022) study, where 57,000 vines were followed across 12 estates of the Bordeaux region for 9 years. Each vine is noted independently every year, which allows the tracking of the disease per individual plant, but also the calculation of a rate per plot with increased consistency. Letter codes per observation per year were then consolidated into a database calculating the incidence of esca per year per plot, our key variable to be explained.

The planting data, including the planting date of each plot, varietal, rootstock, orientation, inter-row and intra-row spacing, soil type and GPS coordinates, were also recorded and completed in the database (See Supplementary Table 1). The soil, spacing, and orientation data were used as inputs to model the fraction of transpirable soil water (FTSW) per plot.

### 2. Weather data

Daily weather data were then gathered from multiple sources to minimise the distance to the plot and maximise the quality of data. Some of the plots have their own stations with high-quality daily measurements for at least temperature and precipitation, and in this case, those were prioritised. The final weather data used in this study was then a combination of a network of 5 private “Château” stations plus one Meteo France station in Pauillac (Supplementary Figure 1, data from <https://meteofrance.com/previsions-meteo-france/pauillac/33250>). A final database was created with full weather data per day per plot (A–L) for the full available timeframe, including daily maximum, minimum and average temperatures, daily precipitation, potential evapotranspiration, and global radiation. For any of the plots where potential evapotranspiration was not available, it was calculated using the Hargreaves formula (Hargreaves and Samani, 1982).

### 3. Phenology models

In addition to having reliable weather data by day of the year, we relativised all measurements to vine phenology stages per varietal per plot. To do so, we calculated phenology stages using a Parker GFV model along with the references published by Parker *et al.* (2013) for veraison and maturity dates, and a GDD<sub>5</sub> model (a Cumulative GDD model with  $T_0 = 5\text{ °C}$ ) for budburst dates (De Cortázar-Atauri *et al.*, 2009b). Both models were programmed into R scripts that went through the given weather data for each one of the plots, running sequentially day by day the temperature summation according to the model, and returning the best date estimates of phenology stages for each year in the database.

### 4. Water balance models

Daily FTSW were calculated using models proposed by Riou *et al.* (1994) and Lebon *et al.* (2003) allowing for the calculation of yearly indexes for the minimum FTSW for each season, and also for the specific phenology periods—flowering to veraison and veraison to maturity.

### 5. Yearly indexes

An initial set of indexes was built for the key climate metrics, averaging temperature measurements per period, and doing a summation for precipitation and GDD, in different periods: per month, per year, per growing season—defined as the months of April (4) to October (10). As such, results are comparable with previous studies (Andreini *et al.*, 2014; Calzarano *et al.*, 2018; Larignon *et al.*, 2009; Larignon, 2009; Martín *et al.*, 2019; Moret *et al.*, 2021; Quaglia *et al.*, 2009). The modelling of phenology stages allowed us then to recalculate indexes around and between phenology stages, creating additional periods “Around Budbreak” (10 days before and the 10 days after calculated budbreak), “Around Flowering” (10 days before and the 10 days after calculated flowering), “Flowering to Veraison”, and “Veraison to Maturity”, based on the modelled phenology dates for each plot. In addition to climate indexes calculated by period and phenology stages, we added calculated indexes for the same periods, such as GDD, Days > 30 °C and minimum FTSW.

### 6. Analytics

The final index database, comprising 108 observations of 130 variables passed through visualisations of data distributions per variable and visual relationships using Poisson and gamma-fitted biplots. A first pass included a series of regressions using stepwise regression algorithms, set to forward and backward variable selection to a hurdle of  $p < 0.05$  (Derksen and Keselman, 1992). Results were consistently tested using the R implementation of the Breusch–Pagan heteroskedasticity test (Breusch and Pagan, 1979).

We then used a generalised linear model, with variable importance estimated by the use of a Conditional Random Forest algorithm (Breiman, 2001; Meinshausen and Ridgeway, 2006; Strobl *et al.*, 2007; Wager and Athey, 2018) that was applied to two databases: the full set of variables, as well as a reduced dataset that did not include monthly indexes. Generalised linear models were then run in R using the lme4 package (Bates *et al.*, 2015; Bates *et al.*, 2008) with a mixed model fit by maximum likelihood (Laplace Approximation), using a gamma link function and the BOBYQA optimiser (Lavrijsen *et al.*, 2020; Schälte *et al.*, 2018). Challenges of collinearity with a reduced dataset remain, as well as the distortion caused by the age of the plots.

With the identification of the disproportionate importance of the age of plots (see Results section), we observed an opportunity to expand our analytical approach to include a cluster analysis and run regressions per cluster. The final approach included a goodness of clustering measurement to estimate the ideal number of clusters using the clusGap R function (Maechler, 2019) and later applying k-means

clustering analysis (Lemenkova, 2019). The two subsets (one for the older plantings and one for the more recent plantings) were then re-analysed using stepwise regression algorithms, set to forward and backward variable selection to a hurdle of  $p < 0.05$ .

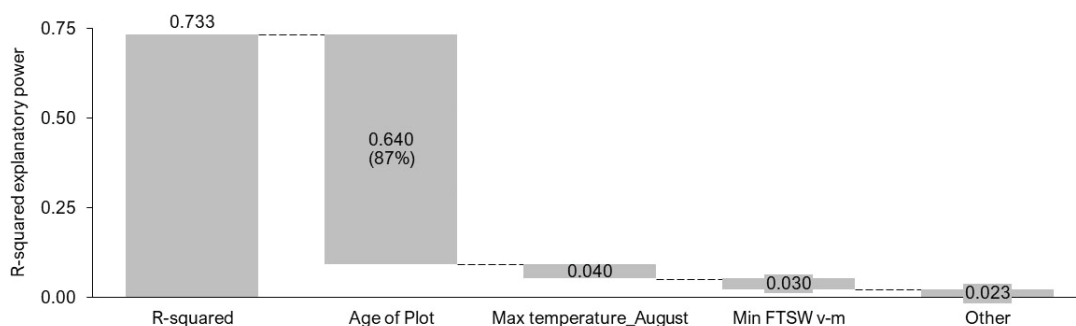
## RESULTS

### 1. Age effects predominated over the long-term

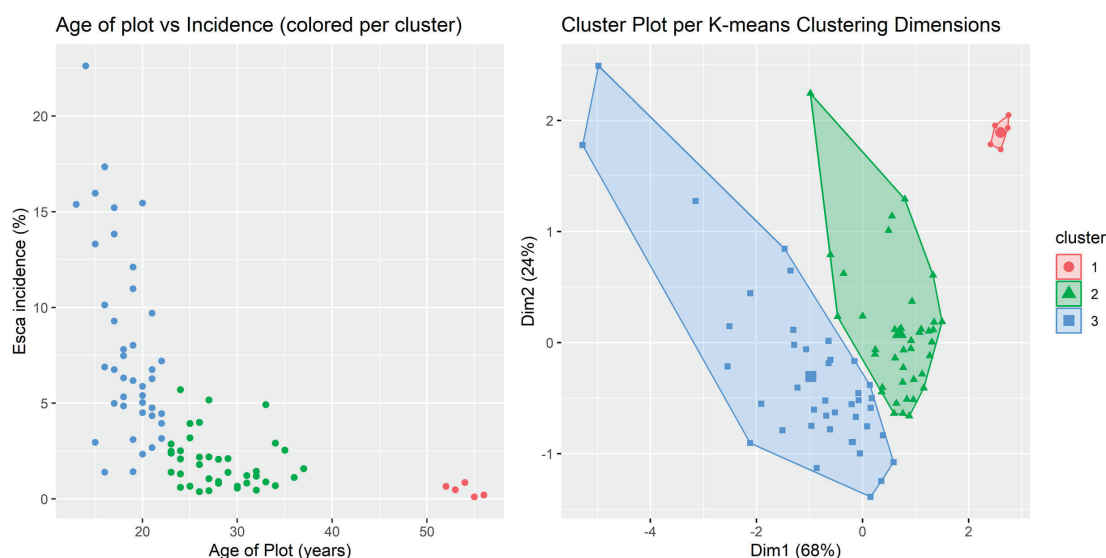
With a series that is sufficiently long to study climate effects and that also used standardised procedures in the same plots over the years, the dataset for this study has the potential to provide a robust view of the correlations between climate and esca incidence. Exploratory data are available in the Supplementary Data (Supplementary Figures 2, 3, and 4),

where some of the relationships can be explored visually. Initial modelling was conducted using the variables in Supplementary Table 2.

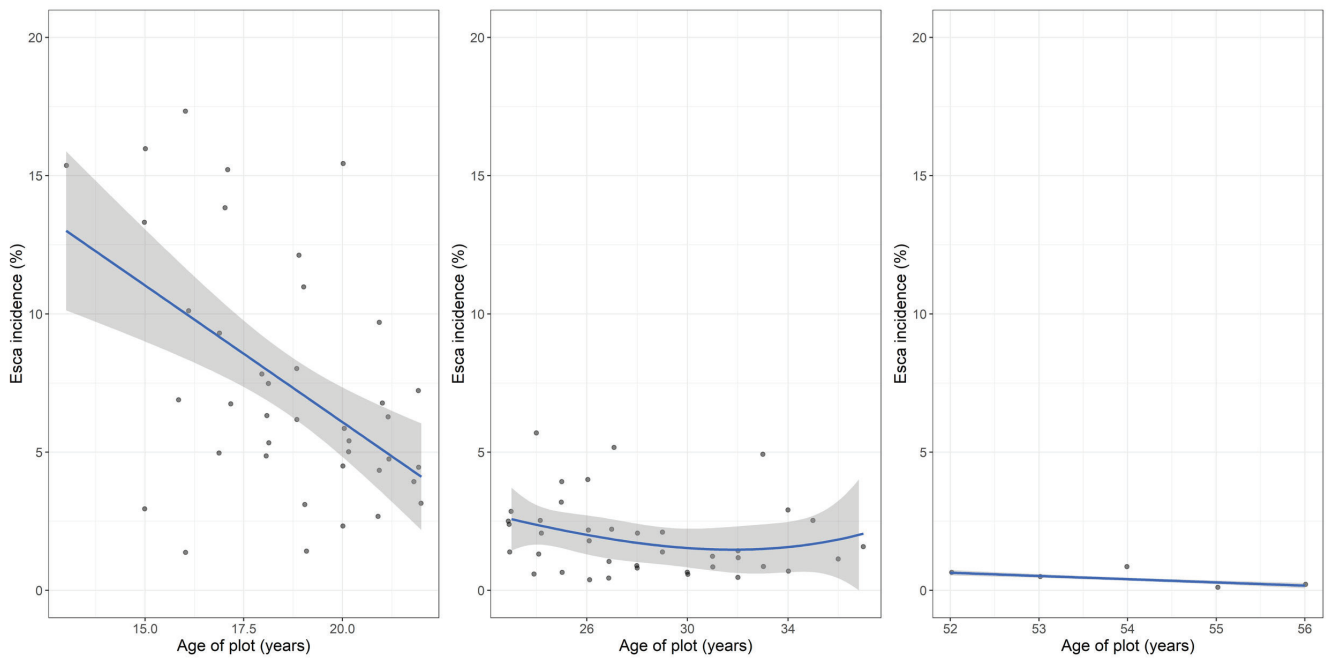
The results of a stepwise modelling, as well as the Generalised Linear Model highlighted two important results: (1) there were statistically significant climate variables that were connected to esca incidence, but (2) there was a disproportionate effect of the Age of Plot variable, with the vast majority (87 %) of the variance explained by that variable, overshadowing the climate parameters (Figure 1, Supplemental Tables 3 and 4). Figure 1 shows the decomposition of the explanatory power of the key variables in the model calculated by squared semi-partial correlation coefficients (Nakagawa and Schielzeth, 2013) for each fixed effect in the generalised linear mixed model (GLMM).



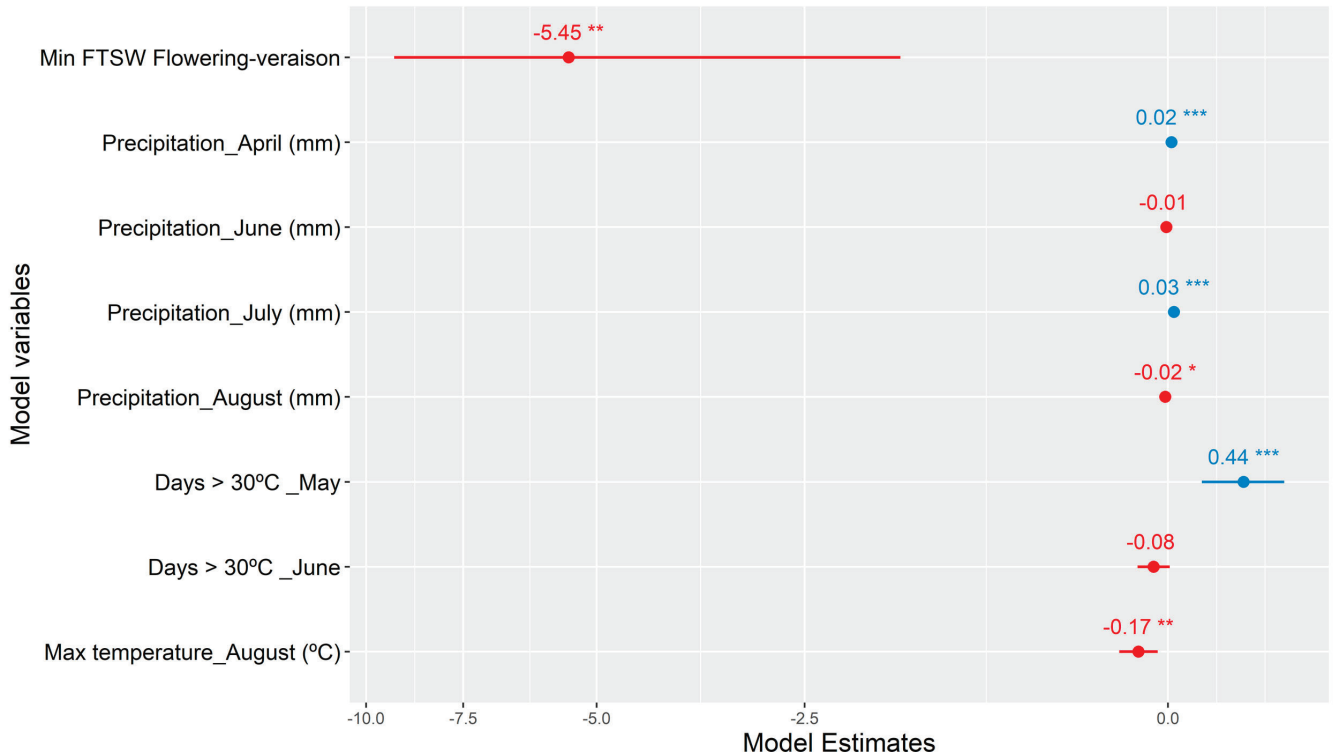
**FIGURE 1.** Generalized Linear Model R-squared decomposition by each of the explanatory variables (Age of Plot, Max Temperature\_August = Maximal Temperature in August of each year, Min FTSW v-m = Minimal FTSW during the period from veraison to maturity of each year). Complete information regarding the model is provided in Supplementary Figures 2, 3, and 4 and Supplementary Tables 3 and 4.



**FIGURE 2.** Three clusters were obtained in terms of incidence vs age of plots for each plot every year from 2012 to 2016 (left) using a K-means clustering algorithm (right). Clusters 2 (older plants) and Cluster 3 (younger plants) were further analysed for their relationships with climate variables separately.

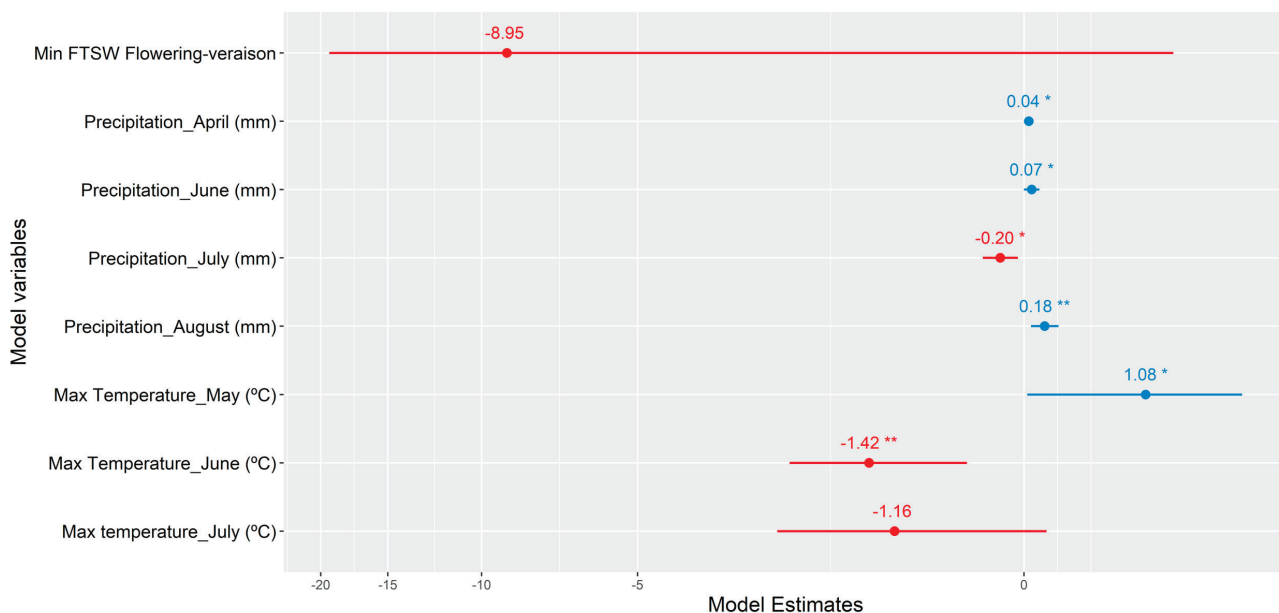


**FIGURE 3.** Esca incidence profile per cluster, in the function of the age of plots (the blue line is a robust fitting model, with 95 % confidence intervals in grey).

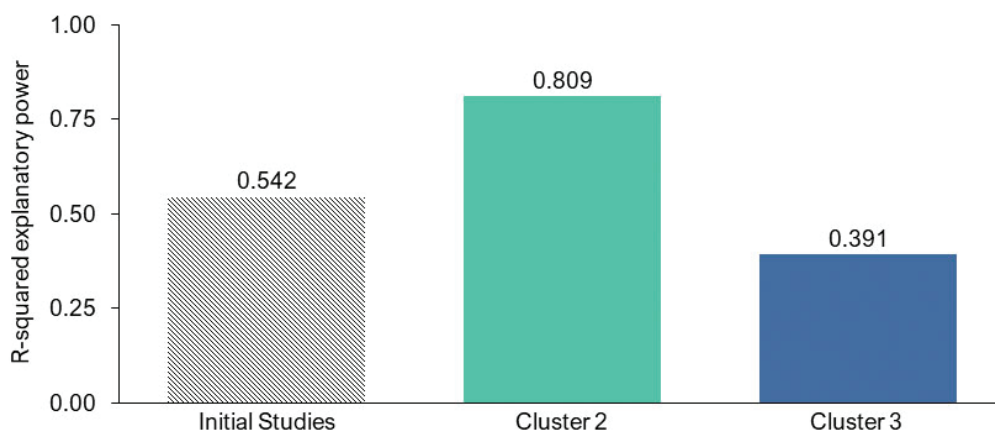


**FIGURE 4.** Cluster 2 (older plantings) Model Estimates for variables with a significant effect. The point represents the mean, and the line the standard error. Significance levels are noted as \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ . Complete information regarding the model is provided in Supplementary Table 5.





**FIGURE 5.** Cluster 3 (more recent plantings) Model Estimates for variables with a significant effect. The point represents the mean, and the line the standard error. Significance levels are noted as \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ . Complete information regarding the model is provided in Supplementary Table 6.



**FIGURE 6.** R-squared for each of the 3 models: the full database, cluster 2 (i.e., the older vines) and cluster 3 (i.e., the younger vines).

Isolating the age of plots effect through clustering, we obtained 3 distinct age clusters (Figure 2). Cluster 1 included the oldest plot which was an age outlier. We focused our analyses on Clusters 2 (old plantings before 1995) and Cluster 3 (more recent plantings since 1995). Figure 3 shows that these two clusters exhibited very different esca incidence vs age relationships and thus they were analysed separately with regard to climate relationships.

## 2. Climate-esca relationship within clusters

Modelling per cluster, the climate-esca relationships were clear and showed estimates with high significance levels for important climate variables (Figure 4, Supplementary Table 5).

The two key clusters analysed (2 and 3) highlighted relationships between the same climate variables and esca incidence. For the plots of cluster 2, those planted

before 1995, climate variables explained most of the esca incidence. As we can see from the details in Supplementary Table 5, the model yielded an R-squared of 0.80 and passed the Breusch–Pagan test for heteroskedasticity. Several different climate parameters exhibited significant relationships with esca incidence (Figure 4). For example, precipitation in April has a positive relationship with incidence—meaning more precipitation in April is correlated with increased esca incidence. On the other hand, “Max Temperature\_August” showed a negative relationship—higher temperatures later in the season are correlated with lower incidence. Warm days earlier in the season also showed a positive relationship (see “Days > 30 °C, May”, Figure 4).

For cluster 3, we can also observe a similar dynamic of early rainfall and high temperatures contributing to esca (Precipitation in April and Maximal temperature in May) but to a lesser extent than for cluster 2, and higher temperatures later in the season contributing negatively (e.g., Maximal Temperature in June) (Figure 5, Supplementary Table 6).

### 3. Within cluster models highlight unknown variables in more recent plantings

Despite the identification of similar climate relationships (e.g., early precipitation and temperatures driving esca incidence, high temperatures late in the season reducing incidence) between the two clusters, in more recent plantings (i.e., Cluster 3) the model failed to explain most of the variance (R-squared of 0.39). The results reinforce the hypothesis that there are likely other factors contributing to the sharp increase in esca incidence for more recent plantings, unrelated to the climate factors considered here. A side-by-side comparison of the different model R-squared results is presented in Figure 6, showing the improvement in explanatory power from initial models to the clusterised analysis for the older vines. In recent plantings, while the same climate indices had an impact, they explained much less of the observed variation.

## DISCUSSION

Our results suggested both April and July precipitation as important factors promoting esca incidence. The effect of precipitation early in the season has been one of the most discussed climate-esca incidence relationships in the literature. It had been found to be strongest in July of each year by Calzarano *et al.* (2018). Another study that tried to go deeper on pedoclimatic conditions, Andreini *et al.* (2014), cited increased “rainfall during fruit growth period” (summation of rainfall from June to August) as increasing esca incidence. Marchi *et al.* (2006) report that precipitations in May and June favour the foliar expression of esca while Murolo and Romanazzi (2014) reach the same conclusion but by considering a longer period from June to September. A correlation between esca expression and precipitation in May was also reported by Larignon (2009) and later revisited in Larignon (2020) (although we did not find a correlation specifically for May precipitation in this study). Taken

together we can generalise the fact that precipitation early in the season has a demonstrable effect on esca incidence.

Regarding temperature, we clearly showed an increased incidence if higher temperatures occurred earlier in the season. This is consistent with Surico *et al.* (2000) which showed the same correlation, early season high temperatures increasing esca expression. But our study here also demonstrated a decreased esca incidence if higher temperatures occurred later in the season. That observation is congruent with Bortolami *et al.* (2021) who showed that esca symptom expression was suppressed under drought conditions. Other works have described similar relationships but in different contexts. Larignon (2009) described a positive correlation between *Botryosphaeria dieback* symptom expression and evapotranspiration early in the season as symptoms increased. Calzarano *et al.* (2018) observed a negative correlation between incidence and a sum of GDD for periods spanning July and August. In contrast, Andreini *et al.* (2014) found no direct correlation between maximum temperature and esca incidence from June to August. These results suggest that temperature effects likely depend both on timing and other climate factors, probably most importantly soil water availability and thus vine water status.

In semi-controlled conditions, Bortolami *et al.* (2021) showed that prolonged water deficit totally inhibited esca leaf symptom development. In this study, we used a calculation of FTSW as a proxy indicator of the water deficit experienced in each plot, each year. Our results are contradictory, sometimes showing positive and sometimes negative correlations between esca incidence and FTSW. This could be due in part to the fact that we do not have a dynamic range of FTSW great enough to robustly resolve any trend (Supplementary Figures 2 and 3). In addition, the fact that we use only punctual end-of-season esca incidence means we cannot resolve within-season temporal relationships. This is an important limitation of this study and future studies that can reveal the precise within-season timing of esca symptom expression relative to climate would be a powerful addition in resolving some of these ambiguities.

In our study, cluster 3 including the younger vines exhibited a lower esca incidence than older plantings. This finding could be surprising because esca is often considered a disease affecting older vines, especially when compared to other wood diseases such as Petri disease or black foot disease (Maluta and Larignon, 1991). However, the youngest vineyards in our study were already approximately 20 years old, being planted between 1995 and 1998 and thus, 13–16 years old at the start of the study and 21–24 years old at the end. Several studies showed that esca decline is associated with medium-young vines which correspond to vines between 15 and 25 years old or to mature vineyards (Fussler *et al.*, 2008; Mondello *et al.*, 2018) and that vines over 25 years old have a lower esca foliar expression but are more affected by eutypiosis. In our study, the “older vines” of cluster 2 were planted before 1995 and mainly in the mid-1980s, so they were already at least 25 years old on average

at the start of the study. It would have been interesting to have very young vines (e.g., 5–15 years) included in the study.

Our main limitation however is our lack of understanding regarding other factors acting on the plant material across time. While our study isolated vineyard age as a non-climate-related variable, the high importance given to that variable in our early models explaining esca incidence suggests that future research should be focused on that area. What are other factors that should be added to the modelling when looking at the future impact of climate on esca incidence—whether that be plant material source, rootstock, soil type, treatments used, or other variables? It is clear that other factors need to be accounted for to have a better view of the future of esca risk in the context of a changing climate.

## CONCLUSION

By using data from long-term, plant-by-plant observations across Bordeaux vineyards our results demonstrated strong climate-esca incidence relationships, connecting the foliar symptoms of the disease to temperature and precipitation dynamics. We also showed that this connection to climate differs in strength between plot age cohorts and that younger plantings may have other factors driving the esca expression that are not captured by our climate models. While the older plots reinforce previous climate-esca relationships where esca incidence is well-modelled by climate parameters alone, this is not the case for younger plots. Factors not captured in this study such as the origins of the planting material, grafting techniques, and vineyard management factors should also be incorporated into future analysis and modelling. The relative contribution of climate and these many factors favouring the propagation of esca remains to be determined. Understanding the factors behind the epidemic can promote better strategies to control this disease.

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