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Necrosis and canker lengths after *Neofusicoccum parvum* inoculation on cuttings from sensitive or resistant powdery and downy mildew genotypes or cultivars

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ABSTRACT

Currently, there is no qualitative resistance to grapevine trunk diseases (GTDs), just vulnerability (possibly quantitative or partial resistance). Similar findings apply to *Botryosphaeria* dieback disease, which involves *Neofusicoccum parvum*. The goals of this study were to compare the susceptibility to *N. parvum* of (i) known susceptible grapevine cultivars (Merlot, Cabernet-Sauvignon, Ugni blanc), (ii) downy and powdery resistant cultivars (Solaris, Voltis, Regent, Artaban, Prior), and (iii) additional grapevine genotypes (BC1-54, BC1-56, BC1-60, BC1-01, BC4-AB and BC5-AB), based on canker and necrosis lengths measured after controlled inoculations repeated over a minimum of two vintages. Cuttings were inoculated with a particularly aggressive *N. parvum* isolate (PER20), known to be aggressive. After four months, the cuttings were sampled and analysed. Necrosis and cankers were measured.

The climate was the characteristic that regulated the measured symptoms across multiple vintages in our pathosystem, regardless of cultivar. This demonstrated that cultivar susceptibility varied depending on environmental factors (PCA analysis explaining 81.8 % of the variability). Furthermore, comparing the symptoms in wood of susceptible and resistant cultivars to downy and powdery mildew (*Plasmopara viticola* and *Erysiphe necator*, respectively) revealed that the vintage substantially affected symptom expression, regardless of the genetic background. For example, the Artaban grape cultivar includes both susceptible (Merlot) and resistant (Regent) cultivars in its pedigree. It showed similar symptom intensities to *N. parvum*, as did these two cultivars. However, the mildew resistance QTLs present in the Regent cultivar (*Ren3*, *Ren9*, and *Rpv3.1*) and the Artaban cultivar (*Run1*, *Ren3*, *Ren9*, *Rpv1*, and *Rpv3*) had no impact on *N. parvum* resistance levels. We also found that canker and necrosis symptoms were not always significantly positively correlated. This depended on the year and grape cultivar. The responses observed in the wood of the downy and powdery mildew-resistant genotypes or cultivar plants under investigation demonstrated that neither offered a significant benefit in terms of reducing symptoms in our experimental conditions.

This system could provide an interesting trade-off between studies on dormant wood (not very variable) and studies in the vineyard (highly variable), enabling us to assess the effectiveness of treatments or to gain a better understanding of the role of the environment.

KEYWORDS: *Botryosphaeriaceae*, grapevine dieback, grapevine trunk diseases, method, resistant cultivars, wood necrosis

INTRODUCTION

Grapevine trunk diseases (GTDs) are a serious worldwide issue resulting in significant productivity losses and causing a major global problem (Fontaine *et al.*, 2016). Additionally, the incidence of these diseases is reported to be increasing. Numerous fungi have been linked to these disorders (Fontaine *et al.*, 2016; Mondello *et al.*, 2018). However, one of the difficulties is that these diseases develop slowly and are sometimes overlooked for several years before symptoms become apparent, potentially leading to the vine's demise. These complex diseases are also affected by environmental factors that impact the physiology and defences of the vine, as well as the biology of the fungi involved (Calvo-Garrido *et al.*, 2021; Larignon *et al.*, 2009; Monod *et al.*, 2025; Mondello *et al.*, 2018; Songy *et al.*, 2019).

It is also crucial to research resistance to these diseases, as well as to examine how powdery and downy mildew-resistant cultivars are resistant to GTDs. There is a wide range of susceptibility to these diseases within the *Vitis vinifera* species (Billones-Baaijens *et al.*, 2014; Bruez *et al.*, 2013; Sosnowski *et al.*, 2022; Travadon *et al.*, 2013). Few studies have specifically documented resistance to these diseases (Csótó *et al.*, 2023). In France and other countries, grape cultivars' susceptibility to GTDs was categorised according to their expression of esca-foliar symptom and associated vineyard losses (Bruez *et al.*, 2013; Grosman & Doublet, 2012; Csótó *et al.*, 2023; Sosnowski *et al.*, 2022; Travadon *et al.*, 2013). These studies generally reveal high susceptibility for Cabernet-Sauvignon (CS) and lower susceptibility for Merlot, including esca and *Botryosphaeria* dieback. However, these findings typically address vine decline diseases as a whole, encompassing various associated pathogens. Furthermore, variability in rootstock, which differs by region and country, is often overlooked.

Among the most aggressive pathogens, the *Botryosphaeriaceae* family is often described as causing vine decline in various woody plants, including vines (Batista *et al.*, 2021; Urbez-Torres *et al.*, 2011). The aggressiveness of *Botryosphaeriaceae* species varies widely (Belair *et al.*, 2022; Bellée *et al.*, 2017; Comont *et al.*, 2024; Garcia *et al.*, 2024; Ji *et al.*, 2023; Pitt *et al.*, 2013; Reveglia *et al.*, 2018). With regard to *Botryosphaeria* dieback, Merlot is considered to be moderately to highly resistant (Travadon *et al.*, 2013), with a susceptibility similar to that of CS (Bellée *et al.*, 2017), and sometimes more susceptible than CS (Billones-Baaijens *et al.*, 2014). Other research, such as Songy *et al.* (2019), provides further insights. However, how can we predict vulnerability to trunk diseases, particularly those caused by *Botryosphaeriaceae* and, more specifically, *Neofusicoccum parvum*? In French vineyards, this fungus is the most prevalent alongside *Diplodia seriata* (Bruez *et al.*, 2013; Comont *et al.*, 2024; Larignon *et al.*, 2009). The species *N. parvum,* which is involved in *Botryosphaeria* dieback and frequently seen in esca, is generally more aggressive than *D. seriata*. *Botryosphaeriaceae* undergo a variety of aggressiveness tests, including those conducted on rooted or non-rooted cuttings, defoliated vine segments, green leafy cuttings non-grafted, or on grafted plants (Bellée *et al.*, 2017; Guan *et al.*, 2016; Travadon *et al.*, 2013; Reis *et al.*, 2019). Although only one study found results with the Tempranillo cultivar, Koch's postulates are difficult to obtain with obvious foliar symptoms (Reis *et al.*, 2016). Most studies use measurements of necrotic lesions beneath the bark, and less frequently, canker size to assess symptoms (Bellée *et al.*, 2017; Billones-Baaijens *et al.*, 2014; Guan *et al.*, 2016; Pitt *et al.*, 2013; Travadon *et al.*, 2013; Urbez-Torres *et al.*, 2011).

Grape varieties that are resistant to powdery mildew and downy mildew at the basal level produce more polyphenols and overexpress defence genes than susceptible cultivars, which could potentially contribute to resistance against *N. parvum* (Ehrhardt *et al.*, 2014; Tardif *et al.*, 2024; Viret *et al.*, 2018).

However, the Merlot cultivar and the American Concord hybrid were found to be equally susceptible to *N. parvum* and *Lasiodiplodia theobromae* (Travadon *et al.*, 2013). A recent study evaluating the susceptibility of various interspecific hybrids in Hungarian vineyards reported that they were less susceptible than *V. vinifera* (Csótó *et al.*, 2023), although there was significant variability in susceptibility. However, how susceptible are other powdery and downy mildew-resistant cultivars to *Botryosphaeriaceae*? Several European research programmes focused on developing resistant cultivars to reduce the use of plant protection products against downy mildew and powdery mildew (Bouquet *et al.*, 2000; Merdinoglu *et al.*, 2018; Rockel *et al.*, 2021; Schneider *et al.*, 2019). These projects usually involve the introduction of the genomes of *Vitis* species from other continents, such as *Vitis amurensis*, *Vitis rupestris*, and *Muscadinia rotundifolia* (Merdinoglu *et al.*, 2018). If grape varieties resistant to powdery and downy mildew were more widely used in vineyards, it would be essential to understand how vulnerable they are to GTDs and the pathogens responsible, in order to ensure the sustainability of these new varieties in the context of global change.

The objectives of this study were to (i) measure the length of cankers and necroses after inoculation with a virulent isolate of *N. parvum* on three susceptible *V. vinifera* cultivars using cuttings over several vintages, and (ii) assess the susceptibility of downy and/or powdery mildew resistant genotypes and cultivars, whether commercial or not, to *N. parvum*.

MATERIALS AND METHODS

1. Plant and fungal material

1.1. Grapevine plants

Three susceptible cultivars of *V. vinifera* (Cabernet-Sauvignon, Merlot, Ugni blanc) and eleven resistant cultivars or genotypes, in which downy mildew and/or powdery mildew resistance quantitative trait loci (QTLs) are present, were propagated in a greenhouse (16 h photoperiod-350 µmol/m^2/s)

from wood cuttings (one-year-old 20 cm canes, including two wood nodes). The plants used for the experiments, Cabernet-Sauvignon (CS, clone 412), Merlot (M, clone 182), and Ugni blanc (UB, clone 459) were provided from a single vineyard in the Nouvelle-Aquitaine region (France). Cultivars BC-4-AB (accession 3083-219) and BC-5-AB (accession 3179-24-7) were obtained from a grapevine breeding project for downy mildew and powdery mildew resistance, conducted by A. Bouquet at INRA Montpellier (Bouquet *et al.*, 2000) and propagated in INRAE in a glasshouse (Villenave-d'Ornon, Nouvelle-Aquitaine). They were backcrossed between *Muscadinia rotundifolia* and *V. vinifera*, as described in Bellée *et al.* (2017).

The cultivars Solaris (clone FR360, QTLs: *Ren3*, *Ren9*, *Rpv3.3*, *Rpv10*), Prior (clone FR600, QTLs: *Ren3*, *Rpv3*), and Regent (clone GF1, QTLs: *Ren3*, *Ren9*, *Rpv3.1*) are the result of German plant breeding programmes and are described on the website of the Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen (https://www.vivc.de) and were cultivated on a plot in the INRAE site (Villenave-d'Ornon, Nouvelle-Aquitaine). The genotypes BC1-54, BC1-56, BC1-60, and BC1-01 were obtained from an INRAE breeding programme in Colmar (a cross between *Muscadinia rotundifolia* and *Vitis vinifera* CS) and maintained in Bordeaux in a glasshouse from 2010 over several years. Genotypes BC1-54, BC1-56 were described as susceptible, and genotypes BC1-60 and BC1-01 as resistant to downy mildew and powdery mildew (potentially with *Rpv1* and *Run1*). The cultivars Artaban (clone N1267, QTLs: *Run1*, *Ren3*, *Ren9*, *Rpv1*, *Rpv3.*1) and Voltis (clone B1266, QTLs: *Run1*, *Ren3*, *Rpv1*, *Rpv3.1*, *Rpv3.3*) came from the ResDur programme carried out at INRAE in Colmar (Schneider *et al.*, 2019) and are resistant to *Plasmopara viticola* and *Erysiphe necator*. The numerous introgressed QTLs originate from various genetic resources, including several species of *Vitis* and *Muscadinia* (*e.g.*, *V. rupestris*, *V. aestivalis*, *V. cinerea*, *V. lincecumii*, *V. amurensis*, and *M. rotundifolia*). For more information on the resistance QTLs mentioned in this study, please refer to the works of Merdinoglu *et al.* (2018) and Adrian *et al.* (2024).

After three weeks, all rooted cuttings were potted in sandy soil (sand (20 %)), blond sphagnum moss, perlite and clay (70 %) in the greenhouse under controlled conditions at 25/20 °C day/night temperature with 75 % relative humidity. Two-month-old plants with 8–10 leaves were used for the experiment. The plants were watered once or twice a week by immersing the pots with added fertiliser (N, P, K (20 %)) with trace elements (B, Cu, Fe, Mn, Mo, Zn).

1.2. Fungal material

One isolate of *Botryosphaeriaceae,* species *Neofusicoccum parvum* (PER20) from the laboratory collection "CoCo" was used (Comont *et al.*, 2024). The isolate grew on malt-agar medium (malt 20 g/L and agar 15 g/L). The isolate was maintained at 23 °C (16 h light/day in a growth chamber), sub-cultured by transferring colonised agar plugs (5 mm diameter) to new medium (malt/agar).

2. Pathogenicity test

Each plant was perforated and inoculated as described in Bellée *et al.* (2017). Briefly, a drill was used to make holes (3 mm in diameter at a maximum depth of 5 mm) between the two nodes of the cutting. A 3 mm mycelium plug (one day old) or malt-agar plug (control) was inserted into the cavity, and each point of inoculation was closed with a deposit of liquid paraffin. For each modality, uninoculated or inoculated with the PER20 isolate, from 10 to 20 plants were used for each cultivar or genotype, depending on the year. The plants were grown for three weeks in a greenhouse (16 h/8 h day/night photoperiod and watered by a drip) and then placed under a plastic greenhouse open and exposed to natural light and ventilation for three months. During this period, temperature and humidity levels were not controlled and corresponded to the climatic data of the site. The development of lesions on the plants was observed four months after inoculation. Stems were cut and bark peeled. The length of the canker lesion on the surface around the point of inoculation and the length of the necrosis in the wood tissue were measured, and re-isolation tests for *N. parvum* were performed as described by Bellée *et al.* (2017).

A canker is defined as the measurement of the inoculation hole and the extension of the lesion to the outside (non-healing and extension of the hole). By contrast, necrosis corresponds to the measurement of the necrotic section under the bark that extends on either side of the inoculation hole (Bellée *et al.*, 2017).

In all trials, the average length of the control plant inoculated with a fungus-free plug was 0.42 ± 0.22 cm for necrosis and 0.22 ± 0.18 cm for cankers (data not shown). Inoculations were performed between mid-March and mid-April in 2010, 2011, 2012, and 2015, and at the end of May in 2019. We characterised the experimental vintage by taking into consideration the number of days during the experiment when the maximum temperature surpassed 30 °C, as well as the cumulative amount of rainfall, despite the plants being protected from rain (in a greenhouse or beneath a plastic greenhouse) (Table 1).

3. Statistics

Statistical analyses were carried out with R 4.3.1 software. Our characteristics of interest were compared to cultivars and vintages using ANOVA and Tukey's test ($p < 0.05$). When the variation tests were significant, a non-parametric test was performed (Kruskal–Wallis, Wilcoxon's test). The tests were considered statistically different when $p < 0.05$. Temperature, canker and length necroses were gathered in a single numerical database before being statistically described using a global principal component analysis (PCA) with R (4.3.1). PCA was performed using Pearson's correlation coefficient. Variables with a $\cos^2 \geq 0.5$ on one of the first two principal components (dimension 1 and 2) were considered as sufficiently well represented by the principal plan generated by this PCA. Correlation analyses were performed on canker and necrosis to indicate vintage-specific results (Pearson's statistical test).

TABLE 1. Climatic characteristics of the different study vintages. Data from the 33550003 Villenave-d'Ornon station of the INRAE agroclimatic network.

Year of experiment	Water cumulated (mm)	Number of days with maximum temperature over 30 °C
2010	112	21
2011	83	6
2012	249.5	4
2015	94	14
2019	222	41

https://agroclim.inrae.fr/climatik/ClimatikGwt.html

RESULTS

1. Multiannual assays on Cabernet-Sauvignon and Merlot

Analysis of cuttings taken from canes of the previous year (N-1) from the same plot over four vintages revealed year-specific variations in the length of necroses and cankers for both cultivars (Figure 1). Year-specific variations were observed between the two cultivars ($p < 0.01$). Necrotic lengths varied from 1.4 cm to 5.6 cm depending on the year, while canker lengths ranged from 0 to 2.8 cm. The annual average length of necroses and cankers was more stable in Cabernet-Sauvignon (CS), whereas Merlot showed greater variation, particularly for the 2011 vintage, even in 2015.

The length of necrosis varied significantly across years for the same cultivar, depending on the year. For example, CS necrosis and canker lengths differed considerably between 2010 and 2015 ($p < 0.001$ and $p < 0.02$, respectively), as well as between 2011 and 2015 ($p < 0.001$). Merlot necrosis lengths showed similar variations between 2010 and 2015 and between 2015 and 2019 ($p < 0.001$).

These results suggested that the variability in responses was greater in Merlot than in Cabernet-Sauvignon, depending on the year. Regarding the average length of cankers and necroses over the four vintages, there was no significant difference between the two cultivars, with necrosis lengths of 3.5 ± 1.68 cm for CS and 3.8 ± 2.32 cm for Merlot. However, for cankers (2.1 ± 1.31 cm for CS and 1.3 ± 1.48 cm for Merlot), we observed a significant difference ($p = 0.0107$), suggesting that Merlot may be more resistant to canker extension than CS.

2. Effect of *N. parvum* on necrosis and canker lengths of genotypes and resistant cultivars to *P. viticola* and/or *E. necator*

2.1. Multiannual effects on Solaris and Regent

The comparison of canker and necrosis lengths on the downy mildew-resistant cultivars Solaris and Regent in 2015 and 2019, with CS as a reference, displayed variability depending on the year (Figure 2).

There was no significant difference in necrosis length between the cultivars in either year. Regarding cankers, the two resistant cultivars differed considerably from CS ($p < 0.02$), exhibiting larger cankers in 2015 and smaller ones in 2019, which suggests a year-specific effect. The two resistant cultivars had no significant effect on wood symptom expression. It should be emphasised that the ratio between necrosis and canker size ranged from 1.68 to 3.64 cm, depending on year and cultivar, with more specific correlations for each cultivar. The correlations were indeed important for CS ($R^2 = 0.76$ and 0.65 in 2015 and 2019), moderate for Regent ($R^2 = 0.47$ and 0.36) and negligible for Solaris ($R^2 = 0.17$ and 0.16).

2.2. Impact of *N. parvum* on two susceptible genotypes to *P. viticola* and *E. necator* (BC1-54 and BC1-56) and two resistant genotypes to *P. viticola* (BC1-60 and BC1-01)

These four genotypes consistently influenced necrosis and canker lengths depending on the year (Figure 3). In 2011, the BC1-56 genotype, which was susceptible to downy and powdery mildews, exhibited significantly larger necroses (4.11 ± 0.61 cm) than the other genotypes, which ranged from 1.71 to 2.37 cm. The year always had a significant impact on the symptoms, particularly on canker length.

2.3. Effect of *N. parvum* on three susceptible cultivars (Merlot, Cabernet-Sauvignon, and Ugni blanc) and two resistant genotypes (BC-4-AB and BC-5-AB)

Large necrotic lengths were observed in Merlot and Ugni blanc across the two vintages, particularly in 2012 (more than 3 cm, Figure 4A; $p = 0.018$). The two resistant genotypes, BC4-AB and BC5-AB, exhibited larger necrosis lengths in 2011. Regarding cankers, the impacts were relatively similar for CS, Merlot, and the two resistant genotypes (BC4-AB and BC-AB5), despite interannual variability (see statistics), with the smallest cankers obtained in 2012 (Figure 4B). Only UB showed important differences between the two vintages. This study suggested that the resistant genotypes BC4-AB and BC5-AB produced similar outcomes to CS. These genotypes (BC-4-AB and BC-5-AB) have been backcrossed twice with Cabernet-Sauvignon.

2.4. Effect of *N. parvum* on two susceptible cultivars and five resistant cultivars commercialised

In 2019, a study compared the effects of *N. parvum* on two susceptible and five resistant cultivars (Figure 5). Only the Merlot and Artaban cultivars had significantly shorter necrosis lengths (2.1 ± 1.2 and 2.6 ± 0.9 cm) than the Cabernet-Sauvignon cultivar (4.31 ± 1.4 cm, $p < 0.001$

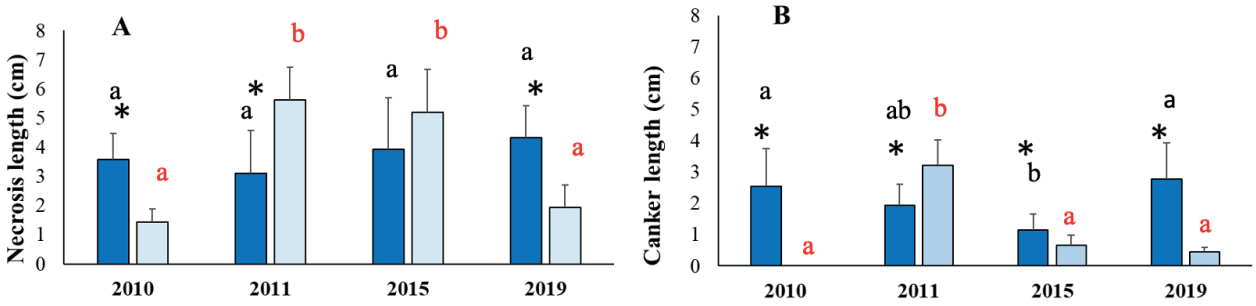


FIGURE 1. Necrosis lengths (A), and canker lengths (B) after inoculation with *N. parvum* of Cabernet-Sauvignon (dark blue) and Merlot (light blue) cuttings in 2010, 2011, 2015, and 2019. Asterisks indicate significance (Tukey, $p < 0.05$) between the two cultivars each year. The letters indicated the differences for each according to the grape cultivar (Kruskal–Wallis, $p < 0.05$), black = Cabernet-Sauvignon; red = Merlot.

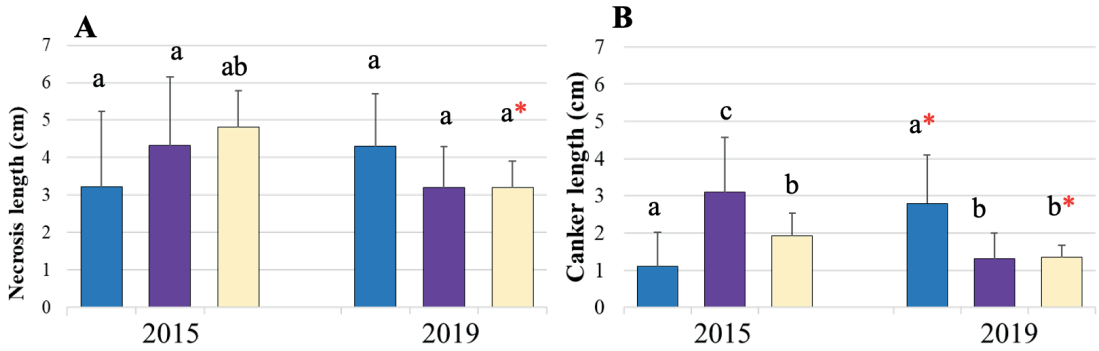


FIGURE 2. Necrosis (A) and canker (B) lengths on Cabernet-Sauvignon (dark blue), Regent (purple), Solaris (yellow) cuttings in 2015 and 2019, after inoculation with *N. parvum*. Letters indicated significant differences *vs* CS, and red asterisks between the two vintages for a cultivar ($p < 0.05$).

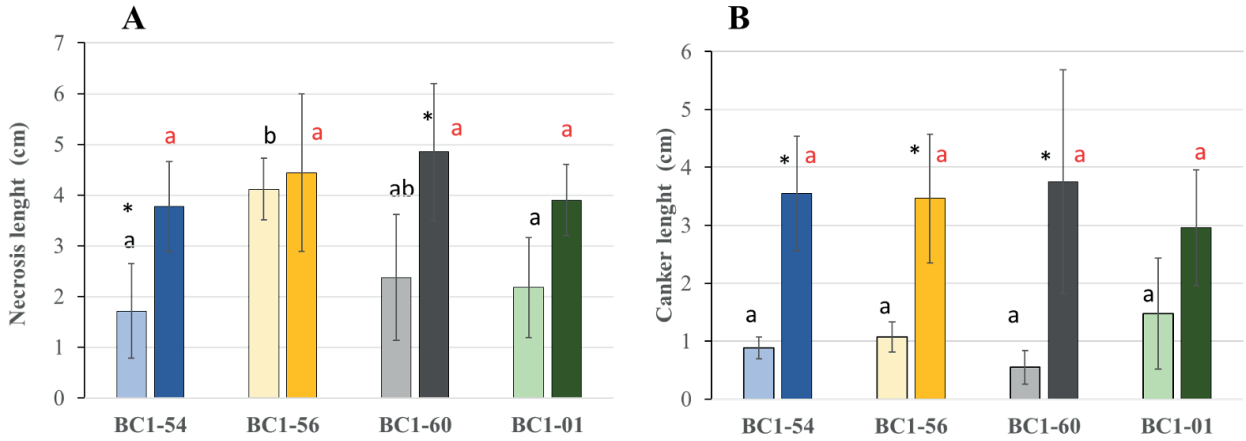


FIGURE 3. Necrosis (A) and canker (B) lengths of different genotypes from a back cross 1 between *Vitis vinifera* and *Muscadinia rotundifolia*, in 2011 (light colour) and in 2012 (dark colour). The letters indicated the significance between the different genotypes in each year (black: 2011, red: 2012). The asterisks indicate the differences for a cultivar between the two vintages of the experiment ($p \leq 0.05$).

and $p < 0.015$, respectively). The other resistant cultivars had intermediate necrosis lengths. The canker lengths of Merlot and Artaban were significantly smaller (from 0.2 to 1.5 cm) than those of Cabernet-Sauvignon (from 1.3 to 3.8 cm, $p < 0.001$ for both). The other resistant cultivars, Prior, Solaris, and Voltis exhibited canker lengths close to those observed on CS, while Regent had intermediate canker lengths.

Regardless of canker lengths, resistant cultivars like Artaban were less susceptible to *N. parvum* than Voltis ($p = 0.009$), Prior ($p = 0.018$), and Solaris ($p = 0.027$). We noted that the Artaban cultivar exhibited symptoms (necrosis and canker lengths) comparable to those displayed by Merlot ($p = 0.812$ and $p = 0.882$ for necrosis and canker, respectively) and Regent ($p = 0.915$ and $p = 0.205$) cultivars. The last two are part of Artaban's pedigree.

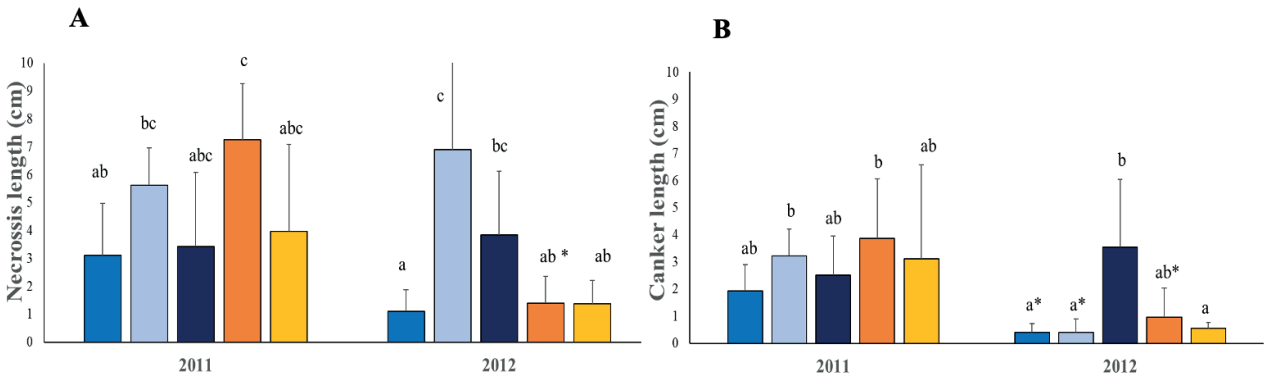


FIGURE 4. Necrosis (A) and canker (B) lengths, after cutting inoculation with *N. parvum*, in Cabernet-Sauvignon (blue), Merlot (light blue), Ugni blanc (dark blue), BC-4-AB genotype (orange) and BC-5-AB genotype (yellow) in 2011 and 2012. The letters indicated the significant difference between cultivars and genotypes for each year ($p \leq 0.05$). Asterisks indicated differences between the two vintages.

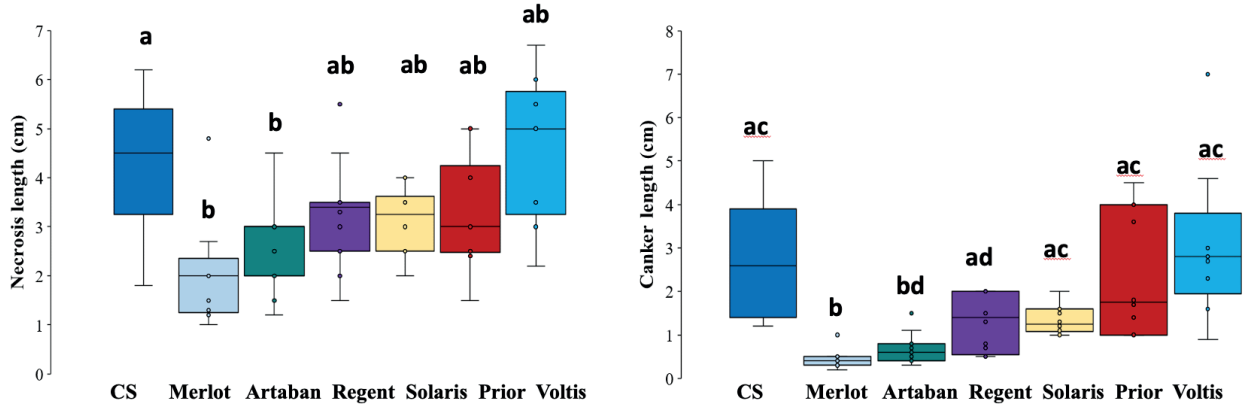


FIGURE 5. Grapevine susceptibility to *N. parvum*, illustrating necrosis and canker lengths (cm) measured four months after inoculation in 2019. Bars with different letters were significantly different at $p \leq 0.05$.

3. Potential effect of environmental temperatures on canker and necrosis lengths

3.1. Correlation between necrosis and cankers

Our multi-annual research findings highlighted the role of the environment on the expression of necrosis and cankers, as well as the influence of the genetic background. Together, all of the grape cultivars and vintages analysed yielded $R^2 = 0.32$ ($p = 0.059$) (data not shown).

However, for a given cultivar, this correlation can vary depending on the vintage. Cabernet-Sauvignon exhibited a greater correlation between canker size and necrosis ($R^2 = 0.43$, $p < 0.001$) than Merlot ($R^2 = 0.12$, $p < 0.001$, Figure 6A).

Examining canker and necrosis sizes over several years, with a minimum of 20 days above 30 °C in 2010 and 2019, and a maximum of 10 days above 30 °C in 2011 and 2012, revealed a clear distinction between Merlot and Cabernet-Sauvignon cultivars (Figure 6B). This was evident for both necrosis and cankers ($p < 0.001$ and $p = 0.002$, respectively).

The resistant cultivars studied in 2019 showed highly variable R^2 values ranging from 0.16 (Solaris) to 0.72 (Prior), which were significant for Regent ($R^2 = 0.36$, $p = 0.004$), Prior ($R^2 = 0.72$, $p = 0.004$), Voltis ($R^2 = 0.63$, $p = 0.005$), and Artaban ($R^2 = 0.30$, $p = 0.01$) (data not shown).

3.2. Principal component analyses representing the effect of temperature on the vintage and the cultivars

PCA analyses summarised results with a particular focus on the impact of cultivars and vintages. Significant differences were evident across the vintages, with dimensions 1 and 2 accounting for 51.76 % and 31.04 % of the variation between the years, respectively. Dimension 2 was used to distinguish the 2010 and 2019 vintages from the others (Figure 7A).

The dimension 2 distinguished three groups based on cultivars. The first group comprised Artaban, Prior, and Voltis, and stood out from the others. The second group exhibited a continuum and comprised Solaris, Regent, Merlot, and Cabernet-Sauvignon. The third group comprised all the BC-resistant genotypes.

Clearly, the results showed that the cultivars were linked to the vintage, especially in the 2011 and 2012 tests of BC genotype (Figure 7A). Similarly, in 2015 and 2019, the resistant cultivars Solaris and Regent (Figure 7B) were projected between the two vintages (Figures 7A and 7B).

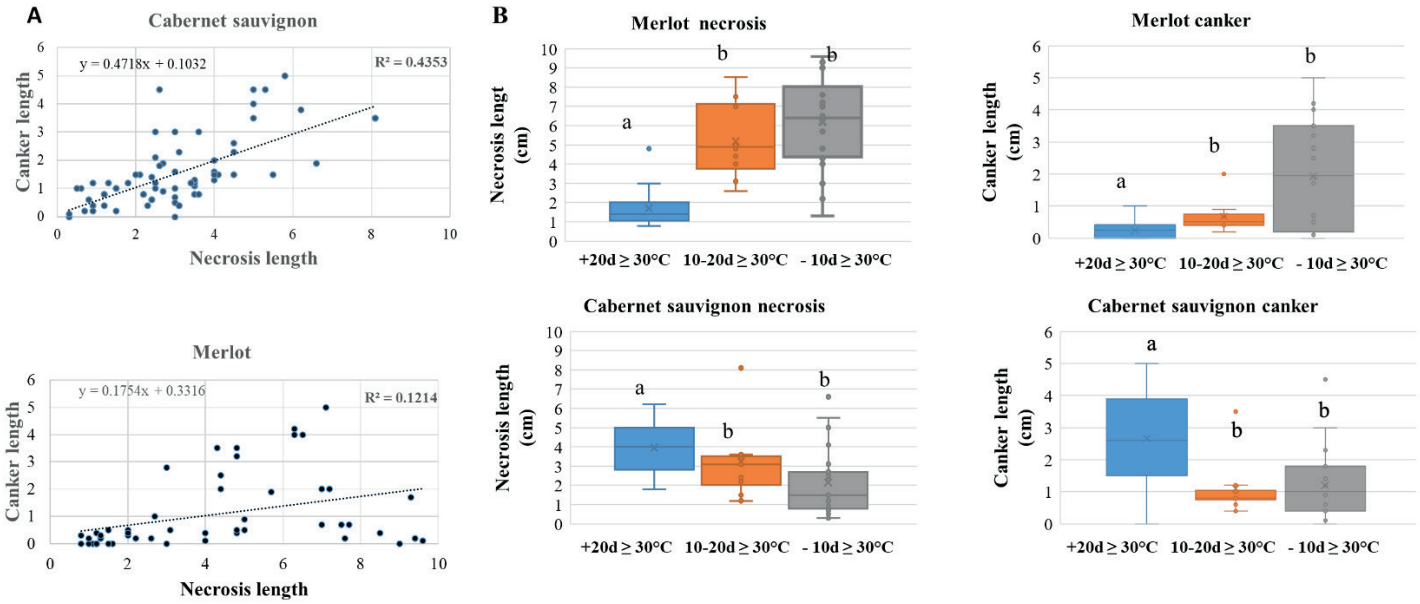


FIGURE 6. Correlations between the canker and necrosis lengths for all vintages of Merlot and Cabernet-Sauvignon (A). Boxplots of necrosis and canker lengths according to the number of days when temperatures were above 30 °C for the Merlot and Cabernet-Sauvignon (B) (blue: more than 20 days above 30 °C; orange: between 10 and 20 days above 30 °C; grey: for less than 10 days above 30 °C).

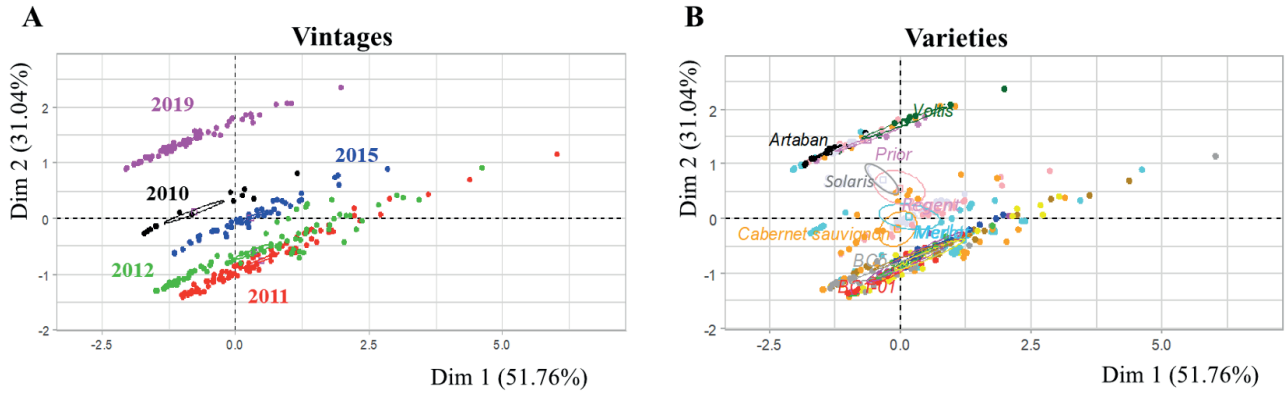


FIGURE 7. Principal component analyses (PCA) of canker and necrosis lengths and temperature from 2010 to 2019 (A), and according to cultivars (B). The variations (%) explained by each PCA abscissa are given in brackets. Squares corresponded to individual cuttings and ellipses to the 95 % confidence intervals calculated for each variable.

DISCUSSION

All the results obtained under semi-controlled conditions (in a tunnel) pointed to variability in the expression of symptoms in the wood after inoculation with an isolate of *N. parvum*. The climate, particularly the temperature, may impact this variation. Indeed, fewer symptoms were observed on the wood in the warmest vintage (as determined by the number of days with maximum temperatures over 30 °C during the experiment), particularly for Merlot in 2010 and 2019 (Table 1, Figure 6B).

Neofusicoccum parvum isolate PER20 grows very well at temperatures between 23 and 28 °C, but growth slows above 30 °C (Bellée *et al.*, 2017). This could explain the findings that support the idea that heat plays a part in fungal development and reduced symptom expression in wood. This is consistent with the lower foliar symptom expression of these decline diseases in warmer years (Monod *et al.*, 2025), which may be influenced by soil moisture and air temperature (Murolo *et al.*, 2014). However, the cultivar's genetic background and susceptibility (*e.g.*, CS) must also be considered. Songy *et al.* (2019) described Cabernet-Sauvignon and Merlot as similar in terms of susceptibility to *Botryosphaeria* disease. Bellée *et al.* (2017) found no significant differences between the two cultivars when using different *Botryosphaeriaceae* isolates. Furthermore, Merlot exhibited smaller cankers than CS in all vintages, with a lower correlation between necrosis and canker. These findings were consistent with those of Travadon *et al.* (2013), who also found Merlot to be more resistant than CS. In terms of foliar symptoms, Merlot is also thought to be more resistant to GTDs than Cabernet-Sauvignon (Lecomte *et al.*, 2024).

This may be in contrast to a year like 2012, which had few warm days (four) and 249.5 mm of rain (Table 1), which promoted the size of cankers and necroses in BC1 genotypes compared to 2011, which had six warm days and little rain (83 mm). However, depending on the year, temperature amplitudes of varying intensity might be recorded in addition to maximum temperatures above 30 °C. While only eight days in 2012 surpassed a temperature amplitude of 15 °C, this figure was 28 days in 2011, which could disrupt fungal growth and/or stress the plant. Furthermore, it is claimed that a fresh and rainy summer can promote the expression of esca symptoms (Surico *et al.*, 2000).

This difference between cultivars and vintages of the same cultivar could be attributed to the transport of water in the vine shoots via the xylem vessels, as well as environmental factors (de Herralde *et al.*, 2006). The importance of xylem structure as a driver of resistance has also been demonstrated for a GTD-causing pathogen *(Phaeomoniella chlamydospora)* (Pouzoulet *et al.*, 2020). In our study, the plants were regularly watered, and temperature could have a significant impact on water transfer. It was noted that the occurrence of external symptoms is not strictly correlated with internal symptoms (Di Marco *et al.*, 2000).

Aside from temperature differences, grape cultivars and genotypes do not always respond equally to the same year. This would be consistent with the variation in leaf symptom intensity observed among *V. vinifera* and hybrid cultivars (Chacon *et al.*, 2020; Travadon *et al.*, 2013). As the relationship between canker size and necrosis varied with year and cultivar, we hypothesise that plant response mechanisms for these two symptoms differed depending on environmental variation and/or genetic background. This point could be reinforced by the different correlations found between cankers and necroses, which varied depending on the cultivars (*e.g.*, Regent-Solaris) and, sometimes, the year. We propose that complex and multigenic types of tolerance are likely involved.

One of our hypotheses was that cultivars or genotypes resistant to downy mildew and powdery mildew may be less susceptible or even resistant to *N. parvum*, which is involved in wood diseases (esca and *Botryosphaeria* dieback), and that resistance QTLs may play a role in limiting the expression of symptoms in wood. These cultivars were studied as part of a discovery process to assess their susceptibility to *N. parvum*/GTDs, given the availability of their powdery and downy mildew resistance QTLs. Our study of six genotypes and five mildew-resistant cultivars with different resistance QTLs (*Rpv1*, *Rpv3-1*, *Rpv3.3*, *Run1*, *Ren3*, and *Ren9*) indicated no *N. parvum*/GTD resistance. This suggests that these QTLs for downy and powdery resistance had no effect on the lengths of necrosis and cankers that *N. parvum* induced. Overall, little or no resistance to canker and necrosis size is conferred by the QTLs of the resistant genotypes and cultivars studied. This confirms that there are currently no cultivars resistant to *Botryosphaeria* dieback or *N. parvum* (Martinez-Diz *et al.*, 2019; Massonnet *et al.*, 2017). However, the prospect of developing resistant cultivars or discovering resistance mechanisms to *Botryosphaeriaceae* or GTDs cannot be fully ruled out. Guan *et al.* (2016) described a potential source of resistance to *Botryosphaeriaceae* in *V. vinifera* subsp. *Sylvestris*. This source was not found in the resistant cultivars investigated, possibly due to *V. vinifera* subsp. *Sylvestris* great susceptibility to *P. viticola* and *E. necator*. The option of crossing with *V. vinifera* subsp. *Sylvestris* remains to pyramid QTLs for resistance to GTDs.

Resistant cultivars to *P. viticola* and *E. necator* produce different quantities and types of polyphenols than susceptible cultivars (Ehrhardt *et al.*, 2014; Tardif *et al.*, 2024; Tomaz *et al.*, 2021). However, the wood polyphenols of the resistant cultivars studied here did not appear to provide resistance to *N. parvum*. These may be consistent with the limited effectiveness of these molecules against wood-decaying pathogens (Lambert *et al.*, 2012).

We conclude that annual temperature had a significant impact on canker and necrosis sizes. This was validated by multiple vintages of studies on rooted cuttings. While the inoculation and fungus isolate can be easily controlled, the study showed that experiments on rooted cuttings subjected to a variable environment in a plastic glasshouse (temperature, humidity) made necrosis and canker development more unpredictable. These changes were undoubtedly linked to both the plant (its genetics, physiology, and stress response) and the pathogen (its biology and stress response).

Finally, all the mildew-resistant cultivars and genotypes tested developed lesions in the wood after inoculation with an isolate of *N. parvum*. This indicated that there was no evidence of quantitative resistance in the analysed cultivars. However, depending on the grape cultivar, rootstock, pathogen, or combination of pathogens, this semi-controlled study approach appeared promising for gaining a better knowledge of the effect of temperature and/or water on the expansion of cankers and necroses. This system would allow for a more in-depth investigation of plant responses to single or combined stresses over multiple vintages. Following the example of Songy *et al.* (2019), who focused on the effects of heat and even water stress on the initiation and progression of GTD in vines. We investigated the ability *of N. parvum* to cause lesions on susceptible and resistant cultivars under various seasonal conditions. Further research incorporating physiological and biochemical data would provide valuable insights into how plants respond to infection in different climatic conditions. As a result, our methodology could be used to study a wide range of biophysical and ecosystem changes that contribute to the advancement of grapevine wood decline diseases.

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