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Plant resilience and physiological modifications induced by curettage of Esca-diseased grapevines

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ABSTRACT

The re-emergence of Grapevine Trunk Diseases (GTDs), mainly Esca, has been observed in most of the world's vineyards during the last two decades. Development of necrosis in grapevine wood, especially white-rot, is typically associated with Esca-diseased plants. One of the different methods being used in attempts to eradicate GTDs is curettage. This old cultural practice, which consists in surgically removing the necrotic wood, specifically white-rot, retaining only the non-necrotic tissue of Esca-diseased grapevine, is used in some European vineyards (Spain, France, Italy, Portugal), and is being increasingly reintroduced since 10 years ago in France. We, therefore, wanted to study the effect of curettage on vigour, fertility and berry quality, and year after year plant recovery. Our study synthetizes a 3-year experiment on Esca-diseased cv. Sauvignon blanc grapevines curetted in a commercial plot in the Bordeaux region. Asymptomatic control grapevines were compared to Esca-diseased grapevines without curettage (with typical foliar symptoms), and with curetted Esca-diseased grapevines (without foliar symptoms). Even if the curetted grapevines recovered lower vigour and fertility than the control plants, their grape berry quality was comparable, unlike for Esca-diseased grapevines. This cultural practice proved particularly effective in helping Esca-symptomatic grapevines to recover asymptomatic after treatment. Over time, curettage induces the resilience of grapevines, allowing them to recuperate their full physiological functioning, thereby compensating for Esca's detrimental impact on berry quality.

KEYWORDS

curettage; Esca, *Vitis vinifera* L., resilience, yield

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/4478

INTRODUCTION

The major phenomenon of Grapevine Trunk Diseases (GTDs) re-emerged in the late 1990s, and it took a mere two decades for Esca, the most frequent GTD, to become a subject of major concern, endangering vineyard sustainability in France, Europe and worldwide (Bertsch *et al.*, 2013; Fontaine *et al.*, 2016a; Guerin-Dubrana *et al.*, 2019). Esca is caused by a broad range of taxonomically unrelated fungal pathogens, such as *Phaemoniella chlamydospora* (*Pch*), *Phaeoacremonium minimum* (*Pm*), *Fomitiporia mediterranea* (*Fm*), which attack grapevine woody tissues and induce necrosis. It is hypothesised that when fungal colonization and degradation of the wood reach a critical point, the functional tissues, the plant-vessels, are severely damaged, thus interfering with grapevine physiology, and frequently resulting in plant death (Bartoli *et al.*, 2006; Pouzoulet *et al.*, 2014). Whatever the process of wood degradation, Esca decreases vineyard longevity, thereby affecting wine quality (Calzarano *et al.*, 2004; Lorrain *et al.*, 2012), causing huge economic losses throughout the viticulture sector (Hofstetter *et al.*, 2012). In France, over the period 2012-2017, the proportion of unproductive vineyard area was some 12 %, with Esca being the principal cause (Doublet, 2018 (French Ministry of Agriculture, *the "Journées des maladies du bois" at Dijon* - 2020/11/29-31)), the ensuing losses being estimated at some €1 billion.

As regards Esca-pathogens, they progressively develop in the grapevine trunk and arm wood, causing various types of necrosis such as central necrosis, sectorial necrosis, mixed necrosis and white-rot (Larignon and Dubos, 1997; Maher *et al.*, 2012). In Esca-diseased grapevines, as in *Botryosphaeria* dieback (Úrbez-Torres, 2011), another typical symptom is the formation of a brown-orange stripe, presumably reflecting vascular disorder (Lecomte *et al.*, 2012). Depending on the development of wood necrosis, a more or less rapid decline of grapevines is observed, inducing the mild and apoplectic forms frequently reported in the literature (Larignon *et al.*, 2009; Lecomte *et al.*, 2012). The mild expression form of Esca is typically associated with leaf discolourations and/or drying zones between the primary veins (Mugnai *et al.*, 1999). The discolourations may gradually become enlarged around the veins, sometimes becoming necrotic. Although the presence of foliar symptoms does not predict diseased-grapevine mortality, their expression levels are positively correlated with plant mortality, as shown by Guerin-Dubrana *et al.* (2013). The second form of Esca is apoplexy, resulting in the sudden wilting either of a whole arm or of all the grapevine vegetation. This sudden emergence often occurs after a very rainy period, followed by a hot and dry one (Marchi *et al.*, 2006; Mondello *et al.*, 2018; Mugnai *et al.*, 1999). A wide range of leaf symptoms, between the mild and apoplectic forms, is also described by Lecomte *et al.* (2018).

These diseases, especially Esca, are in alarming recrudescence, which triggers great apprehension in the viticulture sector. No effective control treatments have been available ever since the ban in Europe in the early 2000s of the sole pesticide registered to control GTDs: the sodium arsenite (Gramaje *et al.*, 2018; Mondello *et al.*, 2018). To help to find a solution to this worldwide sanitary issue, the present study focuses on the control of wood necroses. This assertion has been supported by Travadon *et al.* (2016) who, in their comparison of two pruning methods, observed that the more wood necroses developed, the more grapevines tended to express Esca-leaf symptoms. Additionally, in Esca-diseased grapevines, Maher *et al.* (2012) estimated that wood necroses formed a continuum within the scion, which constitutes a single unit with a volume of necroses useful in determining the health status of vines. The same authors observed that within grapevines developing the apoplectic form of Esca, the xylem and cambial areas had very advanced peripheral tissue degradations. In grapevines expressing Esca-chronic foliar symptoms, however, the quantity of internal necroses was higher than those obtained for asymptomatic plants. Among these necroses, white-rot represented the ultimate degradation of wood tissues and was strongly associated with Esca. Maher *et al.* (2012) even considered that white-rot in the arm was the best predictor for the chronic form of Esca. Recent findings support the idea that white-rot plays a key role in Esca, with at least 10 % of white-rot of Esca-diseased grapevine trunks. Plant sap flow decreases several weeks before the appearance of any Esca-foliar symptoms (Ouadi *et al.,* 2019). In Esca-diseased grapevines that had recovered healthy after treatment by sodium arsenite, this molecule accumulated in white-rot and eliminated the overabundant fungus, *F. mediterranea* (Bruez *et al.*, 2017). Therefore, by preventing the formation of white-rot in healthy grapevines, or by removing it surgically in

Esca-diseased grapevines, one possible method of controlling Esca can be envisaged. The process of removing wood necroses, particularly white-rot, already described one century ago by Lafon (1921), consisted of removing both infected and dead wood from the living grapevine.

In the present study, by revisiting the old viticultural technique of curettage thanks to new insights and the use of modern equipment, we first verified that removing white-rot from Esca-diseased grapevine helped them to recover. For this study, the symptom expression rate of a plot was observed over 7 years. The grapevine resilience of a panel of curetted-plants from this plot was subsequently studied over a period of 3 years. This was done by measuring various plant physiological parameters, such as plant growth capacity, fertility and berry quality of grapevines. To the best of our knowledge, no scientific study on the physiological consequences of curettage has been published so far (Mondello *et al*., 2018).

MATERIALS AND METHODS

1. Experimental vineyard

A commercial vineyard located at Beguey (near Bordeaux, France: 44°39’04.2”N 0°19’18.3”W) was used to study the effects of curettage on Esca-diseased grapevines, with a vine-plot of *Vitis vinifera* L. cv. Sauvignon blanc being selected for its high susceptibility to GTDs (Bruez *et al.*, 2013). This experimental vine plot, with its vines being omega-grafted onto 101-14 MTG rootstock, was planted in 1994 (row distances × vine = 1.8 × 1 m), and managed with a reasoned viticulture itinerary. The training system was in simple “Espalier” trellis and a Guyot double pruning regime, with a mean of five to seven buds per vine, and with two arms per plant. The plot was chosen for its high rate of Esca-foliar expression. In 2012 and 2013, out of 914 vine stocks that were surveyed each year, the percentages of trunk-affected vine stocks were respectively 43 % and 44.2 % and those of Esca-symptomatic vines were 11.8 % and 14.4 %.

2. Climatic conditions

The synthesis of the climatic conditions (Supplementary Figure 1) of the Bordeaux region where the commercial plot is located was obtained from meteofrance.com website (http://www.meteofrance.com/climat/france/bordeaux)

3. Design and Esca symptom assessment

All Esca symptoms were mapped each year from August 2012 to August 2018 based on a scale already used in a similar study (Lecomte *et al.*, 2018). Grapevines expressing Esca-foliar symptoms were labelled, and symptoms were assigned to two classes according to their severity: mild (corresponding to limited leaf symptoms, mostly discolourations, on one or two arms), and severe ones, with many drying zones and some wilting on one or two arms, as described earlier (Lecomte *et al*., 2012). For the resilience study, all results from 2014 to 2018 were used. For physiology studies, a dataset is corresponding to the evolution of the only 2014-labelled grapevines, and a second dataset of the only 2016-labelled grapevines.

4. Curettage

Curettage was practised about a week before harvest, on vines affected by Esca foliar-symptoms which appeared during the vintage. A thermic chainsaw was used to remove all dead wood and white-rot necrosis from the large pruning-wound scars affecting the inner trunk in the head of the scion (Figure 1). The result was that only the non-necrotic wood tissues remained.

5. Resilience

The first step of this study monitored the health evolution of Esca-symptomatic vine-stocks, with or without curettage. The vine-stocks were classified into three categories (recovered asymptomatic, symptomatic and dead), and their progression year after year was followed. The observations began in 2014 and finished in 2018, with the experimental plot (2424 vine-stocks) being divided into two blocks (Figure 1):

➤ The first block, in which all Esca-symptomatic plants were curetted, is called the “curetted part” (CP). Two batches of curetted vines were identified: batch 2014 (corresponding to the 52 newly symptomatic vine-stocks that were all curetted in 2014), and batch 2016 (corresponding to the 29 newly symptomatic vine-stocks that were all curetted in 2016).

➤ The second block, the “non-curetted part” (NCP), was the control plot, comprised of non-curetted Esca-diseased vines. As in the CP, two batches of symptomatic vines were identified to follow the evolution of Esca: batch 2014 (corresponding to the 79 newly symptomatic vine-stocks in 2014), and batch 2016 (corresponding to the 32 vine-stocks newly symptomatic in 2016).

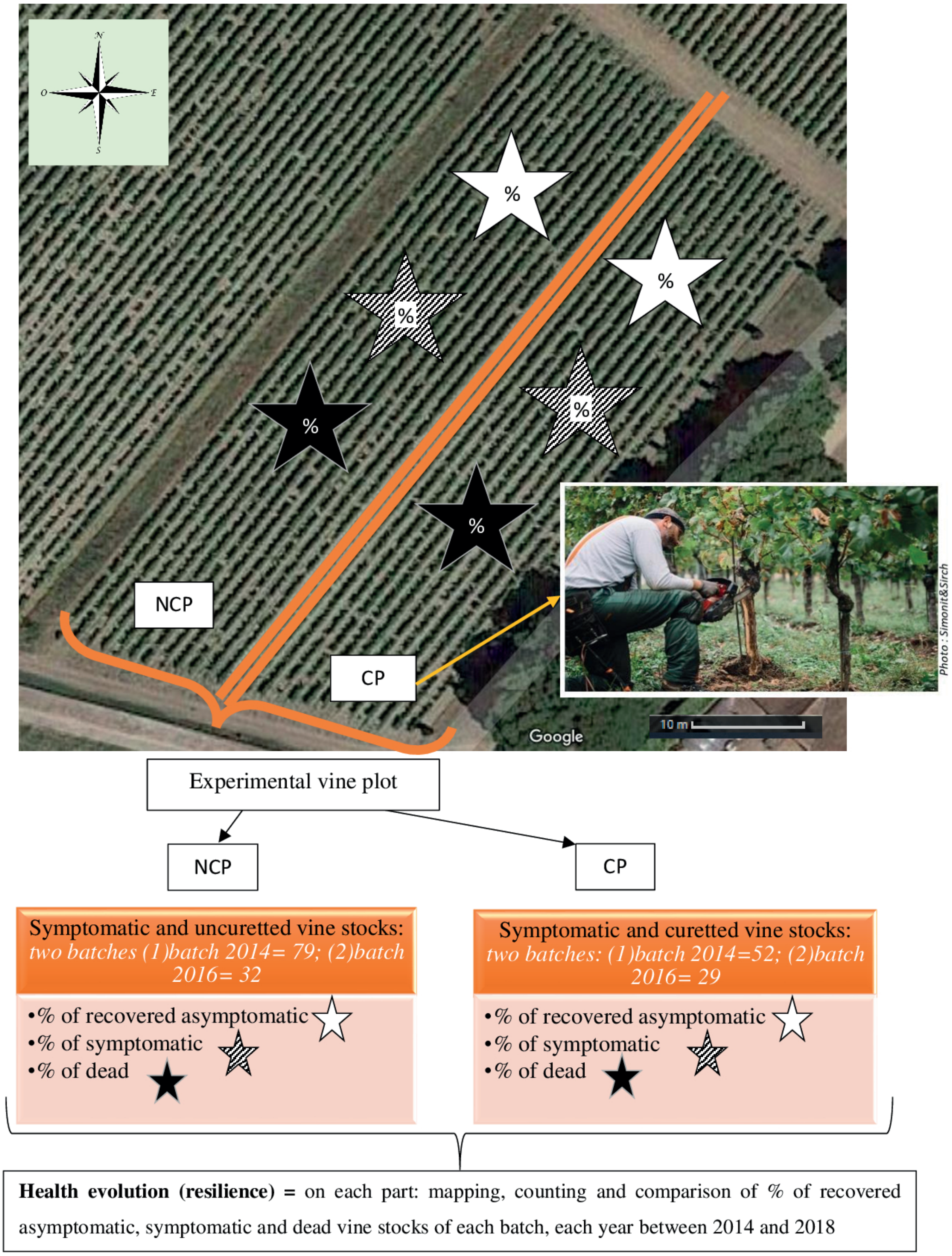
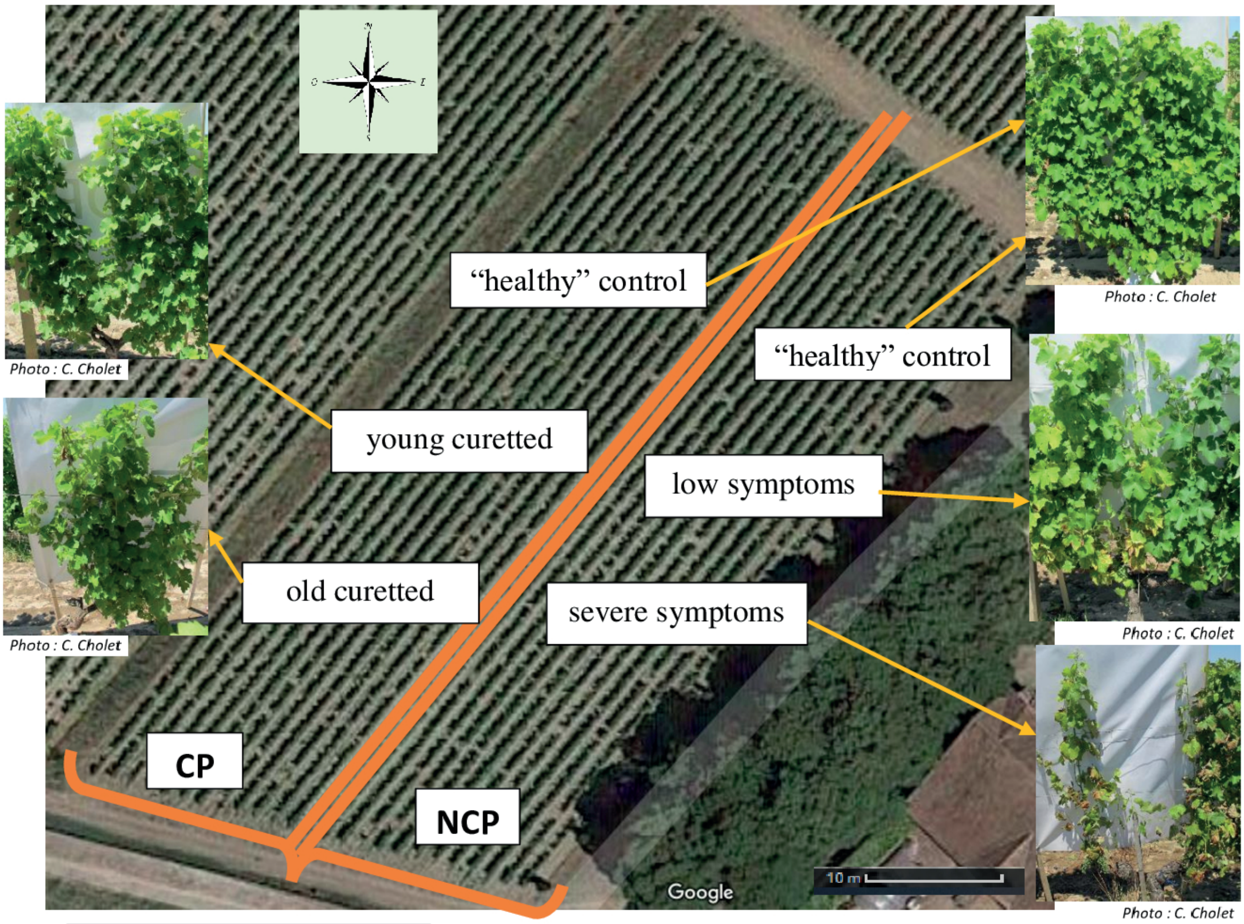


FIGURE 1. Experimental design of resilience.

CP = Curetted part of the plot; NCP = Non-curetted part of the plot. *Photo credit: Simonit&Sirch*



Experimental vine plot ⇨ **Growth capacity, Fertility, Berry quality?**

modality	"healthy" control	young curetted	old curetted	low symptoms	severe symptoms
year	**2016**				
number of vine stocks counted	15	12	12	13	14
number of arms counted	30	22	24	23	25
year	**2017**				
number of vine stocks counted	13	12	12	12	13
number of arms counted	26	22	21	22	19
year	**2018**				
number of vine stocks counted	12	12	12	12	11
number of arms counted	24	22	21	22	18

FIGURE 2. Experimental design of assessment of physiological consequences (growth capacity, fertility, berry quality) of Esca and curettage.

CP = Curetted part of the plot; NCP = Non-curetted part of the plot. *Photo credit: C. Cholet*

6. Physiological parameters

To assess the putative physiological consequences of Esca and curettage (growth capacity, fertility, berry quality), in each part of the same experimental plot, five modalities were identified from August 2016 (Figure 2; Supplementary Figure 2 and Table 1) and studied until the 2018 harvest:

1) Modality control: asymptomatic vine-stocks from 2014 to 2018, located in both CP and NCP (respectively, 7 and 8 vine-stocks).

2) Modality "old curettage": Vine-stocks curetted in 2014, corresponding to Esca-symptomatic vine-stocks with low symptoms (Supplementary Figure 2, in accordance with Lecomte *et al*., 2012) from the CP batch 2014, and recovered healthy after treatment in 2015 and remaining asymptomatic until 2018. This modality allowed us to study the medium-term impact of curettage, three years and more after curettage.

3) Modality "young curetted": Vine-stocks curetted in 2016, corresponding to curetted Esca-symptomatic vines with low symptoms (Supplementary Figure 2, in accordance with Lecomte *et al*., 2012) from the CP batch 2016, and recovered healthy after treatment in 2017 and remaining asymptomatic until 2018. This modality allowed us to study the short-term impact of curettage, the first and second year after curettage.

4) Modality "low symptoms": vines with mild Esca-foliar symptoms (Supplementary Figure 2, in accordance with Lecomte *et al*., 2012) from the NCP batch 2016, and located in the NCP. This modality allowed us to study the impacts of Esca (Supplementary Figure 2).

5) Modality "severe symptoms": vines with severe Esca-foliar symptoms (Supplementary Figure 2, in accordance with Lecomte *et al*., 2012), from the NCP batch 2016 (apoplectic vines were not considered in this modality). This modality allowed us to study the impacts of Esca.

7. Growth capacity

The buds left after pruning, and those that had started budburst, were counted at mid-budburst (stage 09 of BBCH scale) (Lorenz *et al*., 1994) in both 2017 and 2018, for all modalities. This allowed us to calculate the budburst percentage (= capacity of growth-start) for each vine, and for each modality [% B = (total budburst per vine /total post-pruning buds per vine) × 100)]. Like budburst is principally linked with abiotic parameters (Buttrose, 1969; Coombe, 1995; Olivian and Bessis, 1987; Pouget, 1963), and that each modality batch is on the same plot, so under the same abiotic parameters, their differences of budburst start rapidity is due to biotic parameters that can be traduced in growth capacity of each vine stocks. This ratio (% B) allowed us to evaluate and compare the differences in growth start rapidity between modalities, represented by budburst precocity.

The total number of annual shoots was counted at maturity (stage 89) (Lorenz *et al*., 1994) in 2016, and at bloom period (stages 62-63) (Lorenz *et al*., 1994) in both 2017 and 2018, for each vine and modality. This allowed us to determine definitive budburst for each modality [Def. B = (total annual shoots per vine /total post-pruning buds per vine) × 100)]. The difference between budburst percentage and definitive budburst represented the individual growth-capacity differences.

The foliar area of 6 grapevines per modality was photographed in both 2017 and 2018, at "bunch closed" stage (stage 77 (Lorenz *et al*., 1994)), with a white background of 1 meter × 1 meter, always taken in the same time slot. The photographs were processed using freeware ImageJ (v1.51k) (Schneider *et al*., 2012) to measure the total foliar-surface of each vine (area in m^2). Each measurement was repeated three times for each vine. The dataset explored per vine stocks and per arm because both Esca and curettage often lead to the removal of one of the two arms (respectively, by the death of the arm, or suppression of the infected arm).

8. Fertility

The total number of bunches per vine was counted at maturity (stage 89) (Lorenz *et al*., 1994) in 2016, and at bloom period (stages 62-63) (Lorenz *et al*., 1994) in both 2017 and 2018, for each modality. This allowed us to calculate punctual fertility (= total bunch numbers / total shoot numbers) (Olivain and Bessis, 1988).

Fertility was also characterised by berry-weight at harvest in 2016, 2017 and 2018 (stage 89) (Lorenz *et al*., 1994), to understand the impact of curettage on yield. Measurements of berry-weight were made from the sampling of 3 × 100 berries. Berries were destemmed by hand from 8 kg of bunches per modality, harvested on the same day (stage 89) (Lorenz *et al*., 1994), pooled and picked at random.

9. Berry quality

In 2016, 2017 and 2018, 8 kg of bunches per modality were harvested on the same day (stage 89) (Lorenz *et al*., 1994). These harvests took place in the morning, when the temperature was low, to preserve grape organoleptic characteristics. The bunches were harvested. Fresh musts were made in triplicate from the sampling of 3 × 100 berries. Measurements of the technological maturity of fresh must (total acidity and total sugar) were made after 5 h of decantation at 4 °C, using an automatic analyser OenoFoss™ (Foss France) calibrated daily.

10. Statistical analysis

For the Esca resilience study, Chi-square tests ($p = 0.01$ or $p = 0.001$) were used for (CP) and (NCP), to compare the 2015–2018 evolution of asymptomatic, symptomatic or dead distributions per vine category. For each observation year, statistical comparisons of distributions between curetted and control vines were either all highly significant ($p < 0.01$) or even very highly significant ($p < 0.001$).

For the physiological parameters studied, the statistical significance of the differences appearing between each modality was determined using the Kruskal–Wallis test for independent samples, and the Wilcoxon test for dependent samples. Experimental data detected as being significantly different, and the level of that significance, are indicated in the various figures and tables.

RESULTS

1. Resilience

This experimentation concerned the development of foliar and trunk Esca symptoms of Esca-leaf symptomatic vines curetted in 2014 (CP batch 2014), and other vines curetted in 2016 (CP batch 2016), compared with non-curetted vines (NCP batch 2014 and NCP batch 2016).

Two years after curettage, the non-curetted part of the experimental plot vines (NCP) showed a high proportion of Esca-symptomatic vines: 48 % in 2016 for NCP batch 2014 (Figure 3), and 63 % in 2018 for NCP batch 2016 (Figure 4). A medium proportion recovered asymptomatic by themselves without curettage, which is common with Esca-foliar symptoms: 52 % (2016) and 37 % (2018). There was also a low proportion of dead vine: 10 % in 2016 for NCP batch 2014, and 6 % in 2018 for NCP batch 2016, consecutive to health erosion. Conversely, a high proportion of curetted vines no longer showed any Esca-foliar symptoms: 75 % in 2016 for CP batch 2014 (Figure 3), and 79 % in 2018 for CP batch 2016 (Figure 4). Vine-health erosion of NCP vine-stocks was significantly higher than in the CP, whatever the year of curettage. In the NCP, the asymptomatic and Esca-symptomatic vine proportion decreased annually, because the diseased vines declined inexorably, which was not the case in the CP. Four years after curettage (batch 2014) (Figure 3), the percentage of dead vines was limited to 9 % in the CP but had strongly increased to 39 % in the NCP. Moreover, whereas Esca-diseased-vine mortality for the NCP evolved rapidly, this was much slower for the CP.

2. Grapevine growth capacity

Growth capacity was estimated from growth-start precocity, definitive budburst percentage, and foliar area.

2.1. Growth-start precocity

Overall, there were few significant differences between the modalities, either in 2017 or 2018 (Table 1).

Concerning the Esca-symptomatic vines, we observed that the plants expressing Esca-foliar symptoms most strongly were those whose growth started later. Their budburst rate at that stage was the lowest significantly, in both 2017 and 2018 vintages.

Concerning the curetted vines, the "old curetted" (more than 3 years) had budburst rates very similar to those of controls. The "young curetted" (+1 year in 2017, and +2 years in 2018) did not appear significantly different but tended to have a slightly lower budburst rate than controls. That rate was either equal to (2017) or even higher (2018) than that of low Esca-symptomatic vines. The "young curetted" tended to catch up with controls.

2.2. Definitive budburst percentage

Concerning the Esca-symptomatic vines, the more severe the foliar symptoms were, the less significant the budburst rate was. Depending on the vintage, either the growth capacity of the symptomatic vines did not change (2017) compared to the previous count (stage 09), always remaining with a significantly lower definitive budburst percentage than for other modalities (Table 1). Alternatively, a catch-up in growth capacity appeared (+26 % for "low symptoms" modality

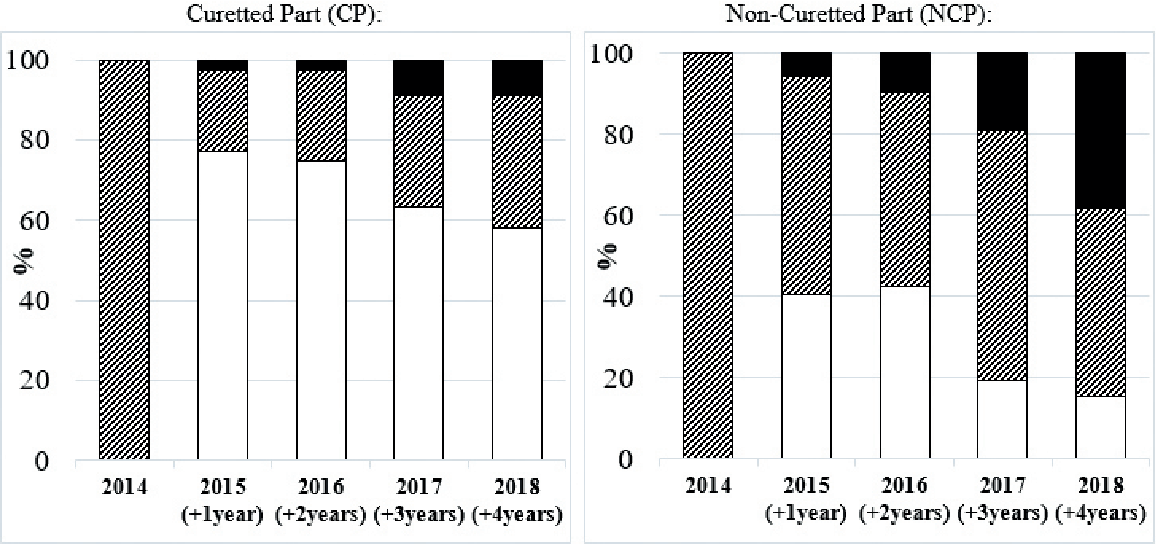


FIGURE 3. Yearly Esca development and health resilience on curetted vines (CP) or health erosion on vines without curettage and used as control (NCP) in 2014.

In white colour: % of asymptomatic vines; in black stripes: % of symptomatic vines; in solid black: % of dead vines.

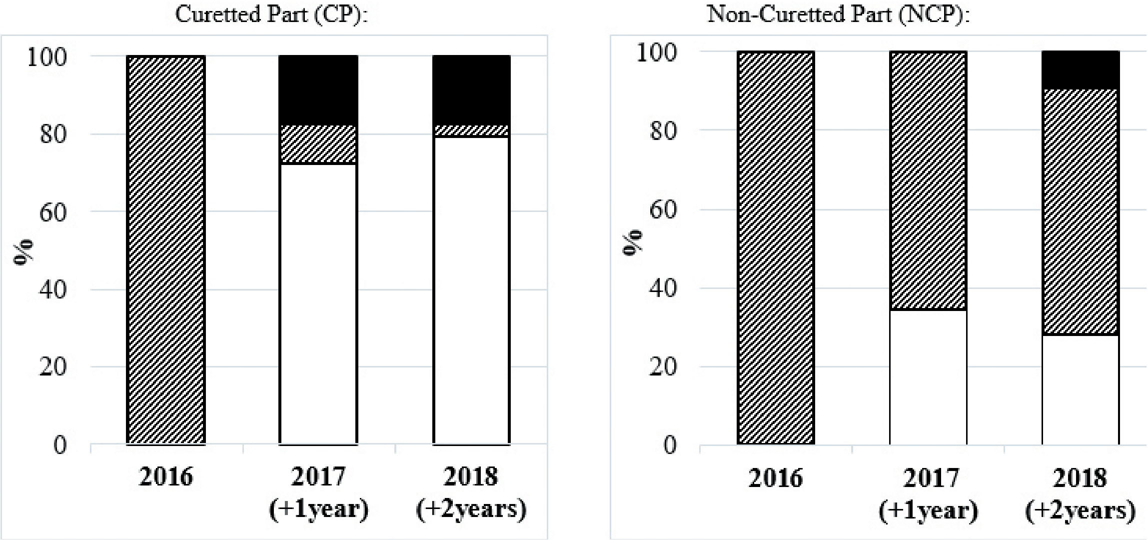


FIGURE 4. Yearly Esca development and health resilience on curetted vines (CP) or health erosion on vines without curettage and used as control (NCP) in 2016.

In white colour: % of asymptomatic vines; in black stripes: % of symptomatic vines; in solid black: % of dead vines.

and +41 % for "strong symptoms" modality (2018)). In addition, the more Esca-foliar symptoms were expressed, the greater the delay in growth. This occurred when there was springtime water constraint (vintage 2017; Supplementary Figure 1).

Unlike the symptomatic vines, whatever the vintage, the curetted vines were comparable to the control vines (Table 1). Compared to the previous count, there was a catch-up in the growth capacity of "young curetted" modality only for 2017 (+16 %), which was not the case for the diseased vines. Thus, the "young curetted" modality had budburst % at stage 53, similar to that of controls (Table 1), despite the spring-time water constraint (Supp. data1). In 2018, the curetted (old or young) vine-stocks remained with lower definitive budburst rates than those of "healthy" control. The growth capacity of those vines had not changed significantly compared to stage 09.

TABLE 1. Comparison of growth capacity between modalities.

modality	year	% of growth starting (%B) at stage 9 of BBCH scale	% of real budburst (Def. B) at stages 62-63 of BBCH scale
"healthy" control	2017	80.78 *sd 14.65* ab	83.9 *sd 22* a
low symptoms	2017	70.64 *sd 26.61* ab	68.45 *sd 23.28* ab
severe symptoms	2017	53.75 *sd 38.89* b	52.77 *sd31.9* b
young curetted	2017	68.21 *sd 31.21* a	84.17 *sd 14.48* *a
old curetted	2017	87.43 *sd 11.14* a	90.6 *sd 13.38* a
"healthy" control	2018	69.69 *sd 15.74* a	100 *sd -21.84* ***a
low symptoms	2018	43.82 *sd 34.12* ab	69.18 *sd 41.26* **b
severe symptoms	2018	33.49 *sd 28.38* b	84.08 *sd 38.2* ***ab
young curetted	2018	62.6 *sd 19.93* ab	68.54 *sd 24.56* b
old curetted	2018	64.73 *sd 19.89* ab	72.4 *sd 19.9* b

(a, b): significant differences between modalities, with the Kruskal–Wallis test with $\alpha \leq 2$ %. (*, **, ***): significant differences between stages, with the Wilcoxon test with $\alpha = 2.5$ %; sd= standard deviation

TABLE 2. Comparison of foliar area for all modalities: per vine, per arm in 2017 and 2018.

Modality	Year	Mean area par vine (cm²)	Mean area per arm (cm²)
"healthy" control	2017	9035.2 *sd 1325.7* a	4512.6 *sd 662.8* ab
low symptoms (NCP)	2017	8011.6 *sd 3318.1* a	4236.4 *sd 2092.7* a
severe symptoms (NCP)	2017	8006.7 *sd 4246.5* a	5643.2 *sd 2295.9* ab
young curetted (CP)	2017	8304.4 *sd 2270.8* a	4585.1 *sd 1489.3* b
old curetted (CP)	2017	7210.7 *sd 1658.4* a	4484.4 *sd 868.1* ab
"healthy" control	2018	10,614.8 *sd 2693.9* a	5681.6 *sd 1557.9* a
low symptoms (NCP)	2018	6837.2 *sd 3837.7* b	5860.5 *sd 2539.3* a
severe symptoms (NCP)	2018	5132.7 *sd 2462.9* b	3509.1 *sd 3010.1* b
young curetted (CP)	2018	7363.5 *sd 2888.7* b	4116.5 *sd 1869.9* b
old curetted (CP)	2018	8001.1 *sd 1539.6* ab	4143.1 *sd 952.3* b

(a, b): significant differences between modalities, with the Kruskal–Wallis test with $\alpha < 5$ % (2017); $\alpha < 0.05$ % (2018). sd = standard deviation. NCP: Non-Curetted Part; CP: Curetted Part

2.3. Foliar area

When we measured total foliar area per vine stock (Table 2), all the modalities tended, more or less significantly, to have a smaller leaf area than control plants (in 2017, control: 9035 cm² > "young curetted": 8304 cm² > "low symptoms": 8011cm² > "severe symptoms": 8007 cm² > "old curetted": 7211 cm². In 2018, control: 9035 cm² > "old curetted": 8001 cm² > "young curetted": 7363 cm² > "low symptoms": 6837 cm² > "severe symptoms": 5133 cm²). Moreover, it was interesting to note that the standard deviation varied according to modalities. In the case of Esca-symptomatic vines, the variability was more marked, with very extensive standard deviations, reflecting a very large heterogeneity of the leaf area measured for those vines ("low symptoms": +/- 3318 cm², "severe symptoms": +/- 4247 cm², "healthy" control: +/- 1325 cm²). That heterogeneity can be linked to the symptomatic vine variability.

Concerning curetted modalities, standard deviations were far less extensive than those of Esca-symptomatic vines, which reflected their leaf-area homogeneity (in 2017, "young curetted": +/- 1489 cm², "old curetted": +/- 868 cm², in 2018, "young curetted": +/-2889 cm², "old curetted": +/- 1540 cm²).

Having first studied foliar area per vine stock, we then examined it per arm (Table 2) because both Esca and curettage often lead to the removal of one of the two arms. Accordingly, we chose to measure leaf area per arm, to verify whether the observations per vine were homogeneous.

When foliar area per arm was measured, significant differences appeared between control and Esca-symptomatic vines. In 2017, "low symptoms" vines had a significantly smaller foliar area on each arm (4236 cm² +/- 1489 cm²), while severe symptom vines had a larger foliar area per arm (5643 cm² +/- 2296 cm²). Inversely, in 2018, "low symptoms" vines had a larger foliar area per arm (5860 cm² +/- 2539 cm²), whereas "severe symptoms" vines had a significantly smaller foliar area on each arm (3506 cm² +/- 3010 cm²). Equally, the standard deviations of Esca-symptomatic vines, contrary to asymptomatic ones, were very large, suggesting great variability in vigour. As "low symptoms" vine had their two arms, their leaf area, whether measured per arm or vine stock, was always smaller than that of control vines. This meant that the vine-stocks with low symptoms had lower overall vigour than control vines. Most "severe symptoms" vine had only one arm out of two. Consequently, their foliar area measured per arm was very similar to that measured per vine (lower than or the same as control). These vines compensated for their absent arm by developing a larger leaf area for the remaining arm.

Concerning curetted modalities, there were no significant differences between control and curetted vines in 2017 ("young curetted": 4585 cm² +/- 1489 cm², "old curetted": 4484 cm² +/- 868 cm²) whereas, in 2018, curetted vines had smaller foliar areas ("young curetted": 4116 cm² +/- 1870 cm², "old curetted": 4143 cm² +/- 952 cm²). Standard deviations of control and curetted modalities were small, suggesting that the foliar area results were similar.

3. Fertility and yield

Fertility and yield were estimated from punctual fertility, bunch and berry size, and berry quality.

3.1. Punctual fertility

Punctual fertility corresponded to the average bunch numbers per vine stock/average annual shoot numbers per vine stock.

Whatever the year studied, the Esca-symptomatic-vine punctual fertility tended to be lower than that of control. The bunch numbers per vines were lower than for control vines, and the average shoot numbers per vine stock tended to be equal to control (Table 3).

Whatever the year of the study, curetted vine bunch numbers per vine and per arm were significantly lower than those of control. "Old curetted" and "young curetted" had either significantly fewer shoots (stem per vine or per arm) (2016, 2018) or a number equal (2017) to that of control. "Old curetted" vines had fewer bunches, but also fewer shoots. Like control, their punctual fertility was relatively constant between study years and exhibited similar levels. "Young curetted" vines had fewer bunches, but as many shoots as control. Like Esca-symptomatic vines, they had significantly lower punctual fertility than control, but this was not Table from one year to the next, tending to increase annually, reflecting an increase in bunch rather than shoot numbers.

3.2. Bunch and berry size

With regard to grape-bunch weights (Table 4), a few tendencies emerged between modalities, irrespective of the particular study year, with bunches and berries in control modality (Table 5) always appearing to be heaviest. This meant that those bunches were composed of a large number of berries.

Concerning Esca-symptomatic modalities, the more symptomatic the vines were, the lighter bunches tended to be. Berries of those symptomatic modalities had, however, the lowest weights. Thus, bunches of very diseased vines were smaller, with small berries, borne in smaller numbers compared to control vines.

The bunch and berry weights of the curetted vines were comparable to those obtained for control, with a tendency to have bunches that were lighter, but heavier than Esca-diseased vine bunches. When we examined the old and young curetted vines, we observed that:

The "old curetted" vines had bunches and berries whose weights were comparable to control.

"Young curetted" vines had bunch and berry weights that, in 2016, tended to be lighter than control (first year of their curettage). This time lag gradually decreased, tending to match the results obtained for "old curetted". Recent curettage affected bunch and berry sizes, which were both smaller. Over time, that morphological difference gradually faded, however, so that two years after curettage, curetted vine bunches were comparable both for bunch and berry weights and for the berry number of control vines.

TABLE 3. Comparison of means: stem numbers per arm; stem numbers per vine; bunch numbers per arm; bunch numbers per vine; punctual fertility.

α*: significant threshold. with the Kruskal–Wallis test*	Modality	“Healthy” control	Young curetted	Old curetted	Low symptoms	Severe symptoms
	year	2016				
	number of vines counted	15	12	12	13	14
	number of arms counted	30	22	24	23	25
α = 1 %	stems/arm	7.60 *sd 1.79* a	6.29 *sd 2.31* ab	5.41 *sd 1.94* b	7.08 *sd 2.04* ab	7.52 *sd 3.02* a
α = 0.5 %	stems/vine	15.20 *sd 2.93* a	10.39 *sd 3.96* b	9.20 *sd 2.25* b	12.54 *sd 4.50* ab	13.43 *sd 5.24* ab
α = 0.5 %	bunches/arm	10.4 *sd 3.64* a	6.59 *sd 3.73* b	6.88 *sd 2.52* a	7.45 *sd 2.82* b	8.35 *sd 5.43* b
α = 0.5 %	bunches/vine	20.80 *sd 5.63* a	11.04 *sd 6.21* b	11.70 *sd 2.71* b	12.77 *sd 5.39* ab	14.14 *sd 8.11* ab
α = 5 %	punctual fertility	1.38 *sd 0.33* a	1.03 *sd 0.37* b	1.29 *sd 0.21* ab	1.02 *sd 0.29* b	1.01 *sd 0.59* ab
	year	2017				
	number of vines counted	13	12	12	12	13
	number of arms counted	26	22	21	22	19
α = 5 %	stems/arm	6.75 *sd 2.45* a	5.29 *sd 2.11* ab	6.68 *sd 2.16* a	4.32 *sd 2.44* b	*3.74* *sd 2.92 b*
α = 0.5 %	stems/vine	12.46 *sd 4.16* a	9.33 *sd 3.01* ab	10.58 *sd 3.26* a	7.92 *sd 3.53* ab	5.46 *sd 3.78* b
α = 5 %	bunches/arm	9.00 *sd 4.34* a	5.97 *sd 3.65* a	7.06 *sd 2.89* a	6.36 *sd 4.74* a	4.68 *sd 3.85* a
α = 1.5 %	bunches/vine	16.62 *sd 7.36* a	10.43 *sd 4.53* ab	10.83 sd *3.81* ab	11.67 *sd 7.36* ab	6.85 *sd 4.98* b
α = 5 %	punctual fertility	1.30 *sd 0.27* a	1.11 *sd 0.37* a	1.06 *sd 0.33* a	1.34 *sd 0.39* a	1.01 *sd 0.63* a
	year	2018				
	number of vines counted	12	12	12	12	11
	number of arms counted	24	22	21	22	18
α = 5 %	stems/arm	6.31 *sd 2.12* a	4.61 *sd 1.86* b	4.22 *sd 1.76* b	4.28 *sd 3.19* ab	5.45 *sd 3.53* ab
α = 5 %	stems/vine	12.62 *sd 3.72* a	7.9 *sd 3.3* b	7.67 *sd 2.58* b	8.21 *sd 5.48* ab	9.23 *sd 5.26* ab
α = 1.5 %	bunches/arm	8.59 *sd 3.45* a	6.41 *sd 3.32* ab	5.71 *sd 2.93* b	5.46 *sd 4.74* b	7.14 *sd 5.49* ab
α = 0.5 %	bunches/vine	17.19 *sd 5.23* a	11.29 *sd 4.47* b	10.00 *sd 3.35* b	10.14 *sd 8.03* b	12.08 *sd 7.66* ab
α = 5 %	punctual fertility	1.38 *sd 0.26* a	1.64 *sd 1.26* a	1.28 *sd 0.24* a	1.07 *sd 0.62* a	1.18 *sd 0.48* a

(a, b): significant differences between modalities, with the Kruskal–Wallis test; sd = standard deviation.

TABLE 4. Comparison of medium weight (g) of 10 bunches at harvest, for all modalities.

Modality	2016	2017	2018
"healthy" control	1764.0 *sd 53.7* a	1764.0 *sd 53.7* ab	1118.5 *sd 14.1* a
low symptoms	1279.1 *sd 169.8* a	1279.1 *sd 169.8* ab	693.4 *sd 28.3* a
severe symptoms	884.6 *sd 367.4* a	884.6 *sd 367.3 b*	717.3 *sd 14.1* a
young curetted	1148.2 *sd 136.2* a	1148.2 *sd 136.2* ab	1185.5 *sd 67.9* a
old curetted	1425.0 *sd 41.0 a*	1425.0 *sd 41.0 a*	1220.2 *sd 51.3* a

(a, b): significant differences between modalities for the same year, with the Kruskal–Wallis test with α = 1 %. sd = standard deviation

TABLE 5. Comparison of medium weight (g) of 100 berries at harvest, for all modalities.

Modality	2016	2017	2018
"healthy" control	142.3 *sd 9.8* a	161.8 *sd 7.7* a	148.7 *sd 3.6* ab
low symptoms	69.0 *sd 6.1* b	151.8 *sd 1.9* a	116.0 *sd 7.2* ab
severe symptoms	102.9 *sd 35.1* a	145.7 *sd 8.4* a	108.7 *sd 2.4* b
young curetted	105.4 *sd 19.9* ab	155.5 *sd 24.4* a	140.5 *sd 16.0* ab
old curetted	130.8 *sd 4.8* ab	151.8 *sd 3.9* a	149.3 *sd 5.9* b

(a, b): significant differences between modalities for the same year, with the Kruskal–Wallis test with α = 5 %. sd = standard deviation

TABLE 6. Mean of sugar content (g/L) and total acidity (g/LH$_2$SO$_4$) at harvest, for all modalities

	Mean sugar (g/L) content at harvest		
Modality	2016	2017	2018
"healthy" control	202.2 *sd 7.5* a	224.2 *sd 3.0* a	184.1 *sd 26.3* a
low symptoms	174.5 *sd 16.6 b*	215.1 *sd 0.8* b	176.8 *sd 22.9* ab
severe symptoms	133.6 *sd 1.1* b	196.9 *sd 3.8* b	151.1 *sd 43.8* b
young curetted	143.3 *sd 4.4* ab	216.9 *sd 1.9* ab	162.7 *sd 21.6* b
old curetted	183.8 *sd 6.2* a	218.8 *sd 3.2 a*	187.7 *sd 1.7* ab
	Mean total acidity (g/LH$_2$SO$_4$) at harvest		
"healthy" control	4.9 *sd 0.2* a	5.9 *sd 0.1* a	6.1 *sd 1.7* ab
low symptoms	5.4 *sd 1.2* a	5.9 *sd 0.1* a	6.5 *sd 1.6* ab
severe symptoms	4.0 *sd 0.0* a	6.0 *sd 0.0* a	7.6 *sd 3.3* ab
young curetted	3.5 *sd 0.1* a	6.4 *sd 1.6* a	7.6 *sd 1.6* b
old curetted	5.3 *sd 0.3* a	5.5 *sd 0.4* a	6.7 *sd 0.7* a

(a, b): significant differences between modalities for the same year, with the Kruskal–Wallis test with, α = 1.5 %. sd = standard deviation

These results showed that, after just one year of treatment, curettage tended to allow vines to recuperate their fruit formation capacity, which was not the case for the non-curetted Esca-diseased vines.

3.3. Berry quality

Independently of the year of observation, berry must quality of control modality was characterised by more sugar and the total acidity level intermediate between all modalities and for each vintage (Table 6). Esca symptomatic modality must was always less rich in sugar, and had an acidity that tended to be either higher than or more or lesser equal to control modality must level, depending on the vintage. Symptomatic vine berries tended to be late in their technological maturity, mainly in relation to their sugar content.

Concerning curetted modalities, "old curetted" must quality was significant compared to that of control modality. Sugar and total acidity levels were the same, whatever the year. For the "young curetted", the technological maturity difference tended to fade gradually. Thus, two years after curettage, must quality has remained intermediate between that of controls and Esca-symptomatic vines. Curettage, then, made it possible to recuperate in medium-term both yield quantity and maturity quality close to those of asymptomatic vines.

DISCUSSION

1. Curettage and diseased wood

Esca-foliar symptoms are positively correlated with extensive wood necroses within grapevines, particularly when white-rot developed to an excess of 10 % woody surface (Guerin-Dubrana *et al*., 2013; Maher *et al*., 2012; Ouadi *et al*., 2019). This necrosis development leads to plant sap flow decrease, even weeks before any Esca-leaf symptoms can be seen, as shown recently by Ouadi *et al*. (2019). Having considered these points, and to help cure Esca-disease grapevines, we choose to study a surgical method to remove wood necroses, mainly white-rot, from plants. Although this is an old arboricultural practice used in (Lafon, 1921), its effects on plant resilience and physiology have never been studied scientifically (Mondello *et al*., 2018).

In the present study, the results showed that curettage enabled Esca-symptomatic vine to exhibit medium-term resilience, with a parallel slowdown in typical Esca-foliar symptoms and vine-death. So, in our study, curettage clearly had a positive effect on promoting vine resilience. By surgically removing the white-rot and dead wood, this practice certainly allows most of the Esca-pathogens inhabiting these degraded-wood structures to be eliminated, thereby restricting their development in the vine stock. For instance, when the white-rot was removed, *F. mediterranea*, which plays a major role in its formation (Fischer and Kassemeyer, 2003; Markakis *et al*., 2017; Martín *et al*., 2019), and is overabundant in this necrosis (Bruez *et al*., 2017), disappeared and just one year later the vines no longer expressed Esca-foliar symptoms.

In the present paper, we hypothesised that curettage allows the vine to compensate for the physiological, yield and qualitative damage previously induced by Esca. To verify this point, the recuperation was studied at three levels: physiological, fertility and berry quality.

2. Curettage and growth capacity

Curetted vines rapidly regained growth capacity similar to that of asymptomatic vines. This capacity had already been restored one year after curettage but, as for the physiological state of the former Esca-diseased vine stock, it was stabilised only three years later. Conversely, we observed that Esca-diseased vine stock presented a delay in growth start, in line with symptom expression levels. As Esca-diseased vines have more necrotic than healthy wood, especially white-rot necrosis, (Maher *et al*., 2012; Ouadi *et al*., 2019), this could explain the delay in growth being triggered by a reduction in starch reserve efficiency. It should also be recalled that Esca-symptomatic leaves present photosynthetic dysfunctions which limit their reserve metabolite production (Magnin-Robert *et al*., 2016; Petit *et al*., 2006; Valtaud *et al*., 2011). The combination of these two causes results in limited starch storage in both of the perennial vine parts (root and trunk) (Lecomte *et al*., 2012; Surico *et al*., 2006; Valtaud *et al*., 2009, 2011).

3. Curettage and vintage constraints

All of the physiological damage induced by Esca that we studied was significantly decreased by curettage. The resulting physiological resilience also allowed improved management of the stress impact of growth-phase vintage conditions. Even in stressful vintage conditions, the curetted vines, unlike the Esca-symptomatic vines, do not see to have been affected.

This confirmed the resilience of these curetted vines because, unlike the Esca-symptomatic vines, they showed only very limited growth delay. This is particularly well illustrated by the 2017 vintage, characterised by increased stressful water-constraint during the growth phase in 2018 (Supplementary Figure 1). The growth capacity of curetted and asymptomatic vines was both strong and rapid, whereas that of the Esca-symptomatic vines was weak and slow.

Vigour delay was also found in foliar area measurements where, similarly, the more the vines expressed Esca-symptoms, the more limited their leaf area was. Whereas curetted vines compensated for their absent arm by developing a larger leaf area for the remaining arm, this was not the case of Esca-symptomatic vines. Even if curettage did not induce any additional leaf-surface development, it did allow vines to recuperate their foliar area homogeneity, unlike non-curetted symptomatic vines. In spring-time, a particularly active growth phase, the water constraint suffered by vines (for all modalities) seemed to slow down or even blocked Esca-symptomatic vine growth (Larignon *et al*., 2009; Lecomte *et al*., 2012), but not that of curetted vines. The non-curetted Esca-diseased vines were unable to grow probably because of their reaction to environmental stress, combined with their diminished growth. The increase in the proportion of Esca-symptomatic or dead vines between 2017 and 2018 is in line with this interpretation. Curettage restored the vine stock's efficient physiological functioning, enabling it to adapt to environmental constraints and to manage the vintage-condition stress consequences, which was not the case for symptomatic vines.

4. Curettage, vine fertility and berry quality

The resilience of the curetted vines was also reflected in berry fertility and quality. Thus, even if the physiological equilibrium of young curetted vines was not initially respected, over time, old curetted equilibrium tended to be restored. Although the fertility resilience of curetted and asymptomatic vines was comparable, that of Esca-symptomatic vines always remained much lower. Equally, whereas berry and must quality of curetted and asymptomatic vines were comparable, that of Esca-symptomatic vines was characterised by retarded ripening. The punctual fertility of Esca-symptomatic vines revealed a physiological disequilibrium in the leaf/fruit ratio. This corresponds to retarded ripening of the current vintage, and to weak starch reserves, which impact the fertility of the following vintage (Li-Mallet *et al*., 2015; Li-Mallet, 2017; Pellegrino *et al*., 2014). Esca-symptomatic vines have necrosed-like foliage, *i.e.*, leaf discolourations and/or drying zones between the primary veins, which explains retarded ripening and induces weak starch reserves. Those weak reserves negatively impact the fertility of the following vintage (Li-Mallet *et al*., 2015; Li-Mallet, 2017; Pellegrino *et al*., 2014).

This disequilibrium was also reflected in the berry and bunch morphology of these Esca-symptomatic vines. The expression level of foliar symptoms was inversely proportional to berry and bunch size, the consequence of (I) spring-time water constraint level (Supplementary Figure 1) during the previous and current vintage (Ojeda *et al*., 2001, 2002); (II) leaf/fruit ratio disequilibrium (Zufferey *et al*., 2015), and (III) weak starch storages. Thus, Esca-symptomatic vines decline more or less quickly, depending on the climatic constraints they undergo (Fontaine *et al*., 2016b), which was not the case on a medium-term perspective (old curetted vines in our study).

The decline of Esca-symptomatic vines affects their physiological functioning, with consequences on berry quality (Calzarano *et al*., 2004), which is more acidic and less sweet. Previous work on berry and must quality of Esca-diseased vines has highlighted similar observations, as well as pointing out greater richness in nitrogen protein and total polyphenols (Calzarano *et al*., 2004; Lorrain *et al*., 2012). The consequences of these oenological parameter modifications raise the question about the wine quality of these bunches and their ageing capacity. As nitrogen is an essential element in the alcoholic fermentation process, modification of the must nitrogen protein composition strongly influence the fermentation process and the aromatic component. Equally, as polyphenolic compounds play an essential role in wine structure and ageing, the polyphenolic-maturity modification would have significant consequences on wine stability and structure (Brossaud *et al*., 2001; Chira *et al*., 2012).

5. Curettage and other methods

To the best of our knowledge, curettage is the only curative method currently used to control Esca in severely attacked vines, having at least 10 % of white rot in the wood. It differs from other methods, such as trunk renewal, since it allows the same plant to recover rapidly, *i.e.*, within one year, after curettage treatment. Recently, Bruez *et al.* (2017) showed that in recovered

Esca-disease vines, sodium arsenite accumulated in the white-rot, eliminating the predominant fungus, *F. mediterranea*. The curettage mode of action is based on the same concept: by eliminating the white-rot and consequently, *F. mediterranea*, from Esca-diseased vines. The result is the same: plants are turning healthy. Among the points that could limit the use of curettage: (I) removing the white-rot from diseased vines requires strong expertise and has to be done by a specialist; (II) as curettage is time-consuming, it could induce high cost, particularly when Esca is widely disseminated in the vineyards. Unfortunately, no data on these specific points are presently available.

CONCLUSION

Esca-symptomatic vines generally showed lower vigour, lower fertility and negatively impacted grape quality. When, however, curettage was practised on those vines, it was very helpful in retarding Esca-diseased vine decline. Curettage also allowed grapevines to recuperate their growth capacity (stem number; foliar area), fruiting (bunch number; punctual fertility), yield capacity (bunch weight) and grape-berry quality (sugar) within just one year. Equally, even in stressed spring conditions, although curetted-vine vigour was lower than that of asymptomatic vines, it remained much better than for Esca-symptomatic vines. Over time, and at least on the mid-term, curettage continued to slow down vine mortality, seemingly allowing curetted vines to compensate for Esca's detrimental impact on physiology functioning, and to recover resiliently.

Curettage is particularly effective by seeming to allow the restoration of must-quality and to minimize the above-mentioned oenological degradations. This method could, accordingly, be considered as a practice that is effective in rapidly reducing the impact of Esca, but its precise cost in severely attacked vineyards has to be determined. Additional experiments need to be done to verify whether curettage also allows wine quality characteristics to be conserved. We are currently investigating the ensuing question of its impact on wine quality and wine ageing capacity.

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