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Research Papers

Recovery after curettage of grapevines with esca leaf symptoms

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Summary. Grapevine curettage was re-introduced in France in the early 2000s, and is important for facilitating recovery of plants from esca disease. This surgical practice involves removal of deadwood of vines with leaf symptoms, focusing on white rot generally observed at the centres of grapevine trunks. Assessment of the efficacy of this practice was initiated in the Bordeaux region in 2014. One 'Sauvignon Blanc' vineyard severely affected by esca was initially surveyed in the summer of 2014, to identify and treat vines with esca foliar symptoms. Annually thereafter, from 2014 to 2018, selected vine stocks were curetted. Two other 'Sauvignon Blanc' vineyards also displaying high levels of esca damage were added to the study in 2015 and 2016. Curettage treatments ceased in 2018, resulting in 11 trials (vineyard × year combinations). In total, 856 vines (422 curetted and 434 control vines) were then surveyed annually up to 2021, for assessments and comparisons of esca development. At each site, plants with esca symptoms recovered well after curettage: on average 85% of all curetted vines became asymptomatic the year immediately after the treatment. Six years after treatment, for curettage campaigns carried out in 2014 and 2015, more than half of the curetted vines were symptom-free, whereas <12% of the control vines were asymptomatic, and gradual loss of efficacy was observed at each site. The mean annual proportion of efficacy erosion was approx. 8% per year. This study highlights the possible short- and mid-term benefits of trunk surgery to enable recovery of esca-affected vines, and for them to recover and remain leaf-asymptomatic for several years.

Keywords. Trunk surgery, plant health recovery, *Vitis vinifera* L., white rot.

INTRODUCTION

Grapevine trunk diseases (GTDs) are a major cause of grapevine decline and death in many grape-growing regions, including the vineyards of European wine-producing countries (Guérin-Dubrana *et al.*, 2019). These fungal diseases affect the perennial parts of vines, causing diverse types of damage in established vineyards (Lecomte *et al.*, 2018). The principal GTDs in mature vineyards are Botryosphaeria dieback, esca disease, Eutypa dieback, and Phomopsis dieback (Larignon and Dubos, 1997; Mugnai *et al.* 1999; Úrbez-Torres, 2011; Baumgartner *et al.* 2013; Gramaje *et al.*, 2018). Esca, in particular, causes major economic losses in France (Bruez *et al.*, 2013). Pruning wounds are the main entry sites for pathogenic fungi, and slow and progressive extension of the infections within the grapevine wood leads to permanent infections of different degrees of latency (Hrycan *et al.*, 2020). These may result in development of cankers or inner necroses of variable size, shape and discolouration (Larignon and Dubos, 1997; Mugnai *et al.*, 1999; Maher *et al.*, 2012, Úrbez-Torres, 2011).

Eutypa, Botryosphaeria and Phomopsis (or Diaporthe) diebacks are generally associated with one or several related xylem-inhabiting fungi (Hrycan *et al.*, 2020). Esca on mature vines is associated, following the definition of Larignon and Dubos (1997), with a large complex of vascular fungi as primary and secondary pathogens, including *Ascomycota* and *Basidiomycota*. The ascomycetes *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum* and other *Phaeoacremonium* spp. were reported as the pioneering pathogens (Larignon and Dubos, 1997), but the other ascomycetes involved in the three dieback diseases (above) may also act as precursors, or co-colonizing fungi, as reported for *Eutypa lata* in temperate climates (Larignon and Dubos, 1997) and *Botryosphaeria* species in dry (e.g. Mediterranean) regions (Luque *et al.*, 2009; Ammad *et al.*, 2014; Choueiri *et al.*, 2014). These fungi can be isolated from wood lesions on the trunks or cordons of esca-infected grapevines (Larignon and Dubos, 1997; Choueiri *et al.*, 2014; Elena *et al.*, 2018). Communities of wood-inhabiting microorganisms, including other fungi or bacteria, can also be identified in individual samples (Larignon and Dubos, 1997, Bruez *et al.*, 2014, 2015), but their roles in host degradation remain unclear. As vines and their necroses age, the basidiomycete *Fomitiporia mediterranea,* which causes white rot ('amadou'), may develop in grapevine wood. Presence of white rot and its progressive development are associated with foliar symptoms of esca, or sudden vine collapse in the summer (Arnaud and Arnaud, 1931; Maher *et al.*, 2012; Ouadi *et al.*, 2019).

In the summer, leaf symptoms of esca, such as gradual discolouration, drying, wilting or leaf fall and collapse, differ between grapevine cultivars. Appearance of orangish longitudinal stripes just under the bark is another typical feature of esca, and is indicative of a probable vascular disorder (Lecomte *et al.*, 2012). This peculiar symptom was first described by Arnaud and Arnaud (1931), but the underlying mechanism remains unknown. Several hypotheses have been proposed, including association of leaf stripe symptoms with sudden xylem disruption in summer, a period generally marked by high temperatures and water deficits (Lecomte *et al.*, 2012), or the presence of vessel occlusions (Pouzoulet *et al.*, 2019).

Eradicative pruning is a classical practice in plant protection (Svihra, 1994; Clark and Matheny, 2010), and removal of infected host branches or wood by cutting has been recommended for the control of many plant diseases or decay (Shigo, 1982). This technique has been used, with various degrees of success, for the management of Dutch elm disease (Gregory and Allison, 1979), oak wilt (Camilli *et al.*, 2007), and played a strategic role in the control of fire blight (Paulin, 1996). In viticulture, trunk renewal is a common method for removing infected vine parts (Sosnowski *et al.*, 2004; Smart, 2015), and involves the re-training of vines from suckers growing at trunk base. Excising cankers with pruning knives is another surgical approach suitable for treating diseases such as anthracnose (Garton *et al.*, 2018) or European canker (Zeller, 1926). In viticulture, the surgical method known as 'curettage' has been used for centuries, as reported by Larignon and Yobregat (2016). This involves removing rotten tissues from trunk wood of diseased vines. Curettage of grapevines has played a key historical role, and was described by Columelle, Pline the Elder, and Palladius in Roman times. Use of curettage was subsequently mentioned by Pierre de Crescens in the Middle Ages, and then by Bidet and Duhamel du Monceau in the 18th Century. It was also an ancient oriental method (Pavlou, 1906; Gaudineau 1959). Curettage was historically performed with metal tools, including pruning or farrier's knives, small hatchets, billhooks or 'gouges', used to remove the necrotic parts of the diseased vines. Given the very long period over which curettage has been used, the practices covered by this term have been highly diverse.

Efficacy of curettage has been little studied, despite its use over many centuries and the likely beneficial effects that such long-term use implies. Some information was provided by Eugène Poussard (Lafon, 1921), a viticulturist who regularly applied the curettage technique (Figure 1) to his vineyards. He reported a high

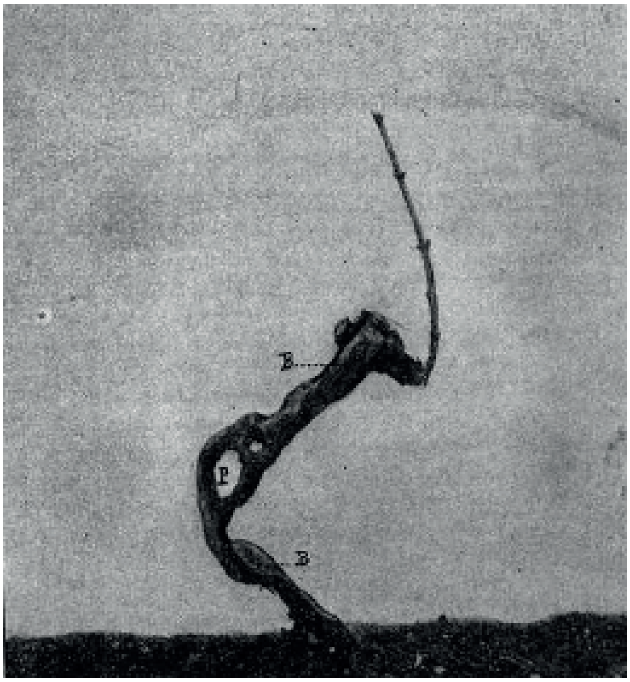

Figure 1. Illustration of a grapevine curetted in 1919, as shown by Lafon (1921).

degree of efficacy because 90 to 95% of the curetted plants were resilient. However, curettage remained a minority practice until its modernization and reintroduction in France at the beginning of the 21st Century (Larignon and Yobregat, 2016). This return has been associated with a combination of factors, including the ban of sodium arsenite, the only curative pesticide previously used to control esca (Bruez *et al*., 2021a), that was forbidden in Europe in the first decade of the 21st century. Development of tools, such as lightweight chain saws, has also opened new possibilities for the use of curettage. Nowadays, necrotic wood is removed with thermal and/or electric saws, and an increasing number of operators are proposing curetting services to vine growers.

Despite the renewed development of curettage, questions about the efficacy of the technique remain unanswered, because very few experiments have been performed to assess curettage efficacy (Mondello *et al*., 2018; Pacetti *et al*., 2021). The present study has provided relevant information about the short and mid-term efficacy of curettage, based on results obtained over 3 to 7 years in three Bordeaux vineyards in which the development of esca-leaf-symptomatic vines was monitored, with or without curettage. The study was undertaken over a long period to ensure that the results were robust. Advantages and limitations of curettage are discussed. Some preliminary results of this study have also been used to assess effects of curettage on vine physiology and berry quality (Cholet *et al*., 2021; Bruez *et al*., 2021b).

MATERIALS AND METHODS

Field study

This study was based on comparisons of esca development in curetted or non-curetted grapevines in three vineyards in the Bordeaux region of France (Table 1). First assessments of the effects of curettage on the grapevines with esca began in 2014 at a commercial and conventional farm in Béguey (Gironde). An initial vineyard of Sauvignon Blanc vines, named 'Cyprès East', was selected for study, based on its high susceptibility to GTDs (Experiment 1). This vineyard had sandy gravel soil, and was planted in 1994 with vines omega-grafted onto 101-14 rootstocks (row width × inter-vine distance = 1.8 × 1 m). The trellising system was typical of the region, with an 'Espalier' Guyot vine form comprising short trunks (60–70 cm) each with two lateral cordons. The vines were trained according to a 'Guyot-double' pruning regime. Experiment 2 was carried out on the same farm and began in 2015, in a second and adjacent vineyard, named 'Cyprès West' with very similar characteristics (Table 1). These two vineyards were located at the bottom of a hill and the vine rows were arranged to follow the topography slope. A third vineyard with the same vine training system was integrated into the study in 2016, at another commercial and conventional farm, Château Couhins at Villenave d'Ornon, close to Bordeaux, on a clay-limestone and soil with flat topography (Experiment 3). There was no known soil-related heterogeneity factor at this location. For all three sites, curettage campaigns were carried out annually and ceased in 2017 or 2018. This gave a total of 11 trials (vineyard × year combinations), and 53 comparisons between treated (curetted) and control vines from 2014 onwards.

Study design

The experimental designs differed between vineyards and years. In 2014, in the 'Cyprès East' vineyard at Béguey (Exp. 1), control and curetted vines were randomly selected in the same part of the vineyard. In 2015, for practical reasons, this vineyard was divided into two equal parts, one for the curetted vines and the other for non-curetted vines (controls). The 'Cyprès west' vineyard (Exp. 2) was also divided in two equal parts. At Villenave d'Ornon (Exp. 3; 'Couhins III-7'), curetted or control vines were randomly selected from vines displaying

Table 1. Main cropping characteristics of the three 'Sauvignon blanc' vineyards used for assessment of 'curettage' effects.

Site Winery	Experiment and *vineyard name*	Rootstock	Date of planting	Trellising system and vine intervals^{a} (m)	No. of rows	Total No. of vines	'Curettage' Year(s)	Rows used for control ( )^{b}	Rows used for curettage ( )^{b}
Béguey Château Reynon	Experiment 1 *Cyprès East*	101-14	1994	Espalier Guyot double 1.8 × 1	25	914	2014	16-25 914 vines	
						2424	2015 to 2018	1-13 (1105 vines)	14-25 (1319 vines)
	Experiment 2 *Cyprès West*	101-14	1996	Espalier Guyot double 1.8 × 1	14	1409	2015 to 2017	1-7 (717 vines)	8-14 (692 vines)
Villenave d'Ornon, Château Couhins	Experiment 3 *Couhins III-7*	Fercal	2000	Espalier Guyot double 1.5 × 1	20	940	2016 and 2017a	1-10 Random selection	
						940	2017b	16-20 (470 vines)	11-15 (470 vines)

^{a} Distances between and within rows (m). ^{b} Total number of vines surveyed.

symptoms, within a ten-row sample, in 2016 and for one trial in 2017 (2017a). For another trial in 2017 (2017b), an additional ten new rows were split into two equal parts, as at Béguey. The rows studied in each vineyard and the number of vines surveyed in each part are indicated in Table 1. All vineyards were managed under conventional sanitary programmes in accordance with IPM guidelines. Esca was the most widely reported disease in these vineyards. Any damage to vine wood was therefore assumed to be mostly due to this trunk disease.

Esca symptom assessment

Esca symptoms on wood and leaves were recorded in late August before harvest: i) to assess the sanitary status of each part of the vineyards, and ii) to select diseased vines with symptoms of similar severity for use in comparisons of the control and curettage treatments. All vines were mapped and symptoms were recorded until 2021, for 2 to 7 years after the first curettage treatment. Symptoms were assessed with severity scales used in previous similar studies (Darrieutort and Lecomte, 2007; Lecomte *et al.*, 2012; Lecomte *et al.*, 2018). Leaf symptoms were assigned to five classes according to severity and position on each vine (Table 2): S1 and S2, corresponding to mild symptoms, limited to the leaves, mostly discolourations, affecting one (S1) or two (S2) cordons, or corresponding to severe symptoms, with many drying zones (S3), wilting (S4) or leaf fall (S5) on one or two cordons. Apoplectic vines (APO) were also observed but were not analyzed in detail in this study. For assessment of the sanitary status of each part of each vine plot, the original vines were grouped into three severity categories: asymptomatic, unproductive (trunk-affected: retrained, restored, replanted, dead, absent or with dead or missing arm, as detailed in Table 2), and leaf-symptomatic vines, as in a previous study comparing trellising systems (Lecomte *et al.*, 2018). For assessments of the recovery from esca of curetted vines, and comparison with the control vines, vines were again assigned to three categories: asymptomatic, symptomatic (on leaves or on wood), or dead vines. Differences between the proportions (%) of all asymptomatic curetted vines observed in each year and those observed the previous year were calculated, and these were used to estimate the annual losses of curettage efficacy.

Curettage

All annual curettage operations were carried out by specialist operators from Simonit & Sirch, France (Table 1). With the exception of one trial at Villenave d'Ornon established in 2017 (Exp. 3, Couhins III-7 vineyard, trial 2017b) with vines identified in summer but curetted in spring 2018, the selected symptomatic vines were curetted in late August, after most esca symptoms had appeared. The objective was a complete decay removal by avoiding weakening the vine structures to support mechanized working. Degraded wood (generally with white rot and dark-brown necroses) was gradually removed with a thermal chainsaw from the tops of trunks or cordons down to the base of the visible necroses. A smaller electric chain saw with a sharpened tip was used to refine the removal of discoloured wood. A small gutter was fitted to direct the flow of rainwater on the base of each open cavity to avoid any waterlog-

Table 2. Esca disease severity indices used to assess the status of each original 'Espalier' grapevine surveyed in this study (from Lecomte *et al.*, 2018).

Code	Meaning
V	Original vine with no damage (leaves and wood)
S	Symptomatic vine with leaf symptoms[a]: S1 = light symptoms (mostly discolorations) on one cordon only S2 = light symptoms (mostly discolorations) on both cordons S3 = severe symptoms (discolorations, drying and some wilting) on one cordon only S4 = severe symptoms (discolorations, drying and some wilting) on both cordons S5 = very severe symptoms of wilting affecting a large number of leaves and grapes on both cordons
APO1 - APO2	Vine showing complete wilting on one or two cordons (apoplexy)
DA[b]	Vine showing portion of dead wood (often a dead cordon)*
U[b]	Vine trained with only one cordon (a dead cordon had been removed)
R[b]	Retrained or restored vine*
D or A[b]	Dead or absent vine
Y[b]	Replanted, regrafted, marcotte, or young vine (any vine planted after the original planting date)

[a] Other or intermediate categories of leaf symptoms were possible, as for example, S1 + S3 for a vine with mild symptoms on one cordon and severe symptoms on the other.
[b] The categories DA, U, R, D or A, and Y refer to all original vines with GTD-affected trunks, and can be grouped into a one category, ("unproductive").

ging. Depending on the robustness of the vine stocks, one or two wooden stakes were positioned at each treatment site to provide support and protection, particularly when the soil was regularly tilled, as at Béguey. Correct curetted vines had as little darkened necrotic areas as possible, each with mostly functional wood exposed to oxidation (Figures 2 and 3).

Statistical analyses

Chi-squared tests were performed on three categories of vines (two degrees of freedom) for all annual comparisons of vine distributions for the assessment of the sanitary status of selected vines prior to treatment or recovery (P = 0.05, 0.01, or 0.001).

Figure 2. Examples of 'Sauvignon Blanc' vines curetted in the Couhins III-7 vineyard.

Figure 3. Illustration of the curettage technique, performed on a grapevine stock with a small chain saw (Photograph Francesco Cecconi).

RESULTS

Sanitary status of vineyards

The numbers of vines examined in each part of each vineyard (control or curettage) ranged from 470 to 1319 (Table 1). In total, 5713 different vine stocks were examined. The proportions of unproductive and esca-symptomatic vines varied from 20 to 66%, depending on the year and the vineyard considered (Suppl. Table 1). These high disease levels facilitated the selection of vines for the experiments. At Béguey, the proportions of unproductive vines were particularly high in the 'Cyprès East' (Exp. 1) and 'Cyprès West' (Exp.2) vineyards, at greater than 50%, about four times the national (French) average (Bruez *et al*., 2013). Annual statistical comparisons of the distributions of percentages of vines in each symptom category showed that the parts of the vineyards used for control and curettage treatments had similar levels of disease development (Suppl. Table 1).

The esca disease status of the vines randomly selected each year for control or curettage treatments in each part of the vineyards is shown in Table 3. The number of vines in the sample displaying very severe symptoms was generally small, except in Exp. 3 for two treatments (curettage sample in the 2016 trial, and control sample in the 2017a trial). Annual statistical comparisons of the distributions of the numbers of vines in each symptom severity category between the control and curettage treatments showed no statistically significant differences ($P > 0.05$) in all the trials except one (Exp. 1, 2015 trial).

Vine recovery

Esca development, recorded each year for the control and curettage treatments, is summarized in Table 4. In total, 422 vines were curetted and examined from 2014 onwards. They were compared to 434 vines used as experimental controls. One year after symptom expression and treatment (y + 1), most of the vines curetted in all 11 trials (358 out of 422), regardless of vineyard site, displayed general decreases in esca expression after curettage treatment relative to the controls. Percentages of curetted vines that became asymptomatic in the year after treatment ranged from 73 to 96% for vines treated just after the appearance of leaf symptoms (ten trials). The exception was in the 2017b trial in the 'Couhins III-7' vineyard (Exp. 3), where only 64% of the vines that were symptomatic in summer 2017 but treated in April 2018 remained symptomatic. For each annual distribution of curetted vines in three severity categories, the category with the greatest number of vines was asymptomatic, for all years of observations except two, for the seventh year after treatment in the oldest trial (Exp. 1, 2014 trial at 'Cyprès East') and for the fourth year after treatment in the 2017b trial in the Couhins III-7 vineyard (Exp. 3). This indicates a decrease in efficacy of curettage over time. Conversely, for control vines, the severity category with the largest number of vines generally corresponded to symptomatic or dead vines. One year after treatment, only 33% of control vines (144 out of 434) were asymptomatic. From 2014 to 2021, the annual records provided 53 comparisons of distributions between control and curettage treatments. Significant differences were observed in all comparisons: 42 of the 53 comparisons revealed highly significant differences at $P = 0.001$, and six of comparisons were significant at $P = 0.01$. The mean annual loss of efficacy of the curettage treatment was almost 7.6%, indicating a gradual decrease in health recovery over time.

Figure 4 summarizes the data for Béguey only (Experiments 1 and 2), a site at which there was a 6-7 year history of treatment. This provides a graphical representation of the fate of vines after curettage treatment, using proportions calculated for the three symptom categories. Despite the variability between years, these data clearly indicate that more than 70% curetted vines did not develop esca symptoms for at least 3 years after curettage. In contrast, despite the well-known variability of esca leaf symptoms between years (Marchi *et al*., 2006), most of the control vines continued to display

Table 3. Esca status of the control and curetted vines used in this study (11 trials). Distributions of vines between the three leaf symptom categories indicated in Table 2. The distributions were compared by pair-wise Chi-square tests ($P = 0.05$).

Experiment *Vineyard*	Year	Treatment	No. of vines	No. of vines per category			Chi-squared test result
				Mild symptoms S1 + S2	Severe symptoms S3 + S4	Very severe symptoms S5 + APO	
Experiment 1 *Cyprès East*	2014	*Control*[a]	*52*	*n. a.*	*n. a.*	*n. a.*	-
		Curettage	79	46	29	4	
	2015	*Control*	*26*	*9*	*16*	*1*	S
		Curettage	28	20	7	1	
	2016	*Control*	*32*	*20*	*10*	*2*	NS
		Curettage	29	25	3	1	
	2017	*Control*	*51*	*26*	*22*	*3*	NS
		Curettage	*36*	12	21	3	
	2018	*Control*	*21*	*10*	*11*	*0*	NS
		Curettage	19	10	8	1	
Experiment 2 *Cyprès West*	2015	*Control**	*16*	*n. a.*	*n. a.*	*n. a.*	-
		Curettage*	25	n. a.	n. a.	n. a.	
	2016	*Control*	*35*	*17*	*13*	*5*	NS
		Curettage	26	13	11	2	
	2017	*Control*	*33*	*11*	*16*	*6*	NS
		Curettage	25	10	14	1	
Experiment 3 *Couhins III-7*	2016	*Control*	*45*	*4*	*35*	*6*	NS
		Curettage	37	4	23	10	
	2017a	*Control*	*93*	*34*	*42*	*17*	NS
		Curettage	88	25	55	8	
	2017b	*Control*	*34*	*0*	*34*	*0*	NS
		Curettage in 2018	30	0	30	0	

[a] In this treatment, symptom severity on the selected symptomatic vines used was not assessed (n.a.).
Control plots are indicated in italics.

esca or died. The recovery of vines treated from 2014 onwards in the three vineyards is further highlighted in Figure 5. The total percentage of curetted vines (all trials combined) that became asymptomatic the year after curettage treatment was nearly 85%. The percentage of asymptomatic vines subsequently decreased to 39%, 7 years after treatment, for vines that were curetted in 2014. In contrast, for vines used as controls in 2014, the proportion that were symptomatic or died remained high: 60% the year after treatment and 96% 7 years later.

DISCUSSION

Curettage has a long history and was used to control GTDs a century ago, as reported by Lafon (1921). Use of this disease management strategy subsequently declined, but interest in the technique has increased in France over the last 20 years. Viticulturists and extension workers from the Loire Valley, around Sancerre (Thibault, 2015), Alsace and the South-West of France have promoted the use of curettage. Over the same period, this curative treatment has also been used in a number of other European countries, including Italy, Spain, Portugal, Germany, Croatia and Hungary (AA.VV., 2017; Mondello *et al.*, 2018). Since 2011, under the impetus of the late Professor Denis Dubourdieu, this technique has been progressively employed in many vineyards in the Bordeaux region. However, no research data were available on the duration of curettage efficacy and on the limitations of this rehabilitated technique. One recent publication (Cholet *et al.*,

Table 4. Fates of curetted and control vines (in italics), by year, after surgical treatment, in three experiments. The bold figures in gray cells indicate the greatest numbers (or percentages) of vines in each annual distribution. All distributions of numbers of vines per symptom category were significantly different using Chi-squared tests: P values are indicated as superscripts above the numbers of asymptomatic vines for each annual distribution of curetted vines: $P = 0.05$ (*), $P = 0.01$ (**) and $P = 0.001$ (***). Percentages values were rounded.

Experiment *Vineyard*	Year	Treatment	No. of vines	Esca health status by year after treatment; No. and % of vines per category[a]																				
				y + 1			y + 2			y + 3			y + 4			y + 5			y + 6			y + 7		
				As	S	D	As	S	D	As	S	D	As	S	D	As	S	D	As	S	D	As	S	D
Experiment 1 *Cyprès East*	2014	Curettage	79	61*** 77%	16 20%	2 3%	59*** 75%	18 23%	2 2%	50*** 63%	22 28%	7 9%	46*** 58%	26 33%	7 9%	41*** 52%	28 35%	10 13%	38*** 48%	30 38%	11 14%	31*** 39%	33 42%	15 19%
		Control	*52*	*21 40%*	*28 54%*	*3 6%*	*22 42%*	*25 48%*	*5 10%*	*10 19%*	*32 62%*	*10 19%*	*8 15%*	*24 46%*	*20 39%*	*9 17%*	*18 35%*	*25 48%*	*5 9%*	*17 33%*	*30 58%*	*2 4%*	*14 27%*	*36 69%*
	2015	Curettage	28	27*** 96%	1 4%	0	25*** 89%	1 4%	2 7%	20*** 72%	4 14%	4 14%	21*** 75%	2 7%	5 18%	19*** 68%	4 14%	5 18%	15*** 54%	7 25%	6 21%			
		Control	*26*	*3 12%*	*23 88%*	*0*	*3 12%*	*22 85%*	*1 4%*	*5 19%*	*14 54%*	*7 27%*	*5 19%*	*13 50%*	*8 31%*	*2 8%*	*10 38%*	*14 54%*	*1 4%*	*7 27%*	*18 69%*			
	2016	Curettage	29	21*** 73%	3 10%	5 17%	23*** 79%	1 4%	5 17%	22*** 76%	2 7%	5 17%	20*** 69%	4 14%	5 17%	18*** 62%	5 17%	6 21%						
		Control	*32*	*11 34%*	*21 66%*	*0*	*10 31%*	*20 63%*	*2 6%*	*11 34%*	*17 53%*	*4 13%*	*8 25%*	*15 47%*	*9 28%*	*4 12%*	*15 47%*	*13 41%*						
	2017	Curettage	36	29*** 81%	4 11%	3 8%	33*** 92%	0	3 8%	30*** 84%	3 8%	3 8%	25*** 70%	8 22%	3 8%									
		Control	*51*	*20 39%*	*28 55%*	*3 6%*	*15 29%*	*30 59%*	*6 12%*	*14 27%*	*25 49%*	*12 24%*	*4 8%*	*34 67%*	*13 25%*									
	2018	Curettage	19	16*** 84%	2 11%	1 5%	13** 69%	5 26%	1 5%	10* 53%	8 42%	1 5%												
		Control	*21*	*3 14%*	*18 86%*	*0*	*4 19%*	*16 76%*	*1 5%*	*3 14%*	*15 72%*	*3 14%*												
Experiment 2 *Cyprès West*	2015	Curettage	25	24** 96%	1 4%	0	23*** 92%	2 8%	0	21*** 84%	3 12%	1 4%	20** 80%	4 16%	1 4%	19** 76%	3 12%	3 12%	18* 72%	4 16%	3 12%			
		Control	*16*	*8 50%*	*7 44%*	*1 6%*	*4 25%*	*11 69%*	*1 6%*	*4 25%*	*10 62%*	*2 13%*	*4 25%*	*10 62%*	*2 13%*	*5 31%*	*6 38%*	*5 31%*	*5 31%*	*3 19%*	*8 50%*			
	2016	Curettage	26	23*** 88%	1 4%	2 8%	22*** 84%	2 8%	2 8%	22*** 84%	2 8%	2 8%	18*** 70%	4 15%	4 15%	17*** 66%	5 19%	4 15%						
		Control	*35*	*11 31%*	*23 66%*	*1 3%*	*7 20%*	*26 74%*	*2 6%*	*8 23%*	*21 60%*	*6 17%*	*2 6%*	*18 51%*	*15 43%*	*2 6%*	*13 37%*	*20 57%*						
	2017	Curettage	25	21*** 84%	1 4%	3 12%	20*** 80%	2 8%	3 12%	14*** 56%	6 24%	5 20%	15*** 60%	4 16%	6 24%									
		Control	*33*	*9 27%*	*23 70%*	*1 3%*	*5 15%*	*23 70%*	*5 15%*	*3 9%*	*18 55%*	*12 36%*	*2 6%*	*17 52%*	*14 42%*									

(Continued)

Table 4. (Continued).

Experiment *Vineyard*	Year	Treatment	No. of vines	Esca health status by year after treatment; No. and % of vines per category[a]																				
				y + 1			y + 2			y + 3			y + 4			y + 5			y + 6			y + 7		
				As	S	D	As	S	D	As	S	D	As	S	D	As	S	D	As	S	D	As	S	D
Experiment 3 *Couhins* *III-7*	2016	Curettage	37	**34***** **92%**	3 8%	0	**33***** **89%**	3 8%	1 3%	**29***** **79%**	6 16%	2 5%	**26***** **70%**	8 22%	3 8%	**24***** **65%**	9 24%	4 11%						
		Control	*45*	*16* *36%*	***27*** ***60%***	*2* *4%*	*13* *29%*	***24*** ***53%***	*8* *18%*	*8* *18%*	***23*** ***51%***	*14* *31%*	*5* *11%*	***24*** ***53%***	*16* *36%*	*2* *4%*	*17* *38%*	***26*** ***58%***						
	2017a	Curettage	88	**83***** **94%**	2 2%	3 4%	**75***** **85%**	9 10%	4 5%	**62***** **70%**	22 25%	4 5%	**52***** **59%**	28 32%	8 9%									
		Control	*93*	*35* *38%*	***40*** ***43%***	*18* *19%*	*23* *25%*	***42*** ***45%***	*28* *30%*	*18* *14%*	***45*** ***54%***	*30* *32%*	*1* *1%*	***46*** ***49.5%***	***46*** ***49.5%***									
	2017b	Curettage	30	**19**** **64%**	10 33%	1 3%	**14*** **47%**	12 40%	4 13%	**14*** **47%**	10 33%	6 20%	8* 27%	**13** **43%**	9 30%									
		Control	*30*	*7* *23%*	***22*** ***74%***	*1* *3%*	*7* *23%*	***22*** ***74%***	*1* *3%*	*8* *27%*	***20*** ***67%***	*2* *6%*	*1* *3%*	***17*** ***57%***	*12* *40%*									
Total		Curettage	*422*	**358** **85%**	44 10%	20 5%	**340** **81%**	55 13%	27 6%	**296** **70%**	87 21%	39 9%	**254** **62%**	99 25%	50 13%	**139** **62%**	53 24%	32 14%	**73** **54%**	39 31%	20 15%	31 39%	**33** **42%**	15 19%
		Control	*434*	*144* *33%*	***260*** ***60%***	*30* *7%*	*113* *26%*	***261*** ***60%***	*60* *14%*	*87* *20%*	***245*** ***56%***	*102* *24%*	*40* *10%*	***218*** ***53%***	*155* *37%*	*24* *12%*	*79* *38%*	***103*** ***50%***	*11* *12%*	*27* *29%*	***56*** ***59%***	*2* *4%*	*14* *27%*	***36*** ***69%***

[a] Vines were classified into three categories: As = Asymptomatic; S = Symptomatic (foliar and wood symptoms); D = Dead.

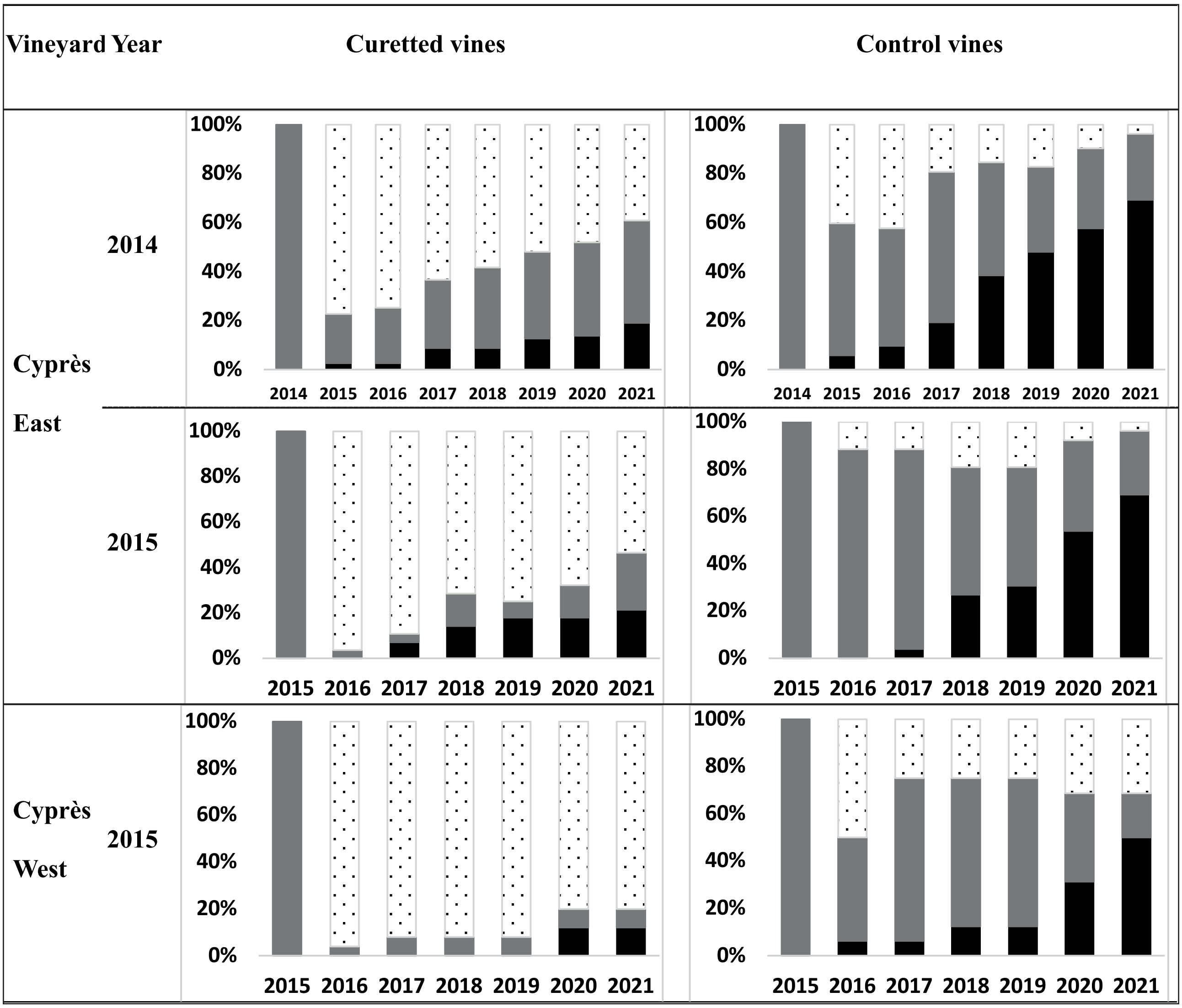

Figure 4. Percent of curetted and control vines, by year, for up to 6 and 7 years after the curettage treatment. The vines concerned were those curetted in 2014 and 2015 at Cyprès East (Exp. 1) and Cyprès West (Exp. 2). The X-axis relates to the rating years and the Y-axis relates to the % in each symptom category: asymptomatic vines are shown in white with black dots, esca-symptomatic are shown in dark gray and dead vines are shown in black.

2021) on data from the present study (Exp.1, in 2014 and 2016) focused on vine vigour, soil fertility, berry quality, and year-to-year plant recovery over a 4-year period. The study presented here is the first report on the short and mid-term recovery of curetted vines with symptomatic esca, and is based on 11 trials.

The annual esca development comparisons between curetted and non-curetted vines began in 2014 in three local vineyards. However, the study was initiated in 2012 in the vineyard 'Cyprès East' at Béguey, in which two preliminary trials were carried out in 2012 and 2013, to establish the curettage technique (results not shown). In 2012, 35 esca leaf-symptomatic vines were curetted but were not compared with control vines. Most of the curetted vines had severe esca leaf symptoms (none of the vines were classified S1 + S2), and were either dead or displaying trunk damage or were symptomatic in the year after treatment. The failure of the treatment in this trial was explained by overthinning of the trunks, lack of support stakes, and high severity of leaf symptoms. Another possible explanation was that, among the vines that were curetted at the start of the curettage campaigns, most would exhibit foliar symptoms with long esca histories. As experiments progress, treated vines

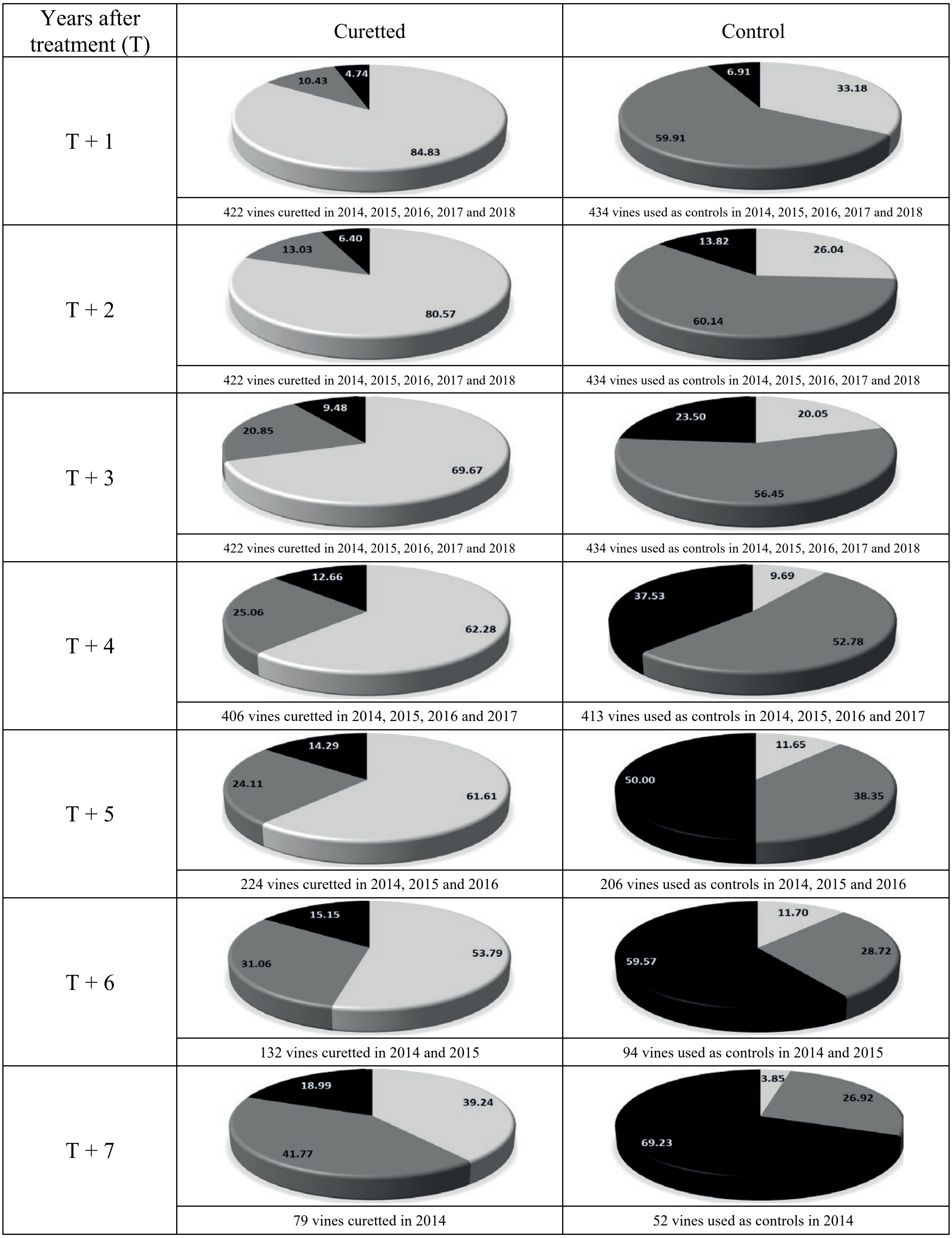

Figure 5. Health status, with respect to esca, for all vines that were curetted or used as experimental controls since 2014 (all experiments combined), 1 to 7 years after curettage treatment (T). Percentages of asymptomatic vines are shown in light gray, those of symptomatic vines are in dark gray, and those of dead vines are shown in black.

could previously have not, or infrequently, expressed symptoms. These explanations are consistent with the observations of Thibault (2015) in the Loire Valley and the outcomes of the "Winetwork" project (AA. VV., 2017). In 2013, a second preliminary trial without experimental controls, carried out on 62 curetted vines with 52 vines showing mild (26 vines) or moderately severe symptoms (26), 81% recovered the year after treatment. After these trials, it was decided to apply curettage primarily on vines with mild or moderately severe symptoms and to protect them with small wooden posts. However, further experiments are required, such as the 2017b trial at Couhins on vines showing uniform disease levels, to establish precise limits of the technique.

The experimental designs of the present study were dependent on curettage operators requiring simple, non-time-consuming procedures with easy-to-use equipment, so it was not possible to set up experiments with blocks within each vineyard. For eight of the trials, vineyards were split into two areas, one for the curettage treatment, and the other as a control. This experimental design was possible due to the severe GTD damage and its relative homogeneity at the sites. The high levels of GTD affected vines facilitated identification of symptomatic vines for the experiments in each year. For three trials, at Béguey in 2014 (Exp. 1) and at Villenave d'Ornon (Exp. 3) in 2016 and in 2017 for the 2017a trial, vines were selected at random within the same rows. Clear, highly consistent trends emerged from the results obtained from both study designs.

From 2014 onwards, curettage led to immediate recovery in symptomatic vines the year after treatment. All annual comparisons showed a general and positive effect of curettage on esca control. Conversely, most of the control vines steadily declined and eventually died. The high mean percent efficacy the year immediately after curettage (close to 85%) was consistent with other reports (Lafon, 1921; Thibault, 2015; De Montaignac, 2019; Pacetti *et al*., 2021). The gradual loss of efficacy that was subsequently observed indicates that curettage can delay vine decline due to esca, but does not completely control the disease. Additional long-term experiments focusing on the durability of vine recovery induced by curettage would be useful, to determine the value this technique over total vineyard lifespans.

Based on the present study, several hypotheses can be formulated to explain curettage-induced recovery of vines with esca symptoms. A high proportion of curetted vines displayed no esca symptoms in the years following treatments. The mechanism(s) involved cannot only be related to the well-known year-to-year variability of esca leaf symptoms (Surico *et al*., 2000; Marchi *et al*., 2006). Mondello *et al.* (2018) showed that suppression of GTD foliar symptoms may reflect temporary increases in oxygenation, the action of elicitors inducing host resistance, or possible interactions with saprobes. However, these studies did not focus on the effect of the removal of dead wood and rotten tissues, including white rot in particular. The hypotheses were based on a study of Calzarano and Di Marco (2007), where no correlation was found between the volume of white rot or wood discolouration in vine trunks and foliar symptoms in esca-affected vines. However, other studies have reported that the volume of degraded wood may be a key factor, at least partly explaining grapevine decline and the expression of esca leaf symptoms (Lecomte *et al*., 2008 a; b; Liminana *et al*., 2009; Maher *et al*., 2012; Travadon *et al*. 2016; Lecomte *et al*., 2018; Bénétreau *et al*., 2019). By removing white rot, curettage eliminates much of the tissue colonized by pathogenic basidiomycetes, including *Fomitiporia mediterranea* in particular, a fungus already reported to play a major role in esca (Maher *et al*., 2012; Bruez *et al*., 2017). Ouadi *et al*. (2019) also suggested that a threshold of at least 10% white rot was potentially a is a potentially good descriptor of the chronic form of esca, and Pacetti *et al.* (2021) observed a significant decrease in the abundance of *Fomitiporia mediterranea* after trunk surgery. Two reviews (Del Frari *et al*., 2021; Moretti *et al*., 2021) have also underlined the key role of *Fomitiporia mediterranea* in esca leaf expression. Curettage surgically removes white rot and part of dead wood, thereby eliminating the GTD pathogens present in damaged wood, mostly caused by *Fomitiporia mediterranea*. This probably leads to decreasen the inoculum pressure of this pathogen, and improves the balance between functional and non-functional wood structures. This physiological and pathological context may explain the remission of leaf symptoms, which may also be related to decreased water or nutrient demands by the remaining pathogens.

Curettage is a technique that should be used as soon as the first esca symptoms are expressed, or on asymptomatic vines before disease expression, such as those with external dead wood 10 years after planting. In practical terms for each vine, curettage first involves identifying a large wound or a necrotic sectoral wood zone as a starting point for opening up the trunk or cordons. The operator then removes the damaged wood, while avoiding interference with functional wood. At the start of the 20th Century, the removal of all dead wood was recommended (Ravaz, 1909; Lafon, 1921), but some operators now consider that the principal objective of curettage is specific removal of white-rot tissues (often in trunk centres), rather than removal of all dead wood.

This procedure is more rapid, is just as effective and does not greatly weaken the stocks (Thibault, 2015). In practice, it is not possible to remove all the dead wood. Curettage may leave some hard wood in place, and cannot eliminate vascular pathogens, such as *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*, which are also involved in the esca complex. These two fungi, and many others, are also often present in functional wood (Bruez *et al*., 2014), and possibly cause the foliar symptoms through the action of metabolites (Andolfi *et al*., 2011), although this view remains controversial. Despite the presence of these fungi and their putative toxic activity, most curetted vines display no leaf symptoms of esca for several years after treatment. This observation is consistent with *Fomitiporia mediterranea* being involved in the mechanism of foliar symptom expression, and lends weight to the theory that toxins (from *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*) are probably not the only elements involved in the development of esca leaf symptoms (Moretti *et al*., 2021).

Esca symptoms are also observed only in the summer, when temperatures are high and water constraints can be strong (Lecomte *et al*., 2012). Beside leaf symptoms, longitudinal stripes affecting host vessels just below the bark have also been observed. These stripes were associated with sudden disruptions of sap routes during a period in which competition for water can be strong, particularly if there are large volumes of necroses and an imbalance between the amounts of functional and non-functional wood (Maher *et al*., 2012). Removal of the white rot helps to decrease inoculum pressure and competition for water and nutrients. This hypothesis is consistent with the general rationale of curative control. Curettage may have other consequences, such as improvements in vine capacity to compartmentalize necroses, or reductions in host energy required for defenses. By opening trunks and removing the tender and spongy tissues characteristic of white rot, curettage may also decrease the amount of water available to mycelia of esca pathogens, particularly of *Fomitiporia medirerranea*, thereby decreasing colonization of functional wood during rainy periods. Curettage should be accompanied by insertion of gutter in the lower parts of curetted wood, to facilitate water flow.

Decreases in inoculum pressure and recovery plant health are not the only benefits of curettage. Cholet *et al*. (2021) showed that curetted grapevines rapidly regained growth capacity similar to that of asymptomatic vines. By extending vine lifespan, this technique enables them to continue to produce high quality grapes, which is essential for wine production. However, previous studies on curettage have shown that this technique is not suitable for all vineyards. The most suitable vine training systems for this technique are those in which the vines have large trunks, such as the 'Espalier Guyot' forms. Other limitations are the costs which have not been economically analysed. Growers may be reluctant to adopt curettage because the increases in lifespan and net returns afforded by this technique have yet to be quantified. The equipment required is not expensive, but the technique is time-consuming and highly dependent on operator skills. Curettage takes at least 10-15 min per vine, with estimated cost of between 18 and 35 euro per h, depending on the operator. Depending on region, the time spent by the operators and the type of operator (company employee or external service provider), the additional cost of curettage ranges from 2.5 to 15 euro per vine (Thibault, 2015; De Montaignac, 2019). However, as curettage results in vine recovery for several years, the net returns are increased before vines need to be uprooted. There are also time lags between the cost and the benefits of curettage procedures. Annual costs must therefore be considered in light of long-term outcomes. Based on this 7-year study, simulations may be able to show whether no action results in significant economic losses, or whether a given investment in curettage is profitable, as was reported by Kaplan *et al.* (2016) for preventative techniques.

The rehabilitation of curettage extends the array of methods available for esca control. Curettage is particularly suitable for grapevines with moderate esca symptoms or for vines with external dead wood. Eugène Poussard recommended performing curettage as soon as leaf symptoms appear, and Lafon (1921) performed this operation in winter. The reduced efficacy of the technique recorded in the present study for Experiment 3 trial 2017b, with vines curetted later than the others, also indicates early implementation of this control strategy. In cases of moderate symptoms not affecting the grapes, curettage may also save part of the harvest. For vines with very severe symptoms, other alternatives must be used, such as trunk renewal (Smart, 2015), trunk renewal plus curettage (cutting off the cordons), or re-grafting (Dal *et al*., 2013). A feature of this mutilating technique is the absence of protection after exposure of the functional wood to the open air. The opened trunks, with their large wounds, would be expected to facilitate new infections and/or development of latent pathogens, which would limit vine longevity. In the past, Ravaz (1909) advised application of tar products on wounded tissues, and Eugène Poussard painted curetted vines with a solution of copper salts (Lafon, 1921). Protection products currently are not applied to the curetted vine tissues, and further research is required to determine if application of these products increases the lifespan of the curetted vines.

In the present study, curettage reduced vine mortality due to GTDs. This practice is a useful GTD control strategy, for vineyards with low training systems, such as 'Espalier Guyot' forms (Pacetti *et al*., 2021), and for vineyards producing wines with high added value or with high expected longevity. Increased understanding of the mechanisms of esca symptom expression will help explain symptom suppression after curettage. This is the first report on the efficacy of this technique based on a mid-term study over 7 years.

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