OENO One vine & wine

DOI: https://doi.org/10.20870/oeno-one.2026.60.1.9459

OPEN ACCESS

VITICULTURE ORIGINAL RESEARCH ARTICLES

Evaluation of the efficacy of curettage and over-grafting in the control of esca disease complex

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Article number: 9459

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Associate editor: Florence Fontaine

Received: 17 June 2025

Accepted: 16 December 2025

Published: 22 January 2026

ABSTRACT

The domestication and cultivation of the vine required pruning techniques that expose the vines to trunk pathogens, thereby facilitating the spread of Grapevine Trunk Diseases (GTDs). Among these, the Esca Disease Complex (EDC), primarily caused by *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*, and *Fomitiporia mediterranea*, brings significant challenges to European viticulture. This study examines the effects of curettage and over-grafting as potential methods for containing the expression of the disease in three vineyards located in Tuscany and Veneto (Italy). For several years, symptomatic vines were treated with curettage (removal of necrotic wood) or over-grafting, and the manifestation of symptoms, recurrence, and mortality rates were compared with those of untreated controls. The results revealed that both techniques reduced the incidence of foliar symptoms and mortality from Grapevine Leaf Stripe Disease (GLSD), one of the diseases in the EDC, which was previously simply referred to as "esca". In the long term, treated vines had a significantly lower probability of resuming symptoms or dying compared to the untreated ones. The effectiveness of over-grafting varied depending on the health status of the rootstock. Overall, both methods offer promising, yet context-dependent, strategies for managing GTDs, highlighting the importance of integrated and site-specific approaches in vineyard disease control.

KEYWORDS: GTDs, esca disease, curettage, over-grafting

INTRODUCTION

The domestication of the grapevine for cultivation purposes forced the growers to contain the vine size, adopting pruning techniques. Unfortunately, pruning cuts create multiple wounds on the old wood, becoming the main entry ports for trunk pathogens (Eskalen *et al.*, 2007). Throughout history, the pruning techniques evolved, and in certain viticultural regions, more conservative pruning techniques applied on specific training systems permitted to minimise the progression of trunk diseases (Dal, 2013).

Nowadays, the management of grapevine trunk diseases (GTDs) is considered one of the most relevant challenges for viticulture; currently, GTDs cause serious concern among winegrowers as they have been a growing problem in all wine-producing regions over the last three decades (Fontaine *et al.*, 2016; Mondello *et al.*, 2018a; Bruez *et al.*, 2021).

Around the world, many different fungus species and families have been identified as responsible for GTDs, but in Europe, the most common is represented by esca disease complex (EDC) (Guerin-Dubrana *et al.*, 2019). Within EDC, *Phaeomoniella chlamydospora (Pch)* and *Phaeoacremonium minimum (Pmin)* are considered the main pathogens associated with the vascular infection, while basidiomycete species cause white rot (esca proper), the most common in Europe being *Fomitiporia mediterranea* (Bertsch *et al.*, 2013). Other species of *Phaeoacremonium* may also be involved in the aetiology of the EDC (Gramaje *et al.*, 2018).

Symptoms associated with *Pch* and *Pmin* may occur only internally, as in brown wood streaks, or both internally and externally, as in Petri disease and grapevine leaf stripe disease (both diseases of the EDC). Specifically, black streaks represent infected xylem vessels. Foliar symptoms appear after the development of internal symptoms; nevertheless, a poor correlation has been found between the degree of wood discolouration or necrosis and the severity of foliar stripes (Calzarano & Di Marco, 2007). Moreover, leaf symptoms do not develop every year in the same vine, indicating that several environmental factors are likely involved in their development (Surico *et al.*, 2008; Maher *et al.*, 2012). Researchers have never described specific symptoms in the roots (Surico *et al.*, 2006).

In particular conditions, another symptom that can often be observed is “apoplexy”, which is characterised by the wilting of one or more shoots or the entire vine. The evolution of the symptoms happens suddenly in the middle of summer. A period with low temperatures and abundant rains followed by heat and drought can easily lead to plant apoplexy (Mugnai *et al.*, 1999; Bigot *et al.*, 2020). After such an event, the affected vines almost die; however, in rare cases, they may grow back the following year without symptoms, although their growth is weak and they do not produce fruit. In the past, because of the association with “esca”, apoplexy was always considered a severe form of the disease (Surico, 2009).

Why do grapevines suffer from GTDs? We should find an answer in the vascular system of the trunks, which presents an architecture that pathogens can easily colonise. Indeed, wide-lumen vessels provide highways for wood-colonising pathogens. In addition, cell walls present scalariform punctuation, allowing the development of pathogenic hyphae from vessels to adjacent fibres, where they can thereafter proliferate (Pouzoulet *et al.*, 2014; Pouzoulet *et al.*, 2017). Shigo and Marx (1977) examined the strategies adopted by woody plants to cope with trunk diseases; starting with the study of wood anatomy, the authors described the structural barriers developed by the plants to limit the spread of microorganisms. The theory proposed by the two researchers was based on the creation of four different typologies of walls, with increasing strength and efficacy, which could counteract the spread of pathogens through the xylem vessels. Two of the walls involve phenols and terpenes, which are generally expressed after host-pathogen interactions, when the transcriptional and biosynthetic machinery of the phenylpropanoid pathway is induced (Pierron *et al.*, 2016). The above-mentioned theory, known as the CODIT model (Shigo & Marx, 1977), contributed to understanding how the pruning techniques could be improved in order to reduce the development of GTDs in the vineyard. Among all the pruning systems, Guyot–Poussard represents the first attempt to adapt the CODIT model in the vineyard (Dal, 2013).

The possibility of adopting surgical techniques such as curettage to remove decayed wood from the grapevine tissues has been re-evaluated as a promising technique to recover plants affected by EDC, as described by Lafon (1921) and later by Bruez *et al.* (2021), Lecomte *et al.* (2018), and Pacetti *et al.* (2021). According to Poussard, who implemented this technique in the late 1800s, wood cleaning resulted in a reduction of up to 90–95 % of the foliar symptoms resumption when applied to remove white rot. Technically, “curettage” consists of the removal of white rot and part of the deadwood to ensure a complete elimination of the tissues decayed by *F. mediterranea*. Although several authors have actually demonstrated the effectiveness of this technique, contributions about medium- to long-term efficacy are still few (Lecomte *et al.*, 2018; Lecomte *et al.*, 2022).

Studies carried out in Australia have shown that the elimination of unhealthy trunk down to 20 to 30 cm above the ground increases the probability of eradicating dieback and canker agents like *Eutypa lata* from the vine (Sosnowski *et al.*, 2011). Trunks can be regenerated starting from shoots originating from latent buds, allowing the vines to yield back to full production within a few years and exploiting the pre-existing root system. This practice, trunk renewal, has also been successfully applied to EDC-affected vines, called *“recépage”* in France, or *“rinnovo del tronco”* in Italy (Egger *et al.*, 1998). Similarly, vines can be over-grafted at the rootstock level to take advantage of the pre-existing root system that has already established and colonised the soil environment (Dewasme *et al.*, 2023).

This study aims to evaluate the effectiveness of curettage and over-grafting in controlling foliar symptoms of EDC under field conditions.

MATERIALS AND METHODS

1. Vineyard sites, experimental design, and treatments compared

Three vineyards were selected (Table 1): two in Tuscany within the PDO (Protected Designation of Origin) area of Bolgheri and one in Veneto in the PDO area "Valpolicella". In Tuscany, the first vineyard (BO1) extends over a surface of 4.5 ha and was planted in 2001 with Cabernet-Sauvignon (*Vitis vinifera* L.) grafted on RSB1, with a density of 6,650 plants/ha (2.20 m × 0.60 m between rows and between vines, respectively). The second vineyard (BO2) covers an area of 6.8 ha and was planted in 2002 with the same variety grafted half on 101.14 and half on 110R, with a plant density of 8,333 plants/ha (2.00 m × 0.60 m between rows and between vines, respectively). Both trails in Tuscany started in 2019, but they ended at different times: BO1 in 2024 and BO2 in 2022. In the Veneto region, the vineyard selected was planted in 2001 with the variety Corvina (*V. vinifera* L.) grafted on 41B, with a plant density of 9,615 plants/ha (1.60 m × 0.60 m between rows and between vines, respectively) and adopting a single Guyot training system (DF1). The vineyard covers a surface of 2.14 ha. The experiment started in 2018 and ended in 2023.

For each site, a completely randomised block design was arranged, with three treatments and four plots of 140–180 vines for each treatment (Figure 1). The first treatment in comparison was curettage, where the vines were subjected to surgery to remove the decayed portion of the wood using a small blade electric chainsaw. This operation was applied by expert operators, who were able to remove precisely both the white rot and the dead wood inside the vertical trunk. Curettage was applied at the end of the winter, between March and the beginning of April, on the vines showing GLSD symptoms in the previous year. The second treatment in comparison was the over-grafting on the rootstock; in this treatment, the vines were cut below the grafting point, taking care to completely remove the white rot by lowering the cutting level. The over-grafting was performed thereafter with a "single slit" technique, inserting two scions inside the slit. To compare the efficacy of the treatments applied, an untreated control was maintained, where the vines were followed for their health fate during the years, monitoring the occurrence of GLSD. The data gathering needed the deployment of a GIS (Geographic Information System), where each vine was identified with a GPS (Global Positioning System) position to monitor symptoms during the years of observation. During the time course of the experiments, a number of parameters have been monitored, including the occurrence of GLSD symptoms over time, the dead vines and also the newly planted vines (Table S1).

Field visits for data collection took place annually at two different times: during the winter, when the treatments in the vineyard were performed, and in the summer, between August and September, when a visit was carried out to ascertain symptoms and to mark the vines for the application of the treatments in the following winter. At both times, data were inserted into the database to track the operations and the monitoring. The assessment of symptoms was conducted through visual inspection. The GLSD category included vines exhibiting leaf tiger stripe, and if more than 50 % of the leaves were affected, the GLSD class was classified as severe. Vines exhibiting apoplexy were identified by the presence of one or more wilted shoots. In the present article, all the symptomatic classes are included in a single one, named "Symptomatic".

2. Statistical analysis

To assess the effectiveness of the two techniques as vineyard management strategies for containing the occurrence of GLSD, we evaluated for each treatment the incidence of symptomatic vines, *i.e.*, the percentage of plants that exhibited symptoms in each season, and dead vines, *i.e.*, the percentage of plants that died.

The angular arcsine transformation of the data was applied prior to ANOVA. This approach addresses potential violations of the fundamental assumptions associated with linear models, such as normality, homoscedasticity, and non-additivity of the model (Ahrens *et al.*, 1990). One-way ANOVA was applied year by year, and when the test was significant, a post-hoc Tukey HSD test was applied to separate the averages ($p < 0.05$).

To examine the effectiveness of the application on the single plan in the long-term period until five years, the treated and untreated vines were monitored throughout the experiment. The status of each vine was yearly classified as asymptomatic, symptomatic, or dead. The chi-square tests were applied to these three categories for the treatment in comparison with each year of the experiment. We compared curettage and over-grafting separately with the untreated; this way, the statistical test has two degrees of freedom (p-value < 0.05, 0.01, or 0.001).

TABLE 1. Location and characteristics of the experimental site.

Vineyard	Rootstock	Variety	Year of planting	Years of healing	Number of treatments	Number of plots	Number of vines for plot	Total vines
BO1	RSB1	Cabernet-Sauvignon	2001	2019–2024	3	4	160	1,920
BO2	101.14 and 110R	Cabernet-Sauvignon	2002	2019–2022	3	4	180	2,160
DF1	41B	Corvina	2001	2018–2023	3	4	140	1,680

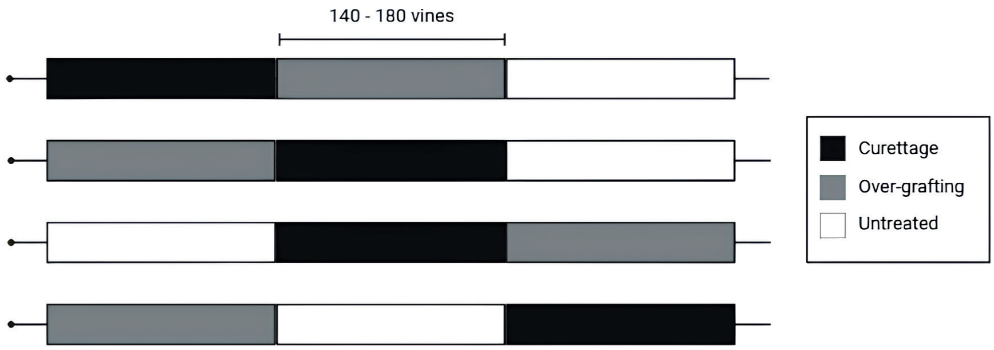

FIGURE 1. Experimental design applied in the vineyards for the evaluation of the treatment efficacy.

RESULTS

In the first vineyard, BO1, curettage and over-grafting demonstrated to be effective in containing GLSD symptoms within the EDC (Figure 2). From the 2021 season, the percentage of plants showing symptoms was significantly lower in the case of curettage or over-grafting than in untreated vines. Despite the significant differences observed after 2021, the post-hoc test did not reveal any difference between curettage and over-grafting. In the last two years of observation, the incidence of symptomatic vines was higher in the case of the untreated control (> 30 %), and it was instead much lower for the two treatments compared (< 10 %). No statistical difference was ascertained between treatments for the incidence of dead vines.

As established in the trial BO1, the effectiveness of both over-grafting and curettage techniques in containing esca was also demonstrated in the vineyard of Vignone (BO2) (Figure 3). Specifically, the percentage of symptomatic vines significantly decreased after the application of both treatments, but this is particularly evident in 2022, after three years of application. Different from the results from vineyard BO2, the percentage of vines that died yearly was significantly higher for the treatments in 2020. Among the dead vines, we also included plants that died for other reasons, not necessarily due to the treatment.

Moving on to the vineyard DF1, in the first year of observation, 2018, although the post-hoc analysis did not separate the means, the plots where over-grafting was applied showed a higher percentage of symptomatic plants compared to the other two treatments (Figure 4). Even though there was such a difference at the beginning of the trial, it is interesting to highlight that within four years, over-grafted vines showed the lowest percentage of esca symptoms, demonstrating once again the effectiveness of this technique in controlling the disease. A similar reduction was also ascertained in the case of the curettage treatment, although the starting situation was much better. The mortality of vines was higher in the case of over-grafting treatment.

During the experimental years, across all three sites, a total of 642 curetted and 686 over-grafted vines were compared with an untreated population of 730 individuals (Tables 2 and 3).

Over the medium-long term, a sharp decline was observed in the percentage of asymptomatic untreated vines, which in some cases fell to less than 50 % of the initial population. This decline was due both to a more consistent recurrence of symptoms and to a high mortality rate (Tables 2 and 3). The treated groups did not exhibit this trend. Actually, during the final year of observation, the proportion of asymptomatic vines was higher across treatments in each site.

The chi-squared test yielded statistically significant results for all years in vineyards BO1 and BO2, while no such significance was found for vineyard DF1 (Table 2). In this latter case, only the population of vines treated during the first experimental year (2018) showed significant differences in some years (Figure 5).

In the two Tuscan vineyards, curettage proved to be successful, as demonstrated by very high recovery rates, around 90 %. Indeed, the percentages of symptomatic and dead vines were significantly lower compared to the control group. Vineyard DF1 was characterised by a lower incidence of the disease, which resulted in some years and for some populations in values similar to those observed for curettage.

As expected, the over-grafting treatment showed percentages of symptomatic vines close to zero. However, this result was counterbalanced by higher average mortality rates, which could exceed 35 % in vineyard BO2 and 20 % in vineyard DF1 (Table 3). The greatest number of deaths occurred in the year following treatment and then remained stable over the medium-long term (Figure 6). Mortality is an important index to consider since both techniques are highly invasive.

Over-grafting resulted in a higher percentage of mortality as compared to curettage; however, there was a huge variability between vineyards. Possibly, this outcome could be explained by the rootstock used. Because of these last results, the owners of the vineyard decided to stop the application of the treatment from the 2022 season, in the vineyard BO2.

DISCUSSION

The initial phytosanitary conditions across the three sites were heterogeneous, with disease incidence ranging from 6 % (DF1) to 25 % (BO2). The high percentage of symptomatic vines monitored in the vineyards allowed a significant number of plants to be treated per plot. In all sites, the techniques demonstrate effectiveness in reducing the occurrence of foliar symptoms of esca.

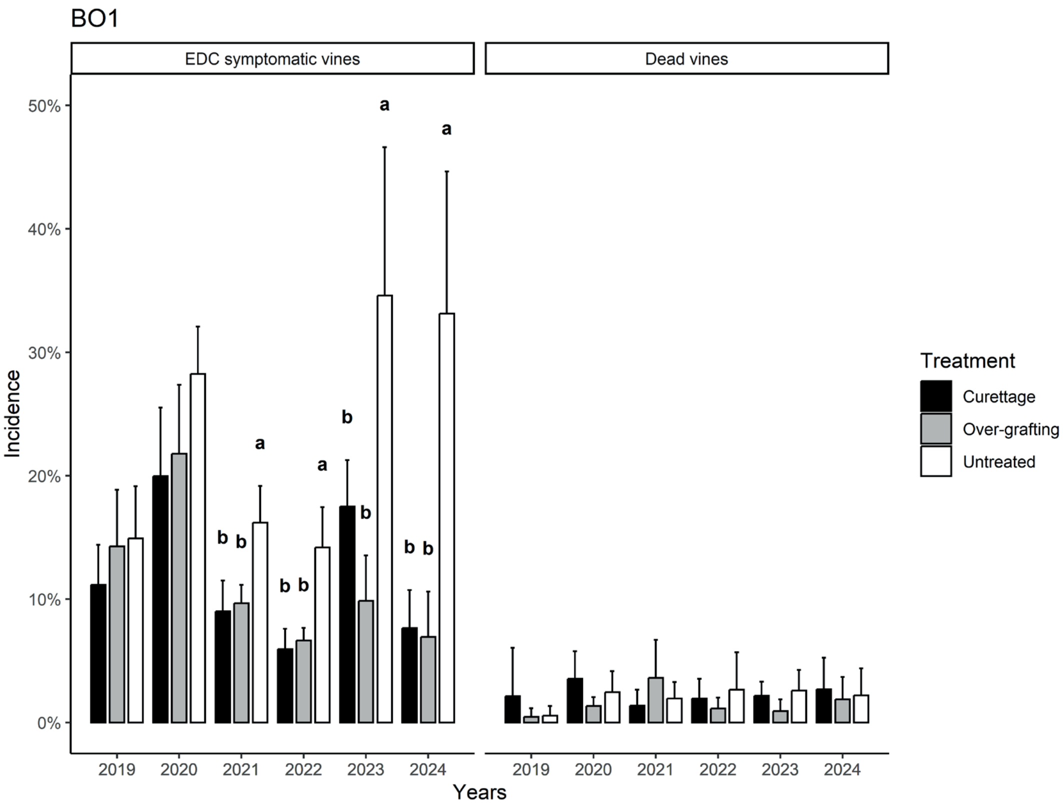

FIGURE 2. Incidence of EDC symptomatic and dead vines in the treatments applied in the vineyard BO1 (Argentiera, Tuscany). One-way ANOVA was applied; when the test was significant, the means were separated with the Tukey HSD test ($p < 0.05$); in each year, different letters identify significantly different means.

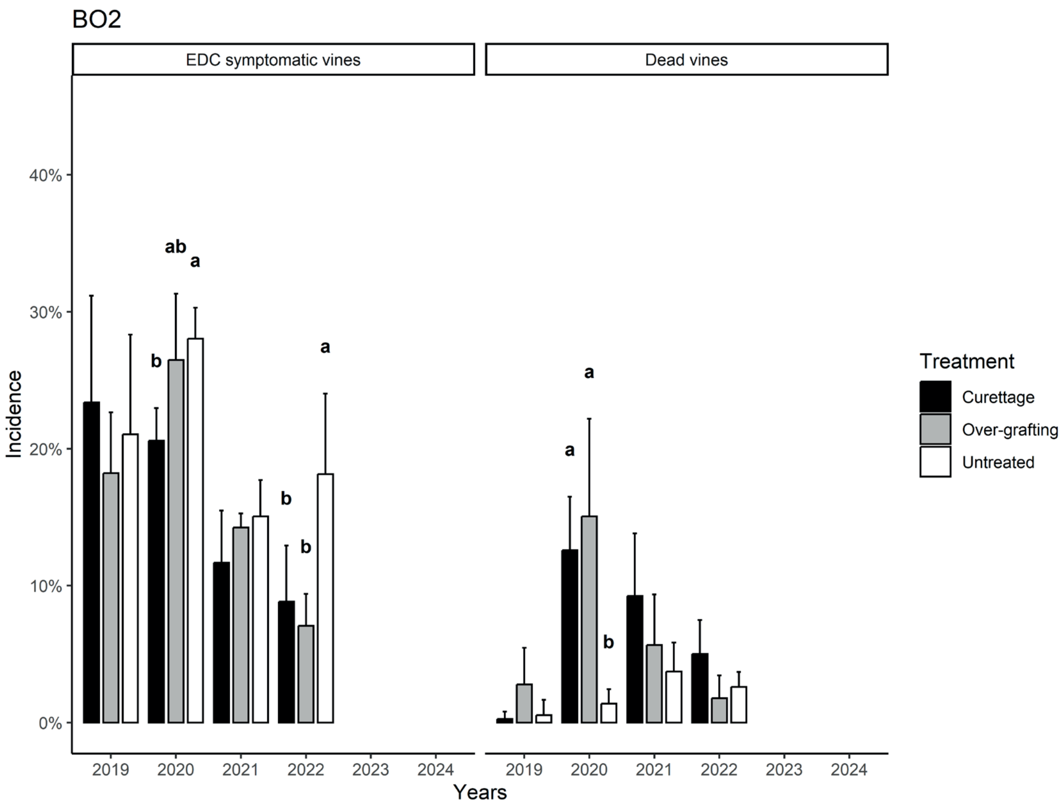

FIGURE 3. Incidence of yearly EDC symptomatic and dead vines in trial BO2 (Ornellaia, Tuscany). One-way ANOVA was applied, and the means were separated with the Tukey HSD test ($p < 0.05$); in each year, different letters identify significantly different means.

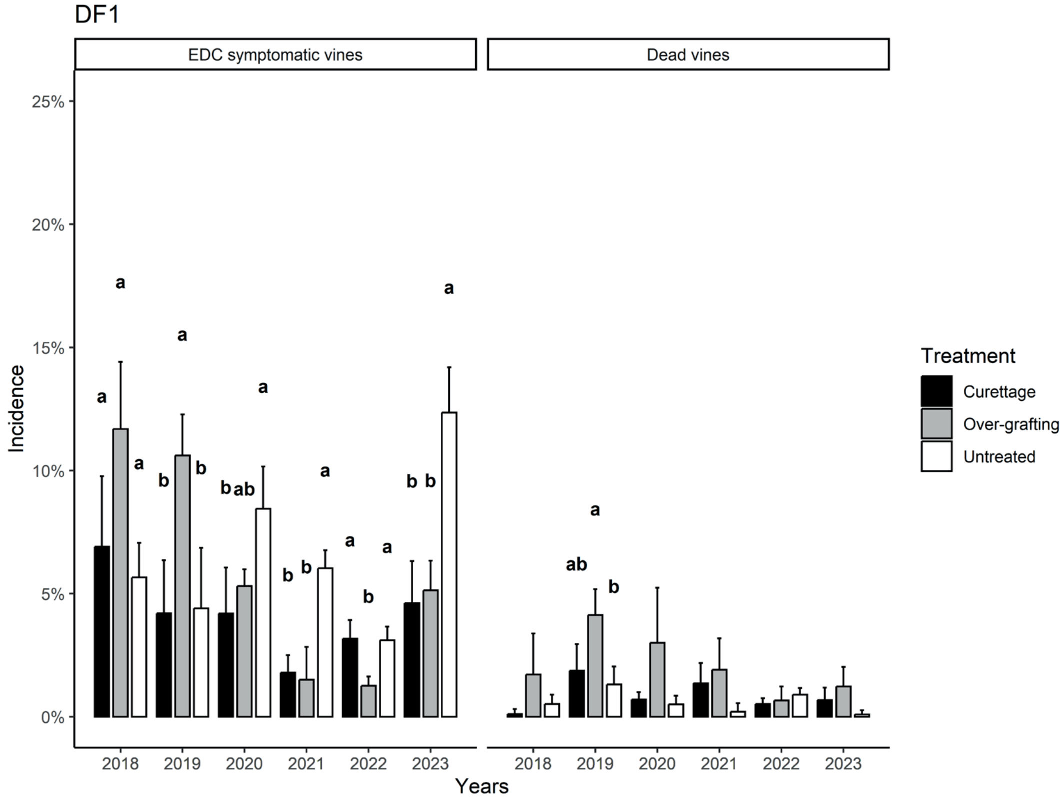

FIGURE 4. Incidence of yearly EDC symptomatic and dead vines in trial DF1 (Romano Dal Forno, Veneto) over five years. One-way ANOVA was applied, and the means were separated with the Tukey HSD test ($p < 0.05$); in each year, different letters identify significantly different means.

Curettage showed a percentage of asymptomatic vines ranging between 80 to 100 %, depending on the vineyard and the year. These results are comparable to those reported by Lecomte *et al.* (2022), who have observed a recovery rate after treatment ranging from 73 % to 96 %. The same authors have also shown a decline in the percentage of asymptomatic vines several years after treatment, suggesting a reduction in the technique's long-term efficacy. Although our study ended only after five years, the reduction of symptomatic plants was not as much as expected by the cited authors. The life span expectancy of vines treated with this technique was found to be longer than compared to the untreated symptomatic plants, as highlighted by the chi-square test applied to the treatments in comparison. Curettage represents an ancient technique that was just recently rediscovered by agronomists to find solutions against the spreading of esca disease (Lafon, 1921). It is well known that the best approach to reduce EDCs spread is prevention in the selection of varieties, the pruning technique and the pruning wound protection since the first year of plantation (Bigot *et al.*, 2020; Mondello, *et al.*, 2018b). Still, as curative tools to maintain the productivity of valuable vineyards, after sodium arsenite was banned for its use against EDC in vineyards, the curettage technique represents one of the few, if not the only, curative tools that can be applied to the vines to stop the development of EDC symptoms. The concept of the technique is the removal of wood affected by white rot. This operation reduces the inoculum pressure, as the decayed wood is the result of the development and activity of some GTD-related fungi on the woody substrate. Comparing the fungal and bacterial microbiome before and after curettage, a substantial change can be observed. In particular, Pacetti *et al.* (2021) recorded a significant decrease in the relative abundance of *F. mediterranea* in the median and sound wood after three months from the surgery. The results reported by the authors of this study suggest an indirect relationship between the activity of *F. mediterranea* and the manifestation of leaf symptoms. Foliar tiger stripes could be mitigated by targeting *F. mediterranea* or other pathogenic fungi associated with white rot in our management strategies; even though there are other theories, further investigations are necessary (Del Frari *et al.*, 2021). These outcomes are in line with the data presented in the present research, where curettage proved to be effective in the containment of leaf symptoms.

The discussion remains open, as other studies have observed a lack of correlation between the manifestation of leaf symptoms and wood decay (Calzarano & Di Marco, 2007). Probably, leaf symptoms cannot be directly connected to the single pathogen *F. mediterranea*; rather, they are linked to a changed balance of the vine microbiome.

Generally, EDC causes the plant to have a less vigorous vegetative development, as well as a reduction in the yield and worsening of the grape quality (Calzarano *et al.*, 2004a;

TABLE 2. Fates of curetted and untreated vines, by year, after curettage treatment, in the three experiments. The bold figures indicate the greatest numbers (or percentages) of vines in each annual distribution. Chi-squared tests were applied for each year to the numbers of vines per category (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$), where As, S and D mean "Asymptomatic", "Symptomatic" and "Dead", respectively. (part 1/2)

Location	Year	Treatment	Number of vines	y+1			y+2			y+3			y+4			y+5		
				As	S	D	As	S	D	As	S	D	As	S	D	As	S	D
BO1	2019	Curettage	65	**58 *** (89 %)**	6 (9 %)	1 (2 %)	**61 *** (94 %)**	4 (6 %)	0 (0 %)	**61 *** (94 %)**	2 (3 %)	2 (3 %)	**56 *** (86 %)**	5 (8 %)	4 (6 %)	**58 *** (89 %)**	2 (3 %)	5 (8 %)
		Untreated	99	54 (55 %)	36 (36 %)	9 (9 %)	66 (67 %)	20 (20 %)	13 (13 %)	66 (67 %)	18 (18 %)	15 (15 %)	42 (42 %)	29 (29 %)	28 (28 %)	38 (38 %)	27 (27 %)	34 (34 %)
	2020	Curettage	133	**118 *** (89 %)**	8 (6 %)	7 (5 %)	**119 *** (89 %)**	5 (4 %)	9 (7 %)	**106 *** (80 %)**	17 (13 %)	10 (8 %)	**118 *** (89 %)**	4 (3 %)	11 (8 %)			
		Untreated	144	106 (74 %)	32 (22 %)	6 (4 %)	108 (75 %)	26 (18 %)	10 (7 %)	58 (40 %)	58 (40 %)	28 (19 %)	63 (44 %)	45 (31 %)	36 (25 %)			
	2021	Curettage	41	**40 ** (98 %)**	1 (2 %)	0 (0 %)	**40 *** (98 %)**	1 (2 %)	0 (0 %)	**41 *** (100 %)**	0 (0 %)	0 (0 %)						
		Untreated	47	35 (74 %)	7 (15 %)	5 (11 %)	24 (51 %)	14 (30 %)	9 (19 %)	19 (40 %)	16 (34 %)	12 (26 %)						
	2022	Curettage	31	26 (84 %)	2 (6 %)	3 (10 %)	**28 *** (90 %)**	0 (0 %)	3 (10 %)									
		Untreated	35	21 (60 %)	8 (23 %)	6 (17 %)	15 (43 %)	10 (29 %)	10 (29 %)									
	2023	Curettage	90	**86 *** (96 %)**	2 (2 %)	2 (2 %)												
		Untreated	60	31 (52 %)	25 (81 %)	4 (13 %)												
BO2	2019	Curettage	54	**50 *** (93 %)**	3 (6 %)	1 (2 %)	**48 ** (89 %)**	4 (7 %)	2 (4 %)	**47 *** (87 %)**	3 (6 %)	4 (7 %)						
		Untreated	78	44 (56 %)	31 (40 %)	3 (4 %)	50 (64 %)	19 (24 %)	9 (12 %)	39 (50 %)	28 (36 %)	11 (14 %)						
	2020	Curettage	45	**41 ** (91 %)**	1 (2 %)	3 (7 %)	**40 ** (89 %)**	0 (0 %)	5 (11 %)									
		Untreated	72	50 (69 %)	16 (22 %)	6 (8 %)	49 (68 %)	14 (19 %)	9 (13 %)									
	2021	Curettage	30	**29 * (97 %)**	1 (3 %)	0 (0 %)												
		Untreated	18	12 (67 %)	4 (22 %)	2 (11 %)												

TABLE 2. Fates of curetted and untreated vines, by year, after curettage treatment, in the three experiments. The bold figures indicate the greatest numbers (or percentages) of vines in each annual distribution. Chi-squared tests were applied for each year to the numbers of vines per category (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$), where As, S and D mean "Asymptomatic", "Symptomatic" and "Dead", respectively. (part 2/2)

DF1	2019	Curettage	44	43 (98 %)	1 (2 %)	0 (0 %)	**42 * (95 %)**	1 (2 %)	1 (2 %)	**42 * (95 %)**	1 (2 %)	1 (2 %)	42 (95 %)	1 (2 %)	1 (2 %)	**38 * (86 %)**	5 (11 %)	1 (2 %)
		Untreated	55	49 (89 %)	4 (7 %)	2 (4 %)	42 (76 %)	10 (18 %)	3 (5 %)	40 (73 %)	11 (20 %)	4 (7 %)	45 (82 %)	3 (5 %)	7 (13 %)	36 (65 %)	11 (20 %)	8 (15 %)
	2020	Curettage	35	32 (91 %)	2 (6 %)	1 (3 %)	33 (94 %)	1 (3 %)	1 (3 %)	33 (94 %)	1 (3 %)	1 (3 %)	32 (91 %)	2 (6 %)	1 (3 %)			
		Untreated	35	32 (91 %)	2 (6 %)	1 (3 %)	29 (83 %)	5 (14 %)	1 (3 %)	31 (89 %)	2 (6 %)	2 (6 %)	26 (74 %)	7 (20 %)	2 (6 %)			
	2021	Curettage	38	34 (89 %)	0 (0 %)	4 (12 %)	34 (89 %)	0 (0 %)	4 (11 %)	33 (87 %)	1 (3 %)	4 (11 %)						
		Untreated	63	59 (94 %)	3 (5 %)	1 (2 %)	58 (92 %)	3 (5 %)	2 (3 %)	46 (73 %)	15 (24 %)	2 (3 %)						
	2022	Curettage	7	6 (86 %)	0 (0 %)	1 (17 %)	6 (86 %)	0 (0 %)	1 (14 %)									
		Untreated	30	27 (90 %)	3 (10 %)	0 (0 %)	24 (80 %)	6 (20 %)	0 (0 %)									
	2023	Curettage	29	27 (93 %)	2 (7 %)	0 (0 %)												
		Untreated	12	11 (92 %)	1 (8 %)	0 (0 %)												
Total		Curettage	642	590 (92 %)	29 (5 %)	23 (4 %)	451 (91 %)	16 (3 %)	26 (5 %)	363 (89 %)	25 (6 %)	22 (5 %)	248 (90 %)	12 (4 %)	17 (6 %)	96 (88 %)	7 (6 %)	6 (6 %)
		Untreated	748	531 (71 %)	172 (23 %)	45 (6 %)	465 (71 %)	127 (19 %)	66 (10 %)	299 (57 %)	148 (28 %)	74 (14 %)	176 (53 %)	84 (25 %)	73 (22 %)	74 (48 %)	38 (25 %)	42 (27 %)

TABLE 3. Fates of over-grafted and untreated vines, by year, after over-grafting treatment, in the three experiments. The bold figures indicate the greatest numbers (or percentages) of vines in each annual distribution. Chi-squared tests were applied for each year to the numbers of vines per category (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$), where As, S and D mean "Asymptomatic", "Symptomatic" and "Dead", respectively. (part 1/2)

Location	Year	Treatment	Number of vines	y+1			y+2			y+3			y+4			y+5		
				As	S	D	As	S	D	As	S	D	As	S	D	As	S	D
BO1	2019	Over-grafting	74	**71 *** (96 %)**	0 (0 %)	3 (4 %)	**69 *** (93 %)**	2 (3 %)	3 (4 %)	**69 *** (93 %)**	2 (3 %)	3 (4 %)	**69 *** (93 %)**	2 (3 %)	3 (4 %)	**69 *** (93 %)**	1 (1 %)	4 (5 %)
		Untreated	99	54 (55 %)	36 (36 %)	9 (9 %)	66 (67 %)	20 (20 %)	13 (13 %)	66 (67 %)	18 (18 %)	15 (15 %)	42 (42 %)	29 (29 %)	28 (28 %)	38 (38 %)	27 (27 %)	34 (34 %)
	2020	Over-grafting	125	**117 *** (94 %)**	0 (0 %)	8 (6 %)	**117 *** (94 %)**	0 (0 %)	8 (6 %)	**116 *** (93 %)**	0 (0 %)	9 (7 %)	**113 *** (90 %)**	3 (2 %)	9 (7 %)			
		Untreated	144	106 (74 %)	32 (22 %)	6 (4 %)	108 (75 %)	26 (18 %)	10 (7 %)	58 (40 %)	58 (40 %)	28 (19 %)	63 (44 %)	45 (31 %)	36 (25 %)			
	2021	Over-grafting	52	**50 ** (96 %)**	0 (0 %)	2 (4 %)	**49 *** (94 %)**	1 (2 %)	2 (4 %)	**47 *** (90 %)**	2 (4 %)	3 (6 %)						
		Untreated	47	35 (74 %)	7 (15 %)	5 (11 %)	24 (51 %)	14 (30 %)	9 (19 %)	19 (40 %)	16 (34 %)	12 (26 %)						
	2022	Over-grafting	32	**31 ** (97 %)**	0 (0 %)	1 (3 %)	**29 *** (91 %)**	0 (0 %)	3 (9 %)									
		Untreated	35	21 (60 %)	8 (23 %)	6 (17 %)	15 (43 %)	10 (29 %)	10 (29 %)									
	2023	Over-grafting	48	**44 *** (92 %)**	0 (0 %)	4 (8 %)												
		Untreated	60	31 (52 %)	25 (42 %)	4 (7 %)												
BO2	2019	Over-grafting	41	**30 *** (73 %)**	0 (0 %)	11 (27 %)	**30 *** (73 %)**	0 (0 %)	11 (27 %)	**29 *** (71 %)**	0 (0 %)	12 (29 %)						
		Untreated	78	44 (56 %)	31 (40 %)	3 (4 %)	50 (64 %)	19 (24 %)	9 (12 %)	39 (50 %)	28 (36 %)	11 (14 %)						
	2020	Over-grafting	78	47 (60 %)	0 (0 %)	31 (40 %)	44 (56 %)	0 (0 %)	34 (44 %)									
		Untreated	72	**50 *** (69 %)**	16 (22 %)	6 (8 %)	**49 *** (68 %)**	14 (19 %)	9 (13 %)									

TABLE 3. Fates of over-grafted and untreated vines, by year, after over-grafting treatment, in the three experiments. The bold figures indicate the greatest numbers (or percentages) of vines in each annual distribution. Chi-squared tests were applied for each year to the numbers of vines per category (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$), where As, S and D mean "Asymptomatic", "Symptomatic" and "Dead", respectively. (part 2/2)

DF1	2019	Over-grafting	87	73 (84 %)	0 (0 %)	14 (16 %)	**71 *** (82 %)**	0 (0 %)	16 (18 %)	**71 *** (82 %)**	0 (0 %)	16 (18 %)	71 (82 %)	0 (0 %)	16 (18 %)	**70 *** (80 %)**	1 (1 %)	16 (18 %)
		Untreated	55	**49 ** (89 %)**	4 (7 %)	2 (4 %)	42 (76 %)	10 (18 %)	3 (5 %)	40 (73 %)	11 (20 %)	4 (7 %)	45 (82 %)	3 (5 %)	7 (13 %)	37 (67 %)	11 (20 %)	7 (13 %)
	2020	Over-grafting	83	65 (78 %)	0 (0 %)	18 (22 %)	65 (78 %)	0 (0 %)	18 (22 %)	65 (78 %)	0 (0 %)	18 (22 %)	**65 *** (78 %)**	0 (0 %)	18 (22 %)			
		Untreated	35	**32 * (91 %)**	2 (6 %)	1 (3 %)	**29 * (83 %)**	5 (14 %)	1 (3 %)	31 (89 %)	2 (6 %)	2 (6 %)	26 (74 %)	7 (20 %)	2 (6 %)			
	2021	Over-grafting	43	40 (93 %)	0 (0 %)	3 (7 %)	40 (93 %)	0 (0 %)	3 (7 %)	**39 ** (91 %)**	1 (2 %)	3 (7 %)						
		Untreated	63	59 (94 %)	3 (5 %)	1 (2 %)	58 (92 %)	3 (5 %)	2 (3 %)	46 (73 %)	15 (24 %)	2 (3 %)						
	2022	Over-grafting	12	12 (100 %)	0 (0 %)	0 (0 %)	12 (100 %)	0 (0 %)	0 (0 %)									
		Untreated	30	27 (90 %)	3 (10 %)	0 (0 %)	24 (80 %)	6 (20 %)	0 (0 %)									
	2023	Over-grafting	11	8 (73 %)	0 (0 %)	3 (27 %)												
		Untreated	12	11 (92 %)	1 (8 %)	0 (0 %)												
Total		Over-grafting	686	588 (86 %)	0 (0 %)	98 (14 %)	526 (84 %)	3 (0 %)	98 (16 %)	436 (86 %)	5 (1 %)	64 (13 %)	318 (86 %)	5 (1 %)	46 (12 %)	139 (86 %)	2 (1 %)	20 (12 %)
		Untreated	730	519 (71 %)	168 (23 %)	43 (6 %)	465 (71 %)	127 (19 %)	66 (10 %)	299 (57 %)	148 (28 %)	74 (14 %)	176 (53 %)	84 (25 %)	73 (22 %)	75 (49 %)	38 (25 %)	41 (27 %)

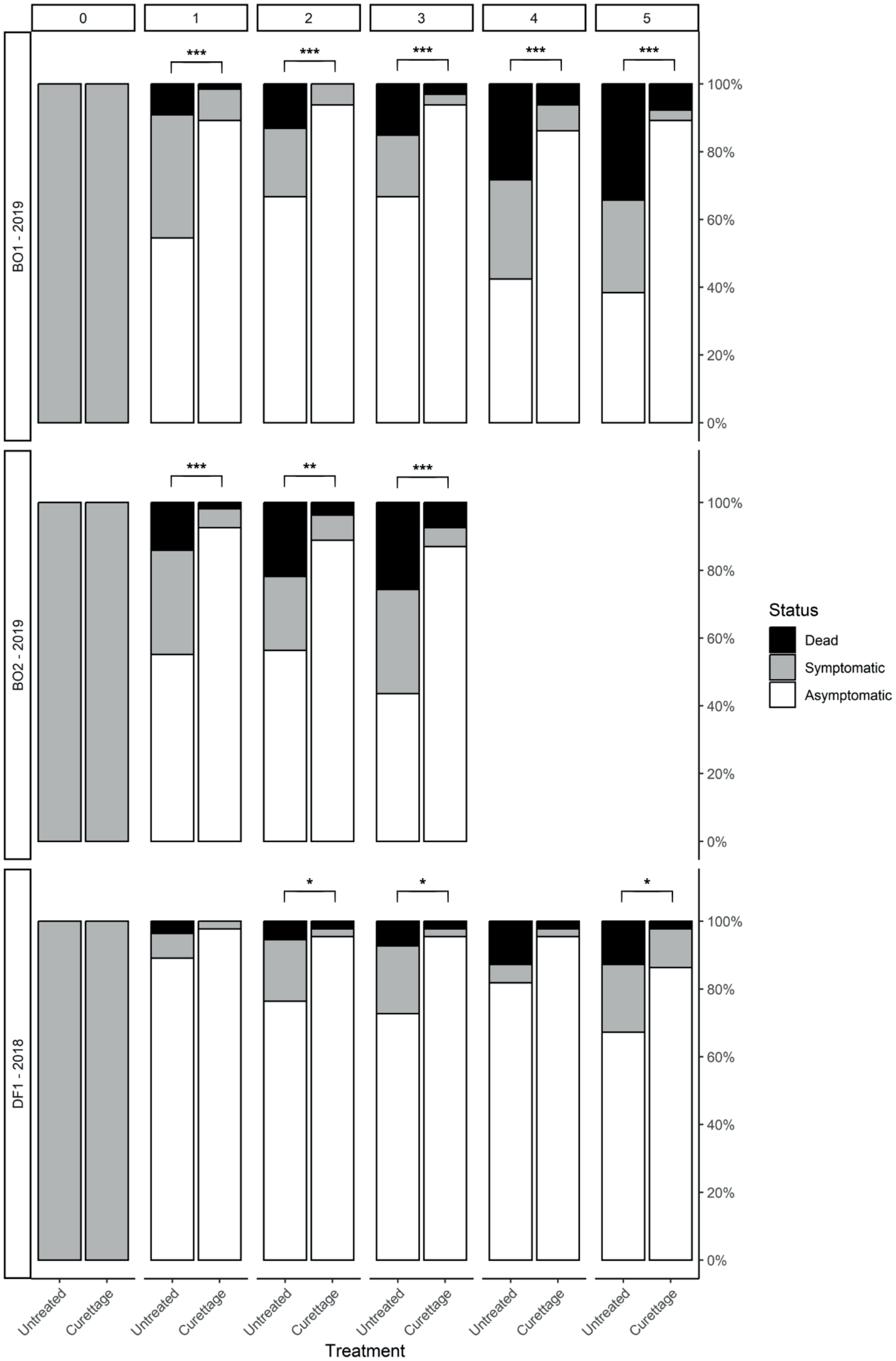

FIGURE 5. Percentage of curetted and untreated vines that remained healthy, died, or showed EDC symptoms, by year, for up to three and five years after the curettage treatment. The vines concerned were those curetted in the first year of the experiment, 2019 for Argentiera, Bolgheri (BO1) and Ornellaia, Bolgheri (BO2), 2018 for Dal Forno, Veneto (DF1).

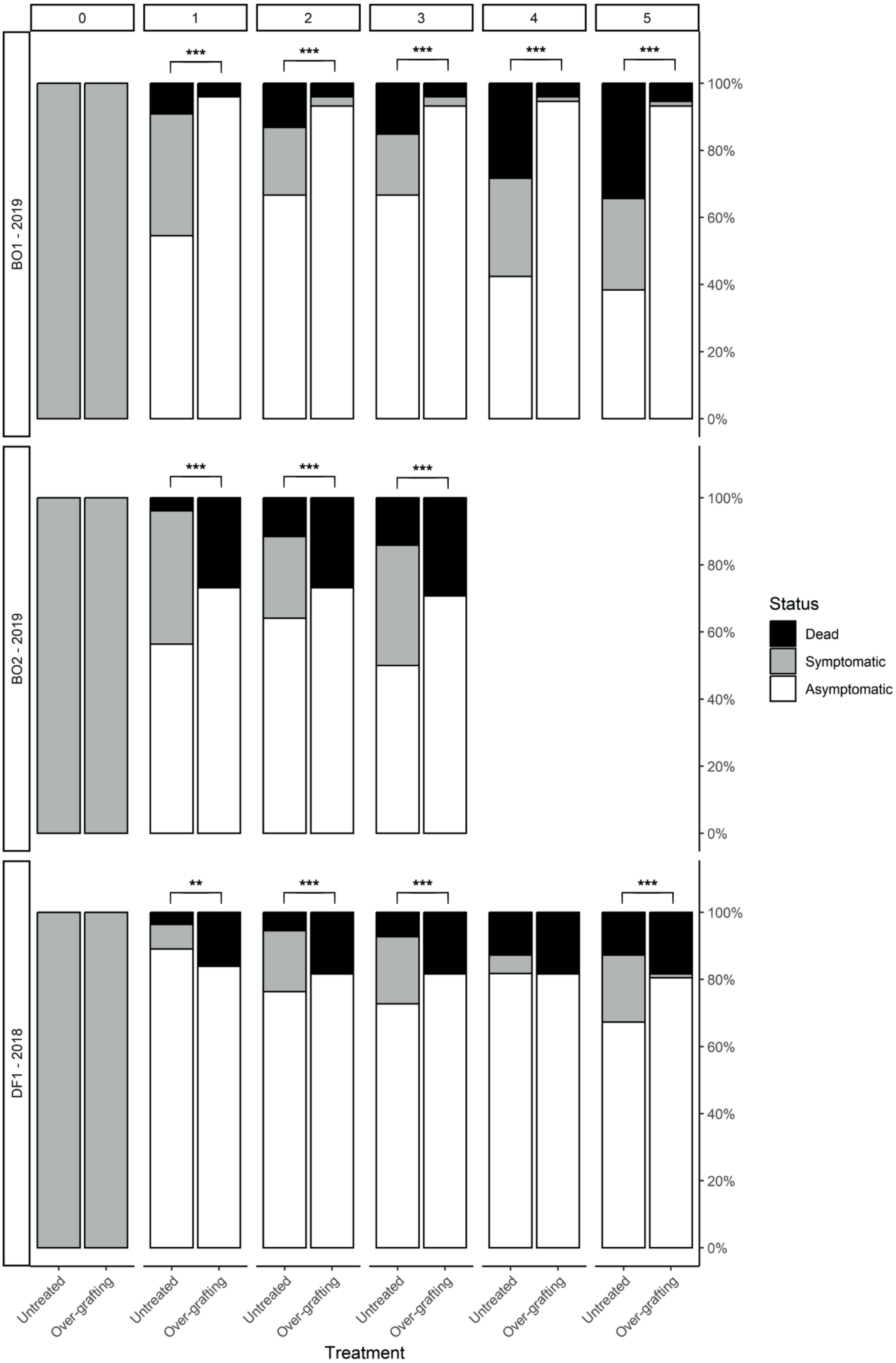

FIGURE 6. Percent of over-grafted and untreated vines that remained healthy, died, or showed EDC symptoms, by year, for up to three and five years after the treatment. The vines concerned were those over-grafted in the first year of the experiment, 2019 for Argentiera, Bolgheri (BO1) and Ornellaia, Bolgheri (BO2), 2018 for Dal Forno, Vento (DF1).

Dewasme *et al.*, 2022), and a delay in ripening (Lorrain *et al.*, 2012). However, when curettage is applied, it is possible to restore the normal capacity of the plant to grow and bear fruit. In a study carried out by Cholet *et al.* (2021), curettage has resulted in the development of a comparable number of shoots, leaf area, bud fertility, and yield. In another study dealing with the same experiment, Bruez *et al.* (2021) also showed that the main quality parameters of musts obtained from treated and healthy vines were not significantly different.

When white rot is highly extensive, to the point of completely compromising the hydraulic system of the vine, an alternative technique that can be adopted to avoid uprooting and replanting is over-grafting. This technique allows the renewal of the stem and the canopy, taking advantage of a well-branched root system. In this way, the new plant is protected from the surrounding grapevine root competition. Empirical experiences of SICAVAC, a technical and research support organisation (Sancerre, France), suggest that vine over-grafting is a viable technique, even though the rootstocks show GTD-like internal symptoms in cross-sections but healthy external wood (Dal, 2013). This solution would be desirable, especially if the variety does not easily produce new shoots, making trunk renewal unfeasible.

Some scientific evidence conducted in Italy has shown the resumption of declining symptoms in vines renewed with symptomatic rootstocks (Calzarano *et al.*, 2004b). Indeed, although remote, the possibility that over-grafted plants will show back symptoms cannot be excluded. Such an event could happen when the infection has also reached the rootstock. In agreement with these considerations, in the present research, we also observed some cases of symptom resumption following the treatments applied. Over-grafting has shown highly variable success rates depending on the trial; what probably affects the data the most is the rootstock used. In fact, a great variability can also be observed between the vineyards BO1 and BO2, where the vineyards were both planted with Cabernet-Sauvignon, adopting a very similar winter pruning and training system, but the rootstock was different. The rootstock RSB1 was used in the BO1 vineyard, and no grafting problems were ascertained, as opposed to the BO2 vineyard, where the weaker rootstock 101.14 resulted in grafting incompatibility with the Cabernet-Sauvignon. Before adopting this solution, it is crucial to take into account the rootstock factor. Further, more targeted studies could clarify the role of the rootstock in the success of this technique.

All this evidence must be considered when thinking about the economic cost of both techniques. When deciding on the intervention, it is important to evaluate the opportunity cost. The symptomatic vines can be replaced with economic costs and a period with less yield; moreover, the young vines need time to create the new root system, competing for space and nutrients with the adult plants. On the other hand, curettage seems to provide promising results, but specialised manpower is required. In case of over-grafting, the chances of failure are not that low, depending on the variety and rootstock. Furthermore, in the year of treatment, the plant does not produce fruit. Grape production is not guaranteed even in the year following the execution, but the vine shows a quicker return to production than newly planted (Dewasme *et al.*, 2023). A key element of over-grafting is that it allows the recovery of plants with severe symptoms, whose trunk is extensively compromised. Both techniques examined in the present research can be expensive, but they can offer a faster solution to wine growers to recover the yield and exploit the root system already developed in the soil.

CONCLUSION

Curettage and over-grafting have been demonstrated to be effective techniques leading to a progressive and significant reduction of the percentage of plants showing EDC foliar symptoms. Therefore, both are to be considered valid strategies for the management of the disease. In particular, over-grafting showed the lowest resumption rates, certainly due to the lower susceptibility to GLSD of the new (green) shoot compared to old plants. As regards this technique, it is important to consider the rootstock of the vineyard, as revealed by the high mortality rate obtained with the trial carried out in the BO2 vineyard. The curettage also proved to be a powerful technique in containing the development of EDC, with results comparable to the over-grafting.

The results obtained in these three experiments proved that both techniques are valid tools to contrast EDC development, adopting the curettage where possible and applying over-grafting where the situation of the wood is more critical. Thus, the integration of the different techniques in the same vineyard could be a combined solution to the disease.

ACKNOWLEDGEMENTS

The authors would like to thank the agronomists of the Argentiera winery, Leonardo Raspini and Nicolò Carrara, the agronomists of the Ornellaia winery, Giuliano Tarchi and Alessio Neri, and the owner, Marco Dal Forno and the agronomist Cesare Spada of Romano Dal Forno winery.

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