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Foliar Applications of Calcium, Magnesium, and Seaweed Mixture to Mitigate Chronic and Apoplectic Forms of Esca Disease and Improve Yield in Vineyards

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Abstract

Esca disease, the most widespread grapevine trunk disease in Europe, is characterized by both chronic and acute forms. In both cases, alterations in the plant's physiological processes are significant and lead to yield losses and/or plant death. Studies have highlighted the effects of a mixture of foliar fertilizers and seaweeds in reducing foliar symptoms and improving both the quantity and quality of yield. These effects have now been evaluated on additional cultivars and in other vineyard areas. Furthermore, for the first time, the activity of the fertilizer mixture in reducing apoplexy and the resulting vine mortality has been assessed. During the 2022–2023 biennium, in four vineyards of the Lambrusco cultivar in the Province of Reggio Emilia, Northern Italy, affected by both chronic and acute forms of the disease, foliar applications of the mixture were carried out at 10-day intervals starting from the "nine leaves unfolded" BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) stage 19 up to the "berries developing color" BBCH stage 83. The results confirmed the activity of the fertilizer mixture in reducing chronic symptoms, which appeared particularly pronounced in 2022, when rainfall quantity and distribution allowed regular development of phenological stages. In that year, in all vineyards, a reduction of approximately 50% and 60% in the incidence and severity of chronic leaf symptoms was recorded. Under these optimal growth conditions, treated vines generally showed superior yield and quality. Conversely, in 2023, characterized by heavy rains, smaller effects on foliar symptoms and no improvements in yield were observed. Applications of the mixture resulted in a significant reduction in apoplexy and, consequently, vine mortality, as verified in 2024. This effect did not appear to be influenced by climatic conditions. This study confirms that applications of the mixture aimed at reducing symptom expression and yield damage are a valid addition to the few available control practices. The positive effects observed on the acute form for the first time require further investigation.

Keywords: grapevine; esca complex; leaf stripe symptoms; apoplexy; leaf fertilizer applications

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1. Introduction

Esca is the most important grapevine trunk disease and is present in almost all grape-growing areas. The main pathogens associated with the disease are vascular fungi, such as *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*, and the white rot agent

Fomitiporia mediterranea [1–4]. The disease can occur in a chronic or an acute apoplectic form, eventually leading to the death of plants [5].

Global figures on the economic impact of esca are hard to find due to the local variations caused by different vineyard management and climatic conditions. However, esca disease causes significant damage in the vineyard (affecting yield quantity and quality and causing vine death), leading to millions in lost revenue, increased management costs, and the need for replanting.

Grapevine trunk diseases are widespread. However, the largest wine-producing countries are reporting the largest production losses. In Europe, the overall incidence of trunk diseases, particularly due to the presence of esca, was 19%, 14%, and 13% in Spain, France, and Italy, respectively [6,7]. In Italy, esca incidence peaks of 60–80% have been observed in old vineyards [4].

In France, the National Institute of Vine and Wine estimated damage due to esca at one billion euros per year, corresponding to approximately €1500 per hectare per year in wine production losses. In California, yield losses of approximately $2000 to $3000 per hectare per year have been recorded [6,7]. The symptoms of the chronic form are characterized by leaf tiger stripes, black measles on the berries, and wilting of shoots and clusters [1]. In Italy, leaf symptoms normally appear from the berries' groat-sized stage (73 BBCH) to harvest maturity. A decrease in both the quantity and quality of yield is the main consequence of disease symptoms [7–9]. The chronic form is also typically characterized by an erratic appearance of canopy symptoms across seasons [5,10]. Finally, asymptomatic plants yield grapes that are not dissimilar, in quality and quantity, from those of plants that have never shown symptoms [8,9,11,12].

The acute apoplectic form can result in the death of the vine. In some cases, apoplectic vines may partially grow in the following season but without producing any significant yield. Apoplexy usually occurs in midsummer and generally consists of a sudden, rapid, basipetal, and complete collapse of the canopy and bunches [2].

Apoplexy was the first symptom historically described as being associated with the disease. Surico (2009) reported that the association between apoplexy and esca was already present in popular knowledge in Smyrna and Puglia. Viala was the first to propose this association at the scientific level [13]. Apoplexy is more serious than the chronic form, with a major impact on vineyards, as it can cause total loss of yield and, in most cases, the death of the plant [14,15]. Nevertheless, the study of apoplexy is more challenging than that of the chronic form due to its rapid and sudden development, which is compounded by the inability to reliably predict which vines are likely to be affected by apoplexy during the season [16]. However, multi-year observations of plants infected with esca have made it possible to identify alterations in plants that subsequently develop apoplexy within the same season. Such alterations involve photosynthesis, defense and detoxification systems, and proteomics and lipid composition of the leaf [12,14,16–18].

Therefore, monitoring these parameters could be useful for developing correlations and, hopefully, a prediction model for apoplexy. What we now know is that there is an increased risk of developing apoplexy in vines affected by white rot agents [19].

Currently, control strategies against the disease are limited and aimed at: (i) preventing infections of pruning wounds [20–22]; (ii) surgically removing wood infections [23–25]; and (iii) improving the resistance of the host plant and/or promoting a lower expression of symptoms [9,26].

In recent years, a formulation based on calcium, magnesium, and seaweed has been successfully studied for the chronic form of the disease. Fertilizer applications have been hypothesized to reduce the plant's excessive defense response to the various factors associated with the onset of symptoms, thereby reducing their severity [27,28]. Moreover, treated vines showed increased calcium druse content in leaves, likely helping to prevent the development of leaf tiger stripes [9]. The formulation was shown to interact with the physiology of the infected plant while improving the yield and quality of treated grapevines [9,29–31].

Given that the aforementioned applications targeted the chronic form and that control of the acute form is currently not possible, this study evaluated the potential of a calcium, magnesium, and seaweed-based formulation against the acute form in vineyards of the Lambrusco cultivar in the wine-growing area of the Province of Reggio Emilia, north-central Italy. Furthermore, to confirm results previously obtained with other cultivars and in other wine-growing areas, the effect of applications of the fertilizer mixture on foliar symptom expression in the chronic form, as well as on the quantity and quality of yield, was assessed.

2. Materials and Methods

2.1. Vineyards

This study was conducted in four vineyards of the cv. Lambrusco affected by both chronic and acute forms of esca. The vineyards are located in the Province of Reggio Emilia, Emilia-Romagna region, north-central Italy. Field details are provided in Table 1. The four vineyards are situated in the same viticultural district, in a plain area, a short distance apart, with the same loam soil tending towards silty and a Kober 5BB rootstock.

Table 1. Characteristics of the cv. Lambrusco experimental vineyards.

Vineyard Location	Planting Year	Training System	Planting System (m × m)	Vineyard Area (m^2)	Total Vine Number	Experimental Single Plot Area (m^2)	Experimental Single Plot Vine Number
Rio Saliceto	2000	GDC (Geneva double curtain)	3 × 2	5868	978	978	163
Luzzara	2005	Spurred cordon	2.8 × 1.2	2318	690	386	115
Fabbrico	2010	Spurred cordon	3 × 1.7	4192	822	699	137
Correggio	2007	Double Guyot	2.7 × 1.25	3341	990	557	165

In these vineyards, fertilization is not carried out due to the characteristics of the soil and the cultivar. The vineyards are not equipped with irrigation systems, nor are they irrigated occasionally. Pest and disease control are managed following IPM (integrated pest management) protocols.

2.2. Experimental Plan and Foliar Applications

In September 2021, the year before the applications, the four vineyards were surveyed for disease symptoms. Each vineyard was divided into six plots, similar in size and number of plants, with each plot representing a replicate of each treatment: (1) plants to be treated with the fertilizer mixture; (2) untreated control. Thus, each treatment had three replicates (three plots) positioned throughout the vineyard according to a randomized block design.

For each vineyard, the size and number of vines in a single plot are reported in Table 1. The total area (m^2) of each of the four vineyards was divided into 6 plots of equal size. Then, the area of each plot was divided by the planting system (m × m) to obtain the number of plants in the plot (Table 1).

In all vineyards, applications of a fertilizer mixture based on calcium, magnesium, and seaweed extracts were carried out in 2022–2023. The fertilizer mixture was applied at 10-day intervals, starting from the “nine leaves unfolded” (19 BBCH) stage up to the “berries developing colors” (83 BBCH) stage [32]. In 2022, 9 applications were carried out in all vineyards, while in 2023, 8 applications were carried out in the Rio Saliceto and Luzzara vineyards and 7 applications in the Fabbrico and Correggio vineyards. The dates of application are reported in Table 2. In each application, the fertilizer mixture was applied at 4 hL ha^{-1}.

Table 2. Application data of the foliar fertilizer mixture of calcium, magnesium and seaweed extract in 2022 and 2023 in the experimental vineyards.

Application Number	Rio Saliceto		Luzzara		Fabbrico		Correggio	
	2022	2023	2022	2023	2022	2023	2022	2023
1	9 May	26 May	10 May	18 May	16 May	29 May	10 May	06 May
2	20 May	9 June	23 May	26 May	25 May	11 June	23 May	18 May
3	2 June	19 June	2 June	7 June	4 June	19 June	4 June	28 May
4	10 June	27 June	12 June	15 June	12 June	26 June	16 June	9 June
5	24 June	8 July	24 June	24 June	19 June	2 July	28 June	29 June
6	5 July	18 July	8 July	8 July	27 June	11 July	5 July	26 July
7	16 July	28 July	19 July	18 July	10 July	28 July	15 July	8 August
8	26 July	3 August	26 July	27 July	22 July	/	25 July	/
9	5 August	/	4 August	/	1 August	/	4 August	/

/ = Application not made.

The leaf fertilizer mixture contained 466 g of $CaCl_2$, 403 g of $Mg(NO_3)_2$, 75 mL of *Fucales* seaweed extract, and 466 mL of distilled H_2O for 1 L of solution. Details of the fertilizer mixture are also reported in Calzarano et al., 2014; 2017 [9,29].

Foliar applications were carried out with an air blast sprayer in the Rio Saliceto vineyard, distributing a volume of 10 hL ha^{-1}, and with pneumatic sprayers in the Luzzara, Fabbrico, and Correggio vineyards, with volumes of 5, 5, and 4 hL ha^{-1}, respectively.

In all vineyards, for each year of the study, rainfall and temperatures from April to September were collected from the il Meteo website (iLMeteo s.r.l., https://www.ilmeteo.it/, accessed on 24 June 2025) (Table S1).

2.3. Assessment of Foliar Symptoms of the Chronic Form of Esca

Chronic foliar symptom assessments were carried out in each of the six plots of each vineyard on 14, 15, 16, and 17 September 2021 at Rio Saliceto, Luzzara, Fabbrico, and Correggio, respectively. The chronic foliar symptom assessments were also carried out in 2022 and 2023, when the fertilizer applications were performed, and in 2024, when no applications were made.

In all vineyards, every year, the assessments were carried out between 14 and 20 September, immediately before harvest, when the incidence and severity of symptoms reach their maximum. In each plot, the percentage incidence was calculated by dividing the number of vines with tiger-stripe foliar symptoms by the total number of vines in the plot and multiplying the result by 100. The tiger-stripe foliar symptom severity was recorded using an arbitrary scale of 0 to 5, where 0 = no leaf symptom; 1 = 1–10%; 2 = 11–30%; 3 = 31–50%; 4 = 51–70%; and 5 = 71–100% [9]. Foliar symptom severity was calculated from the McKinney index: $\sum N \times 100/(Y \times Z)$, where $\sum N$ = sum of the severity values for symptoms; Y = number of standing vines; and Z = maximum value of symptoms [9].

2.4. Assessment of Apoplexy and Death of Plants

In the different plots of the surveyed vineyards, and in each year of the study, apoplectic and dead plants were assessed. As for chronic symptoms, the 2021 assessments aimed to determine the incidence of apoplectic plants in the different vineyard plots before the start of the 2022 and 2023 fertilizer applications. In all cases, the apoplexy assessments were carried out at harvest, at the same time as the chronic symptom assessments.

In this period, in fact, it is possible to calculate the annual incidence of apoplexy, which appears at veraison and progresses until the harvest.

Vines were considered affected by apoplexy if they had been growing normally during the season until veraison but, at the time of assessment, showed completely dried shoots, leaves, and bunches. For each plot, the incidence of apoplexy was calculated by dividing the number of plants affected by apoplexy by the total number of plants present at the time of the survey and multiplying the result by 100. In 2022 and 2023, the assessment made it possible to calculate the annual incidence of apoplectic plants by comparing treated and untreated plots.

In 2024, in each plot, the overall mortality due to the disease over the four years of investigation was determined. Actual mortality was calculated by considering as dead those vines affected by apoplexy in 2021 or 2022 that did not regrow in 2023 or 2024. Expected mortality was calculated by considering as dead all vines that had shown apoplectic symptoms during all four years of the study, therefore also including apoplectic plants in 2023 and 2024 in the calculation.

2.5. Quantity and Quality of the Yield

The yield samples were collected to determine the quantity and quality parameters only in the years when fertilizer applications were carried out. The yield of 12 apparently healthy vines, located in the central rows of each plot (3 vines per row) of each treatment, was weighed to calculate the average yield per plant. Sample collection was carried out at harvest, on 22 and 23 September 2022 and on 25 and 26 September 2023 in the Rio Saliceto and Luzzara vineyards, and on 25 and 26 September 2022 and on 27 and 28 September 2023 in the Fabbrico and Correggio vineyards, respectively.

In both years, a 1.8 kg berry sample (150 g per vine) was selected from the 12 vines in each plot, alternately taken from the wings, tips, and centers of clusters located approximately midway along the vine shoots. The harvested berries had the same sun exposure and were likely to have a similar maturity level. Thus, three berry samples (as three replicates) of 1.8 kg were obtained for each of the two treatments. Each berry sample was weighed on an analytical balance, then ground in a mortar and pressed to separate the must from the skins and seeds. The obtained must was analyzed for soluble solids (°Brix), pH, and total acidity (g L^{-1}) using the Fehling, acid/base titration, and potentiometric methods, respectively. The analyses were carried out according to the methods of the Official Gazette of the European Communities, Commission Regulation (EEC) No. 2676/90 (Official Journal L 272, 3 October 1990) [33].

2.6. Statistical Analysis

In each vineyard, for each assessment year and treatment, the following parameters were subjected to statistical analyses using one-way analysis of variance (ANOVA): (i) the disease incidence and severity of foliar symptoms of the chronic form of esca, (ii) the annual incidence of apoplexy, (iii) the actual and expected mortality, and (iv) the yield and the grape quality parameters of apparently healthy vines. When significant differences were detected between the analyzed parameters, the separation of the means was carried out using Tukey's honest significant difference (HSD) test, for $p = 0.05$. Statistical analyses were performed using XLSTAT v.2016 (Addinsoft, Paris, France).

3. Results

3.1. Assessment of the Foliar Symptoms of the Chronic Form of Esca

In 2021, the year before the fertilizer mixture applications, the incidence and severity of foliar symptoms did not significantly differ between the plots meant to be treated in the following two-year period and the untreated control plots in all vineyards (Table 3). The highest levels of incidence and severity were observed in the Rio Saliceto vineyard (approximately 21% and 15%, respectively) and the Correggio vineyard (approximately 18% and 13%, respectively) (Table 3). The lowest levels of symptom expression (7% incidence and 5% severity) were recorded in the Luzzara vineyard, while these values were close to 11% and 6% in the Fabbrico vineyard (Table 3).

Table 3. Incidence and severity of foliar symptoms of the chronic form of esca in vines treated with fertilizer mixture and in untreated vines in the experimental vineyards from 2021 to 2024.

Vineyard	Assessment Year	Treatment	Incidence (%)	Severity (%)
Rio Saliceto	2021	1—treated	21.81 a	15.88 a
		2—untreated	20.58 a	14.65 a
	2022	1—treated	6.17 b	3.91 b
		2—untreated	12.35 a	9.92 a
	2023	1—treated	15.02 b	6.67 b
		2—untreated	24.07 a	17.12 a
	2024	1—treated	24.69 a	17.10 a
		2—untreated	24.90 a	17.53 a
Luzzara	2021	1—treated	7.02 a	4.50 a
		2—untreated	7.31 a	5.03 a
	2022	1—treated	5.26 b	2.92 b
		2—untreated	11.40 a	7.08 a
	2023	1—treated	7.31 b	2.87 b
		2—untreated	13.74 a	9.59 a
	2024	1—treated	12.87 a	8.95 a
		2—untreated	13.16 a	9.30 a
Fabbrico	2021	1—treated	11.76 a	7.01 a
		2—untreated	10.54 a	5.74 a
	2022	1—treated	7.11 b	2.75 b
		2—untreated	13.97 a	8.28 a
	2023	1—treated	15.44 a	10.34 a
		2—untreated	15.93 a	11.67 a
	2024	1—treated	16.18 a	9.85 a
		2—untreated	16.67 a	11.32 a
Correggio	2021	1—treated	17.78 a	13.01 a
		2—untreated	18.79 a	13.38 a
	2022	1—treated	8.48 b	5.54 b
		2—untreated	15.35 a	11.43 a
	2023	1—treated	23.84 a	16.57 a
		2—untreated	23.23 a	17.37 a
	2024	1—treated	23.84 a	16.69 a
		2—untreated	24.24 a	17.25 a

In each vineyard, for each year of assessment, in each column, values followed by the same letter do not differ statistically according to Tukey's honest significant difference (HSD) test at $p = 0.05$.

In 2022, in all vineyards, the incidence and severity of foliar symptoms were statistically lower in vines treated with the fertilizer mixture compared to untreated ones, according to Tukey's honest significant difference (HSD) test at $p = 0.05$ (Table 3). In the Rio Saliceto, Luzzara, Fabbrico, and Correggio vineyards, the percentages of reduction in foliar symptom expression in treated vines, with respect to the untreated control vines, were similar for both incidence (50.04%, 53.86%, 49.11%, and 44.76%) and severity (60.59%, 58.76%, 66.79%, and 51.53%) (Table 3).

In 2023, a significant reduction in foliar symptom expression was confirmed in the vines treated with the mixture in the Rio Saliceto and Luzzara vineyards, compared to untreated controls: 37.6% and 46.8% for incidence, and 61.04% and 70.07% for severity, respectively (Table 3). However, in the same year, the incidence and severity of foliar symptoms in the Fabbrico and Correggio vineyards did not differ between treated and untreated vines (Table 3).

In 2024, in all vineyards, in the absence of treatments, no differences in symptom incidence or severity were observed in the plots previously treated with the mixture compared to the untreated control plots (Table 3).

Figures 1 and 2 show the canopy symptoms of the chronic form of esca in untreated control vines from the cv. Lambrusco experimental vineyards.

Figure 1. Tiger-stripe foliar symptoms on vines of the cv. Lambrusco infected with the chronic form of esca disease in the experimental vineyards. (**Left**) Chronic leaf symptoms at the pre-bunch closure stage. (**Right**) Advanced chronic leaf symptoms with dryness near the harvest.

Figure 2. Tiger-striped leaves and wilting of clusters on untreated vines of the cv. Lambrusco in the experimental vineyards.

3.2. Assessment of Apoplexy and Death of Plants

In 2021, the first year of the survey, the incidence of apoplectic plants in the plots chosen for mixture applications in subsequent years did not differ from that in the untreated control plots. The average incidence was 3.70%, 0.59%, 1.96%, and 2.22% in the plots selected for applications and 4.12%, 0.88%, 2.21%, and 2.63% in the untreated control plots in the Rio Saliceto, Luzzara, Fabbrico, and Correggio vineyards, respectively.

In all vineyards, in both years of mixture application (2022 and 2023), a significantly lower annual incidence of apoplectic vines was observed in treated vines compared to untreated vines (HSD test; $p = 0.05$) (Figure 3). In the Luzzara vineyard, the annual incidence of apoplexy in untreated vines was 2.34% in 2022 and 1.46% in 2023, while no apoplectic vines were ever observed in the treated plots (Figure 3). The highest annual incidence of apoplexy in treated vines, 0.61% in both 2022 and 2023, was observed in the Correggio vineyard, while even lower incidence percentages were recorded in treated vines in the Rio Saliceto and Fabbrico vineyards (Figure 3).

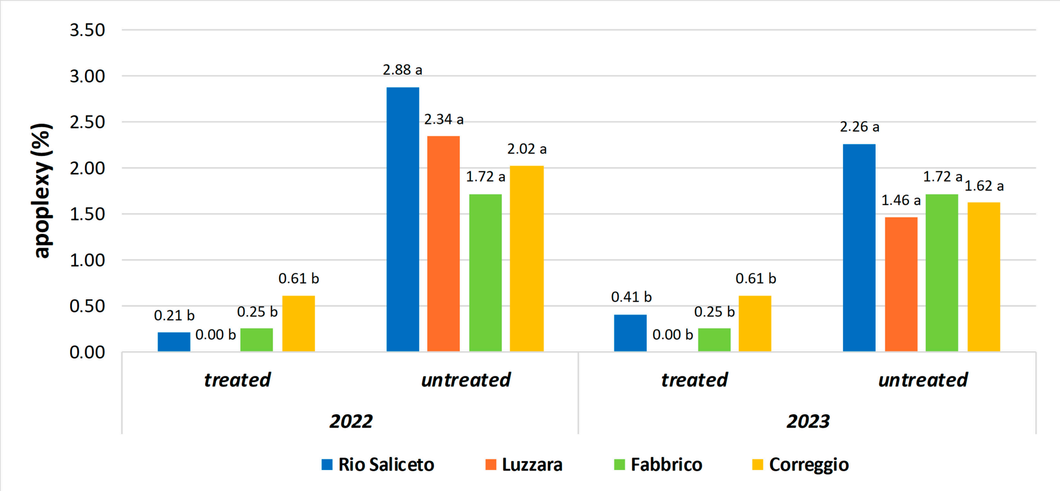

Figure 3. Mean annual incidence of apoplexy in vines treated with fertilizer mixture and in untreated vines of experimental vineyards. In each vineyard, Tukey's honest significant difference (HSD) test at $p = 0.05$ compared the fertilizer mixture treatments to the untreated controls. In each vineyard, the values under comparison followed by the same letter do not differ statistically.

Actual mortality levels due to apoplexy, calculated in the last year of the study (2024), considering as dead those vines affected by apoplexy that did not regrow in the following two years, were always significantly lower in vines treated with the fertilizer mixture than in untreated vines (HSD test; $p = 0.05$) (Figure 4). The only exception was observed in the Correggio vineyard, where the actual mortality (2.85%) for treated vines was not statistically lower than that (4.65%) of untreated vines (Figure 4).

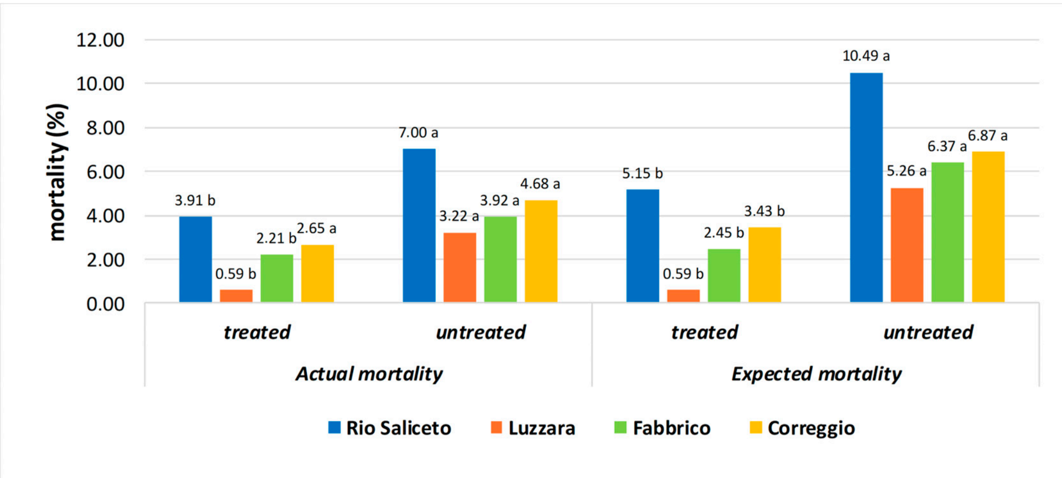

Figure 4. Mean actual and expected mortality in vines treated with fertilizer mixture and in untreated vines of experimental vineyards in 2024. In each vineyard, Tukey's honest significant difference (HSD) test at $p = 0.05$ compared the fertilizer mixture treatments to the untreated controls. In each vineyard, the values under comparison followed by the same letter do not differ statistically. All comparisons were statistically significant, except for actual mortality at Correggio vineyard.

However, in all vineyards, the expected mortality, representing all vines affected by apoplexy over the four years of the study, was always significantly lower in treated vines than in untreated ones (HSD test; $p = 0.05$) (Figure 4). The highest expected mortality was found in the untreated vines of the Rio Saliceto vineyard, at 10.49%, compared with 5.15% in the treated vines (Figure 4). A particularly marked reduction in expected mortality was observed in the treated vines of the Luzzara vineyard, with a value of 0.59%, compared with 5.26% in the untreated control (Figure 4). In the Fabbrico and Correggio vineyards, expected mortality reductions of 60% and 45% were observed in treated vines compared to untreated ones, respectively (Figure 4).

In all vineyards during the four years of the study, none of the vines affected by apoplexy regrew in subsequent years.

Figure 5 shows apoplectic symptoms in untreated control vines, while Figure 6 shows rows of vines treated with the fertilizer mixture without symptoms of esca on the canopy.

Figure 5. Apoplectic vine of the cv. Lambrusco in the experimental vineyards.

3.3. Quantity and Quality of the Yield

In 2022, in the Rio Saliceto, Luzzara, and Fabbrico vineyards, the mean yield per vine of apparently healthy vines treated with the fertilizer mixture was significantly higher than that of untreated vines (HSD test; $p = 0.05$) (Table 4). These higher yields per plant were 1.81 kg, 1.16 kg, and 1.06 kg in the Rio Saliceto, Luzzara, and Fabbrico vineyards, respectively (Table 4). Conversely, in the same year, in the Correggio vineyard, no differences were detected between the yields of treated and untreated vines, and the average yield per plant was 4.44 kg in both groups (Table 4).

In 2023, the effect of fertilizer applications on yields was no longer detectable, as no differences were observed between treated and untreated vines in any of the four vineyards (Table 4).

In 2022, a significantly higher soluble solids content was detected in the treated vines in the Rio Saliceto, Luzzara, and Fabbrico vineyards (HSD test; $p = 0.05$) (Table 4). In all vineyards, treated vines had soluble solids contents approximately 1°Brix higher than those of the untreated plots, while total acidity was statistically lower (Table 4). Differences in soluble solids content and total acidity were not detected in the Correggio vineyard in 2022 (Table 4).

In 2023, with respect to yield, no differences were recorded between treated and untreated vines in any of the quality parameters measured in the four vineyards (Table 4).

Figure 6. Rows of vines of the cv. Lambrusco treated with the fertilizer mixture and free of esca symptoms on the canopy near the harvest.

Table 4. Mean yield and quality parameters of the berries of apparently healthy vines treated with fertilizer mixture and of untreated apparently healthy vines of experimental vineyards in 2022 and 2023.

Vineyard	Assessment Year	Treatment	Yield (kg Vine^{-1})	Soluble Solids (° Brix)	pH	Total Acidity (g L^{-1})
Rio Saliceto	2022	1—treated	15.97 a	20.07 a	3.16 a	10.67 b
		2—untreated	14.16 b	19.07 b	3.06 a	11.87 a
	2023	1—treated	13.18 a	20.73 a	3.05 a	10.07 a
		2—untreated	13.16 a	20.33 a	3.03 a	10.43 a
Luzzara	2022	1—treated	9.03 a	20.20 a	3.16 a	9.00 b
		2—untreated	7.87 b	19.33 b	3.11 a	9.53 a
	2023	1—treated	6.63 a	20.13 a	3.05 a	10.83 a
		2—untreated	6.78 a	20.27 a	3.07 a	10.85 a
Fabbrico	2022	1—treated	9.02 a	21.73 a	3.28 a	10.20 b
		2—untreated	7.96 b	20.60 b	3.16 b	11.20 a
	2023	1—treated	7.58 a	19.73 a	3.17 a	10.87 a
		2—untreated	7.39 a	19.87 a	3.21 a	10.83 a
Correggio	2022	1—treated	4.44 a	18.80 a	3.17 a	10.70 a
		2—untreated	4.44 a	18.93 a	3.17 a	10.73 a
	2023	1—treated	3.76 a	19.90 a	3.06 a	10.90 a
		2—untreated	3.75 a	20.10 a	3.04 a	10.50 a

In each vineyard, for each year of assessment, in each column, values followed by the same letter do not differ statistically according to Tukey's honest significant difference (HSD) test at $p = 0.05$.

4. Discussion

The chronic form of esca disease causes yield losses mainly due to the occurrence of foliar symptoms [34]. This form often coexists with the acute form, which exhibits apoplectic symptoms, causing sudden shoot dieback, leaf drop, and, in most cases, plant death. In some cases, apoplectic vines may recover and produce new growth the following season [16].

Despite advances in understanding the disease's etiology and epidemiology, an effective control strategy has not yet been achieved [35,36]. However, protecting pruning wounds with Trichoderma has been shown to reduce infection occurrence over the years [22]. Furthermore, a foliar fertilizer mixture based on calcium, magnesium, and seaweed has been shown to reduce the occurrence of foliar symptoms in the chronic form of the disease [9,29,37]. The mixture does not act directly on the pathogens but instead affects mechanisms associated with foliar symptom expression [9]. Its activity leads to a significant reduction in yield losses, which are associated with the incidence and severity of leaf symptoms [8,38,39].

The present study tested the effects of fertilizer mixture applications on both chronic and acute form symptoms, as well as on yield quantity and quality, in a wine-growing area and cultivar that had never previously been investigated.

In 2022, in all experimental vineyards, rainfall from April to September was balanced and well distributed throughout the different growth stages, favoring the regular development of plant physiological processes [40]. Therefore, it is possible that these favorable conditions improved the activity of the mixture. In all vineyards, a significant reduction in chronic foliar symptoms in the treated vines was observed. Furthermore, except in the Correggio vineyard, this effect also enabled a significant increase in both the quantity and quality of yield.

In the 2023 growing season, the amount of rainfall was significantly higher than in 2022. This rainfall was particularly heavy in the first part of the season, likely favoring a higher occurrence of leaf symptoms compared to the previous year, thus confirming the relationship between rain and foliar symptoms [41–45].

Furthermore, the amount and frequency of rainfall from bud break to veraison likely hampered balanced plant vegetative development precisely during the period in which the processes leading to symptom onset take place [46]. Finally, due to the heavy rainfall, fewer applications, and in some cases at longer intervals, were made in 2023. It cannot be excluded that these negative conditions caused a lower effect of the fertilizer mixture on chronic symptoms in 2023 compared to 2022. However, even in 2023, in two of the four investigated vineyards, chronic symptoms in treated vines were significantly lower than those in untreated ones.

Finally, the unfavorable vegetative conditions due to the large amount of rainfall in 2023 were probably the main reason for the lack of improvement in yield quality and quantity observed in 2022.

A reliable hypothesis associated with the onset of foliar symptoms is linked to toxic substances produced by pathogens in the wood and translocated to the leaf via the transpiration stream [47]. Assumptions regarding the mechanisms of action of the fertilizer mixture against toxic substances and their effects have been discussed in previous studies and are briefly summarized below:

- Reduction in transpiration due to the CO_2-stabilizing effects of calcium oxalate druses within the leaf, which were found to a greater extent in treated leaves [9,48]. This reduction, by decreasing sap flow, leads to lower translocation of toxic substances towards the leaf [4,18,49].

- Strengthening of the cell wall due to the decomposition of calcium oxalate in the druses and the production of H_2O_2, reducing the diffusion of phytotoxic substances [9,17,50]. This effect is also explained by higher peroxidase activity, such as lipoxygenase, in leaves that then show symptoms [46].
- Reduction in the plant's oxidative response, triggered by toxic metabolites from infected wood. This is likely involved in the onset of leaf symptoms [51]. The calcium in the fertilizer mixture, translocated into the leaves by the seaweed component, can lead to accumulations of calmodulin, which can modulate the plant's response and reduce toxic effects [9,52–54].
- Induction of *trans*-resveratrol biosynthesis at early growth stages, before the appearance of symptoms on the canopy and up to pre-bunch closure. This could be hypothesized as a further mechanism of action of the fertilizer mixture [9,29]. It is also relevant to note that the treated vines, which remained asymptomatic due to the applications of the mixture, accumulated the calcium distributed during the pea-sized berry stage. During this stage, the peak of *trans*-resveratrol was recorded in the treated leaves, indicating the possible synergy of different mechanisms of action of the mixture [29,37].

Unlike chronic symptoms, in this study, the effect of the fertilizer mixture on apoplexy did not appear to be influenced by the different climatic conditions that occurred in the two years of applications. In fact, the annual incidence of apoplexy was significantly lower in treated vines compared to untreated vines in both 2022 and 2023. This effect was observed in the mortality assessed in 2024. Actual mortality was significantly lower in treated vines in all vineyards, except in the Correggio vineyard, where the reduction was not statistically different.

However, expected mortality, which included vines affected by apoplexy for only one year and therefore potentially able to recover, was significantly reduced in treated vines in all vineyards. As none of the vines affected by apoplexy recovered during the four years of the study, it can be stated that, in this experiment, expected mortality corresponds to actual mortality.

In any case, as this study reports for the first time the effects of the fertilizer mixture on reducing the acute form of esca, further studies should be conducted to confirm these effects in the vineyard and to investigate the mechanisms of action.

It cannot be excluded that the mechanisms of toxic translocation and detoxification described for the chronic form may also be involved in the acute form of the disease. The involvement of phytotoxins or toxic substances specifically related to the apoplexy symptom has not yet been described, although it may be considered a plausible supposition [55].

Before apoplexy, superoxide dismutase (SOD), responsible for detoxifying ROS, is drastically reduced [16,55,56]. Given the importance of calcium in activating SOD, it should be verified whether the mixture applications could facilitate SOD activity, supporting the plant against the effects of a strong defense reaction that can lead to the onset of apoplexy [57].

In the early growth stages, up to fruit set, the leaves of asymptomatic diseased vines treated with the fertilizer mixture had a higher water index than asymptomatic untreated leaves, indicating greater water-holding capacity [37]. Therefore, it will be possible to investigate whether this effect can effectively reduce the risk of complete weakening of the translocation system, particularly in plants affected by white rot, which are more susceptible to the acute form of esca disease [58,59].

A week before the onset of apoplectic symptoms, a high concentration of *trans*-resveratrol was found in the herbaceous organs of the plant [56], probably as an ineffective response to toxic substances. As described above for the chronic form of the disease, a similar increase in *trans*-resveratrol was found in the leaves of asymptomatic plants at early growth stages ("fruit set" and "pea-sized berries"), following applications of the fertilizer mixture [29]. Therefore, the early induction of *trans*-resveratrol biosynthesis, reported above as one of the possible mechanisms of action of the fertilizer mixture in containing the chronic symptom, could also be one of the putative mechanisms underlying the observed reduction in apoplexy of treated vines.

5. Conclusions

The fertilizer mixture of calcium, magnesium, and seaweed extracts confirmed its efficacy in reducing canopy symptoms in vines affected by chronic esca. This efficacy was more evident in seasons with rainfall that allowed the regular development of plant growth stages. Conversely, heavy rainfall in the early part of the season, from May to July, likely created less favorable conditions for the plants, just when the processes associated with the subsequent onset of foliar symptoms were active. Under these adverse conditions, a reduced effect of the fertilizer mixture on foliar symptoms and the absence of quantitative and qualitative improvements in yield were observed.

The effects of the mixture in reducing apoplexy, reported for the first time in this study, need to be confirmed with further research conducted in different grape-growing areas and cultivars, as well as by investigating the mechanisms of action. However, from this early evidence, the activity of the mixture against the acute form of esca appeared consistent and seemed less influenced by seasonal conditions.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy16040403/s1, Table S1: Total monthly rainy days and rainfall in the 2022–2024 growing seasons at experimental vineyards.

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