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Effect of Different Foliar Fertilizer Applications on Esca Disease of Grapevine: Symptom Expression and Nutrient Content in the Leaf and Composition of the Berry

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Abstract: Esca disease, the most common grapevine wood disease in Europe, causes yield losses correlated with the foliar symptoms' expression. In two vineyards located in the Abruzzo Region of Italy, each of which were investigated for esca symptoms after 1994, different applications of macro- and microelements were performed in two consecutive growing seasons. The main aim of the work consisted of verifying the effects of the fertilizer applications on the foliar symptoms' expression, in order to deepen knowledge of the nature of the symptom, which is still unclear. For each treatment, in each year, the leaf content of macro- and microelements and the composition of berries and musts were assessed. The effects of these applications on vegetative growth and yield quantity were also verified. The trials were carried out on symptomatic, asymptomatic and apparently healthy vines. All applications, in particular those with microelements, increased the foliar symptoms' expression, and a greater vegetative growth was detected only in vines treated with NPK fertilizers. The symptoms' increase was always associated in the leaf with a decrease of calcium, and to a lesser extent, magnesium, reinforcing the hypothesis of the plant's hypersensitive reaction in the development of foliar symptoms, given the role of calcium in the defense response. The vineyards were in nutritional balance regardless of the fertilizer applications. The general increase in foliar symptoms and the decrease in sugars in the musts of asymptomatic treated vines underlined the importance of the vegetative-productive balance, in Esca infected vineyards especially, in order to limit the symptoms' expression and the decrease in yield.

Keywords: grapevine esca disease; leaf macro and microelement applications; leaf symptom incidence and severity

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1. Introduction

Grapevine wood diseases (GTD) have significantly increased in recent decades in almost all vine-growing areas [1,2]. Esca complex is the most widespread GTD in Europe. The disease is mainly caused by the tracheomycotic fungi *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*, and by the basidiomycete *Fomitiporia mediterranea*, often with the involvement of Botryosphaeriaceae species [1,3]. The woody tissues of diseased vines show longitudinal brown streaking and/or necrotic areas produced by tracheomycotic fungi, while a white rot is caused by basidiomycete fungi. The typical foliar symptoms of the disease may appear on the foliage as tiger stripes, while the berries can show purple spots, cracks and drying up. A wilt of shoots occurs in late spring and summer, eventually affecting the entire branch [4,5].

In the nursery, the brown wood-streaking inside the cuttings is the result of pathogen infection occurring at different stages of the grapevine's propagation process [6]. The presence of brown wood-streaking in the cuttings, often as latent infections, is one of the major signs of young plants' decline and Petri disease in the vineyard. In any case, plants already contaminated by tracheomycotic pathogens are more likely to have reduced productivity and longevity and become a major potential source of inoculum [7]. The infections of planting material have led to the assessment of different tools in order to reduce the contaminations of rootstock mother vines and cuttings, such as the use of Trichoderma, hot water (HWT) and electrolyzed acid water (EAW) [8,9]. In particular, a protocol for the management of grapevine rootstock mother vine for the definition of a management strategy in the nursery was recently proposed [10].

In the vineyard, the infections caused by tracheomycotic fungi, mainly through pruning wounds, lead to the appearance of grapevine leaf stripe symptoms on the leaf, which are associated with grapevine leaf stripe disease (GLSD). In most cases, the woody tissues of infected vines are characterized by the concomitant presence of white rot, and the disease is traditionally reported as "esca of grapevine" [11]. This is the most common disease of the esca complex, one where the white rot causes serious worsening of plant phytosanitary status, both due to the wood's deterioration and a greater severity of expression of the leaf's symptoms, given the correlation between foliar symptoms and white rot [12,13].

In the vineyard, plants contaminated in the nursery or infected by pruning wounds can show foliar symptoms and wilt of shoots after a variable number of years. Yield losses are directly correlated with the symptoms' incidence and severity [14,15], but not necessarily with the amount of necrotic wood in the trunk and shoots [16].

The incidence and severity of foliar symptoms are associated with meteorological factors, the rainfall in June and July in particular. The amount of rainfall has been demonstrated to be correlated with the severity of foliar symptom expression [17]. Furthermore, the cultural practices aimed at reducing infection occurrence and its effect on the plant seem to play a role in the expression of foliar symptoms [1,18]. The factors and treatments briefly described above may affect the expression of foliar symptoms. The nature of the mechanisms associated with foliar symptoms is still debated. Symptoms have been correlated with the toxic metabolites produced by the pathogens in the infected wood and translocated to the leaf through the transpiration stream [19–21].

These metabolites would trigger a hypersensitivity reaction in the plant, with the development of interveinal chlorosis, and then the typical tiger stripe leaf symptom [5,22–25]. In the symptomatic leaves, the synthesis of phytoalexins increase with the increase of the leaf necrotic area. This would agree with the synthesis of antimicrobial compounds observed after the occurrence of necrosis as a result of the hypersensitivity reaction [26].

Further hypotheses on the nature of foliar symptoms have either focused on sap flow alterations and occlusions of gum and tylose vessels, or nongaseous embolisms and the role of infections on the year's shoots [27–30].

The impossibility of eradicating the disease in the infected plant, the correlation between symptom expression and yield loss, and the lack of quantitative and qualitative decreases in asymptomatic vines compared to healthy vines [13,31] have stimulated studies aimed at understanding the mechanisms that regulate the expression of foliar symptoms, seeking to improve the ability of infected plants to reduce or mask the expression of the disease in the leaf [32].

In particular, one study assessed the effect of foliar applications with different nutrients on the disease's foliar symptoms' appearance, and on the quality parameters of the asymptomatic bunches of symptomatic vines. The results highlighted the increase in incidence and severity of foliar symptoms in the treated vines, and no difference in the main quality parameters between asymptomatic bunches of symptomatic treated and untreated vines was found [33].

A higher calcium content in the leaves of asymptomatic diseased vines was then recorded [34]. This result led to further studies consisting in leaf applications of a mixture of calcium, magnesium and seaweed in the growing season up to the pre-bunch closure. The applications significantly reduced the expression of foliar symptoms, and this reduction was probably due to different modes of action of the mixture [35–37]. The role of calcium, magnesium and sodium have recently been investigated and highlighted as different possible mechanisms of interaction, when the mixture applications interact on naturally asymptomatic vines or on vines asymptomatic due to the applications [11].

These outcomes and the need to further investigate the nature of the mechanisms involved in the foliar expression of the disease have led us to provide unpublished data recorded in 2006 and 2007, in order to provide more information on this topic. In fact, the nature of the leaf symptom has not yet been clarified. The present study reports the effects of the applications of different foliar fertilizers on foliar symptom expression. Furthermore, analyses were made on the contents of macro- and microelements in the leaves and berries of diseased and apparently healthy vines, which were either treated or untreated with fertilizers. Finally, assessments were carried out on the effect of the applications on vegetative growth and on the quantitative and qualitative yield parameters.

2. Materials and Methods

2.1. Leaf Nutrient Applications

This study was carried out in 2006 and 2007 in two 28-year-old vineyards monitored for esca foliar symptoms since 1994. The vineyards were located in Controguerra and Giulianova, Province of Teramo, Abruzzo Region, central Italy. The Controguerra and Giulianova vineyards were trained on overhead trellis systems, Geneva Double Curtain (GDC) and Tendone, respectively.

In the period 1991–2021, the average annual rainfall was 925 mm at Controguerra and 696 mm at Giulianova. The annual highest incidence of leaf symptoms was recorded in both vineyards in 1999, 35.6% in Controguerra, and 25.9% in Giulianova. Both vineyards had a clay-calcareous soil, with an alkaline pH of 8.45 and 8.36 at Controguerra and Giulianova, respectively.

In 2006, for each vineyard, two plots were set up, each plot consisting of 309 vines in 8 rows and 584 vines in 8 rows, in Controguerra and Giulianova, respectively. Foliar applications were performed in one of the two plots (the other one as control) and with the same products used in a previous study conducted in 2004 and 2005 [33]. The present study included a smaller number of applications compared to that of 2004 and 2005, and the end of applications occurred at BBCH growth stage 77, "berries beginning to touch", instead of, as in 2004 and 2005, growth stage 85, "softening of berries" (Table 1).

In 2007 the number of applications was further reduced in both vineyards, as compared to 2006, as indicated below. In the Controguerra vineyard, 3 plots, each consisting of 209 plants in 6 rows, were considered; two plots were treated with NPK ternary fertilizer (23-11-11) and microelement humates, respectively, while the third plot was the control (Table 2). One of the 2 plots of Giulianova vineyard was treated with 23-11-11; the other one was the control (Table 2).

The growth stages were described according to Lorenz et al., 1995 [38].

The harvest was made on 18 and 19 September 2006, and on 19 and 20 September 2007, at Giulianova and Controguerra vineyards, respectively.

The applications were carried out with an air blast sprayer, distributing the fertilizers in a water volume of 500 L ha^{-1}. The details of the products used are described in Calzarano et al., 2007 [33].

Table 1. Field application details in Giulianova and Controguerra vineyards in 2006.

BBCH Growth Stages	Application Data	Nutrients	Dose (kg/L ha^{-1})
Five leaves unfolded—15	19 May	Iron humate	1
Nine or more leaves unfolded—19	26 May	NPK 15-36-13 Microelement humate "S" bioactivator	1 1 0.3
Nine or more leaves unfolded—19	31 May	Iron-humate	1
Inflorescens fully developed; flowers separated—57	8 June	NPK 15-36-13 Microelement humate "S" bioactivator	1.5 1.5 0.4
Fruit set: young fruit begin to swell—71	20 June	NPK 23-11-11 Microelement humate "S" bioactivator	1.5 1.5 0.4
Berries groat-sized—73	30 June	NPK 23-11-11 Microelement humate "S" bioactivator	1.5 1.5 0.4
Berries pea-sized—75	11 July	NPK 12-18-32 Ca-Mg-B solution "S" bioactivator	1.5 1.5 0.4
Berries beginning to touch—77	21 July	NPK 8-16-50 "S" bioactivator	1.5 0.4

BBCH: Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie.

Table 2. Field application details in Giulianova and Controguerra vineyards in 2007.

BBCH Growth Stages	Application Data	Nutrients			Dose (kg ha^{-1})
		Controguerra Vineyard (Plot 1)	Controguerra Vineyard (Plot 2)	Giulianova Vineyard	
Berries groat-sized—73	3 July	NPK 23-11-11	Microelement humate	NPK 23-11-11	1.5
Berries pea-sized—75	17 July	NPK 23-11-11	Microelement humate	NPK 23-11-11	1.5
Berries beginning to touch—77	31 July	NPK 23-11-11	Microelement humate	NPK 23-11-11	1.5

BBCH: Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie.

2.2. Leaf Symptom Surveys

The Controguerra and Giulianova vineyards were surveyed every year, from 1994 to 2007, assessing both the incidence and severity of esca symptoms. Thanks to this multi-year survey, it might be possible to classify different types of vines, discriminating asymptomatic vines, as diseased vines that have shown symptoms in at least one surveyed growing season, from apparently healthy vines that were never symptomatic in all the years of survey [11]. Therefore, the effects of the foliar applications were assessed only on diseased vines, which are (i) the symptomatic vines, and (ii) the asymptomatic vines that have shown the symptoms on their canopy in at least one of the years of the survey.

An arbitrary seven-point symptom scale (as reported in Calzarano et al., 2007) was used to evaluate the levels of symptom severity [33].

The first four classes of the scale corresponded to progressively-increasing levels of extension of leaf symptoms on the canopy; class 5 included vines affected by apoplexy, but potentially able to vegetate the following season. The plants that were certainly dead because they were no longer vegetating for two consecutive seasons were included in class 6; class 7 referred to the vines included in class 6 the previous year of survey.

The apoplectic and dead vines were excluded from the calculation of incidence and severity of foliar symptom assessment.

In each year and for each plot of each vineyard, the incidence of the foliar symptoms of esca was calculated by dividing the number of symptomatic vines by the total number of diseased plants. The foliar symptom severity was calculated using the formula $\sum N \times 100/(Y \times Z)$, where $\sum N$ = sum of the severity values for symptoms; Y = number of infected plants (asymptomatic and symptomatic); and Z = maximum value of symptoms [39].

Leaf symptom surveys were carried out every year in September, immediately before harvesting, when the incidence and severity of symptoms reached their maximum seasonal levels. In particular, the surveys were carried out on 18 and 19 September 2006, and on 19 and 20 September 2007, in Controguerra and Giulianova vineyards, respectively.

2.3. Leaf and Grape Berry Sampling

In both years of the study, in each plot of the Controguerra vineyard, the bunches of 6 vines located in the central part of the plot were collected and weighed in order to estimate the grape yields. Moreover, samples of leaves and berries were collected for chemical analysis. In both years of trials, in each plot and for each vine type, leaves were sampled from 3 groups of 3 vines each, at the beginning of BBCH growth stage 83, "berries developing color". From each of the 3 vines of each group, 6 leaves were taken from the median part of the primary shoots of the two differently exposed parts (3 leaves exposed to east and 3 to west) of the canopy. Thus, 3 samples were obtained for each type of vine, each consisting of 18 leaves. In 2007, in symptomatic vines, from each of the 3 vines of each group, a 6-leaf sample was collected from both symptomatic and asymptomatic shoots. Therefore, 6 samples of 18 leaves were obtained for each type of vine, 3 samples from symptomatic and 3 samples from asymptomatic shoots.

At 89, the "berries ripe for harvest" stage, from each plot and for each of the 3 types of vines, berries were collected from 3 groups of 6 vines each. From each of the 6 plants of the group, 1 kg of berries were taken from the wings, tips and central portions of the bunch. Thus, 3 samples were obtained for each type of vine, each consisting of 6 kg of berries.

In 2006, the sampling of leaves and berries were carried out on (i) symptomatic, (ii) asymptomatic diseased vines and (iii) apparently healthy ones, treated and untreated.

In 2007, (i) symptomatic and asymptomatic leaves of symptomatic vines, treated with 23-11-11 NPK fertilizers or with microelement humates, (ii) symptomatic and asymptomatic leaves of untreated symptomatic diseased vines and (iii) leaves of untreated apparently healthy vines were collected. The berries were collected from the same types of vines: symptomatic and treated with 23-11-11 NPK fertilizers or with microelement humates, symptomatic and untreated, and apparently healthy and untreated. In both 2006 and 2007, the berries collected from symptomatic vines were free of lesions and were taken from a part of the plant that did not show foliar symptoms.

2.4. Chemical Analysis of Leaf and Grape Berry

The collected leaves were immediately washed with deionized water, blotted dry, and dried in an oven at 70 °C. The leaf samples of the different types of vines were then analyzed to determine the contents of nitrogen, phosphorus, potassium, magnesium, calcium, iron, manganese, zinc and sodium.

The harvested berries were weighed on an analytical balance and then ground in a mortar. Half of the sample was used for the analysis of the same macro- and microelements assessed in the leaves. The other half was pressed to obtain must from the skins and seeds. The must was analyzed for total acidity, malic acid, tartaric acid, soluble solids and pH.

The analyses of soluble solids, pH, and total acidity in musts were carried out following the methods of the Official Journal of the European Communities Regulation (EEC) No. 2676/90, Official Journal L 272, 3.10.1990 [40].

The materials and methods used for the determination of macro- and microelements in leaves and berries, and for malic and tartaric acid in musts are reported in Calzarano et al., 2009 [34].

2.5. Effect of Applications on Vegetative Growth

The effect of foliar applications on the vegetative growth of the plants was evaluated in the Controguerra vineyard in both years of the study.

In 2006, the leaf areas of treated healthy, asymptomatic, and symptomatic vines were compared with the leaf areas of the corresponding untreated types of vine. In symptomatic vines, only the surfaces of leaves harvested from asymptomatic shoots were evaluated.

In 2007, the leaf areas of asymptomatic leaves of symptomatic diseased vines treated with either NPK 23-11-11 fertilizers or with microelements, asymptomatic leaves of untreated symptomatic diseased vines, and leaves of untreated, apparently healthy, vines were compared.

For each treatment, 24 primary shoots were chosen, 12 exposed to east and 12 to west. The shoots were collected at the beginning of BBCH growth stage 83, "berries developing color". The leaves of the primary shoots were collected and photographed separately from the leaves of the secondary shoots.

Each leaf area was recorded and processed by Image-pro plus version 7.0 (Media Cybernetic Inc., Silver Spring, MD, USA). The leaf areas of each primary shoot of each treatment, and separately, of each secondary shoot, were then added up to obtain the total leaf area of each shoot.

2.6. Statistical Analysis

In both vineyards and for all the years of the trial, foliar symptom incidence and severity which were recorded in control plots were compared with those plots subjected to foliar fertilizer applications, by means of Chi-square tests at $p \leq 0.05$, according to the method reported in Calzarano et al. (2007) [33].

For each vineyard and year of the trial, a one-way analysis of variance (ANOVA) was applied to compare the effects of applications on macro- and microelements contents recorded from each type of vine in leaves and berries, and on the parameters of must. When significant differences among the investigated parameters occurred, Tukey's honest significant difference (HSD) test was performed at $p = 0.05$.

In 2006, the leaf areas of each type of untreated vine were compared with the leaf areas of the corresponding type of treated vines. In 2007, the leaf areas of untreated apparently healthy vines were compared with the leaf areas of untreated, symptomatic vines or symptomatic vines treated with either NPK 23-11-11 fertilizer or microelements. Data of the comparisons of these pairs were subjected to analysis of variance and Student's *t*-test at $p = 0.05$.

Statistical analysis was performed using SAS 9.3 statistical program (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Incidence and Severity of Esca Foliar Symptoms

In both vineyards and for each year of the trial, the foliar application of nutrients generally increased the incidence and severity of the foliar symptoms of esca.

In 2007, in the Controguerra vineyard, the plot treated with NPK 23-11-11 foliar fertilizer showed a statistically insignificant increase of foliar symptom incidence (2.1%) compared with the untreated control. On the contrary, the increase in symptom severity (1.4%) was significant in comparison with the control (Figures 1 and 2; Table 3). In both years of the trial, the increase in incidence and severity of leaf symptoms observed in the other treatments was significant in comparison with the control (Figures 1 and 2; Table 3).

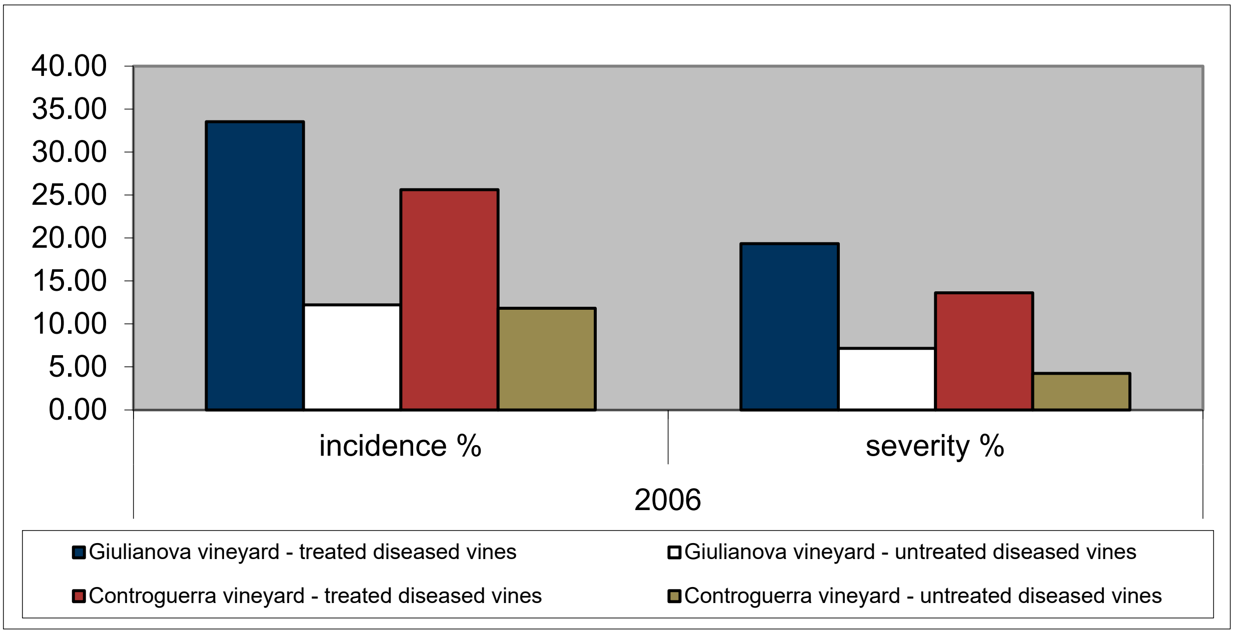

Figure 1. Incidence and severity of esca's leaf symptoms on vines treated and untreated with nutrients in the 2006 growing season.

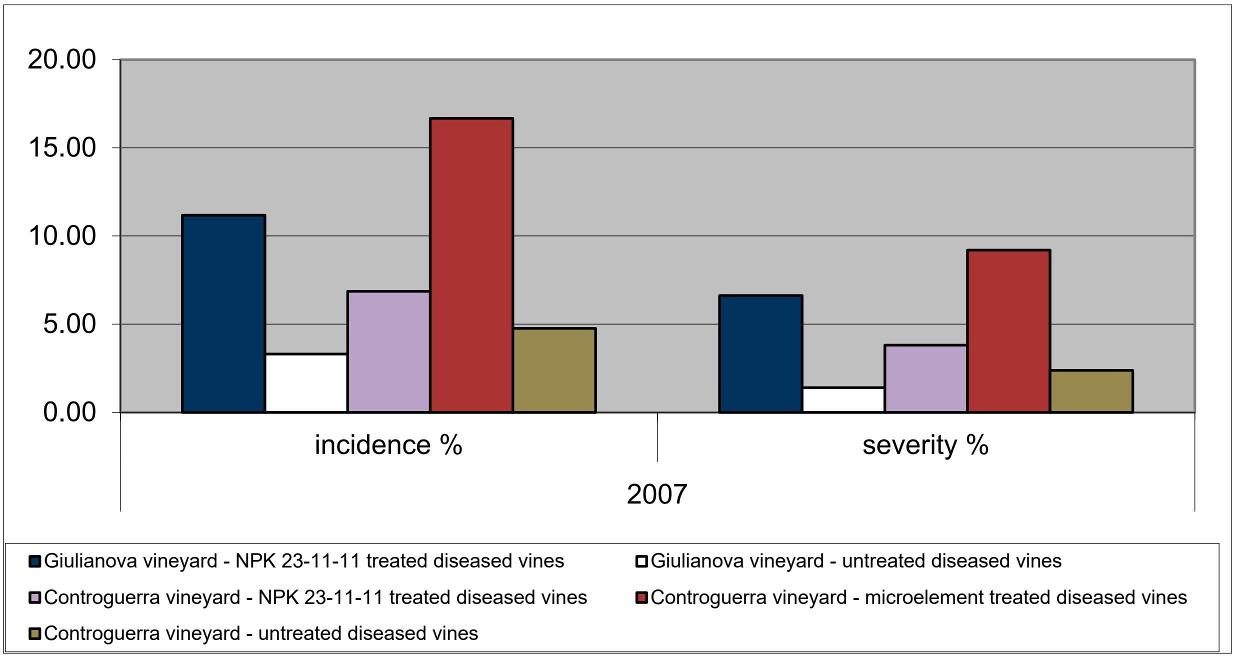

Figure 2. Incidence and severity of esca's leaf symptoms on vines treated and untreated with nutrients in the 2007 growing season.

In 2007, in the Controguerra vineyard, the highest incidence and severity of leaf symptoms were recorded in the plot treated with microelements, with 16.7% and 9.2%, respectively, while in the plot treated with NPK 23-11-11 fertilizer, and in the untreated plot, incidence and severity were 6.9% and 3.8%, or 4.8% and 2.4%, respectively (Figures 1 and 2; Table 3).

Table 3. Significance of the differences in incidence and severity of the foliar symptoms of esca between vines treated with nutrients and untreated vines according to χ^2 tests, at $p = 0.05$, in 2006 and 2007, in Controguerra and Giulianova vineyards.

Vineyard	Comparison among Treatments	2006		2007	
		Incidence %	Severity %	Incidence %	Severity %
Controguerra	Treated diseased vines/untreated diseased vines	0.0004	<0.0001	/	/
	Microelement treated diseased vines/untreated diseased vines	/	/	0.0014	<0.0001
	NPK 23-11-11 treated diseased vines/untreated diseased vines	/	/	0.4495	0.0002
Giulianova	Treated diseased vines/untreated diseased vines	<0.0001	<0.0001	/	/
	NPK 23-11-11 treated diseased vines/untreated diseased vines	/	/	0.0043	<0.0001

In 2006, in the untreated control plot, the incidence and severity of symptoms in both Controguerra (11.8% and 4.2%) and Giulianova (12.2% and 7.2%) vineyards were higher than those in the untreated plots in 2007 (Figures 1 and 2; Table 3). Accordingly, in 2006, in the treated plots, the highest values of incidence and severity of the two-year trial period were observed both in Giulianova (33.5% and 19.3%) and in Controguerra (25.6% and 13.6%), respectively (Figures 1 and 2; Table 3).

3.2. Macro- and Microelements in the Leaf

3.2.1. Analysis of the 2006 Growing Season

In 2006, in the untreated control plot of the Controguerra vineyard, a higher and statistically significant calcium content was observed in the leaves of both asymptomatic diseased vines (31.24 g kg^{-1}) and apparently healthy vines (30.53 g kg^{-1}), compared to that recorded in the leaves of symptomatic diseased vines (24.42 g kg^{-1}) (Table 4).

Table 4. Macro- and microelements in symptomatic leaves from symptomatic vines and in leaves from asymptomatic and apparently healthy grapevines, treated and untreated, in 2006.

Leaf		N	P	K (g kg^{-1})	Ca	Mg	Fe	Mn	Zn (mg kg^{-1})	Na
Untreated vines	Symptomatic	21.01 a	1.94 a	5.99 a	24.42 b	2.79 a	139.92 a	60.02 a	24.03 a	95.33 a
	Asymptomatic	21.23 a	1.98 a	8.81 a	31.24 a	2.82 a	121.02 a	67.91 a	23.82 a	104.32 a
	Healthy	21.81 a	1.91 a	8.97 a	30.53 a	2.80 a	110.11 a	54.83 a	24.22 a	93.27 a
Treated vines	Symptomatic	24.02 a	2.15 a	6.16 a	19.61 b	2.29 a	102.03 a	45.63 a	26.12 a	94.24 a
	Asymptomatic	21.32 a	2.21 a	6.68 a	23.64 b	1.93 a	105.02 a	52.44 a	29.52 a	93.56 a
	Healthy	22.63 a	2.25 a	8.83 a	21.81 b	2.02 a	99.24 a	53.63 a	24.74 a	90.04 a

For 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 the treated plot, a decrease of calcium in the leaf of all types of vines was observed, one which no longer differed from each other (Table 4).

The contents of the other elements did not register significant differences in the leaves of the different types of vines, both in the treated and in the untreated plots (Table 4).

3.2.2. Analysis of the 2007 Growing Season

In 2007, the leaves of asymptomatic shoots of untreated symptomatic vines showed a significantly higher calcium content (37.06 g kg^{-1}) than both the leaves of untreated symptomatic shoots (28.52 g kg^{-1}) and the leaves of untreated apparently healthy vines (31.13 g kg^{-1}) (Table 5). The magnesium content was significantly higher in the leaves of untreated apparently healthy vines (5.57 g kg^{-1}) and the leaves of asymptomatic shoots of untreated symptomatic vines (4.94 g kg^{-1}) than in the leaves of untreated symptomatic shoots (3.67 g kg^{-1}). (Table 5).

Table 5. Macro- and microelements in symptomatic and asymptomatic leaves from treated and untreated symptomatic grapevines and in leaves from untreated apparently healthy grapevines in 2007.

Leaf		N	P	K (g kg^{-1})	Ca	Mg	Fe	Mn (mg kg^{-1})	Zn	Na
NPK 23-11-11-treated diseased vines	Symptomatic shoot	14.41 a	1.76 a	4.17 a	29.02 bc	3.55 b	119.00 ab	64.82 a	25.42 c	120.03 a
	Asymptomatic shoot	15.02 a	1.56 ab	3.60 ab	33.53 ab	3.54 b	118.03 ab	67.05 a	24.91 c	112.01 ab
Microelements-treated diseased vines	Symptomatic shoot	15.34 a	1.34 bc	3.54 ab	24.82 c	2.97 b	132.06 a	68.13 a	52.31 a	102.11 bc
	Asymptomatic shoot	16.43 a	1.24 c	3.13 ab	31.43 bc	3.24 b	89.92 c	64.72 a	40.53 b	92.42 c
Untreated diseased vines	Symptomatic shoot	17.62 a	1.40 bc	3.71 ab	28.52 bc	3.67 b	113.11 b	64.51 a	32.12 bc	101.03 bc
	Asymptomatic shoot	16.10 a	1.31 bc	3.21 ab	37.06 a	4.94 a	110.06 b	68.20 a	31.73 bc	89.14 c
Untreated healthy vines		16.24 a	1.28 bc	2.77 b	31.13 bc	5.57 a	87.43 c	48.23 b	25.64 c	104.06 bc

For each column, values followed by the same letter do not differ statistically according to Tukey's honest significant difference (HSD) test at $p = 0.05$.

The contents of iron, manganese and zinc in the leaves of untreated and apparently healthy vines was lower, in most of the cases, than in the leaves of symptomatic and asymptomatic shoots of untreated symptomatic vines (Table 5).

The content of calcium was lower in the leaves of asymptomatic shoots of symptomatic vines both those treated with NPK 23-11-11 fertilizers (33.53 g kg^{-1}) and those treated with microelements (31.43 g kg^{-1}), in comparison to the content of untreated asymptomatic shoots. Because of this decrease in calcium, the calcium content did not differ in leaves of treated symptomatic and asymptomatic shoots. The calcium content of the treated shoots also did not differ from the content of the untreated and apparently healthy vines (Table 5).

The leaf contents of iron, manganese and zinc did not differ between NPK 23-11-11-treated symptomatic vines and untreated symptomatic vines (Table 5). Conversely, the content of iron (132.06 mg kg^{-1}) and zinc (52.31 mg kg^{-1}) significantly increased in the leaves of symptomatic shoots treated with microelements, compared to (i) the leaves of symptomatic untreated shoots (113.11 mg iron kg^{-1} and 32.12 mg zinc kg^{-1}), (ii) the leaves of untreated healthy vines (87.43 mg iron kg^{-1} and 25.64 mg zinc kg^{-1}) and (iii) the leaves of asymptomatic shoots treated with microelements (89.92 mg iron kg^{-1} and 40.53 mg zinc kg^{-1}) (Table 5).

The content of manganese in the leaves of all symptomatic vine shoots, treated and untreated, was higher than that of untreated and apparently healthy vine leaves (Table 5). With regard to the other elements analyzed, significantly higher contents of phosphorus, potassium and sodium were found in the leaves of symptomatic shoots treated with NPK 23-11-11 fertilizers, compared to the content recorded in the untreated healthy vines (Table 5).

3.3. Macro- and Microelements in the Berry

3.3.1. Analysis of 2006 Growing Season

In 2006, in the untreated control plot of the Controguerra vineyard, significantly higher nitrogen content was detected in the berries of symptomatic vines (9.58 g kg^{-1}) compared to the berries of asymptomatic (6.02 g kg^{-1}) and apparently healthy (6.42 g kg^{-1}) vines

(Table 6). An evident trend to higher contents of phosphorus and potassium in the berries of untreated symptomatic vines could also be noticed (Table 6).

Table 6. Macro- and microelements in berries without lesions from symptomatic vines and in berries from asymptomatic and apparently healthy grapevines, treated and untreated, in 2006.

Berry		N	P	K	Ca	Mg	Fe	Mn	Zn	Na
				($g\ kg^{-1}$)				($mg\ kg^{-1}$)		
Untreated vines	Symptomatic	9.58 a	1.52 a	12.13 a	1.75 a	0.58 b	22.12 a	12.21 a	9.77 a	38.02 a
	Asymptomatic	6.02 c	1.36 ab	10.40 ab	1.10 a	0.46 bc	19.61 ab	7.42 b	6.18 b	29.44 a
	Healthy	6.42 bc	1.18 abc	10.60 ab	0.88 a	0.41 c	16.11 c	7.28 b	6.37 b	27.06 a
Treated vines	Symptomatic	8.11 ab	1.09 abc	9.87 bc	1.81 a	0.84 a	18.42 bc	6.48 b	6.49 b	38.51 a
	Asymptomatic	5.38 c	0.86 bc	8.74 bc	1.26 a	0.45 bc	19.34 ab	4.89 b	6.36 b	35.26 a
	Healthy	4.90 c	0.75 c	8.26 c	1.17 a	0.43 c	16.15 c	4.23 b	4.50 c	37.03 a

For each column, values followed by the same letter do not differ statistically according to Tukey's honest significant difference (HSD) test at $p = 0.05$.

Magnesium (0.58 g kg^{-1}), iron (22.12 mg kg^{-1}), manganese (12.21 mg kg^{-1}) and zinc (9.77 mg kg^{-1}) were also found to be significantly higher in the berries of untreated symptomatic vines than in the berries of untreated and apparently healthy vines (0.41 g kg^{-1}, 16.11 mg kg^{-1}, 7.28 mg kg^{-1}, and 6.37 mg kg^{-1}), respectively (Table 6). The content of iron recorded in the berries of untreated asymptomatic vines was also significantly higher compared to the berries of untreated apparently healthy vines (Table 6).

In 2006, in the treated plot of the Controguerra vineyard, the nitrogen, phosphorus, and potassium contents were generally lower in all vine types than in the ones of the untreated plot (Table 6). However, the nitrogen amount found in the berries of symptomatic vines (8.11 g kg^{-1}) was significantly higher than in those of the asymptomatic (5.38 g kg^{-1}) and apparently healthy (4.90 g kg^{-1}) vines (Table 6). The potassium content in the berries of treated symptomatic vines was significantly lower than the ones of untreated symptomatic vines. (Table 6).

In the treated plot, the iron, manganese and zinc content in the berry also tended to be lower in comparison to the levels observed in the untreated plot. In particular, the contents of these microelements were significantly lower in the berries of symptomatic vines with respect to the contents observed in the corresponding untreated vines (Table 6). The content of magnesium in the berries of symptomatic treated vines was significantly higher than the levels in both the berries of untreated symptomatic vines and of the berries of all the other types of vines, both treated and untreated (Table 6).

3.3.2. Analysis of 2007 Growing Season

In 2007, berries from untreated symptomatic vines had generally higher contents of nitrogen, phosphorus, potassium, iron and manganese than those of berries from untreated apparently healthy vines and vines treated with NPK 23-11-11 or microelement fertilizer (Table 7). No difference was found among the berries of the different types of vines for calcium, magnesium and zinc (Table 7). The amount of sodium was statistically higher in the berries of untreated healthy vines than in the berries of untreated symptomatic vines or those treated with NPK 23-11-11 or microelements (Table 7).

Table 7. Macro- and microelements in berries without lesions from treated and untreated symptomatic vines and in berries from untreated apparently healthy grapevines in 2007.

Berry	N	P	K (g kg^{-1})	Ca	Mg	Fe	Mn	Zn (mg kg^{-1})	Na
NPK 23-11-11 treated symptomatic vines	3.93 b	0.92 ab	9.48 b	0.83 a	0.60 a	26.03 a	5.68 c	5.73 a	26.14 b
Microelements treated symptomatic vines	3.77 b	0.83 b	9.91 b	0.78 a	0.50 a	19.21 b	7.84 b	5.75 a	32.15 b
Untreated symptomatic vines	5.72 a	1.11 a	12.8 a	1.02 a	0.57 a	31.53 a	9.86 a	5.27 a	26.33 b
Untreated healthy vines	3.62 b	0.87 b	10.29 ab	1.05 a	0.56 a	28.32 a	9.56 ab	5.11 a	51.82 a

For 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.

3.4. Yield Amount and Must Parameters

In 2006, the yield of untreated asymptomatic and apparently healthy vines was very similar, 14.3 and 14.0 kg plant^{-1}, whereas the yield of untreated symptomatic vines was 4.8 kg plant^{-1}. Applications of foliar fertilizers increased the yield in all the vine types. In the treated vines the yield was 16.6, 17.0, and 7.0 kg plant^{-1}, in healthy, asymptomatic and symptomatic vines, respectively.

In 2007, the yield of untreated healthy vines was 13.4 kg plant^{-1}, while that of untreated symptomatic vines and symptomatic vines treated with NPK 23-11-11 fertilizer or treated with microelements was 3.8, 6.4 and 4.1 kg plant^{-1}, respectively.

In 2006, in the untreated plot, the content of soluble solids in the musts of healthy (199 g L^{-1}) and asymptomatic (190 g L^{-1}) vines did not differ from each other, whereas in the musts of symptomatic vines, the content of soluble solids (150 g L^{-1}) was significantly different (Table 8). Consequently, a higher level of total acidity was detected in the musts of symptomatic vines, due to the higher content of malic acid, as compared to the musts of the other vine types (Table 8).

Table 8. Chemical analyses of musts from berries without lesions of symptomatic vines and from berries of asymptomatic and apparently healthy grapevines, treated and untreated, in 2006.

Berry		Soluble Solids (g L^{-1})	Total Acidity (g L^{-1})	Tartaric Acid (g L^{-1})	Malic Acid (g L^{-1})	pH
Untreated vines	Symptomatic	150 b	8.18 a	4.73 bc	4.46 a	3.34 c
	Asymptomatic	190 a	6.26 b	4.45 c	2.81 b	3.54 abc
	Healthy	199 a	5.53 bc	4.43 c	2.50 b	3.57 ab
Treated vines	Symptomatic	151 b	6.13 b	4.94 ab	2.99 b	3.39 bc
	Asymptomatic	156 b	5.08 cd	5.34 a	1.42 c	3.48 abc
	Healthy	189 a	4.53 d	4.71 bc	1.23 c	3.63 a

For 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 2006, in the treated plot, a clear lowering of the levels of soluble solids was detected in the musts of asymptomatic vines (156 g L^{-1}), while in the musts of healthy and symptomatic vines, no variations were observed with respect to the soluble solids detected in the corresponding types of untreated vines (Table 8).

In 2006, in the musts of all the treated vines, the total acidity was significantly lower than that of the corresponding types of untreated vines. This decrease was due to the decrease in the malic acid content (Table 8).

In 2007, the soluble solid content in the musts of symptomatic vines, both treated and untreated, was lower than that of untreated healthy vines (188 g L^{-1}) (Table 9). In particular, the lowest content of soluble solids was observed in the musts of symptomatic vines treated with NPK 23-11-11 fertilizer, at 149 g L^{-1}, and the highest content in the musts of symptomatic vines treated with microelements, at 178 g L^{-1} (Table 9). In 2007, the total acidity did not differ in the vines of all different treatments, despite the differences in the contents of malic acid highlighted among the treatments (Table 9).

Table 9. Chemical analyses of musts from berries without lesions of symptomatic vines, treated and untreated, and from berries of apparently healthy grapevines, in 2007.

Berry	Soluble Solids (g L^{-1})	Total Acidity (g L^{-1})	Tartaric Acid (g L^{-1})	Malic Acid (g L^{-1})	pH
NPK 23-11-11-treated symptomatic vines	149 d	5.50 a	5.20 a	0.56 a	3.23 a
Microelements-treated symptomatic vines	178 b	5.45 a	5.28 a	0.21 b	3.30 a
Untreated symptomatic vines	165 c	5.80 a	5.47 a	0.28 ab	3.23 a
Untreated healthy vines	188 a	5.67 a	5.40 a	0.14 b	3.33 a

For each column, values followed by the same letter do not differ statistically according to Tukey's honest significant difference (HSD) test at $p = 0.05$.

3.5. Leaf Area Measurements

In 2006, in the Controguerra vineyard, the area of the leaves collected from both primary and secondary shoots of all types of treated vines was significantly higher than the leaf area of the corresponding untreated vines (Tables 10 and 11), with the exception that the leaf areas of asymptomatic leaves of secondary shoots of treated symptomatic vines did not differ statistically from the leaf areas of asymptomatic leaves of secondary shoots of untreated symptomatic vines (Tables 10 and 11).

Table 10. Average leaf area (cm^2) in apparently healthy, diseased asymptomatic, and symptomatic vines, treated with nutrients and untreated, in Controguerra vineyard in 2006 and 2007.

	Leaf Area	Treatment	Healthy Vines	Asymptomatic Vines	Symptomatic Vines
2006	Primary shoot leaves	Untreated	27920	28469	27778
		Treated	35817	34790	33790
	Secondary shoot leaves	Untreated	30723	28674	27938
		Treated	42662	41827	37206
2007	Primary shoot leaves	Untreated	26228	/	25921
		NPK 23-11-11	/	/	33932
		Microelements	/	/	25960
	Secondary shoot leaves	Untreated	31630	/	31362
		NPK 23-11-11	/	/	42284
		Microelements	/	/	31689

In 2007, both in primary and secondary shoots, the leaf area of untreated healthy vines did not differ from the asymptomatic leaf areas of untreated symptomatic vines, or from those treated with microelements. Conversely, both in primary and secondary shoots, the leaf areas of asymptomatic leaves of symptomatic vines treated with NPK 23-11-11 fertilizer showed values significantly higher than those of the leaves of untreated healthy vines (Tables 10 and 11).

Table 11. Statistical analyses of average leaf area of nutrient treated and untreated vines of Controguerra vineyard carried out by means of pair comparisons according to Student's *t*-test at $p = 0.05$.

Leaf Area		Comparison among Treatments	*p* Value
2006	Primary shoot leaves	Untreated healthy vines/Treated healthy vines	0.01324
		Untreated asymptomatic vines/Treated asymptomatic vines	0.04044
		Untreated symptomatic vines/Treated symptomatic vines	0.04503
	Secondary shoot leaves	Untreated healthy vines/Treated healthy vines	0.04232
		Untreated asymptomatic vines/Treated asymptomatic vines	0.04280
		Untreated symptomatic vines/Treated symptomatic vines	0.20361
2007	Primary shoot leaves	Untreated healthy vines/Untreated symptomatic vines	0.60323
		Untreated healthy vines/NPK 23-11-11 treated symptomatic vines	0.00127
		Untreated healthy vines/Microelement treated symptomatic vines	0.89887
	Secondary shoot leaves	Untreated healthy vines/Untreated symptomatic vines	0.91395
		Untreated healthy vines/NPK 23-11-11 treated symptomatic vines	0.02981
		Untreated healthy vines/Microelement treated symptomatic vines	0.98092

4. Discussion

The difficulty in carrying out an effective control strategy against the esca complex of grapevine diseases, and the need to do so with eco-compatible means, have stimulated studies investigating the host-pathogen-environment interactions in the esca–vine pathosystem and the effects of cultural practices on the susceptibility of the host plant to the disease [5,41].

The present work has to be considered as a part of the studies on the influence of nutrition on esca-infected vines. The data were collected in 2006 and 2007 and are now discussed in light of the findings that have emerged in later years, in order to acquire additional knowledge about the complex interaction between the disease's chronic form and the host plant.

The vines of the Controguerra vineyard were in nutritional balance, as assessed in a study carried out in 2009 on the same esca-infected vineyard [34]. Therefore, the nutrient applications carried out in the present study were aimed at elucidating the possible role of the macro- and microelements in the expression of the foliar symptoms of esca, and not at correcting any nutrient deficiency. The effects assessed on the treated vines were due to the application of the macro- and microelements, without any further fertilization.

In agreement with other studies, the applications of foliar nutrients carried out in 2006, as well as those in 2007 based only on NPK fertilizers, significantly increased both the vegetative growth and the incidence of foliar symptoms [33]. The increase in foliar symptoms in the plots treated with NPK fertilizers may be linked to an increase in sap flow and transpiration due to a higher metabolic activity, with a higher occurrence of phytotoxic compounds translocated from the infected wood to the leaf [42].

The treated plants showed a decrease of their calcium content, both in the leaves of asymptomatic vines, in 2006, and in leaves of asymptomatic shoots in symptomatic vines, in 2007. Therefore the calcium content of these vines, contrary to what observed in the untreated vines, no longer differed from the content found in the leaves of the other types of vines. So, the increase in foliar symptoms in vines treated with NPK fertilizers could have been due both to the higher metabolic activity induced by the applications and to decreased calcium. A decrease in calcium content could weaken the role of calcium in reducing the intensity of the plant's oxidative response [43–45]. Studies showed higher calcium content in leaves of asymptomatic vines than in leaves of other types of vines [34]. Furthermore, the applications of a fertilizer based on calcium, magnesium and seaweed significantly reduced the expression of foliar symptoms, with the development of morphological barriers in the leaf, such as calcium oxalate druses and higher contents of flavonoids, and an increase of *trans*-resveratrol in the leaves of diseased asymptomatic vines. These results strengthened the hypothesis of a strong defense response, as hypersensitivity reaction, associated with the expression of leaf symptoms [11,35–37,46]. In fact, calcium, through its calmodulin component and the development of morphological barriers in the leaf mesophyll, could modulate and reduce the reaction of the plant to toxic compounds associated with the occurrence of foliar symptom [35,43].

Studies on esca-infected vines showed higher magnesium contents in the leaves of untreated asymptomatic vines, compared to the leaves of symptomatic untreated vines [34]. The increase in magnesium was less constant than that found for calcium. In 2007, all applications provided an increase in foliar symptoms and a significant decrease of magnesium content in asymptomatic leaves of symptomatic grapevines. In 2006, the treated plot showed both an increase in symptoms and a decrease in magnesium in asymptomatic vine leaves. Therefore, the reduction of foliar symptoms might be also associated with magnesium as an essential constituent of chlorophyll. Moreover, the magnesium deficiency causes symptoms of interveinal chlorosis in the leaves similar to those leading to tiger-stripe in esca-infected vines [47,48]. Furthermore, magnesium could play a role in the detoxification of phytotoxins, as demonstrated in Eutypa dieback, where the eutipine is transformed into the non-toxic compound eutipinol via Mn^{2+} and Mg^{2+} [49].

In 2007, the applications of microelements did not cause differences between symptomatic treated and untreated vines as to vegetative growth. These results are consistent with the finding in 2006 that only NPK applications provided a higher vegetative growth, because, in that year, the microelements were regularly applied. Therefore, the increase of foliar symptom expression observed in vines only treated with microelements might be correlated to the reserve reconstitution in the wood and not to an increase of metabolic activity, as hypothesized for NPK fertilizers. In vine plants, the translocation of nutrients and carbohydrates towards the woody organs occurs after fruit setting [50]. A greater availability of nutrients in the areas of the trunk colonized by pathogens, as hypothesized in plants treated with microelements, might favor the pathogens through a greater possibility of the production of phytotoxic compounds, increasing the chance of leaf symptom expression.

In 2007, in vines treated with microelements, their elevated availability may have caused their increased translocation to the trunk, compared to what had been observed in untreated vines or in those treated with NPK fertilizers. In particular, the iron might have played a major role in the plant–pathogen interaction, favoring the production of compounds toxic for wood or leaves by both tracheomycotic and white rot pathogens [3,51].

However, it might not be excluded that, in 2006, the applications of both microelements and NPK fertilizers and the resulting increase in vegetative growth could have proportionally reduced the content of microelements in the leaf, reducing as well their effect on the increase of foliar symptoms. Indeed, the iron and zinc amounts recorded in 2007 in the symptomatic leaves of plants treated with microelements only were not recorded in 2006 in the symptomatic leaves of plants treated with both NPK and microelements.

In 2006, the iron, manganese and zinc contents did not differ between untreated diseased asymptomatic or symptomatic vine leaves, and the corresponding treated ones. Additionally, in 2007, no differences in the content of iron, manganese, or zinc were detected between diseased vine leaves treated with NPK and untreated ones. This may suggest an accumulation of microelements in diseased vine leaves, one observed in untreated diseased vines too, and still occurring in vines treated with NPK, despite the higher vegetative growth induced by the applications of this fertilizer. Most likely, the high vegetative growth may have caused a "dilution" effect, resulting in a decrease in the contents of microelements in the leaf [52]. The foliar accumulation of microelements in the diseased vines could be linked to the plant's response to the disease, given the involvement of microelements in enzymatic reactions in the plant defense mechanisms [48].

In 2007, diseased vines only treated with microelements showed higher contents of iron and zinc in the leaves of symptomatic shoots, compared to the leaves of asymptomatic shoots. Therefore, at high availabilities of microelements, diseased vines seemed to accumulate microelements in symptomatic shoots. This condition could be associated with the involvement of microelements in the synthesis of phenolic phytoalexins [48], strengthening the hypothesis of the foliar symptoms of esca as a hypersensitivity reaction in the plant's defense response, as an oxidative burst triggered by the phytoxic compounds, excluding their direct effect on the leaf [5,22,35,53,54]. Studies have also demonstrated that phytoalexins were synthesized in symptomatic leaves after the development of foliar necrosis, that is after the hypersensitivity reaction, and increased proportionally with the symptoms increase, without any effects on the reduction of the leaf symptoms' expression [26,55].

These studies are in agreement with the present work, in which foliar symptoms increased significantly in the treated vines, and with microelements in particular.

The higher potassium amount observed in 2007 in the leaves of symptomatic vines treated with NPK fertilizers may also be linked to the involvement of potassium in the stomatal opening, and therefore in the transpiration, which in turn is altered in symptomatic as compared to asymptomatic leaves [56,57].

In 2007, at veraison, the leaves of asymptomatic and symptomatic shoots treated with NPK fertilizers had significantly higher sodium content than did the leaves of corresponding untreated shoots treated with microelements and the leaves of apparently healthy untreated vines. Further studies on untreated vines showed higher sodium amounts in the leaves of symptomatic vines compared to the leaves of apparently healthy vines, albeit twenty days after veraison. These results were obtained for two consecutive years, both much rainier than 2007 [11].

Heavy rains and applications of NPK fertilizers increased foliar symptom expression, probably increasing the movement of fungal toxic compounds to the leaves [17]. Therefore, it may be hypothesized that diseased vines, the symptomatic ones in particular, are more likely to accumulate sodium in the leaf. The sodium accumulation in symptomatic leaves might be linked to the rapid and temporary increases of reactive oxygen species (ROS) involved in the hypersensitivity reaction and in the synthesis of secondary metabolites observed immediately after Na^+ increases [58]. Furthermore, an increase of foliar symptoms in vines affected by the decay of kiwifruit, a disease very similar to esca, was hypothesized as caused by an increase in sodium content [59]. However, since these results were not detected in 2006, the role of sodium in mechanisms leading to symptom expression needs further investigations.

The higher nitrogen amounts in the berries of untreated symptomatic vines, detected in both years of trials, could be associated to the incomplete ripening with a significant decrease in sugar, in agreement with what has emerged in other studies on symptomatic vines [14,31,60].

On the other hand, in 2006, the decrease in sugars recorded in the musts of treated compared to untreated asymptomatic vines did not appear to be correlated to the amount of nitrogen in the berry. In fact, the content of nitrogen in the treated vines was not different in asymptomatic and healthy grapevine berries. Analyses of musts carried out in studies on the nutrition of esca-infected vines showed that the musts of asymptomatic unfertilized vines did not differ in sugar content compared to the ones of apparently healthy vines [14,31]. Therefore, the decreases in sugars in the musts of treated asymptomatic vines could be caused by the applications of macro- and micronutrients. As reported above, the vines of the Controguerra vineyard were in nutritional balance [34], and the imbalance caused by the applications might be particularly important in esca-infected vines, because the disease itself causes physiological imbalance in the plant [61,62].

The higher contents of potassium in berries of symptomatic vines, in 2006 and 2007, and magnesium, in 2006, might be associated with the buffering of high acidity during ripening [63]. In 2007, the lack of significant difference in magnesium contents among the different types of vines could instead be attributed to the drop in total acidity, given the decrease in malic acid, most likely determined by the high temperatures due to lack of rain during the last ripening period.

In 2006, a decreasing trend of nitrogen, phosphorus, and potassium contents in the berry of treated vines was observed. However, the differences between the different types of vines recorded in the untreated plots were still present. Additionally, in 2007, the berries of the symptomatic vines treated with either NPK fertilizers or microelements had lower contents of the three macroelements, as compared to the untreated symptomatic vines. The decreases of nitrogen, phosphorus and potassium recorded in 2006 in the berries of treated plot, and in 2007 in the berries of vines treated with NPK 23-11-11 fertilizers, could be linked to the higher vegetative growth of the vines, with a possible dilution effect, with consequent lowering of the contents of these three elements [52]. This dilution effect cannot be hypothesized in 2007 for the applications of microelements only, since no increase in vegetative growth was observed. Therefore, the decreases of nitrogen, phosphorus and potassium in the berries of vines treated only with microelements were probably due to other factors, such as the antagonism between macro- and microelements [64].

As described above, in 2007, the vines treated with microelements showed their accumulation at veraison in the leaves of symptomatic shoots. In the berries of these vines, the iron and manganese contents were lower, and the zinc content was not different than those of the berries of untreated symptomatic vines. In 2006 the contents of iron, manganese and zinc were lower in the berries of treated symptomatic vines, compared to the berries of untreated symptomatic vines, although there were no differences between the treatments in the leaf at veraison. In both years of the study, the lower contents of microelements in the berries of treated symptomatic vines may be due to their failure to translocate, during the ripening, from the leaves to the berries, in order to support the plant's defense response. This hypothesis can be corroborated by the dynamic of the disease incidence in the Controguerra vineyard, as characterized by a significant increase in foliar symptoms, from veraison to ripening.

In 2007, the symptomatic vines untreated or treated with NPK fertilizers had similar sugar contents in their musts, which also was similar to the contents detected in symptomatic vines in other studies [14,31]. On the contrary, the sugar in the musts of the vines treated with microelements was higher, although it did not reach the standard levels observed in healthy plants of the cultivar. This qualitative improvement is probably due to both the lack of a higher vegetative growth and a better maturation promoted by microelements [65].

5. Conclusions

Applications of foliar nutrients, based on both NPK fertilizers and microelements, increased the incidence and severity of the expression of esca's foliar symptoms. In the case of applications of NPK fertilizers, the increase was associated with the greater vegetative growth as a consequence of a higher plant metabolic activity in the treated plants. A higher transpiration rate could be an effect of this increased activity, with an increase in translocation to the leaf of phytotoxic compounds produced by esca pathogens and consequently an increase of foliar symptoms. This hypothesis cannot be assumed for the applications of microelements, because a subsequent increase in vegetative growth was not observed.

The increase of foliar symptoms as a result of the application of microelements might therefore be explained by hypothesizing their increased availability in the woody areas colonized by the pathogens. The microelements, through different interactions with the pathogen, could increase the synthesis of phytotoxic compounds subsequently translocated to the canopy via the transpiration stream, thus increasing the expression of leaf symptoms.

Furthermore, the accumulation of microelements in the treated symptomatic leaves could be linked to the enzymatic reactions underlying the response of the leaf to phytotoxic metabolites. A high availability of microelements could therefore increase the hypersensitivity reaction and oxidative burst triggered by phytotoxins, hypothesized as a mechanism involved in the expression of foliar symptoms.

The applications of foliar nutrients led to decreases in calcium in the leaves, confirming what had emerged in other studies relating to its role in reducing the strong plant-defense response leading to symptom development. The decrease in calcium was often matched with decreases in magnesium, and the latter's role in the plant's defense response may not be excluded. The applications of microelements increased the sugar contents in symptomatic vines, but without reaching the levels observed in healthy plants of the cultivar. Furthermore, the sugar improvement of treated plants was ineffective because of the increase of foliar symptoms.

The results obtained in this study indicated that a condition for the correct management of vineyards affected by esca is to keep a vegetative–productive balance, one of the most important conditions that can be imposed to limit the effects of the chronic form of a disease affecting a crop subject to the risk of overfeeding. The investigated vineyards were in nutritional balance. Therefore, the applications may have created some imbalances, which was probably not so important for the healthy plants, which in turn produced regularly, but one sufficient to create further problems in plants already affected by esca. In other studies carried out in the same vineyard, the yield of asymptomatic diseased vines did not show significant decreases in sugars, as compared to the apparently healthy vines. In the present work, asymptomatic vines treated with the nutrients showed a drastic decrease in sugar content, as always observed in symptomatic vines.

Keeping esca-infected vineyards in nutritional balance is not a real means of control of the disease but can contribute to the reduction of the incidence of the disease, probably also producing a better response of the plant to any treatments aimed at reducing infections and the effects of the disease.

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