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Foliar treatment of esca-proper affected vines with nutrients and bioactivators

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Summary. Foliar treatment with nutrients and bioactivators was carried out in two vineyards affected with esca proper in 2004 and 2005. Changes in the foliar symptoms and in the quality of berries without lesions from treated symptomatic vines were assessed. Treated vines unexpectedly had a higher incidence and a greater severity of symptomatic leaves than untreated plants, most likely because physiological processes were stimulated by the treatments, possibly also because treatments facilitated the movement of toxins produced by the wood fungi of esca. However it cannot be excluded that the increase in foliar symptoms was due to the forced nutrition causing an imbalance between the various elements, and altering the mechanisms that vines use for the remission of foliar symptoms. This supposition seemed corroborated by the observation that treated vines diseased with esca proper had a weaker defense response than untreated diseased vines, and that treated diseased vines had lower levels of nitrogen and microelements, which are respectively involved in osmoregulation and as cofactors of enzymes involved in the defense response of the plant. The main quality parameters of berries without lesions from treated and untreated symptomatic vines were very similar.

Key words: esca-disease, microelements, macroelements, leaf symptoms, fertilization.

Introduction

Esca proper, the classic form of esca (Graniti *et al*., 2000; Surico *et al*., 2006), is widespread in the Abruzzo region of Italy, particularly in older vineyards, i.e. of more than 20 years. Some vineyards, grown with the cv. Trebbiano d'Abruzzo and affected with esca proper, have been under observation for more than a decade and various studies on the etiology and epidemiology of the disease, on the damage caused to the yield, and on strategies of control have been carried out (Di Marco *et al*., 2000; Calzarano *et al*., 2001; 2004a; 2004b; Cesari *et al*., 2005). One of the more important findings was that the causal agents of esca have been provisionally identified by several authors (Mugnai *et al,* 1996b; Larignon and Dubos, 1997; Larignon and Dubos, 2001; Lecomte *et al*., 2005a; 2005b). Of particular importance among the findings was also that the quality and yield of healthy vines is very similar to that of diseased but asymptomatic vines (Calzarano *et al*., 2001; 2004a). It had also been found that when vines are subjected to trunk renewal followed by trunk injection with specific fungicides they resume growth and have a lower incidence of foliar symptoms (Calzarano *et al*., 2004b).

In spite of these encouraging results, esca control strategies are still insufficient. Since winegrow-

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ers must cultivate in the presence of diseased plants, and since diseased vines as long as they are asymptomatic have much the same yield and quality as healthy plants, the main aim of this study was to examine whether the leaf symptoms of esca could be alleviated by applying bioactivators and nutrients to the canopy of vines with esca proper. For this reason, the mortality of vines with esca proper was monitored over a 12-year period, in order to determine the longevity of vines after they became diseased. If the vines survived the disease for a long time it would make it all the more interesting to suppress the foliar symptoms and restore the vine to full productive capacity (Cesari *et al*., 2005). We also compared the quality and composition of berries without lesions from treated symptomatic vines with those of berries without lesions from untreated symptomatic vines, as well as with berries from untreated healthy vines. Together with the amounts of the various macro- and microelements, these evaluations addressed the main parameters that determine the quality of grapes.

Materials and methods

Monitoring of foliar symptoms and mortality

The study was carried out in two vineyards of the cv. Trebbiano d'Abruzzo affected with esca proper. Both vineyards were 32 years old and located in the wine growing area of the province of Teramo, Abruzzo. The vineyards used different trelling techniques: the "Geneva Double Curtain" (GDC) in the Controguerra vineyard, and the "Tendone" system in the Giulianova vineyard.

The vineyards were monitored for the foliar symptoms of esca and for esca-related mortality from 1994 to 2005. Vines were identified by numbering them according to their position in the rows, and the appearance and severity of any foliar symptoms was monitored. Since the foliar symptoms of esca can fail to appear on diseased vines for a whole season, or for some seasons in succession (Calzarano and Di Marco, 1997; Cesari *et al*., 2005; Marchi *et al*., 2006), during which time these vines appear to be completely healthy and normal, foliar symptoms were monitored for 12 years in order to securely identify all diseased vines and distinguish them from truly healthy plants. In this way the effect of treatment were evaluated on vines that were certainly affected with esca, namely only on those vines that showed foliar symptoms in one or more years during measurements. On the basis of the yearly measurements, the vines were scored according to a seven-point scale (Table 1) that quantified the severity of foliar symptoms, including vines affected with apoplexy and still capable of vegetating the next season (class 5), and dead vines (class 6); class 7 comprised vines that died the preceding year, and thus includes those already scored in class 6. Mortality was assessed in both treated and untreated (control) plots, in order to exclude vines that died after the onset of foliar symptoms. The annual incidence of mortality and cumulative death was also measured during the 12-year observation period.

Surveys of foliar symptoms were carried out in September 2004 and 2005, following treatment during the vegetative season, and when visible leaf symptoms are most conspicuous. The incidence of esca in each vineyard was calculated by dividing the number of vines with visible symptoms by the total number of diseased plants. Percent severity of expression was calculated using the formula $\Sigma N \times 100 / (Y \times Z)$, where ΣN=sum of the severity values for symptoms; Y=number of plants observed

Table 1. Scale used for classification of grapevines in relation to the severity of foliar symptoms, apoplexy, or death.

Class	Value	Severity of foliar symptoms, apoplexy, and death
1	0.5–1.5	10–30%
2	2–2.5	40–50%
3	3–3.5	60–70%
4	4–4.5	80–100%
5	5	Apoplexy
6	6	Death
7	7	Death in the preceding year

(asymptomatic and symptomatic); Z=maximum value of symptoms (McKinney, 1923).

Foliar treatment

In each vineyard, two plots were laid out having the same number of plants and the same number of rows. Foliar fertilizers and bioactivators were applied in 2004 and 2005 to one plot in each vineyard; the other was left untreated (Table 2).

Foliar fertilization was performed using different preparations during the vegetative season, having regard to the absorption curve of the phenological growth stage (Fregoni, 1998). Treatments were with a sprayer set to 500 l ha^{-1}. The calendar of applications, micro- and macroelements and bioactivators compounds used are shown in Table 3, while the formulations are described in Tables 4 and 5, except the "S" Bioactivator which contained total nitrogen 3% (organic nitrogen 1%).

The phenological growth stages at which treatments were applied were the same as those described by Lorenz *et al*. (1995).

Chemical analysis of berries

Sample collection

In the Controguerra vineyard berries were sampled from both the treated and the untreated plots; in both cases the berries sampled were without lesions and came from the parts of symptomatic vines that did not show any foliar symptoms. From the untreated plot berries were also harvested from healthy vines. Each group (symptomatic treated, symptomatic untreated, healthy), was again divided into 3 subgroups, consisting of 6 vines each. From each vine a total of 1 kg of berries was harvested at maturity from the top, wings and central parts of the symptom-free bunches, giving a total of 6 kg of berries per subgroup. Each 6-kg sample was crushed and a homogenous fraction (1 kg) was taken from the fluid obtained and under constant stirring, before determination of the macro- and microelements. Chemical determination, described below, was carried out on the remaining fraction, which was subjected to manual pressing, separating the must from the grape skins and the pulp.

Determination of total acidity, pH, reducing sugars, and organic acids

Total acidity, pH, and reducing sugars were determined on the must samples following the official method of the EC 2676/90, attachments 13, 24, and 5 respectively (A.A., 1990). The content of organic acids was determined on a 10-ml aliquot of must obtained immediately after harvesting, homogenized with an Ultra-Turrax (Ika, Heidelberg, Germany) and filtered through paper filters. The filtrate was centrifuged at 1800 *g* for 3 min and 1 ml of the supernatant was removed and diluted with 9 ml 0.08 M H_2SO_4. The diluted extract was then centrifuged for 3 min at 1800 *g* and the supernatant analyzed for organic acid using an HPLC (Perkin Elmer, Series 200 System, Monza, Italy) equipped with a diode array detector set to 210 and 220 nm. The analysis was carried out using an ionic exchange column (Aminex HP87 H Ion Exclusion, BIO RAD, Milan, Italy; 300 mm × 7.8 mm) at 55°C, with a pre-column (Cation H Cartridge, BIO RAD, Milan, Italy; 30 mm × 4.6 mm) and a mobile phase of 0.08 M H_2SO_4. The flow rate was 0.6 ml min^{-1} and the elution time 22 min. With this method the peaks for tartaric and malic acid were well-resolved with retention times of 8.83 and 9.70 min (SD, 0.018 and 0.031; CV% 0.2 and 0.3). Analyses were carried out in duplicate injecting 20 μl for each analysis. The calibration curves were obtained by injecting 20 μl of the standard at known concentrations in 0.08 M H_2SO_4. Five standards were prepared, starting at a concentration of 1 mg ml^{-1} and diluted in a 1:2 ratio.

Table 2. Plot details in the vineyards evaluated.

Vineyard location	Plot	Trellis system	Number of rows	Total number of vines	Area (m^2)
Controguerra	Treated	GDC	8	309	3000
Controguerra	Untreated	GDC	8	309	3000
Giulianova	Treated	Tendone	8	584	5000
Giulianova	Untreated	Tendone	8	584	5000

Table 3 - Details of foliar treatment application.

Phenological growth stages	Treatment data		Nutrients and bioactivators	Dose
	2004	2005		
Five leaves unfolded	May 21	May 19	Iron-humate	1.0 l ha^{-1}
Nine or more leaves unfolded	May 28	May 27	15-36-13 Microelement-humate "S" bioactivator	1.0 kg ha^{-1} 1.0 kg ha^{-1} 0.3 l ha^{-1}
Nine or more leaves unfolded	May 31	June 3	Iron-humate	1.0 l ha^{-1}
Inflorescens fully developed; flowers separated	June 9	June 14	15-36-13 Microelement-humate "S" bioactivator	1.5 kg ha^{-1} 1.5 kg ha^{-1} 0.4 l ha^{-1}
Fruit set: young fruit begin to swell	June 22	June 27	23-11-11 Microelement-humate "S" bioactivator	1.5 kg ha^{-1} 1.5 kg ha^{-1} 0.4 l ha^{-1}
Berries groat-sized	June 30	July 4	23-11-11 Microelement-humate "S" bioactivator	1.5 kg ha^{-1} 1.5 kg ha^{-1} 0.4 l ha^{-1}
Berries pea-sized	July 9	July 15	12-18-32 Ca-Mg-B solution "S" bioactivator	1.5 kg ha^{-1} 1.5 kg ha^{-1} 0.4 l ha^{-1}
Berries beginning to touch	July 23	July 29	8-16-50 "S" bioactivator	1.5 kg ha^{-1} 0.4 l ha^{-1}
Berries begin to develop colour	August 10	August 16	"S" bioactivator	0.4 l ha^{-1}
Berries developing colour	August 19	August 23	"S" bioactivator	0.4 l ha^{-1}
Softening of berries	August 30	September 1	"S" bioactivator	0.4 l ha^{-1}

Determination of macro- and microelements

Each 1-kg sample of pressed grape berries was homogenized in an Ultra-Turrax, and an aliquot of 100 ml was frozen at -18°C and lyophilized (Christ, Alpha 2-4, Osterode am Harz, Germany). The powder obtained was ground in an agate ball mill (Retsch, PM 200, Haan, Germany), before drying in a dessicator under vacuum. An aliquot of each powder (500 mg) was mineralized in hermetically sealed Teflon tubes with 7 ml 65% HNO_3, 0.5 ml 50% HF, and 9.0 ml 95% H_2O_2. The sample was digested in a microwave oven (Millestone, ETHOS900, Shelton, CT, USA). The cooled digestion solutions were brought to 25 ml with Milli Q H_2O and stored in polyethylene containers. Solutions were analyzed in an atomic absorption spectrophotometer with an acetylene flame (Perkin Elmer, Analyst 700, Waltham, Massachusetts, USA), with appropriately diluted solutions. The standards used were of the highest quality available.

Ca, Mg, Cu, Zn, Mn, Fe, and K were determined in emission. Phosphorus was determined in the same digestion solutions using colorimetry with a molybdate reagent measuring absorption at 525 nm and a standard curve obtained with a phosphate solution (Murphy and Riley, 1962).

Nitrogen was measured with a HCNS Fisons, EA 1108 (Milan, Italy) on 1–2 mg powder that was

Table 4. Composition of foliar fertilisers as Iron humate (Fe-H), Microelement humate (M-H) and Ca-Mg-B solution.

Nutrient	Fe-H[a]	M-H[a]	Ca-Mg-B
Organic matter	30.0	30.0	
Organic nitrogen	0.5	0.5	
Alkaline humates	5.0	5.0	
Humic acids	3.5	3.5	
Fulvic acids	2.7	2.7	
Iron sulphate	5.0	5.0	
Boric acid		1.5	0.6
Zinc sulphate		0.7	
Manganese sulphate		0.9	
Calcium oxide			8.0
Magnesium oxide			4.0

[a] C/N ratio 34.8

accurately weighed. All analyses were carried out in duplicate and the average value was calculated.

Statistical analysis

Foliar symptoms in the treated plots of both vineyards were compared statistically with those in the untreated plots, considering both symptomatic and asymptomatic vines, but excluding healthy and dead vines.

As regards the incidence of vine plants with esca symptoms, one-way frequency analysis was performed using treatment as a group variable and the presence of symptoms in a binomial scale. The results of the analysis were expressed using a χ^2 test along with the associated P value.

As regards the severity of the foliar symptoms, frequency analysis was performed using treatment as the group variable and the occurrence of symptoms was scored in a categorical scale using the same scale as that used to score the symptoms. The results of this analysis were also expressed using a χ^2 test along with the associated P value.

The parameters of berries without lesions from treated and untreated symptomatic vines of the vineyard of Controguerra, as well as the yield from the healthy Controguerra vines at harvest, were subjected to variance analysis and Duncan's test (P=0.05).

Statistical analyses were performed using SAS Version 8.1 software (SAS Institute, Cary, NC, USA).

Table 5. Composition of foliar fertilisers N-P_2O_5-K_2O.

Nutrient	15-36-13	23-11-11	12-18-32	8-16-50
Nitrate	5.4	8.4	9.0	8.0
Ammonia	5.4	8.4	3.0	
Urea	4.2	6.2		
Phosphoric anhydride	36.0	11.0	18.0	16.0
Potassium oxide	13.0	11.0	32.0	50.0

Results

Mortality

Mortality was evaluated yearly in both vineyards for 12 years from 1994 to 2005. Starting from 1999 at Controguerra and from 1997 at Giulianova a trend of increasing mortality was found (Fig. 1). At Controguerra mortality increased from 0.54% in 1999 to 2.7% in 2005, and at Giulianova from 0.61% in 1997 to 6.77% in 2003; the annual mortality in this second vineyard peaked at 2003 and remained stable in the two years thereafter. The cumulative mortality (the sum of all vines that died up to and including a given year) increased at an exponential rate and in 2005, the last year of monitoring, reached 10% at Controguerra and 27.17% at Giulianova.

Foliar symptoms

The incidence and severity of foliar symptoms was almost always higher in the plots treated with the bioactivators and nutrients than in the untreated plots (Fig. 2).

In the Controguerra vineyard, the incidence of foliar symptoms in the treated plot was 42.67% in 2004, in the control plot it was 25.13%; in 2005 the incidence of foliar symptoms was very similar between plots: 36.17% in the treated plot and 36.7% in the untreated plot. The severity of symptom expression in this vineyard was also higher in the treated than in the untreated plot in both years: 24.86%, compared with 12.39% for the untreated plot in 2004; and 19.29%, compared with 15.42% for the untreated plot, in 2005.

In the Giulianova vineyard, the effect of foliar treatment was similar to that at Controguerra; the incidence of foliar symptoms was higher in the treated plot in both 2004 and 2005: 6.95% and 15.63%, compared to 4.65% and 11.91% in the untreated plot. Symptom severity was also higher in

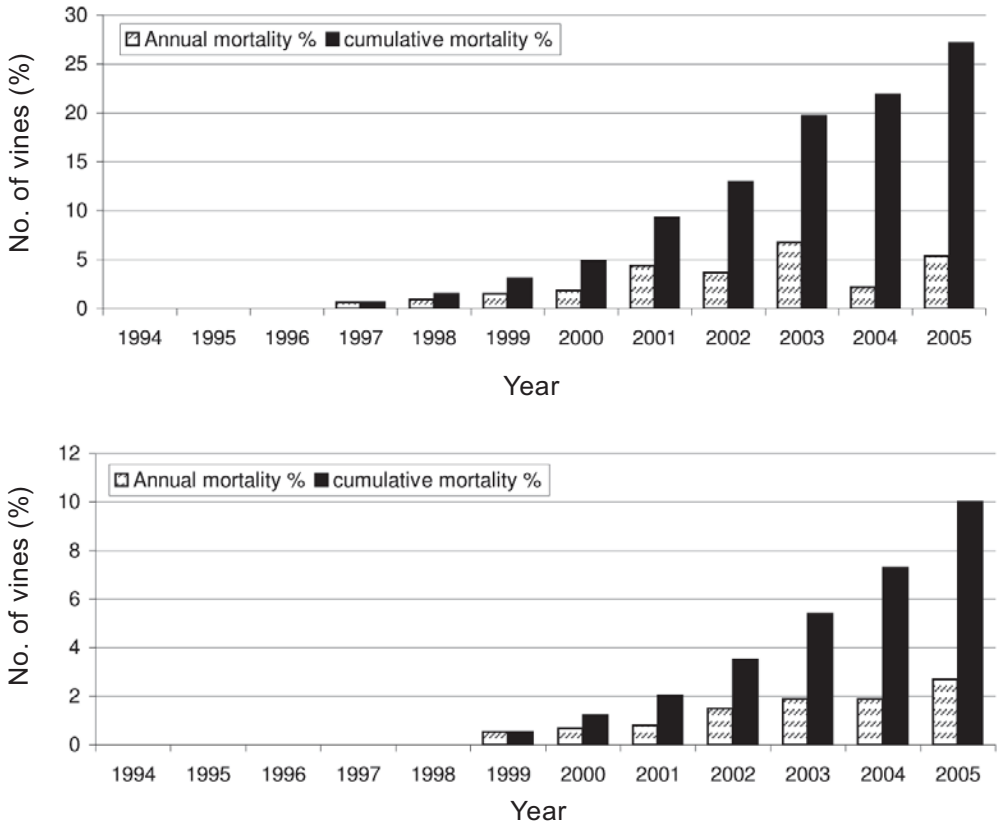

Fig. 1. Annual and cumulative mortality on vines affected with esca proper in the Controguerra (top) and Giulianova (bottom) vineyards.

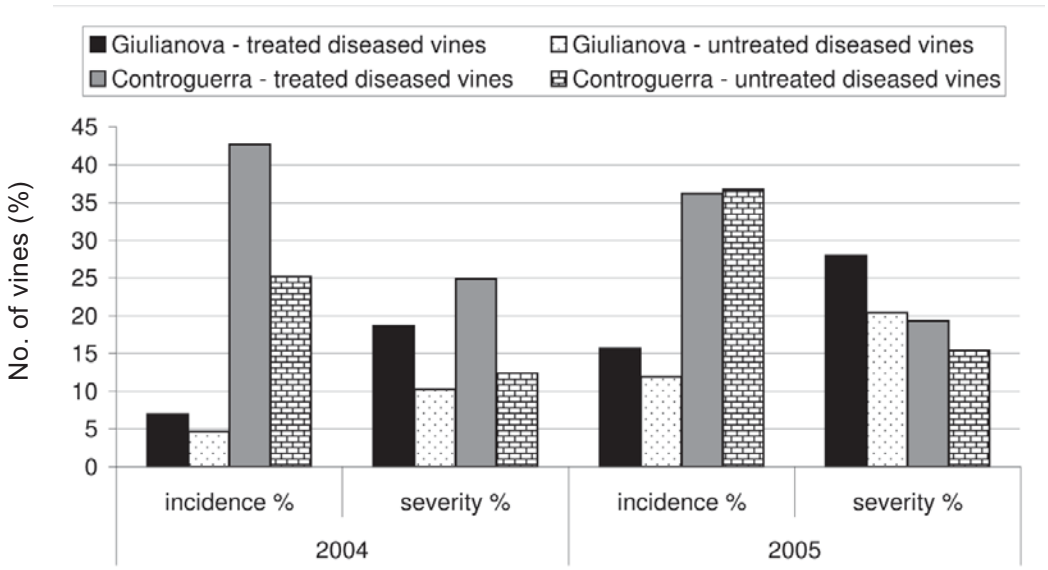

Fig. 2. Incidence and severity of foliar symptoms on vines affected with esca proper treated with nutrients and bioactivators, and untreated vines.

treated plot in these two years (18.68% and 27.98% in the treated plot, compared with 10.23% and 20.36% in the untreated plot).

Frequency analysis of symptomatic and asymptomatic vines revealed that these differences between the control and treated plots were significant, except in 2005 at Controguerra, when incidence and severity of foliar symptoms did not differ significantly between plots. Nonetheless, the differences found between the treated and the control plots showed that the treated plot still tended to have more foliar symptoms (Table 6).

Chemical analysis of yield

Berries without lesions harvested from symptomatic vines at maturity yielded musts with very similar quality characteristics in both the control and the treated plot at Controguerra in 2004 and 2005. Any differences between reducing sugars, pH, total acidity, and the acidic fractions were not statistically significant between plots (Table 7). Unlike symptomatic plants, however, whether treated or untreated, healthy grapes had sugar levels that conformed to the standard of the cultivar in 2005, and had lower levels of total acidity and of the acidic fraction than the symptomatic grapes, which did not ripen sufficiently.

Berries from treated symptomatic vines had lower levels of nitrogen, iron, manganese, and copper than berries from untreated symptomatic vines; the shortfall of these nutrients in 2004 and 2005 was 1.24 and 2.04 g kg^{-1} for nitrogen, 3.35 and 4.58 mg kg^{-1} for iron, 1.10 and 1.55 mg kg^{-1} for manganese, and 1.48 and 4.08 mg kg^{-1} for copper. In 2004, these differences did not reach statistical significance for nitrogen and copper but they were significant for manganese and iron. In 2005 the differences were greater, and were significant in all cases. In berries from healthy vines, levels of these elements were almost always lower than in berries from symptomatic plants, and in most cases these differences between symptomatic and healthy plants were significant. In particular, the content of iron and copper was slightly lower in healthy than it was in diseased vines in 2005 (Table 8).

Table 6. Comparison of the incidence and severity of foliar symptoms between treated and untreated vines affected by esca proper according to the FREQ and CATMOD Procedure (SAS).

Vineyard location	2004		2005	
	Incidence %	Severity %	Incidence %	Severity %
Controguerra	0.0003	0.0001	0.9972	0.1541
Giulianova	0.0137	0.0288	0.0036	0.0434

Values are expressed as χ^2 and do not express statistically differences over 0.05.

Table 7. Chemical analysis carried out in the Controguerra vineyard on musts from berries without lesions of treated and untreated symptomatic vines affected with esca proper and from berries of healthy vines.

Vine group[a]	Reducing sugars g l^{-1}		pH		Total acidity g l^{-1}		Tartaric acid g l^{-1}		Malic acid g l^{-1}	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Treated symptomatic vines	177.47 a[b]	149.43 b	3.48 a	3.20 a	6.53 a	8.80 a	3.65 a	5.20 a	3.47 a	4.52 a
Untreated symptomatic vines	180.00 a	142.53 b	3.35 a	3.13 a	6.53 a	9.17 a	3.75 a	5.24 a	3.25 a	5.25 a
Healthy vines	187.00 a	190.00 a	3.61 a	3.41 a	4.92 b	4.29 b	2.20 b	3.27 b	3.74 a	2.62 b

[a] The statistical analysis separately compared musts from each grapevine group in every year.
[b] Different letters within columns mean significant differences according to Duncan' s Test (P=0.05).

Table 8. Chemical analysis carried out in the Contraguerra vineyard on berries without lesions of treated and untreated symptomatic vines affected by esca proper and on berries of healthy vines.

Vine group[a]	N g Kg^{-1}		P g Kg^{-1}		K g Kg^{-1}		Ca g Kg^{-1}		Mg g Kg^{-1}		Fe mg Kg^{-1}		Mn mg Kg^{-1}		Cu mg Kg^{-1}		Zn mg Kg^{-1}	
	□2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Treated symptomatic vines	6.03 a[b]	8.33 b	1.41 a	1.40 a	12.67 a	13.03 b	1.83 a	2.40 a	0.53 a	0.55 a	10.67 b	16.74 b	3.67 b	3.73 b	14.36 a	13.54 b	6.46 a	7.71 a
Untreated symptomatic vines	7.27 a	10.37 a	1.43 a	1.44 a	12.19 a	13.49 b	1.63 a	2.71 a	0.53 a	0.63 a	14.02 a	21.32 a	4.77 a	5.28 a	15.84 a	17.62 a	6.25 a	6.89 a
Healthy vines	4.74 b	6.16 c	1.28 a	1.20 a	11.89 a	15.57 a	1.32 a	1.17 b	0.61 a	0.65 a	13.56 a	11.88 c	3.99 b	5.57 a	10.91 b	7.81 c	4.65 b	4.78 b

[a] The statistical analysis separately compared berries from each grapevine group in every year.
[b] Different letters within column indicate significant differences according to Duncan' s Test (P=0.05).

Discussion

Between 1994 and 2005 vine mortality in the two vineyards, both of which were 32 years old, went from 0.5 to 6.7% (this last at Giulianova in 2003). Since in all likelihood the disease began well before 1994 (Larignon and Dubos, 2000), it is clear that vines with esca proper still retain a long lifespan. In general, interventions to reduce the incidence and severity of foliar symptoms in vines would thus be useful, since vines that are diseased but without symptoms vegetate and produce fruit in a relatively normal manner, so that losses in yield from such vines would be limited (Calzarano *et al*., 2001; 2004a). Moreover, treatments aimed at suppressing foliar symptoms may be associated with vine trunk renewal and the injection of specific fungicides into the residual stump (Calzarano *et al*., 2004b).

Contrary to what was expected, treatment with bioactivators and nutrients in most cases exacerbated both the incidence and the severity of the foliar symptoms of esca proper. This may have been because the bioactivators and nutrients stimulated physiological processes such as photosynthesis, respiration, and stomatal movement in the vine leaves, leading to higher levels of toxins (Abou-Mansour *et al*., 2004). It has previously been suggested that toxins produced in infected wood may cause foliar symptoms (Evidente *et al*, 2000; Tabacchi *et al*., 2000). Leaf fertilizers may also have caused hypernutrition and this may have produced the more pronounced foliar symptoms than were found in the untreated plants. In fact, when the leaves of untreated vines were evaluated for their nutritional status during the study period, neither the healthy, nor the symptomatic, nor the asymptomatic vines showed any evident signs of nutrient deficiency as defined by Fregoni (1998) (Calzarano, unpublished data). Foliar fertilizer sprays may have caused nutrient levels to rise to toxic levels or to the point where the equilibrium of the various microelements was disrupted. It is known that an excess of certain nutrients can lower resistance to stress (Marschner, 1995), a condition which is particularly evident when nitrogenous fertilizers are used (Huber and Watson, 1974). It is therefore possible that the excess of nutrients interfered with mechanisms whereby vines diseased with esca normally to suppress or conceal the foliar symptoms of esca proper. Such mecha-

nism of symptom remission may be correlated with a stress response.

That the berries without lesions from symptomatic vines at Controguerra had a higher nitrogen content than healthy vines may be due to a defense reaction in the diseased vines. Higher levels of stress proteins, and of proline, signaled for osmotic stress, could be correlated with a more generic response to oxidative stress (Xioung and Zhu, 2002). Furthermore, berries from treated symptomatic vines were found to have lower levels of nitrogen than berries from untreated symptomatic vines. This would seem to indicate that the nitrogen content in berries from treated symptomatic vines was most likely not due to the occurrence of foliar chlorosis nor to the greater amount of nitrogen supplied by the fertilizer. In fact, in this case, it would have been possible to measure the higher levels of nitrogen in the berries of treated symptomatic plants, than those from untreated symptomatic, due to the traslocation of nitrogen from chlorotic areas of striped leaves (higher in treated plants) and to the further amount administered by foliar dispersion. This suggest that the higher nitrogen levels in the berries of symptomatic plants than those from healthy vines was most likely a physiological accumulation, part of a defense response, which was less efficient in plants receiving an excess of nutrients and/or suffering from a loss of nutritional equilibrium on account of the treatments.

Besides the lower nitrogen content, levels of the microelements Fe, Cu, and Mn were also lower in the treated symptomatic vines than in the untreated symptomatic vines. These microelements serve as cofactors for enzymes involved in the regulation of oxidative stress and in the synthesis of phenolic phytoalexin (Marschner, 1995). The lower levels of these elements would appear to indicate a lower responsiveness of the vines, which may be due to the nutritional treatment.

That the levels of microelements in berries from both treated and untreated symptomatic vines were higher in 2005 than in 2004 may have been due to a delay in fruit maturation which occurred in 2005 because the period of May to July of that year was drier than the corresponding period in 2004. The microelement content of berries peaks at veraison, and then decreases steeply at maturation (Fregoni, 1998). The lower amount of water available in the first part of the vegetative season in 2005 evidently had a greater effect on the diseased vines, which had greater difficulties in effecting water transit because their vascular system had been compromised. Healthy vines reached maturity as normal in 2005, as was revealed by the levels of sugars and organic acids in their berries, as well as by a lower content of microelements. However abundant rainfall during the first 10 days of August 2005 may also have favored the movement of Fe, Mn, and Cu from the wood to the foliage of diseased vines and contributed to the higher levels of microelements found. In several ligneous forest species, wood diseased with fungal pathogens contained higher quantities of minerals than healthy wood, due to the activation of defense mechanisms (Shigo and Sharon, 1970). A similar process may occur in plants diseased with esca, where the causal fungi produce low molecular weight chelators (LMWC) that sequester metals such as iron. Ferric iron is then reduced to ferrous iron, and a portion of the reduced iron is translocated towards the foliage (Di Marco, 2001; Goodell *et al*., 1997).

The lower levels of microelements in berries from treated symptomatic vines, compared with untreated symptomatic vines, were possibly caused by treated vines exhibiting a weaker response to esca as a result of the treatment itself. Microelement content was lower in 2005 than in 2004. In 2005 the incidence of foliar symptoms did not increase in the treated plot, though there was a tendential increase in symptom severity. This suggests that the response capacity of diseased vines is not the only factor involved in the expression of foliar symptoms. The availability of water and nutrients at certain phenological growth stages may also have a key role in amplifying or reducing biotic stress when esca is present. When stress is high on account of a number of factors acting together, the defense response of diseased vines may become overstretched and insufficient to trigger the mechanisms that control the foliar symptoms of esca.

The original aim of the study was to improve the quality of berries without lesions from symptomatic diseased vines. This aim was not attained, since the quality parameters of such berries, including the sugar content and the content of organic acids, remained similar to berries from untreated diseased vines despite the treatment giv-

en to the leaves. In conclusion, then, foliar treatment with bioactivators and nutrients did not have any positive effect on grapevines affected with esca. However this does not signify that a more targeted application of selected elements may not succeed in bringing about a reduction in foliar symptoms. In the absence of findings from earlier studies, the data of the present study suggest that way in which the defense mechanism of grapevines copes with the foliar symptoms of esca is an appropriate subject of further experimental inquiry.

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