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Effect of esca on the quality of berries, musts and wines

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Summary. Variations in the composition of berries, musts and wines in three groups of vines: 1. vines with foliar esca symptoms; 2. asymptomatic esca diseased vines; and 3. healthy vines, were studied in 2001 and 2002 in two "Trebbiano d'Abruzzo" vineyards in the Abruzzi region, Italy, to determine the effect of esca. Vines were grouped by foliar symptoms that had been recorded in annual inspections for 10 years: symptomatic vines by definition showed foliar symptoms in the sampling years (2001 and 2002); asymptomatic esca diseased vines showed no symptoms during the sampling years though they were known to be diseased because they had had foliar symptoms in at least one previous annual inspections; healthy vines were those with no symptoms at any time during the 10-year survey. The quality of berries, musts and wines obtained from vines with trunk renewal that had been restored, and of healthy unrenewed vines was compared in another vineyard of the same cultivar. Fairly similar results were obtained over the two sampling years, with a strong reduction in sugar levels of the must from symptomatic vines, leading to a lower ethanol content in the wine made from that must. These findings confirmed those of the preliminary investigation carried out in 2000. The yield pressed from symptomatic vines also had significantly higher levels of malic acid (causing higher total acidity), and of total nitrogen, potassium and total polyphenols. Berries from symptomatic vines had much higher levels of trans-resveratrol, possibly because of the spots on the leaves and berries. Small and non-significant differences between the yields of healthy vines and asymptomatic diseased vines were found, suggesting a slight loss in the quality of musts and wines from asymptomatic diseased vines. The yield of trunk-renewed vines was similar to that of healthy unrenewed vines, despite an increase of trans-resveratrol in the berries and of total nitrogen in the must of the trunk-renewed vines.

Key words: chemical analysis, trunk renewal, foliar symptoms, asymptomatic infection, nitrogen.

Introduction

Both the traditional and the more innovative means to control esca, especially esca proper, are currently ineffective (Di Marco *et al.*, 2000).

A new attempt to control esca, consisting of sanitary measures plus with chemical treatment, has been reported from the same area as that in which the present investigation was carried out; however it will need to be tested more widely to determine its effectiveness in different vine-growing areas and to allow a treatment protocol to be standardised (Calzarano *et al.,* this issue).

The lack of an effective means of control enhances the relevance of studies of the loss in quality caused by esca. Loss in quality and loss in quantity, i.e. yield, together comprise the total damage caused by esca in a vineyard.

While loss in yield is easy to calculate during vintage every year, the loss in quality is much more difficult to determine since it necessitates a complete chemical characterisation and comparison of

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the yield obtained from healthy and infected (including asymptomatic infected) vines selected according to foliar symptoms observations during many consecutive annual surveys (Calzarano and Di Marco, 1997; Mugnai *et al*., 1999; Surico *et al*., 2000).

It is well known that vines infected with "esca proper", the prevalent form of esca in the area near Teramo, Italy, where the present study was conducted, will have a complete remission of their foliar symptoms in the years, following a year (or years) during which they were asymptomatic; therefore annual symptom inspections over a number of years are necessary to establish which vines are infected, whether symptomatic or not, and which are healthy.

The correct classification of vines was particularly important in this study whose aim was firstly to compare yield composition from temporarily asymptomatic but infected vines with that from truly healthy vines, and secondly to determine analytically the loss in quality of the yield of symptomatic vines.

The present work is the continuation of the preliminary investigations begun in 2000. Chemical data on berries, musts and wines obtained in 2001 and 2002 from esca-infected vineyards monitored for 10 years are reported.

Materials and methods

Survey of foliar symptoms and collection of samples

Vines infected with esca were identified by visual inspection of foliar symptoms and the inspection was carried out in three 24 year-old cv. Trebbiano d'Abruzzo vineyards located at Controguerra, Giulianova and Propezzano in the wine-growing area of the Province of Teramo. The Controguerra vineyard had 1125 vines, trained to the Geneva Double Courtain (GDC) trellis system, and an area of 9,000 m^2; the Giulianova vineyard 2000 vines, trained to the Tendone trellis system, and an area of 18,000 m^2, while in the Propezzano vineyard, which was trained to the Tendone system a plot of 4,500 m^2 with 500 vines was laid out for study. The mean yield per vine was 20 Kg with Tendone trellis system, and 10 Kg with GDC. In the vineyards foliar symptoms were recorded every year for 10 years from 1993 to 2002. All vines were numbered according to their place in the row and any symptomatic vines were recorded each year in September, when foliar symptoms are most conspicuous in this area and for this cultivar.

In 2001 and 2002, after nine and ten years of consecutive annual inspections respectively, most vines infected with esca were presumed to have been identified. At the moment of grape cluster sampling in 2001 and 2002, therefore, vines in the Controguerra and Giulianova vineyards fell into three groups:

- vines with foliar symptoms (symptomatic vines);
- asymptomatic vines, but known to be diseased because they had shown symptoms in at least one of the inspection years (infected/asymptomatic vines);
- vines presumed healthy because they never showed esca symptoms during the inspection period (healthy vines).

During the same inspection period, from 1993 to 2002, the Propezzano vineyard was also inspected annually for foliar symptoms of esca, but in this vineyard, all vines that had been symptomatic in any of the inspection years, before 1996, were subjected to trunk renewal in the winter of 1996. In this vineyard, during 2001 and 2002, the vines in the test plot were classified into two groups:

- vines with trunk renewal that were presumed restored since they had had no foliar symptoms in any of the four years after renewal;
- unrenewed vines that had never shown foliar symptoms and were consequently presumed to be healthy.

For the 2001 and 2002 vintages, each vineyard was divided into 6 plots, each having a similar surface. From each plot, 10, 15 and 100 Kg batches of grape clusters were harvested from each vine group for the study of berries, musts and wines, respectively, giving 6 replicate samples of each.

Clusters were harvested from vines located in different parts of the plots; only clusters from the middle of the vine-shoots of each vine were collected to minimise natural variations between clusters due to cluster positioning along the shoot.

Must and wine making and chemical analysis

Immediately after harvesting each 15 Kg (must) batch of grape clusters was separately crushed with a stalk-remover grape crusher and pressed with a vertical oleo-dynamic hydraulic press. Must yield was standardised to 65% (w:v). The 6 replications of musts from each 15 Kg batch of grape clusters, from the 6 plots in each vineyard, were analysed for chemical parameters as shown in Table 1. Analyses were carried out immediately after crushing.

Each 100 Kg batch of grape clusters, to be used for wine making, was crushed and pressed separately as above. Ten g hl^{-1} of commercial non-aromatic yeast and 5 g hl^{-1} of potassium metabisulphite were added to the resulting musts. These musts were fermented at a controlled temperature ranging from 18 to 20°C. At the end of fermentation the wine was decanted and 2 g hl^{-1} of potassium metabisulphite added. Wine was cooled to at 1–2°C to promote tartrate precipitation, filtrated on paper filter, and then bottled in 0.75 l bottles. After 8 months of in-bottle ageing, 6 bottles of each vine group, were analysed as shown in Table 1. Analysis was in accordance with the methods of the Official Gazette of the European Communities (1990).

Sample extraction and chromatographic analysis

Berry samples

From each 10 Kg batch of grape clusters, berries were extracted and analysed immediately after harvesting. One hundred grams of berries was randomly taken from each batch and mixed with a 200 ml chloroform/ethyl acetate (50:50 v:v) solution for 1 min, thereafter the mix was homogenised with ultra-turrax for 5 min. The homogenised substance was transferred to a centrifuge tube and centrifuged at 6000 rpm for 3 min, 10 ml of the organic layer was vacuum-dried with a Rotavapor (Steroglass, Milan, Italy) at 40°C. The extract was dissolved in 1 ml absolute ethanol (Sigma-Aldrich, Milan, Italy) and filtered through a 0.45 μm filter (Millipore, Billerice, MA, USA). The filtrate was collected and stored at 4°C until used in HPLC analysis. During the entire operation light exposure was avoided throughout to prevent isomerisation of trans-resveratrol to the cis form.

Wine samples

Wine samples were extracted using a liquid-liquid procedure. Samples were acidified to pH 2.0 using HCl 10 M. Acidified wine (5 ml) was treated with 5 ml diethyl ether on an orbital shaker at 210 rpm for 25 min, the organic layer was removed, and a further 5 ml diethyl ether was added to the sample and treated in the same manner. The organic phases were combined and vacuum-dried with a Rotavapor (Steroglass) at 40°C. The extract

Table 1. Chemical analysis carried out on cv. Trebbiano d'Abruzzo berries, musts and wines during the trial.

Parameter	Sample	Unit of measurement	Method of analysis
trans-Resveratrol	Berries and Wines	$\mu g\ g^{-1}$; $\mu g\ ml^{-1}$	HPLC
(+)-Catechins	Berries and Wines	$\mu g\ g^{-1}$; $\mu g\ ml^{-1}$	HPLC
(-)-Epicatechins	Berries and Wines	$\mu g\ g^{-1}$; $\mu g\ ml^{-1}$	HPLC
Reducing sugars	Musts	$g\ l^{-1}$	Fheling
Total nitrogen	Musts	$mg\ l^{-1}$	Kjeldal
Total acidity	Musts and wines	$g\ l^{-1}$	Acid/base titration
Malic acid	Musts and wines	$g\ l^{-1}$	Enzymatic
Tartaric acid	Musts and wines	$g\ l^{-1}$	Spectrophotometric
Lactic acid	Wines	$g\ l^{-1}$	Enzymatic
Ashes	Musts and wines	$g\ l^{-1}$	Gravimetry
Potassium	Musts and wines	$mg\ l^{-1}$	Atomic absorption
Iron	Musts and wines	$mg\ l^{-1}$	Atomic absorption
Magnesium	Musts and wines	$mg\ l^{-1}$	Atomic absorption
pH 20°C	Musts and wines	-	Potentiometric
Total polyphenols[a]	Musts and wines	$mg\ l^{-1}$	Folin-Ciocalteau
Ethyl alcohol	Wines	% vol.	Distillation
Neat extract	Wines	$g\ l^{-1}$	Gravimetry
Residual sugars	Wines	$g\ l^{-1}$	Fheling

[a] Total polyphenols were expressed as gallic acid equivalent.

was dissolved in 1 ml absolute ethanol (Sigma-Aldrich) and stored at 4°C until used in HPLC analysis. During this operation light exposure was also avoided throughout to prevent isomerisation of trans resveratrol to the cis form.

HPLC analysis

Trans-resveratrol, (+)-catechin, and (-)-epicatechin were determined using RP-HPLC (Table 1). The chromatographic equipment consisted of a Perkin Elmer Series 200 System. The column was a RP C18 Spherisorb 5 μm, 250×4 mm (Brownlee, Applied Biosystems, Foster City, CA, USA). Detection was with a UV-VIS, dual channel and a diode array detector (DAD).

Analytical conditions, adapted from Jeandet *et al.*, 1995, were: solvent A, H_2O-CH_3COOH (5%); solvent B, acetonitrile. The gradient was from 10% to 70% solvent A in 28 minutes at a flow rate of 1 ml min^{-1}.

The relevant eluting peaks, trans-resveratrol, (+)-catechin, (-)-epicatechin, and cis resveratrol were monitored at both 307 and 280 nm.

Linear calibrations were obtained for trans-resveratrol, (+)-catechin and (-)-epicatechin and used for quantification. Peaks were identified using retention time and spectra comparison. Standard spectra were collected during calibrations.

Statistical analysis

The statistical analysis was carried out in each vineyard separately. Data from each vineyard were statistically evaluated by separately comparing the results for berries, musts and wines in the six plots.

The statistical analysis comprised analysis of variance and Tukey's test to detect significant differences between berries, musts and wines from each group of vines identified during the two-year study. All the analytical parameters in Table 1 were statistically tested for the vintages of both 2001 and 2002.

Results

Analysis of berries

Berries harvested from symptomatic vines in the Controguerra and Giulianova vineyards had higher levels of resveratrol both in 2001 and 2002 than either healthy or diseased asymptomatic vines (Fig. 1).

The levels of (-)-epicatechin in the berries did not vary significantly between vineyards or between years or vine groups, but (+)-catechin levels presented a more complex picture. (+)-Catechin levels likewise did not vary between vineyards or vine groups in 2001, but in 2002 in the Giulianova vineyard (+)-catechin levels were significantly higher in berries from the symptomatic vines (Table 4).

Analysis of musts

In both 2001 and 2002, there was a strong decline in the reducing sugars of the musts of symptomatic vines from the Controguerra and the Giulianova vineyards as compared with musts from the other vine groups. Reducing sugars in musts from diseased asymptomatic vines were only slightly and non-significantly lower than those in musts from healthy vines (Table 2).

In both vineyards and in both years of the study, the must of symptomatic vines had significantly higher levels of total acidity, malic acid and total polyphenols than the must of other vines. In the must of symptomatic vines, tartaric acid levels were also significantly higher (though somewhat less so) in the Controguerra vineyard, and even in the Giulianova vineyard, though here the difference was not significant. Must pH did not vary significantly between vine groups in all vineyards, or between test years (Table 2).

In the 2001 and 2002 must samples, total nitrogen and ashes were significantly higher in musts from symptomatic vines from both vineyards; and so was the level of potassium in 2001, in both vineyards, but in 2002, though potassium was still significantly higher in the Controguerra vineyard, the increase in the Giulianova vineyard was now lower, and no longer significant (Table 4).

In both vineyards and both test years, iron and magnesium levels in the musts from symptomatic vines were significantly lower than were levels of these metals in the other vine groups (Table 4).

Analysis of wines

The resveratrol content of wines tended to level off in both vineyards in both years and was in any case low and similar between vine groups (Fig. 1).

The wines of both vineyards from all groups of vines had higher concentrations of (+)-catechins than did the corresponding musts, whose(+)-cate-

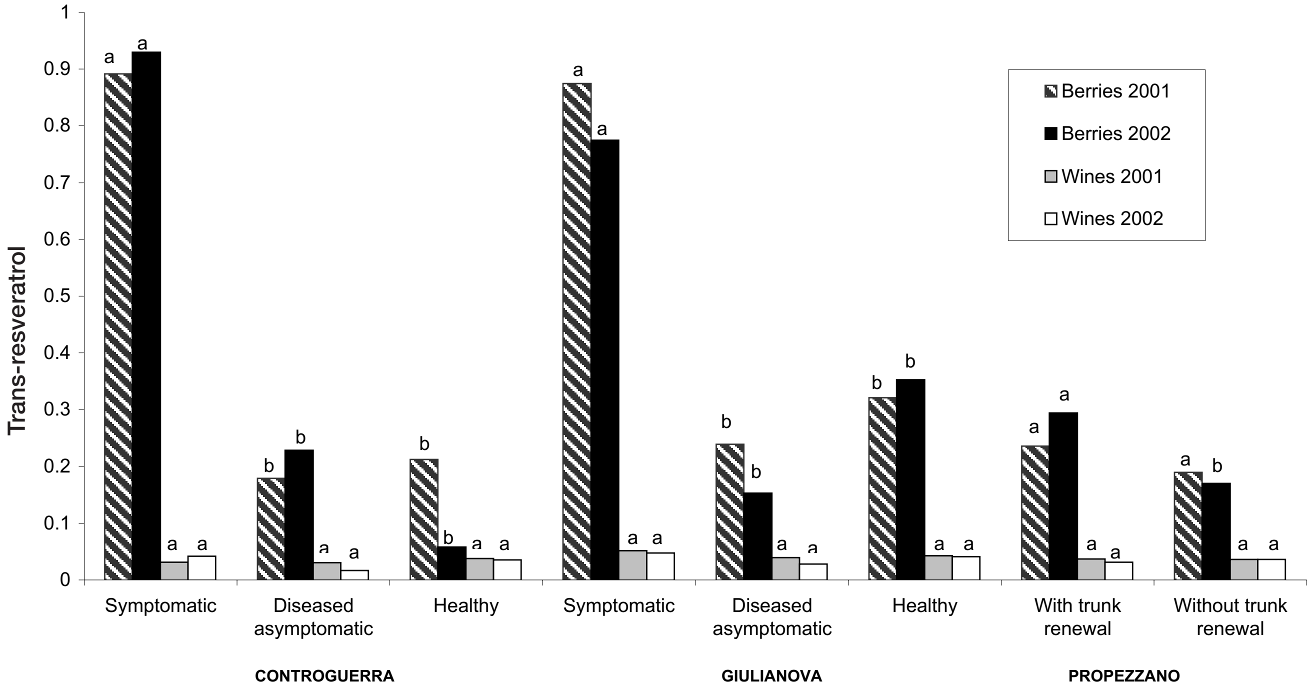

Fig. 1. Trans-resveratrol content of berries (μg g^{-1}) and wines (μg ml^{-1}) obtained from different groups of vines located in three vineyards (cv. Trebbiano d'Abruzzo) in the Province of Teramo (Italy). The statistical analysis separately compared berries and wines from each grapevine group in every vineyard and year. Values followed by the same letter do not differ statistically according to Tukey's test (P=0.05); each value is the mean of 6 replicates of the grape data and 6 replicates of the wine data.

chins values did not differ significantly between vine groups, vineyards or test years. (-)-Epicatechin did not vary significantly between any of the wines (Table 5).

The alcohol content of wines from asymptomatic diseased vines from the Controguerra vineyard in 2001 was lower (-0.36% vol.) than that of wines from healthy vines: the fact that sugar levels varied non-significantly between musts did not translate into significant differences in alcohol content between the resulting wines.

Between wines from healthy vines and wines from symptomatic vines the difference in alcohol content was larger and became significant (-3.08% vol.). The corresponding data for alcohol content from the Controguerra (2002) and Giulianova (2001 and 2002) vineyards were very similar (Table 3).

The neat extract followed the same trend as that described for alcohol content in the different groups. Residual sugar values of all wines were normal but lactic acid levels were significantly higher in wines from symptomatic vines in both vineyards (Table 3).

The other parameters followed the same trend as those of the musts in both vineyards (Tables 3 and 5). All the data on wines for 2001 and 2002 overlapped.

Analysis of berries, musts and wines from renewed vines

In the Propezzano vineyard resveratrol levels in berries from restored vines with trunk renewal were non-significantly higher than those from healthy unrenewed vines in 2001, but in 2002 this difference became significant (Fig. 1).

The musts of vines with trunk renewal had higher total nitrogen than healthy unrenewed vines, but both musts and wines did not differ between trunk renewed and healthy unrenewed vines in any of the other parameters (Tables 6 and 7). Here too data for 2001 and 2002 were very similar.

Table 2. Chemical analysis carried out in two vineyards (cv. Trebbiano d'Abruzzo) on musts from esca-infected vines with and without foliar symptoms and from healthy vines (Part a).

Vineyard location	Vine group[a]	Reducing sugars g l^{-1}		pH		Total acidity g l^{-1}		Tartaric acid g l^{-1}		Malic acid g l^{-1}		Total phenols mg l^{-1}	
		2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Controguerra	Symptomatic	152.23 a[b]	140.48 a	3.16 a	3.14 a	9.12 a	9.50 a	5.43 a	5.80 a	4.60 a	4.90 a	144.23 a	137.21 a
"	Diseased/Asymptomatic	196.31 b	180.14 b	3.17 a	3.33 a	6.23 b	6.30 b	5.11 a	5.20 a	2.43 b	2.84 b	100.11 b	94.89 b
"	Healthy	200.11 b	187.46 b	3.21 a	3.35 a	5.55 b	5.80 b	4.43 b	4.85 b	2.08 b	2.75 b	110.22 b	111.01 b
Giulianova	Symptomatic	170.35 a	180.42 a	3.14 a	3.06 a	8.86 a	8.40 a	5.33 a	5.70 a	4.28 a	4.30 a	177.02 a	164.07 a
"	Diseased/Asymptomatic	212.56 b	206.82 b	3.16 a	3.12 a	7.12 b	6.90 b	5.23 a	5.50 a	3.11 b	3.15 b	75.76 b	62.66 b
"	Healthy	214.13 b	213.20 b	3.15 a	3.10 a	6.64 b	6.50 b	5.12 a	5.45 a	2.25 b	2.75 b	77.89 b	61.99 b

[a] The statistical analysis separately compared musts and wines from each grapevine group in every vineyard and year.
[b] Values followed by the same letter do not differ statistically according to Tukey's test (P=0.05); each value is the mean of 6 replications of the grape data and 6 replications of the wine data.

Table 3. Chemical analysis carried out in two vineyards (cv. Trebbiano d'Abruzzo) on wines from esca-infected vines with and without foliar symptoms and from healthy vines (Part a).

Vineyard location	Vine group[a]	Ethyl alchool % vol.		Residual sugars g l^{-1}		Neat extract g l^{-1}		pH		Total acidity g l^{-1}		Tartaric acid g l^{-1}		Malic acid g l^{-1}		Lactic acid g l^{-1}	
		2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Controguerra	Symptomatic	8.76 a[b]	8.42 a	1.33 a	1.20 a	16.50 a	16.00 a	3.01 a	3.23 a	7.30 a	7.60 a	3.13 a	3.35 a	2.55 a	2.70 a	0.60 a	0.50 a
"	Diseased/Asymptomatic	11.48 b	10.65 b	1.26 a	1.75 a	17.85 b	17.25 b	3.13 a	3.12 a	6.86 b	7.00 ab	2.98 a	2.90 a	1.23 b	1.41 b	0.30 b	0.28 b
"	Healthy	11.84 b	11.41 b	1.78 a	2.65 a	19.23 c	18.45 b	3.12 a	3.24 a	6.90 b	5.90 b	2.65 a	2.75 a	1.22 b	1.35 b	0.24 b	0.30 b
Giulianova	Symptomatic	9.98 a	10.51 a	2.65 a	1.35 a	16.02 a	16.35 a	3.21 a	3.05 a	8.30 a	8.00 a	3.23 a	2.94 a	2.95 a	2.84 a	0.54 a	0.62 a
"	Diseased/Asymptomatic	11.90 b	11.65 b	2.25 a	2.40 a	19.60 b	19.20 b	3.33 a	3.10 a	7.23 b	7.80 a	3.01 a	2.85 a	1.87 b	1.84 b	0.23 b	0.35 b
"	Healthy	12.24 b	12.14 b	2.88 a	1.80 a	20.25 b	20.20 b	3.23 a	3.11 a	7.15 b	7.80 a	3.13 a	2.70 a	1.85 b	1.75 b	0.21 b	0.30 b

[a] See Table 2.
[b] See Table 2.

Table 4. Chemical analysis carried out on two vineyards (cv. Trebbiano d'Abruzzo) on musts from esca-infected vines with and without foliar symptoms and from healthy vines (Part b).

Vineyard location	Vine group[a]	Total nitrogen mg l^{-1}		Ashes g l^{-1}		Potassium mg l^{-1}		Iron mg l^{-1}		Magnesium mg l^{-1}		(+)-Catechin[c] $\mu g\ g^{-1}$		(-)-Epicatechin[c] $\mu g\ g^{-1}$	
		2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Controguerra	Symptomatic	440 a[b]	420 a	3.60 a	3.46 a	1210 a	1100 a	2.12 a	2.68 a	66 a	65 a	1.99 a	1.78 a	1.18 a	0.85 a
"	Diseased/Asymptomatic	165 b	140 b	3.40 ab	3.26 ab	1050 b	985 b	3.54 b	3.86 b	76 ab	74 b	1.84 a	3.61 a	2.01 a	3.25 a
"	Healthy	134 b	112 b	3.11 b	2.78 b	998 b	1010 b	3.67 b	3.20 b	81 b	78 b	2.16 a	1.85 a	2.22 a	1.77 a
Giulianova	Symptomatic	401 a	392 a	3.74 a	3.62 a	1165 a	1105 a	3.03 a	3.82 a	66 a	68 a	3.11 a	3.89 a	1.98 a	1.50 a
"	Diseased/Asymptomatic	173 b	168 b	3.71 a	3.54 a	1010 b	1040 a	4.87 b	5.60 b	89 b	77 ab	3.27 a	2.19 b	1.21 a	0.90 a
"	Healthy	177 b	168 b	3.38 b	3.02 b	1012 b	1065 a	5.15 b	4.60 b	89 b	86 b	2.98 a	2.01 b	1.32 a	1.70 a

[a] See Table 2.
[b] See Table 2.
[c] Determinations of these parameters were carried out on the berries.

Table 5. Chemical analysis carried out in two vineyards (cv. Trebbiano d'Abruzzo) on wines from esca-infected vines with and without foliar symptoms and from
healthy vines (Part b).

Vineyard location	Vine group[a]	Ashes g l^{-1}		Potassium mg l^{-1}		Iron mg l^{-1}		Magnesium mg l^{-1}		(+)-Catechin[c] $\mu g\ ml^{-1}$		(-)-Epicatechin[c] $\mu g\ ml^{-1}$	
		2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Controguerra	Symptomatic	1.97 a[b]	1.84 a	812 a	710 a	1.12 a	1.34 a	52 a	48 a	22.78 a	14.75 a	3.76 a	2.85 a
"	Diseased/Asymptomatic	1.77 a	1.66 a	801 a	690 ab	1.66 b	1.76 b	62 b	52 ab	23.89 a	24.81 a	2.11 a	3.42 a
"	Healthy	1.87 a	1.62 a	788 a	650 b	1.63 b	1.64 b	70 b	65 b	26.26 a	21.67 a	5.33 a	6.04 a
Giulianova	Symptomatic	1.83 a	1.76 a	780 a	740 a	1.25 a	1.40 a	48 a	46 a	26.16 a	30.17 a	3.45 a	3.18 a
"	Diseased/Asymptomatic	1.76 a	1.68 a	712 b	710 a	1.78 b	1.94 b	69 b	56 b	32.64 a	33.03 a	3.78 a	5.22 a
"	Healthy	1.73 a	1.69 a	710 b	720 a	1.88 b	1.86 b	68 b	70 c	21.22 a	24.77 a	4.87 a	7.53 a

[a] See Table 2.
[b] See Table 2.
[c] See Table 4.

Table 6. Chemical analysis of musts and wines from restored vines with trunk renewal and healthy vines without trunk renewal from a cv. Trebbiano d'Abruzzo vineyard at Propezzano (Teramo) with an esca presence (Part a).

Vineyard location	Sample	Vine group[a]	Reducing sugars g l -1		pH		Total acidity g l -1		Tartaric acid g l -1		Malic acid g l -1		Lactic acid g l -1		Total alcohol % vol		Residual sugars g l -1		Neat extract g l -1		Total phenols mg l -1	
			2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Propezzano	Musts	With trunk-renewal	186.23a[b]	175.82a	3.01a	2.98a	8.63a	8.50a	5.98a	5.80a	4.78a	4.30a									71.78a	66.69a
"	"	Without trunk-renewal	184.11a	172.40a	3.03a	2.95a	8.50a	8.30a	5.78a	5.70a	4.89a	4.12a									59.31a	41.17a
"	Wines	With trunk-renewal				3.04a	3.02a	8.11a	8.20a	3.27a	3.10a	2.87a	2.60a	0.48a	0.40a	11.01a	10.37a	1.23a	1.20a	18.60a	18.30a	
"	"	Without trunk-renewal				3.03a	3.03a	8.12a	8.20a	3.34a	3.20a	2.80a	2.45a	0.50a	0.36a	10.96a	10.10a	1.22a	1.35a	18.45a	18.30a	

[a] See Table 2.
[b] See Table 2.

Table 7. Chemical analysis of musts and wines from restored vines with trunk renewal and healthy vines without trunk renewal from a cv. Trebbiano d'Abruzzo vineyard at Propezzano (Teramo) with an esca presence (Part b).

Vineyard location	Sample	Vine group[a]	Total nitrogen mg l -1		Potassium mg l -1		Magnesium mg l -1		Iron mg l -1		Ashes g l -1		(+)-Catechin[c] $\mu g\ g^{-1}/\mu g\ ml^{-1}$		(-)-Epicatechins[c] $\mu g\ g^{-1}/\mu g\ ml^{-1}$	
			2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Propezzano	Musts	With trunk-renewal	386 a[b]	336 a	1187 a	1120 a	63.00 a	67.00 a	5.90 a	6.00 a	3.01 a	3.21 a	1.98 a[c]	1.46 a[c]	2.56 a[c]	2.48 a[c]
"	"	Without trunk-renewal	298 b	224 b	1200 a	1145 a	58.00 a	61.00 a	5.87 a	5.54 a	3.13 a	3.12 a	2.03 a[c]	1.68 a[c]	2.55 a[c]	1.41 a[c]
"	Wines	With trunk-renewal			710 a	740 a	50 a	52 a	2.14 a	2.14 a	1.76 a	1.84 a	33.35 a	38.35 a	1.80 a	1.90 a
"	"	Without trunk-renewal			722 a	725 a	46 a	47 a	2.54 a	2.30 a	1.74 a	1.74 a	36.11 a	31.48 a	1.86 a	1.26 a

[a] See Table 2.
[b] See Table 2.
[c] See Table 4.

Discussion

Resveratrol increased in the berries of symptomatic infected vines, but not in the berries of asymptomatic infected vines. This suggest that resveratrol does not migrate from the wood towards the grapes, for if it did, resveratrol would have increased equally in these two vine groups. All infected vines, whether symptomatic or asymptomatic, have more or less the same level of disease, with the same extensive inner wood necrosis, where resveratrol levels are higher than in healthy wood (Amalfitano *et al*., 2000).

The resveratrol increase in the berries of symptomatic vines could be due to leaf necrosis causing a migration of resveratrol from the symptomatic leaves to the grapes, or to black measles on the berry skin. Measles and leaf necrosis are known to be the external symptoms of a parasitic process affecting the vine wood inside the plant, and such abiotic injuries cause resveratrol production to increase (Jeandet *et al*., 1995).

Variations in (+)-catechin and (-)-epicatechin levels in the berries were not correlated with esca symptoms: nevertheless, the contrasting data obtained in 2002 in the two vineyards need to be further investigated to determine whether these molecules have a role in symptom occurrence (Mugnai *et al*., 1999).

The strong decrease in reducing sugars, occurring only in musts from symptomatic vines was also found in the preliminary investigation carried out in the same vineyards in 2000. Compared with that preliminary investigation, the present study found that the difference between reducing sugars in symptomatic vines and those in asymptomatic diseased or healthy vines had become greater (Calzarano *et al*., 2001). This decrease was possibly due to a loss in functionality of the tiger-striped leaves starting with veraison, leading to a reduction in hexosis migration towards the grapes. The decrease in reducing sugars of the must from symptomatic vines was similar in both the Giulianova and the Controguerra vineyard in both years of the study.

Total acidity, which is prevalently due to malic acid augmentation, was higher in the must of symptomatic vines. The flow of hexosis towards the grape pericarp cells, which begins at the onset of ripening, also occurs in the symptomatic vines. Possibly, the migration of sugars towards the grapes continues, though more slowly, after the veraison, when foliar symptoms start to appear on some of the vine leaves. Malic acid, on the other hand, is produced and then stored in the berry mesocarp (Hale, 1962; Koch and Alleweldt, 1978; Coombe and Matile, 1980). Mesocarp consists of "specialized storage containers" that take over and sequester imported components from the peripheral parts of the berry (Kriedemann, 1969; Steffan and Rapp, 1979). The same function is also performed by "non-storage-type" cells which, under stress conditions that lower the photosynthesis, accumulate metabolites, including sugars. In both cases the sugars can be easily remetabolised in order to guarantee vital cellular functions (Ruffner, 1982). A pathological state usually leads to an increase in respiration: the increase in malic acid and decrease in sugars, as observed in musts from symptomatic vines, suggests that the sugars serve as respiration substrates, producing malic acid via glycolysis (Stafford and Loewus, 1958; Beckman, 1964; Hardy, 1967; Daly, 1976; Asahi *et al*., 1979).

Although a reduced respiratory metabolism in diseased plants, with the lower photosynthetic rate accompanying it, has been associated with water stress, this metabolism can be involved here. In that case a decrease of malic acid respiration would be expected, and the higher malic acid concentration in musts from symptomatic vines should then be explained as a ripening delay.

In the musts of symptomatic vines, the smaller increases in tartaric acid, compared with malic acid, may depend on the lack of correlation of tartaric acid metabolism with the respiration increase and hence on the overall deterioration of acidic metabolism in these plants (Fregoni, 1999).

The rather similar pH values recorded in the different vine groups in spite of the greater total acidity in symptomatic vines might be due to the fact that vines selectively absorbed cations to produce salts, buffering the acidity and adjusting the pH to normal values; the musts of symptomatic vines showed greatly increased potassium levels, possibly because potassium malate and tartrate are formed by the mechanisms described.

The significant increases in nitrogen levels in symptomatic vines from both the Giulianova and the Controguerra vineyards, recorded in 2000 and again in 2001 and 2002, may depend on protein hydrolysis occurring in diseased leaves generating aminoacids that migrate to the grapes (Calzarano *et al*., 2001). The high nitrogen level in musts from symptomatic vines may also account for a significant increase in polyphenols reported to occur in those musts (Kliewer, 1977). Higher nitrogen levels might also be caused by the foliar symptoms themselves, whose functional effect is similar to partial defoliation (Hunter *et al*., 1991).

A correlation between increased water stress and higher levels of polyphenols is often reported in the literature, but such a correlation hardly appears possible in esca-infected vines (Nadal and Arola, 1995; Ojeda *et al*., 2002). The foliar symptoms thought to be caused by water stress are probably due to enzymes or toxins, since water stress is not a consequence of esca (Mugnai *et al*., 1999).

The somewhat higher levels of ashes in symptomatic vines appear to be related to the increase in potassium, but might also be due to other as yet undetermined cations.

Unlike the measurements mentioned so far, iron and magnesium levels in musts from symptomatic vines were significantly lower: perhaps because they are less mobile since iron is involved in cytochrom structures and magnesium in chlorophyll structures, and because they are lost as a result of the formation of foliar necrosis. Alternatively, iron and magnesium may be required by the leaves, which mobilise them from the grapes to remedy the deficiency in these metals caused by injury.

Chemical analysis from wines was in line with that from musts as a normal evolution during the wine making process. The higher level of lactic acid in wines from symptomatic vines corresponded to the higher nitrogen levels observed in the musts and the wines of these same vines. Must microorganisms took advantage of the increased amounts of nutrients: yeasts-driven fermentation occurred at a faster rate in must from symptomatic vines, and the lactic bacteria were promoted by lactic acid production.

The lower levels of resveratrol and the level-off of this substance in white wines, as compared with the berries, reflected white wine making techniques, which require berry skin removal (Siemann and Creasy, 1992).

The significantly higher nitrogen levels in the musts of trunk-renewed vines from the Propezzano vineyard might be related to the greater nitrogen absorption by these vines in order to regenerate tissues in the epigeal portion of the vine and to restore the imbalance between this portion and the root system. This finding did not confirm measurements carried out during the preliminary investigation of 2000, which found no increase in the nitrogen levels of musts from trunk-renewed vines, and therefore it needs to be confirmed.

Resveratrol levels in the berries from trunk-renewed vines were higher. The reason for this is difficult to see, though a dependence on nitrogen augmentation might be suggested; however, further investigation is required to fully understand this increase.

In conclusion, almost all the parameters studied were consistent and similar over the two years of the study, though there was some disagreement between this study and the report of 2000, and many questions raised will have to be answered by an extension of the investigation. In order to understand the kinetics of the molecules involved in producing vine and wine quality and to further understanding of esca-induced modifications in vines/wines it appears interesting to study samples collected from different plant parts at different times of the growing season.

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