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Preliminary evaluation of variations in composition induced by esca on cv. Trebbiano d'Abruzzo grapes and wines

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Summary. The present study examined the effect on quality caused by esca in both grape clusters and wines. In the last year (2000) of an eight-year survey of esca foliar symptoms, the vines of two cv. Trebbiano d'Abruzzo vineyards fell at the moment of grape clusters sampling, into 3 groups: 1. vines with esca symptoms; 2. vines that happened to be asymptomatic but that were known to be diseased because they had show leaf symptoms in at least one other survey year and 3. healthy vines. In a third vineyard, in the same growing area, grape clusters were sampled from vines with trunk renewal that had been restored and from unrenewed vines that had never shown esca leaf symptoms and were consequently presumed to be healthy. Preliminary results on grape clusters in the first two vineyards showed differences in composition between symptomatic vines on the one hand, and asymptomatic/diseased vines and healthy vines on the other, with symptomatic vines having lower levels of reducing sugars and a higher nitrogen concentration. As a result the alcohol content of wines from symptomatic vines was about 1% v:v lower than that of wines from the other two groups of vines. Grape clusters and wines from diseased/asymptomatic vines and healthy vines did not differ in the compositional parameters used in the test except for total polyphenols which gave contrasting data among the first two vineyards. In grape clusters and wines from the third vineyard the preliminary results likewise revealed no difference between vines with trunk renewal and unrenewed healthy **v**ines. This finding would seem to corroborate the practice of trunk renewal as a means of restoring both vines and the quality of grape clusters. Research is continuing to evaluate the reliability of these results.

Key words: chemical analysis, grape clusters, must, trunk renewal, quality.

Introduction

Esca is a disease caused by a number of fungi that invade the plant and produce different types of wood deterioration. In the context of the diversity of symptoms that can be attributed to esca, it has recently been postulated that esca consists of particular syndromes each of which causes specific symptoms and damage. Of these syndromes, which differ in their causal organism and in the age of the vines affected by them, the syndrome most similar to our usual notion of esca is "esca proper". Esca proper usually affects adult vines, and occurs when mitosporic fungi in the genera *Phaeomoniella* and *Phaeoacremonium* and the basidiomycete *Fomitiporia punctata,* acting either in combination or in succession, bring about discoloration of the wood and white rot, foliar symptoms or apoplexy (Graniti *et al.,* 2000). In Abruzzo, an important wine-growing area in central Italy with some 35,000 ha under vines, the majority of vineyards consist of adult

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vines, and indeed the prevalent form of esca here is "esca proper" (Calzarano, unpublished data). Esca-diseased vines suffer direct damage following the death of entire vines (apoplexy) or the wilting of some canes and clusters, but probably also indirect damage since, at least the clusters from vines with foliar symptoms (but not wilting) and the wine made from them are probably of poor quality. However, the only non-disruptive way to identify esca in vines is by visual inspection for foliar symptoms, but when vines become infected, they do not regularly show symptoms every year. Infected vines sometimes go through a growing season or seasons during which the foliar signs of esca fail to appear, and those vines will seem esca free in that season or those seasons. Therefore to identify positively all those vines that are certainly infected with esca, and to distinguish infected, but apparently healthy because asymptomatic vines, from truly healthy vines, at least 5–6 years of continuous annual inspection are required (Calzarano and Di Marco, 1997; Mugnai *et al.*, 1999; Surico et al., 2000).

This paper presents preliminary data on grape and wine quality in vines growing in vineyards with an esca presence that have been monitored for eight years.

Materials and methods

Recording foliar symptoms and collection of samples

Vines infected with esca were identified in three commercial cv. Trebbiano d'Abruzzo vineyards, 22 years old, located at three different localities in the wine-growing area of the Province of Teramo: Controguerra, Giulianova and Propezzano. The first two vineyards had 2000 vines and an area of 18,000 m^2 each; one was grown with the spur-pruned cordon trellis system, the other with the Tendone system. In these vineyards leaf symptoms were recorded every year for eight years, from 1993 to 2000. All vines were numbered according to their place in the row, and any symptomatic vines were recorded each year in September, when visible leaf symptoms in this geographical area are most conspicuous.

In this way after eight years of consecutive annual inspections most vines infected with esca were identified. At the end of this inspection period, vines could be divided into three groups:

- vines with visible leaf symptoms in the last inspection year (2000);
- vines without visible symptoms in the last inspection year, but known to be infected because they had shown symptoms in at least one previous inspection year;
- vines presumed healthy because they never showed any esca symptoms in the inspection period.

In the last year of the inspection period (2000), when the grapes were ready for harvesting, grape clusters from 100 vines per vine-group (symptomatic, diseased/asymptomatic and healthy vines) located at different places in the vineyard were collected for sampling. Only clusters from the middle of the vine-shoots of each vine were collected to secure a sample without the natural variations between clusters arising from their position along the shoot. From each vine-group in each vineyard, 100 kg of grapes (1 kg of grapes from each vine) was collected for chemical analysis.

In the third vineyard, which was grown according to the Tendone trellis system, a plot of 9000 m^2 including 1000 vines was delimited and all the vines in it monitored for the same eight-year period from 1993 to 2000. In the spring of 1996, the fourth year of the monitoring period, all vines that had been symptomatic in any previous inspection year or years had their trunk renewed. At the end of the inspection period in 2000, 100 kg of grapes was collected from 100 of these "renewed" vines that had not been symptomatic in any of the four years after trunk renewal. A further 100 kg of grapes were harvested from 100 unrenewed vines that were presumed healthy, having been asymptomatic in all eight years of the monitoring period.

Chemical analysis of grape clusters

Immediately after harvesting, each group of grape clusters was crushed with a stalk-remover-grape crusher and pressed with a vertical oleodynamic hydraulic press. Must yield was standardised to 65% (w:v). Sixty-five l of must was obtained from each vine group, and from each of these quantities of must, 6 0.5-l bottles were filled, each of which was a repetition. Repetitions of each group of vines were analysed to obtain the determinations shown in Table 1. Analyses were carried out in accordance with the methods of the Official Gazette of the European Communities (1990). Total polyphenols, expressed as mg l^{-1} gallic acid, were evaluated in accordance with the method of Singleton and Rossi (1965).

Wine making and chemical analysis of the wines

Sixty litres of the remaining must per group was made into wine separately at a large-scale experimental winery, the Centro Ricerche Viticole ed Enologiche d'Abruzzo. After pressing, the must from each vine group was clarified by adding 8 g hl^{-1} gelatin and 20 g hl^{-1} silicasol. Eight g hl^{-1} sulphur dioxide was then added. After the skins were separated, the must was inoculated with 20 g hl^{-1} DV 10 yeast, and 30 g hl^{-1} thiamine was added as yeast nutrient. At the end of fermentation in October, the must was decanted and 4 g hl^{-1} sulphur dioxide was added. In the following month the product underwent the usual transformation and, after being decanted once again, was bottled. In May, after a short period of in-bottle aging, 10 wine samples in 10 0.75-l bottles (1 sample per bottle) were drawn off from each type of must. Each of the samples was a repetition, and its chemical composition was analysed as shown in Table 1.

Statistical analysis

Statistical analysis carried out comprised analysis of variance and Tukey's test, to show, separately in each vineyard and for each parameter evaluated, significant differences between grape clusters or wines from each group of vines identified in 2000.

Results

In the grape data from the first two vineyards (Table 2), grapes from symptomatic vines showed a significant decrease in reducing sugars and a significant increase in total nitrogen compared with grapes from the other two vine groups. There was no difference in these two variables between grapes from asymptomatic/diseased and healthy vines. The three grape-cluster groups tested did not differ significantly in total acidity or pH. Alcoholic content of wines from symptomatic vines was about 1% (vol.) less than that of wines from diseased/asymptomatic or healthy vines. The total acidity of the wines was greater than that of the originating grape clusters in both symptomatic and healthy vines from Controguerra, and in healthy vines from Giulianova, but the pH of all wine groups was unchanged from their respective cluster groups. Residual sugar levels of all wine groups were always within normal range.

In the Giulianova and Controguerra vineyards total polyphenols increased in the grape clusters from some vine groups and the wines produced from them, but the groups with polyphenol increases in the vineyards were not the same. In the Giulianova vineyard the increase occurred in the grape clusters of the symptomatic and diseased/asymptomatic groups of vines and the wines pressed from them. In the Controguerra vineyard, by contrast, total polyphenols increased in the grape clusters of the symptomatic and healthy vines and their resulting wines.

In the third vineyard (at Propezzano) none of the variables tested differed between restored vines with trunk renewal and healthy unrenewed vines (Table 3).

Discussion

The lower levels of reducing sugars in the clusters of the Controguerra and Giulianova vineyards occurred only on vines with actual leaf symptoms;

Table 1. Chemical analysis of cv. Trebbiano d'Abruzzo grapes and wines collected from symptomatic, diseased/asymptomatic and healthy vines, and from restored vines with trunk renewal and healthy unrenewed vines in vineyards with esca.

Parameter	Sample	Unit of measurement	Method of analysis
Reducing sugars	Grapes	g l^{-1}	Feheling
Total nitrogen	Grapes	mg l^{-1}	Kjeldal
Total acidity	Grapes and wines	g l^{-1}	Acid/base titration
pH 20°C	Grapes and wines	-	Potentiometric
Total polyphenols	Grapes and wines	mg l^{-1} [a]	Spectrophotometric
Ethyl alcohol	Wines	% vol.	Distillation
Residual sugars	Wines	g %	Enzymatic

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

Table 2. Chemical analysis carried out on two vineyards of the cv. Trebbiano d'Abruzzo, located in Controguerra and Giulianova (Teramo, Italy), on grape clusters and wines obtained from esca-infected vines with and without foliar symptoms, and from healthy vines.

Vineyard location	Sample	Vine group[a]	Reducing sugars g l^{-1}	Total nitrogen mg l^{-1}	Total acidity g l^{-1}	pH	Total polyphenols mg l^{-1}	Ethyl alcohol % vol.	Residual sugars g (%)
Controguerra	Grapes	Symptomatic	135.3 a[b]	298.6 a	6.6 a	3.18 a	560 b		
"	"	Diseased/asymptomatic	170.3 b	153.5 b	6.1 a	3.17 a	481 a		
"	"	Healthy	175.5 b	155 b	6.5 a	3.27 a	580 b		
"	Wine	Symptomatic			7.2 a	3.24 a	491 b	9.35 a	0.13 a
"	"	Diseased/asymptomatic			6.1 a	3.21 a	437 a	10.48 b	0.13 a
"	"	Healthy			6.9 a	3.29 a	503 b	10.46 b	0.68 a
Giulianova	Grapes	Symptomatic	139.9 a	339.7 a	5.6 a	3.03 a	490 b		
"	"	Diseased/asymptomatic	184.8 b	243.7 b	5.6 a	3.18 a	469 ab		
"	"	Healthy	190.5 b	257.6 b	6.5 a	3.13 a	408 a		
"	Wine	Symptomatic			5.3 a	3.23 a	445 a	10.41 a	0.11 a
"	"	Diseased/asymptomatic			5.4 a	3.29 a	411 a	11.76 b	0.09 a
"	"	Healthy			7.6 b	3.21 a	395 a	11.56 b	0.14 a

Table 3. Chemical analysis of grape clusters 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.

Vineyard location	Sample	Vine group[a]	Reducing sugars g l^{-1}	Total nitrogen mg l^{-1}	Total acidity g l^{-1}	pH	Total polyphenols mg l^{-1}	Ethyl alcohol % vol.	Residual sugars g (%)
Propezzano	Grapes	With trunk renewal	174.2 a[b]	253.7 a	6.1 a	3.21 a	458 a		
"	"	Without trunk renewal	182.3 a	261.8 a	6.1 a	3.19 a	470 a		
"	Wine	With trunk renewal			6.2 a	3.21 a	401 a	10.75 a	0.09 a
"	"	Without trunk renewal			6.1 a	3.21 a	391 a	11.24 a	0.11 a

[a] The statistical analysis compared both grape clusters and wine from each grapevine group in each vineyard.
[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 repetitions of the grape data and 10 repetitions of the wine data.

this suggests that these lower levels were caused not so much by wood deterioration as by a reduced photosynthesis in the necrotic leaf blades. The lower level of reducing sugars in the clusters of symptomatic vines corresponded to a lower alcoholic content of the wine obtained from those grapes.

The substantial increase in nitrogen compounds that occurred in the clusters of symptomatic vines is difficult to explain. Perhaps the aminoacids were used as an alternative source of energy to the sugars before hydrolysis of the proteins in the leaves, or the protein component in the wood was degraded by the fungi, releasing nitrogen substances into the xylem (Rayner and Boddy, 1988; Graniti *et al.,* 2001). Total nitrogen levels in the clusters increase when vines are infected with powdery mildew: this increase could be explained as due to an interference of the fungus with the metabolism of nitrogen substances in the plant, or as the direct synthesis of these compounds by the fungi (Piva *et al.,* 1997; Piva *et al.,* 1999).

Total acidity and pH were similar in all three vine groups in both the Controguerra and Giulianova vineyards. This is unlike what occurs with other grapevine diseases, such as downy mildew, powdery mildew or grey mould, in which the pathogens often bring about an increase in the total acidity of the grape clusters (Pallotta *et al.,* 1995; Piva *et al.,* 1999). The explanation for this difference between esca and other diseases is probably to be looked for in the different course of all these diseases. *Oidium tuckeri* and *Botrytis cinerea* affect yield directly, whereas variations caused in the composition of grape clusters by esca pathogens result indirectly from the activity of these pathogens inside the vine plant.

The fact that total acidity of some wines was higher than that of the clusters from which those wines derived could be due to the greater lactic acid produced by lactic bacteria, at the end of alcoholic fermentation, in the presence of residual sugars liable to fermentation.

It remains difficult to see how the conflicting results on total polyphenol levels in the two vineyards can be explained. In the Giulianova vineyard infected vines, both symptomatic and asymptomatic, recorded higher levels of total polyphenols, as generally occurs in vines that activate their defence mechanisms in response to pathogen attack (Pezet and Pont, 1988; Hoos and Blaich, 1990; Jeandet *et al.,* 1995.). In the Controguerra vineyard, it was the symptomatic and the healthy vines that showed higher polyphenol levels. An explanation of this difference will require further and more extensive study.

Perhaps it is relevant here that phenolic compounds synthesized by the vines as defence barriers go up in those particular tissues that undergo pathogen attack. In the case of esca, that means that they go up within the wood of the plant (Amalfitano *et al.,* 2000). However, the variations in composition determined in the present study were calculated from the clusters, which were not themselves attacked directly by the fungi.

As regards the other parameters evaluated, the results suggest that diseased/asymptomatic vines went through the ripening process regularly, producing grapes with characteristics similar to grapes from healthy vines. No difference was detected in the third vineyard between restored vines with trunk renewal healty and vines without. This suggests that trunk renewal is an effective way to restore the quality of yield.

Further studies with a wider spectrum of analysis, to be carried out in the next few years, are already under way to verify the results obtained and the explanations suggested.

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